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Welcome to the 4th International Congress of Molecular Medicine

Dear Colleagues,

On behalf of the Organizing Committee, it is my great pleasure to welcome you to the 4th International Congress of Molecular Medicine.

The congress has brought together recognized scientists and physicians from all around the world to discuss trends, technologies and clinical applications in Molecular Medicine. For the clinician, this is an opportunity to focus on advances in the basic sciences forming the basis of clinical practice. For the scientist, there is a perspective to appreciate the wide-reaching clinical relevance of scientific discovery.

Istanbul is such a vibrant and fascinating city, ancient and modern, religious and secular, Asian and European, mystical and earthly. Therefore, we hope that during the congress you will enjoy a rich scientific program and feel the spirit of Istanbul.

We encourage every participant to be active and use this occasion to exchange ideas, and build collaborations; and we hope that your stay in Istanbul will be academically, educationally and socially rewarding.

On behalf of the Organizing Committee,

Prof. Turgay Isbir
Chairperson of the Turkish Society of Molecular Medicine
Department of Medical Biology, Faculty of Medicine, Yeditepe University, Istanbul, Turkey

Oral Presentations

(Alphabetically by presenter's surname)

1
APOPTOTIC TENDENCY AND GENOMIC ABNORMALITIES OF SMOOTH MUSCLE CELLS IN THORACIC AORTIC ANEURYSMS

Ceyda Açılan[1], Müge Serhatlı[1], Zelal Adıgüzel[1], Ömer Kaçar[1], Altug Tuncer[2] and Kemal Baysal[1,3]

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Background: During the development of aortic aneurysms, degradation of extracellular matrix and loss of smooth muscle cells (SMCs) through apoptosis appear to be the major factors that lead to structural deterioration, leading to progressive dilation. Reactive oxygen species (ROS) are produced well above physiological levels in aneurysm tissues and are known to regulate both of these changes. Here, we hypothesized that: (i) SMCs are more susceptible to apoptosis, (ii) at least some cells undergo apoptosis in response to elevated ROS in the aortic wall and (iii) p53 may be an important player in ROS-induced apoptosis. *Methods:* Cell death in response to H_2O_2 was measured by WST-1 assay and apoptosis was confirmed by AnnexinV/PI, DNA condensation and live-cell microscopy. DNA damage was measured by micronuclei frequency and compared to the percentage of binucleate cells, age, gender, hypertension or aortic diameter. The role of p53 was tested by siRNA silencing. *Results:* While the tendency for apoptosis did not appear to be significantly different when compared to normal cells, the percentage of micronuclei was higher in aneurismal SMCs. Moreover, cell death was unchanged when p53 was reduced. *Conclusion:* Apoptosis of SMCs can still take place in the absence of p53 and there is increased DNA damage in aneurysm samples.

2
FACTOR V LEIDEN AND NATURAL SELECTION

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Background: Factor V Leiden ([FVL] 1691G-A) is a thrombophilic single-point mutation causing activated protein C resistance. Theoretically, high prevalence of the mutation makes it plausible to ask whether FVL has caused selective advantages during evolution. The Turkish population seems to be a good candidate to study the possible selective disadvantages with significantly high FVL. *Results:* Frequency was 11.5 and 8.0% in newborns (57 FVL in 494) and adults (357 FVL in 4,431), respectively. The difference was statistically significant (p=0.015). A question arises: "Did some of the infants with FVL mutation die of clinical conditions related to thromboembolism before reaching adult age and without receiving a specific diagnosis?". This may explain the frequency difference between the newborns and adults. We compared the effect of FVL in thrombotic children with and without FVL. Kaplan–Meier analysis revealed that FVL affects morbidity (p<0.05). Also, FVL increased the risk for pediatric stroke significantly. FVL was also increased among the elderly, aged over 70 years old (29 FVL in 266 (11.5 %)). The difference between the two groups was not significant (p=0.8; p=0.13). *Conclusion:* If these data and hypothesis are verified by other studies, it will be a very important finding to explain advantageous role of FVL for natural selection from an evolutionary point of view.

3
RECOMBINANT FERROPORTIN INFLUENCES THE PARASITE LOAD AND IMMUNE RESPONSES IN BALB/C MICE INFECTED WITH *LEISHMANIA MAJOR*

Mohammad Hossein Alimohammadian[1], Aras Rafiei[2], Seyed Mohammad Reza Fatemi[2], Farhad Riazi-Rad[1], Haiedeh Darabi[1], Vahid Khazee[1] and Soheila Ajdary[1]

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Inoculation of inbred mice by *Leishmania major* results in two distinct patterns. In contrast to resistant mice, BALB/c mice show susceptibility to *Leishmania major* with visceral manifestation, anemia and death. The purpose of this study was to express the recombinant ferroportin (rFpn), a major regulator of iron homeostasis, in enterocytes of *L. major*-infected BALB/c mice. cDNA was prepared from total RNA of ferroportin (Fpn) extracted from zebrafish duodenum. The PCR products were cloned in Topo TA vector and sub-cloned into pEGFP–N1vector. The resulted plasmid (pEGFP-ZFpn) encapsulated in chitosan/alginate nanoparticles was administered orally to *L. major*-infected BALB/c mice. Treated and untreated mice were analyzed for blood hematocrit and serum iron, pathogenicity and cytokine production by ELISA. The results obtained showed high hematocrit and iron levels, a decrease in footpad size and a significant reduction in parasite load in treated mice.

Moreover, lower levels of IL-4 and IL-10 and higher ratios of IFN-/IL-4 were shown in treated mice in comparison to the untreated mice. These data suggest that the expression of rFpn has the ability to increase blood hematocrit and iron, decrease the pathogenicity of the *L. major* infection and suppress the Th2 response and may be used to control leishmaniasis.

4

DNA FINGERPRINTING – A NEW APPROACH IN FORENSIC IDENTIFICATION

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It has long been the ambition of a forensic scientist to be able to identify the origin of blood and body fluid stains in order to establish the relationship between a victim and a suspect with the same degree of certainty as the popular technique of fingerprinting can. The discovery of the "wonder molecule" DNA in the second half of last century and the recognition that it is virtually the universal genetic material made it imperative that, sooner or later, this knowledge would be applied extensively. The methods used in DNA profiling are conventional techniques of molecular biology. The source of DNA can be whole blood, blood stains, semen stains, vaginal swabs, saliva bone marrow, hairs and other muscle tissues. The cells, essentially, must be nucleated as the source of DNA is in the nucleus. There are a number of alternative techniques each most appropriate with a specific source for DNA extraction. For cases of murder and rape and specifically for multiple rape cases, where a single locus probe is used, DNA fingerprinting is the answer to the problem.

5

ANALYSIS OF TAU, MBP, MOG, NFL AND GFAP PROTEINS FROM CEREBROSPINAL FLUIDS OF CLINICALLY DIFFERENT MULTIPLE SCLEROSIS PATIENTS USING COMPUTATIONAL CLASSIFICATION METHODS

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Background: The complex pathophysiology of multiple sclerosis (MS) combined with unpredictable prognosis require identification of disease-specific diagnostic and prognostic biomarkers. The aim of this study was to investigate the biomarker potential of candidate inflammatory and neurodegenerative proteins for different clinical subtypes of MS. *Methods:* Cerebrospinal fluid (CSF) and serum samples were collected from relapsing-remitting (RRMS) (n=67), clinically-isolated syndrome (CIS) (n=46), primary-progressive (PPMS) (n=22) patients and control non-MS subjects (n=22). Western blot analyses were performed for GFAP, MOG, NFL, Tau and MBP proteins. Protein levels were compared with ANOVA and the K nearest-neighbor (KNN) algorithm was further used to assess the predictive use of these proteins for clinical subtype classification. *Results:* Tau, GFAP, MOG and NFL protein concentrations were significantly different in MS-subtypes compared to the control group ($p<0.001$), whereas MBP protein was not. When these proteins were used together for classification, kNN resulted to 94.25%±6.44% accuracy for differentiating MS from non-MS subjects. *Conclusion:* Our study showed the biomarker potential of those proteins in classifying the MS-subtypes and predicting the prognosis increases when they are investigated altogether. Furthermore, prognosis of CIS to RRMS transition can be predicted by evaluating Tau, MOG and NFL protein concentrations in CSF.

6

ANTIOXIDANTS: THEY MAY BE USEFUL, BUT…

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A molecule with antioxidant properties *in vitro* might have alternative or additional properties *in vivo*. For example, estrogens have been implied as antioxidants for lipoprotein and neural protection. However, estrogens act mainly through receptor-mediated signaling and not by the weak antioxidant properties of the molecule. Retinol, an antioxidant *in vitro*, acts in its association with opsin, to produce a reversible complex, rhodopsin needed for the process of vision. Genistein, soy isoflavonoid, inhibits protein-tyrosine kinase and topoisomerase-II and induces G_2 phase cell arrest. Curcumin anti-inflammatory properties after O-conjugation are lost, but not its antioxidant property. Tea polyphenols EGCG, EGC and EC are candidates for tea beneficial effects. Except for EGCG, all other catechins are highly conjugated in plasma. Flavonoid-rich foods cause a plasma antioxidant burst by increasing the level of uric acid. Flavonoids and their

metabolites are unlikely to act as major antioxidants *in vivo*. Their concentrations *in vivo* are sufficiently high to have activity at the receptor, enzyme, miRNA and transcription factor levels. In cells, production of reactive oxygen species is precisely regulated with specific targets and redox-dependent pathways, affecting diverse processes from tumorigenesis to ageing. Excess or uncontrolled administration of antioxidants may result in their malfunctioning.

7

DIAGNOSTIC IMPLICATIONS OF PUTRESCINE, SPERMIDINE AND SPERMINE IN PLEURAL EFFUSIONS

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Background: Pleural effusions are caused by a wide variety of diseases. It is important to elucidate their precise etiology to differentiate benign from malignant effusions. The polyamines are important molecules governing cell proliferation, survival and apoptosis. Consistent with their elevated levels in cancer, it seemed reasonable that patients with active cancer may have elevated levels of these compounds in some of their body fluids. The aim of the present study was to investigate the diagnostic efficacy of measuring pleural effusion concentration of the polyamines (putrescine, spermidine and spermine) for the discrimination of malignant and benign pleural effusions. *Patients and Methods:* Pleural effusions were collected from 138 consecutive patients in whom the diagnosis was confirmed with cytological and/or histological examinations. Cytological samples were classified as malignant (n=78) or benign (n=60). Polyamine concentrations were measured using the ion exchange chromatography method. *Results:* The results showed that the levels of the three polyamines were significantly higher in malignant pleural effusions when compared to the benign effusions. *Conclusion:* The polyamines putrescine, spermidine and spermine are of great value in the diagnosis of malignancy and may be used as an adjunct to cytological findings in determining malignant pleural effusions.

8

METALLOTHIONEIN (MT I & MT II) GENE EXPRESSION IN HEPATOCELLULAR CARCINOMA

Hala Hamouda[1], Amal Baalash[1], Saber Ismail[2], Osama Negm[2], Sherif El Saadany[2], Gamal Byoomy[2], Amal Abdel Hameed[3], Manal Hamouda[3] and Ghada Ismail[4]

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Background: Available information has suggested that metallothioneins (MTs) may play an important role in the carcinogenic and apoptotic processes of some tumors. This study aimed to detect the association between the levels of zinc, copper and metallothioneine as well as metallothioneins (MTs) I and II mRNA expression and the development of hepatocellular carcinoma. *Patients and Methods:* This study was carried out on 45 patients with liver diseases (15 patients with chronic hepatitis, 15 with liver cirrhosis and 15 with HCC) as well as 15 healthy controls. We measured copper, zinc and metallothionein (MT I and MT II) mRNA levels serum for all subjects; also the tissue levels of Cu, Zn and metallothionine were estimated in all patients. *Results:* The results showed a significant decrease in serum and tissue levels of zinc and MT and an increase in copper levels in all patient groups. More changes were documented in HCC patients. The PCR results of MT gene expression showed a significant decrease in MT I and MT II mRNA expression in HCC patients compared to other groups. *Conclusion:* Serum zinc, copper and MT levels may be used as non-invasive biochemical markers for the early detection of the progression of chronic liver diseases.

9

A MULTIPLEX ALLELE-SPECIFIC POLYMERASE CHAIN REACTION FOR THE DETECTION OF FACTOR V LEIDEN AND PROTHROMBIN G20210A

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Background: The aim of this study was to determine the frequencies of factor V Leiden and prothrombin G20210A point mutations in the Iranian population with Azeri Turkish origin. *Methods:* A total of 120 unrelated individuals from the general population were randomly selected and examined for factor V Leiden and prothrombin G20210A mutations using a multiplex allele-specific polymerase chain reaction assay. *Results:* The frequency of prothrombin G20210A mutation was 2.08%, meaning that, out of 240 chromosomes, 5 chromosomes had prothrombin G20210A mutation. The distribution of prothrombin 20210 GG, GA and AA genotypes and prothrombin 20210A allele were 37 (92.5%), 3 (7.5%), 0 (0%) and 3(3.75%) in males and 78 (97.5%), 2 (2.5%), 0(0%) and 2(1.25%) in females, respectively. Factor V Leiden was not found in the tested group. Analysis of the observed frequencies in the studied group indicated that there is no

statistically significant difference between females and males, regarding prothrombin G20210A mutation ($p>0.05$). *Conclusion:* This is the first study of its own kind in this population and implies that the frequency of factor V Leiden G1691A (R506Q, FV-Leiden) allele is extremely low but the prothrombin G20210A mutation is more frequent in the tested group.

10
FACTOR XIII GENE VAL34LEU MUTATION IN THE IRANIAN AZERI TURKISH HEALTHY CONTROLS

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Background: The present study investigated the factor XIII gene Val34Leu mutation in the Iranian Azeri Turkish females. *Methods:* Dde I-based RFLP-PCR was performed to determine the factor XIII gene Val34Leu mutation. *Results:* The observed frequencies of G (Val) and T (Leu) allele and G/G (Val/Val), G/T (Val/Leu) and T/T (Leu/Leu) genotype distributions of factor XIII gene Val34Leu were 165 (84.18%), 31 (15.82%), 67 (68.37%), 31 (31.63%) and 0(0%) in the tested population, respectively. Factor XIII gene Val34Leu genotypes showed an excellent fit to the Hardy-Weinberg equilibrium in the tested population ($\chi^2=3.45<3.84$, p-value (with 2 degrees of freedom)=0.177 >0.05). Factor XIII G (Val) and T (Leu) allele frequencies were 0.84 and 0.16, respectively. *Conclusion:* Our findings suggested that the factor XIII Val34Leu mutation has a very low prevalence in the tested population. These results are reported for the first time for the Iranian Azeri Turkish females, which may be considered as a control group for studies relating the prevalence of factor XIII gene Val34Leu mutation with human primary hemostatic, spontaneous bleeding and thrombotic disorders.

11
ANALYSIS AND MODELLING OF CANCER USING ARTIFICIAL NEURAL NETWORK BASED BIOINFORMATICS

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Cancer is a complex disease. While it may present at a given site there may be a myriad of forms presenting at that site. For example, in breast cancer, using genomic profiling, in excess of 80 sub-types have been identified. In such a complex disease state there is no "one treatment fits all" solution. The ability to characterise the disease for each patient may offer the potential to assess the molecular sub-type of the disease, determine the patient's prognostic outcome or determine the likelihood of the patient responding to a particular therapy. The post-genomic era offers a significant potential for the characterisation of many diseases. Methodologies such as mass spectrometry-based proteomics and gene expression arrays, offer the potential for characterisation of disease-derived samples using a large number of proteins or genes. This depth of information, while providing a comprehensive overview of a disease state also proves problematic in its complexity. One has to search through potentially hundreds of thousands of pieces of information for consistent features that address a clinical question in the population. The human mind is very good at finding patterns in a system but is not able to conduct the task repetitively for large numbers of parameters. Conversely, computers are very good at searching for features in such data spaces, but previously defined statistical methods are not able to cope with the high complexity. Here we present the application of artificial neural networks (a form of artificial intelligence having the characteristics of both human pattern recognition and computer automated searching) to finding genomic solutions to questions in cancer.

12
SMALL INTERFERING RNAS (SIRNAS), RIBOZYMES, CATALYTIC DNA, LENTIVIRAL VECTORS AND STEM CELL BASED GENE THERAPY IN HIV/AIDS

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Background: Catalytic nucleic acid, siRNA, ribozyme (Rz) and DNA-enzyme (Dz) based approaches offer new promise to selectively target any RNA. They have significant advantages over protein based approaches that often result in suboptimal immune response, high toxicity and tolerance. Small interfering RNA technology is expected to revolutionize therapy because of its efficacy, specificity and no immune response. *Methods:* Hammerhead Rzs, which are small hairpin RNAi constructs, were synthesized and cloned into an expression vector and lentiviral vectors for gene therapy. *In vitro* RNA (Tat, Rev) were cleaved by Rzs and Dzs. Hematopoietic stem cells were prepared from fetal liver and transduced with VSV-G pseudotyped lentiviral vectors. *Results:* Rzs cleaved the target RNA in a sequence-specific

manner and stem cell derived T cells and macrophages showed protection against HIV-1 challenge. *Conclusion:* Short catalytic RNA and DNA are effective in controlling HIV-1 challenge and siRNAS were very effective. These antiviral approaches may be combined to obtain a greater level of protection. They may be exploited against HIV or any other viral pathogen.

13

DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS OF SH-SY5Y CELL MODELS TO ELUCIDATE NEURODEGENERATIVE MECHANISMS

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Background: The central nervous system carries complex procesesses and requires methods to understand the aberrant mechanisms that define the deviation from normal physiology. Liquid chromatography (LC) coupled to high-definition mass spectrometry (MS) has found increased use in neuroproteomics with an aim to pinpoint altered pathways and disease-specific biomarkers and to find new drug targets. This study involved an *in vitro* neuroblastoma cell disease model aiming to differentiate the specific pathways related to Alzheimer's, Parkison's, and Huntington's diseases. *Methods:* LC-MS-based bottom-up, label-free differential proteomics expression analysis was applied to electron transport complex specific neurotoxin-induced neuroblastoma cells. The generated tryptic peptides were fractionated by nanochromatography and amino acid sequences for the identification, while absolute quantification was achieved by the MS and MS/MS experiments. *Results:* A total of 400 high-confidence protein identifications were achieved and absolute amounts were quantified. Automated classification of identified proteins based on biological processes, molecular function and cellular compartmentation showed that there are cell-model-specific alterations in the pathways related to energy metabolism, stress response, regulation and others. *Conclusion:* Neurotoxin-treated neuroblastoma cells are viable *in vitro* mitochondrial dysfunction models to study neurodegeneration. LC-MS/MS is a powerfull method to quantify protein expression changes and can help understand the pathways involved in neurodegenerative mechanisms.

14

INHIBITION OF *CANDIDA RUGOSA* LIPASE BY SECONDARY METABOLITES EXTRACTS (PHENOLIC COMPOUNDS AND SAPONINS) OF *ACHILLEA SANTOLINA*

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Background: The polyphenols compounds and saponins possess many beneficial properties, such as reducing the risk of cancer and obesity. To explore these properties, the effect of phenolic and saponins extracts of *Achillea santolina* on the activity of *Candida rugosa* lipase was investigated. *Methods:* The phenolic compounds and the saponins were extracted by ethyl acetate and butanol, respectively. Lipase inhibition was determined using a spectrophotometric method. *Results:* UV-analysis of the polyphenols extracts from *Achillea santolina* indicated that the total phenol content was 6.35±0.30 mg/g for ethyl acetate fraction (EAF) and 0.82±0.03 mg/g for butanol fraction (BF) of gallic acid equivalent, while the saponin content was 21.92±2.09 mg/g in BF of digitonin equivalent. The effect of these extracts on the enzymatic activity of lipase showed a significant inhibitory potency with values of IC_{50} and Ki of 0.376±0.005 g/l and 0.023±0 g/l, respectively for EAF and 1.187±0.030 g/l and 0.071±0.002 g/l, respectively for BF. Both EAF and BF presented uncompetitive inhibition. *Conclusion:* We suggest that these extracts of *Achillea santolina* may possess antiobesity and antiacnes properties.

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INHIBITION OF *CANDIDA RUGOSA* LIPASE BY PHENOLIC OF *MARRUBIUM VULGARE*

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Background: Many studies have focused on polyphenols from teas and herbal sources for lipase inhibition to treat obesity. As a result, the effect of *Marrubium vulgare* phenolic extracts on the activity of *Candida rugosa* lipase and their antioxidant activity were investigated in this study. *Methods:* The phenolic compounds were extracted by ethyl acetate and the enzymatic inhibition of lipase was determined using a spectrophotometric method. *Results:* UV analysis of the polyphenols extracts from *Marrubium vulgare* indicated that the total phenol content was 1.52±0.09 mg/g for the ethyl acetate fraction of gallic acid equivalent, while the flavonoid content was 0.53±0.05 mg/g for the ethyl acetate fraction of quercetin equivalent.

Furthermore, these extracts showed a hydrogen-donating ability in the presence of DPPH stable radical with an EC_{50} value of 12±1.3 μg/ml in the ethyl acetate fraction. The effect of these extracts on the enzymatic activity of lipase showed a significant inhibitory potency with IC_{50}=0.453± 0.064 g/l and Ki=0.640±0.170 g/l in the ethyl acetate fraction and the extract presented competitive inhibition. *Conclusion:* We suggest that these extracts of the plant *Marrubium vulgare* may possess antiobesity and antiacnes properties.

16
SOLUTE CARRIER TRANSPORTERS: PHARMACOGENOMICS RESEARCH OPPORTUNITIES IN AFRICA

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Membrane transporters play a critical role in drug response as they provide the target for many commonly-used drugs and are major determinants of drug absorption, distribution and elimination. Most of them belong to one of the two major super-families of membrane transport proteins, the ATP-binding cassette (ABC) transporters and the solute carrier (SLC) transporters. They are subject to both genotypic and phenotypic polymorphism and variation in drug transporters may be the reason for inter-individual variability in pharmacokinetic disposition, efficacy and toxicity of drug transporter substrates. The growing number of publications reporting genetic population data for the SLC transporters, in particular, shows their importance, as well as, the increased interest in investigating them in most recent pharmacogenetics/genomics research projects. However, there is little or no genetic data available from the Southern and sub-Saharan African populations for the SLC transporters. Excellent research opportunities to investigate the genetic diversity of the SLC transporters and its pharmacogenetic implications are, therefore, available in this region of the world.

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INDUCTION OF APOPTOSIS IN HUMAN LYMPHOBLASTOID CELLS BY DIGALLIC ACID FROM *PISTACIA LENTISCUS* L FRUITS

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Background: The digallic acid (DGA) purified from Pistacia lentiscus L fruits was investigated for its antiproliferative and apoptotic activity on human lymphoblastoid cells. We attempted to characterize the apoptotic pathway activated by DGA. *Materials and Methods:* Apoptosis of TK6 cell line was detected by DNA fragmentation and PARP cleavage and by evaluating caspase activity. *Results:* Apoptosis was observed after 24 and 48 h incubation of the TK6 human lymphoblastoid cells with the tested compound. In fact, DNA fragmentation was observed, after treatment with the tested compound and it was confirmed by PARP cleavage. Caspase-3 and caspase-8 activity was induced by DGA, showing the highest activity for a compound concentration of 10 μg/ml. *Conclusion:* DGA exhibited an apoptosis induction effect in cells with officious tumor suppressive p53 gene, revealing, thus, its potential as a cancer-preventive agent.

18
GATA3 ANTAGONIZES PROSTATE CANCER PROGRESSION

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The identification and treatment of prostate cancer progressing to castration-resistant stage is a major challenge. Using a prostate-specific *Pten*-inactivation model, we showed that the transcription factor Gata3 is lost during tumor progression. To understand the consequences of this loss, we inactivated *Gata3* in the adult prostate, which revealed roles in androgen receptor (AR) regulation and maintenance of epithelial differentiation. *Gata3*-mutant prostates mislocalized AR to the cytoplasm and progressively lose their glandular epithelium in a manner reminiscent of *AR*-deficient prostates. To determine the role of Gata3 in tumors, we developed a conditional mouse model to re-express GATA3. Strikingly, enforced GATA3 expression in *Pten*-deficient prostates antagonized tumor progression by preventing polarity loss, EMT and cellular dedifferentiation,

when GATA3 was present in the nucleus. In contrast, prostatic ducts expressing cytoplasmic or no GATA3 developed carcinoma. We showed that GATA3 prevents PI3K/Akt pathway up-regulation. Remarkably, similar mislocalizaion of GATA3 was observed in human prostate tumors, where GATA3 was lost or sequestered to the cytoplasm in 85% of castration-resistant tumors. In addition, GATA3 expression levels at the hormone-sensitive stage held a predictive value for prostate cancer recurrence. Together, these results conclusively identified GATA3 as a critical player in prostate homeostasis and cancer progression.

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ANTIGENOTOXIC AND ANTIOXIDANT ACTIVITIES OF ISORHAMNETIN 3-O NEOHESPERIDOSIDE FROM *ACACIA SALICINA*

Ines Bouhlel[1], Ines Skandrani[1], Aicha Nefatti[1], Kita Valenti[2], Kamel Ghedira[1], Anne Marie Mariotte[3], Isabelle Hininger-Favier[4], Francois Laporte[2], Marie G. Dijoux-Franca[5] and Leila Chekir-Ghedira[1]

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Background: The isorhamnetin 3-O neohesperidoside (I3ON), isolated from the leaves of *Acacia salicina*, was investigated for its antioxidant and antigenotoxic activity. To further explore the mechanism of action of I3ON, we characterized the expression profiles of genes involved in antioxidant protection and DNA repair in the human lymphoblastic cell line K562 exposed to H_2O_2. *Materials and Methods:* The antioxidant activity of isorhamnetin 3-O neohesperidoside (I3ON), isolated from the leaves of *Acacia salicina*, was determined by the ability of this compound to inhibit lipid peroxidation. Antigenotoxic activity was assessed by using the comet assay. The protective effect exhibited by this molecule was also determined by analysis of gene expression as a response to an oxidative stress, using a cDNA microarray. *Results:* The IC_{50} value of the inhibitory activity toward lipid peroxidation by I3ON was 0.6 mM. This compound was also able induce an inhibitory activity toward H_2O_2-induced genotoxicity. Transcription of several genes related to the antioxidant system (HMOX2 and TXNL) and to the DNA repair pathway (XPC, POLD1, POLD2, PCNA, DDIT3, APEX and LIG4) was up-regulated after incubation with I3ON. *Conclusion:* I3ON, isolated from the leaves of A. salicina, is able to protect cells against oxidative stress.

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HUMAN A-AMYLASE GENES AND THEIR SIGNIFICANCE IN UNDERSTANDING THE ROLE OF AMYLASE IN THE DIGESTION OF STARCH

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α-Amylase catalyses the first step in the digestion of starch, a main source of dietary carbohydrate in humans. During evolution, significant starch consumption probably did not occur until the development of agricultural societies. Native starch granules are relatively difficult to digest and granules of different botanical origin can differ greatly in their susceptibility to attack by amylase. With the advent of cooking, starch digestion would have become much easier and starch was then able to become a favourable source of dietary energy. It has been suggested that selective pressures have acted on amylase. Two distinct but related genes code for amylase. AMY1 produces the enzyme present in human saliva and AMY2 produces the enzyme synthesised in the pancreas and secreted into the duodenum in pancreatic juices. AMY1 is also expressed in lactating mammary gland and active α-amylase is present in human milk. An interesting feature of amylase genes is that they are present in multiple copies and the number of copies varies between individuals and population groups. It has been claimed that individuals that are characterised by a high starch diet have a greater number of gene copies. The physiological role of salivary amylase still remains to be fully understood, however.

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EFFECT OF SURVIVIN PROMOTER (-31 G/C) GENE VARIATION IN ORAL CARCINOMA

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Background: Most cancer types show alterations in genes, including the regulation of growth control and apoptosis. Survivin is a multifunctional protein, member of the inhibitor of apoptosis protein family, involved in the regulation of the cell cycle and the inhibition of the apoptotic pathway. The present study was designed to investigate the role of survivin gene promoter (-31 G/C) polymorphism in oral carcinogenesis. *Materials and Methods:* Survivin gene promoter (-31 G/C) genotypes were determined using polymerase chain reaction-restriction fragment lenght polymorphism analysis. A total of 61 patients with oral cancer and 68 healthy individuals were enrolled in the study. *Results:* The survivin (-31 G/C) gene polymorphism GG,GC,CC genotype frequencies for controls and patients were 54.4%, 35.3%, 10.3%, and 42.6%, 49.2%, 8.2%, respectively. There were no significant differences in the distribution of survivin (-31 G/C) genotypes between patients and controls. Stratification analysis was also performed using prognostic parameters and it was noted that carrying the C allele was increased in patients with advanced tumor stage and this difference was statistically significant (p=0.022, OR=1.736, 95% CI: 1.025-2.940). *Conclusion:* These results suggest that the survivin (-31 G/C) gene polymorphism may be associated with advanced tumor stage in oral cancer patients. Further studies in a larger population are needed to confirm these results.

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POLYMORPHISMS OF V762A OF *PARP1* AND V384D OF *HMLH1* ARE ASSOCIATED WITH INCREASED COLORECTAL CARCINOMA RISK IN KOREAN WOMEN

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Alterations of DNA repair genes are associated with the development of colorectal carcinoma (CRC). We tested the association between the CRC risk and polymorphisms of V762A of poly ADP-ribose polymerase 1 (*PARP-1*) and V384D of human mutL homolog 1 (*hMLH1*) in 507 Korean CRC patients and 736 healthy controls. The polymorphisms were analyzed using high-resolution melting PCR. The V762A polymorphism of *PARP1* was not associated with the overall CRC risk. However, both TC (OR, 1.83; 95% CI, 1.20-2.79) and CC genotypes (OR, 1.75; 95% CI, 1.02-2.98) were associated with the CRC risk but not with the TT genotype in women (p for interaction, 0.01). The V384D of *hMLH1* polymorphism was not associated with the overall CRC risk. The interaction of TA/TC (TA genotype of V384D of *hMLH1* and TC genotype of *V762A* of *PARP1*) was associated with increased risk both in the overall study group (OR, 2.01; 95% CI, 1.06-3.81) and in women (OR, 3.87; 95% CI, 1.43-10.50) but not with the TT/TT genotype. These results suggested that V762A of *PARP1* may be associated with the CRC risk in women, while V384D of *hMLH1* and V762A of *PARP1* may interact to increase the CRC risk both in the overall population and in Korean women.

23
TREATMENTS OF OVARIAN CANCER CELLS WITH PRODRUGS RESULTED IN AN INHIBITION OF THEIR GROWTH IN THE PRESENCE OF ENGINEERED STEM CELLS EXPRESSING CYTOSINE DEAMINASE AND CARBOXYL ESTERASE *VIA* TUMOR-TROPIC EFFECT

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Background: Recent studies have shown that genetically engineered stem cells (GESTECs) to produce suicide enzymes that convert non-toxic prodrugs to toxic metabolites selectively migrate toward tumor sites and reduce tumor growth. In the present study, we evaluated whether these GESTECs are capable of migrating to human ovarian cancer cells and examined the potential therapeutic efficacy of the gene-directed enzyme prodrug therapy against ovarian cancer cells *in vitro*. *Methods:* Ovarian cancer SKOV-3 and engineered stem cells were cultured in DMEN with 10% FBS. After evaluation of *cytosine deaminase (CD)* and *carboxyl esterase (CE)* gene expression in the stem cells using RT-PCR, we confirmed the expressions of chemoattractant molecules, such as SCF, CXCR4, c-kit, VEGF and VEGFR2 in ovarian cancer cells. To determine the migration ability of these engineered stem cells in comparison to primary cells, we performed a modified transwell assay. Using a co-culture system and MTT assay, we examined the therapeutic efficacy of the engineered stem cells with the prodrug 5-FC to selectively target endometrial cancer cells *in vitro*. *Results:* GESTECs (HB1.F3.CD or HB1.F3.CE cells) engineered to express a suicide gene (*CD* or *CE*) selectively migrated toward ovarian

cancer cells. A [^{3}H] thymidine incorporation assay was conducted to measure the proliferative index. Treatment of a human epithelial ovarian cancer cell line (SKOV-3, an ovarian adenocarcinoma derived from the ascites of an ovarian cancer patient) with the prodrugs 5-FC or camptothecin-11 (CPT-11) in the presence of HB1.F3.CD or HB1.F3.CE cells resulted in the inhibition of ovarian cancer cell growth. *Conclusion:* The results of this study have shown that GESTECs expressing *CD/CE* have a potent advantage of selective migration toward ovarian cancer cells. Moreover, these engineered stem cells resulted in an anti-proliferative effect on ovarian cancer cells, suggesting that these GESTECs expressing suicide genes combined with the application of prodrugs may have a therapeutic potential to selectively treat ovarian cancers.

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MATRIX METALLOPROTEINASE-7 GENE POLYMORPHISM AND THE RISK OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN TURKISH POPULATION

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Background: Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide. Among the various proteinases, matrix metalloproteinases (MMPs) digest the extracellular matrix of the lung and play a significant role in the development of COPD. MMPs play a key role in tissue remodeling and repair and there is significant evidence that members of the MMP family may also play an important role in COPD pathology. *Methods:* A total of 85 COPD patients and 85 healthy individuals were enrolled in this study. Genomic DNA was extracted from peripheral whole blood with a Roche High Pure Preparation Template Kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. MMP7 -181 A/G polymorphism was determined by RT-PCR and melting-curve analysis, using fluorescence-labeled hybridization probes (LightCycler; Roche Diagnostics, Mannheim, Germany). *Results:* There were significant differences in the distribution of MMP7 genotypes between the COPD patients and the controls (p=0.049, χ^2=6.012). MMP7 AA genotype was significantly associated with an increased risk of COPD in the study groups (OR=1.8; 95% CI: 1.033-3.135; p=0.033). *Conclusion:* These findings suggest that MMP7 -181 A/G polymorphism may be associated with an increased risk for COPD. This study will be performed in a larger patient series in the near future.

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HSSB2, A NOVEL SINGLE-STRANDED DNA BINDING PROTEIN IMPLICATED IN THE DNA DAMAGE RESPONSE

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Single-stranded DNA binding (SSB) proteins are ubiquitous and essential for a variety of DNA metabolic processes including DNA replication, recombination, transcription and repair, as well as for the recruitment of other repair proteins to sites of DNA damage. SSB proteins from the three domains of life are evolutionarily conserved and utilise oligonucleotide-binding (OB) domains for DNA binding. We recently described two new human SSBs (hSSB1 and 2), with a domain organisation closer to the archaeal SSB than to the eukaryotic replication protein A (RPA) (Richard *et al*. (2008) Nature *453*: 677-681). While hSSB1 is critical for the cellular response to double-stranded DNA breaks, our recent data indicated that hSSB2 is involved in the cellular response to ultraviolet radiation and, hence, in the Nucleotide Excision Repair (NER) pathway. We have probed the molecular mechanism of hSSB2 by surface plasmon resonance, NMR and biochemical approaches. Unlike RPA, hSSB2 showed non-cooperative, distributive binding, which was similar to its archaeal counterpart (Cubeddu and White (2005) JMB *353*: 507-516). We showed that hSSB2 binds with high affinity to bulky lesions that are processed by NER, indicating a likely role in the detection of damage. Like many early participants in the damage response, hSSB2 may be involved in tumorigenesis and may significantly affect the response of patients to certain cancer therapies.

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INFLAMMATION RESULTS IN LOSS OF ANDROGEN RECEPTOR (AR), AND CONSEQUENTLY UNCONTROLLED PROLIFERATION IN PROSTATE CELLS

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Inflammation is associated with the development of carcinoma and a decrease in androgen receptor (AR) expression or responsive factors in the presence of testosterone cause prolonged activation of a redox-sensitive transcription factor, nuclear factor kappa B (NFkB). This initiates and amplifies an inflammatory cascade within the prostate and results in sustained oxidative damage. The inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) accelerate the loss of AR and NKX3.1 expressions, which is found in pre-invasive prostate cancer. Therefore, an inflammation model of prostate using androgen-responsive LNCaP cells was established to investigate the inflammatory proliferative atrophy (PIA) and the subsequent molecular alterations in cancer development. In our model, the U937 monocyte cell line was used for cytokine secretion, and further, conditioned media were used to feed LNCaP cells to create an microenvironment of inflammatory prostatitis. Significant down-regulation of AR, loss of AR-mediated transactivation, down-regulation of p53 as well as increases in p-Akt and B-catenin stabilizations were observed. At certain doses of TNF-α, AR-regulated apoptosis was down-regulated, while DNA damage and proliferative capacity were increased, suggesting that the PIA favors genomic instability and the cellular heterogeneity that eventually makes cells more resistant to apoptosis.

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CANCER EPIGENETICS AND THERAPEUTIC INTERVENTIONS

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Epigenetics is defined as a set of heritable changes in gene expression that are not accompanied by the gene's nucleotide sequence. During the past decade, the somatic mutation theory of cancer has been revolutionized, as it became evident that epigenetic modifications play a role as equally important as genetics in cancer development. Cancer cells have genome-wide aberrations in terms of epigenetic alterations, including global hypomethylation, promoter-specific hypermethylation, histone deacetylation and global down-regulation of miRNAs. These different modifications are closely interconnected. DNA hypermethylation and histone modifications involved in chromatin remodeling are the most studied epigenetic mechanisms. Modifications of DNA and histones are reversible, making them good targets for therapeutic intervention. Several inhibitors of histone deacetylation or DNA methylation are approved for hematological malignancies by the US Food and Drug Administration and have been in clinical use for several years. Combining traditional cancer therapy with the use of epigenetic therapy holds a strong potential for the successful treatment in many types of cancer. Also, the development of biomarker panels based upon changes in the epigenetic pattern between non-malignant and malignat genomes is extremely important in the selection of the right therapy.

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APPROACHING SPORADIC OVARIAN CANCER BY EXPLOITING DEFECTS IN HOMOLOGOUS RECOMBINATION

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Background: Adjuvant chemotherapy in the treatment of ovarian cancer consists of cisplatin, carboplatin, doxorubicin, 5-fluorouracil, gemcitabine and/or paclitaxel. Most epithelial ovarian cancers have defects in homologous recombination. Poly(ADP-ribose) polymerase (PARP) inhibitors take advantage of these defects in homologous recombination and may sensitize standard chemotherapy in the treatment of ovarian cancer. *Methods:* Ovarian cancer cells were incubated with standard chemotherapeutic agents, PARP inhibitors alone (Olaparib, Veliparib and AG143461) or in combination for 72 h. The IC_{50} values we calculated and compared through Sigma Plot. Additionally, the amount of PAR in each cell line was measured and compared to the IC_{50} values. *Results:* The ability of each class of drug to inhibit ovarian cancer growth after being sensitized by PARP inhibition was as follows: Paclitaxel 94×; platinum agents 1,644×; doxorubicin 35.7×; 5-and fluorouracil 6,800×. Gemcitabine was inhibited by PARP inhibitors 0.27×. Linear regression of the IC_{50} values of PARP inhibition and the endogenous quantity of PAR indicated no correlation between the two. *Conclusion:* PARP inhibitors increased the efficacy of cisplatin, carboplatin, doxorubicin, 5-fluorouracil and paclitaxel in the treatment of ovarian cancer, while antagonizing gemcitabine. There was no correlation between PAR and the IC_{50} values of PARP inhibitors.

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TARGETTING DNA REPAIR TO TREAT BREAST CANCER

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Background: Most chemotherapeutic agents used to treat breast cancer either damage DNA, inhibit the production of DNA or inhibit the duplication of DNA. Poly(ADP-ribose)

polymerase (PARP) is important in the repair of DNA. In this study, we used Olaparib, Veliparib and AG143461, alone or in combination with chemotherapy, to treat breast cancer. PARP inhibitors have a high therapeutic index as single agents. *Methods:* Twenty human breast cancer cells representing the four Perou subtypes (er^+/pg^+ slow growing, er^+/pg^+ fast growing, $her2/neu^+$ and triple negative) of breast cancer were treated with standard chemotherapy agents alone or in combination with PARP inhibitors for 72 h. Each experiment was repeated 10 times and the respective IC_{50} values obtained. *Results:* PARP inhibitors greatly enhanced the efficacy of alkylating agents, topoisomerase inhibitors, platinum-type alkylating agents and microtubule inhibitors against all four Perou subtypes of breast cancer. Anti-metabolites with the exception of 5-FU were antagonized by PARP inhibitors in their ability to inhibit breast cancer. *Conclusion:* PARP inhibitors effectively enhanced the ability of DNA and chromosome damaging agents to inhibit breast cancer, while inhibiting the ability of anti-metabolites to treat breast cancer.

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KAEMPFEROL GLYCOSIDES: EMERGING STARS IN THE FLAVONOID GALAXY?

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Flavonoids, a group of more than 4,000 polyphenolic compounds that occur ubiquitously in most plants, have been shown to exhibit physiological, pharmaceutical, antioxidant, antiviral as well as anticancer activities. Flavonols comprise one of the most important flavonoid classes, mainly due to their abundance in the diet of Western populations. Recently, it has been demonstrated that the flavonols, kaempferol and its glycosides, may specifically inhibit the serine/threonine kinases, known as p90 ribosomal s6 kinases (RSKs), which are involved in signal transduction through the MAPK/ERK pathway. Kaempferol glycosides have already been reported to interfere with the cell cycle, the DNA synthesis pathway and to induce apoptosis in human leukemic cell lines. A semisynthetic derivative kaempferol glycoside, named *Tac*, that has been studied by our group demonstrated as well promising *in vitro* and *in vivo* anticancer activity against solid tumors *via* a mechanism involving the MAPK pathway and, more specifically, RSKs and GSK 3α/β. Further analysis of the mechanism of action of Tac suggests that it shares similarities with anthracyclins and flavopiridol, linking, thus, RSKs to DNA damage and CDKs. Taking into consideration all these novel data, it would be of great interest to further investigate whether these agents represent a new class of targeted cancer chemotherapeutics.

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MIDBODIES ACCUMULATE IN STEM CELLS AND CANCER 'STEM CELLS' AND CONTRIBUTE TO PLURIPOTENCY AND TUMORIGENESIS

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The midbody (MB) is a nonmembranous organelle formed between daughter cells during cell division and is required for their final separation during cytokinesis. Here we show that MBs persist in cells long after division, but their fate is unclear. We also show that MBs segregate asymmetrically to the daughter cell with the older centrosome. They selectively accumulate in stem cells in mouse and human tissues, and in cultured stem cells and induced pluripotent cells *in vitro*. Differentiation of stem cells is accompanied by an eight-fold decrease in MB+ cells, which is mediated by autophagic degradation of MBs *via* the autophagy receptor, NBR1 and the MB ligand, Cep55. Reprogramming of fibroblasts to induced pluripotent stem (iPS) cells is accompanied by an eight-fold increase in MB+ cells, which involves evasion of autophagosome encapsulation of MBs. Experimental elevation of MBs in fibroblasts accelerates reprogramming to iPS cells. The subpopulation of MB+ cancer cells shows enhanced *in vitro* tumorigenesis (three-dimensional growth) and co-segregates with cancer 'stem' cells. We conclude that MBs contribute to properties of stem cells and cancer 'stem cells', possibly by scaffolding stem cell molecules (example, CD133/prominin) and activities.

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COMPARING THE OXIDATIVE STRESS INDEXES OF OBESITY IN OBESE AND NON-OBESE PEOPLE

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Obesity is a principal causative factor in the development of metabolic syndromes and the major risk factor for cardiovascular diseases. The present study aimed to assess the systematic oxidative stress, as reflected by plasma activities of antioxidant enzymes in erythrocytes, namely glutathione

peroxidase (GPX), superoxide dismutase (SOD) and total antioxidant capacity of plasma by FRAP method, total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) of plasma. In this case control study, twenty-five overweight volunteers (BMI>30 kg/m^2) were studied. Twenty-five non-obese volunteers were used as controls. The results showed that the total antioxidant capacity of plasma, SOD activity and plasma HDL were lower in the overweight group in comparison to the control group ($p<0.05$), but plasma levels of TC and LDL were higher in the overweight group than in the control group ($p<0.05$). Differences in plasma TG and GPX levels between the overweight and the control group were not statistically significant. The study findings demonstrated the presence of oxidative stress in the plasma of obese people. This is probably the result of an imbalance in oxidant/antioxidant homeostasis.

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ACUPUNCTURE AND SPORTS INJURIES

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Acupuncture is a therapeutic procedure that is applied upon penetration to the specific parts of the body with fine needles. There are some studies suggesting that traditional acupuncture is more effective on pain relief than pharmacological treatment. Despite the pain reduction achieved with acupuncture, the qualitative immunorepressive effect of acupuncture is not yet understood. According to initial results regarding the calcitonin gene-related peptide (CGRP), acupuncture has a significant role in expanding a vessel special to neuropeptides, involved in the regulation of acute, subacute and chronic inflammation. In an effort to provide strong evidence for the immunological effects of acupuncture, clinical studies, which have provided measurement of inflammation with various mediators and assessed the signs of inflammatory and surrounding findings, may provide some explanation of the influence mechanisms of acupuncture. Many scientific studies have been published focusing on the analgesic effect of acupuncture. In many types of pain, the acupuncture is more effective than placebo in a meaningful way, and its efficiency in chronic pain has been compared with morphine in controlled studies. Evaluating the effect of acupuncture on a broad spectrum of sports injuries, such as femoral adductor syndrome, tennis elbow or lateral epicondylitis, osteoarthritis, patellar tendonitis and plantar fasciitis, may render acupuncture a method of alternative treatment for sport injuries.

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MITRAGYNINE PREVENTS THE DEVELOPMENT OF TOLERANCE FOLLOWING MORPHINE TREATMENT

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Aim: Morphine is one of the most effective analgesics available to date. However, the effectiveness of morphine lessens significantly with repeated doses. The objective of this study was to investigate the role of mitragynine in reducing the tolerance to morphine which will result in effective pain relief. *Methodology:* Mitragynine was administered with morphine and the effect on morphine tolerance was investigated. Male ICR mice were randomly divided into groups of 7 animals each. The animals were administered with morphine alone or morphine with mitragynine for nine consecutive days *via* intraperitoneal injection. The antinociceptive effect was estimated using hot-plate test (Ugo Basile Model 7280; 50±0.2˚C). *Results:* Comparing the treated group, a combination of mitragynine and morphine gave a significant increase ($p<0.05$) in the latency period. The antinociceptive action was maintained for nine days compared to group treated with morphine alone ($p<0.05$). Western blotting and densitometry analysis showed that there was a significant elevation of CREB protein ($p<0.001$) in animals treated with morphine alone compared to the other groups. In animals treated with mitragynine, the elevation of the protein was prohibited. *Conclusion:* The results suggested that mitragynine is able to prevent the development of tolerance to morphine and sustain its antinociceptive action.

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EFFECTS OF VITAMIN D AND DOXORUBICIN ON EXPRESSION OF INTERFERON REGULATORY FACTOR-4 AND VITAMIN D RECEPTOR IN ANAPLASTIC LARGE CELL LYMPHOMA

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Anaplastic large cell lymphomas (ALCLs) are T-cell lymphomas (TCLs) that depend on interferon regulatory factor-

4 (IRF4), an oncogenic transcription factor, for their growth. As IRF4 expression in myeloid cells is down-regulated by 1,25-dihydroxy-Vitamin D3 (VD3), we hypothesized VD3 might inhibit IRF4 expression and proliferation in ALCL. Normal T cells required stimulation to induce IRF4 expression. In contrast, IRF4 was constitutively expressed in ALCL. In SUDHL-1, an ALCL cell line with low IRF4 expression and high vitamin D receptor (VDR) expression, VD3 (100 nM) inhibited IRF4 expression by 63% and decreased proliferation by 40% (p=0.006, t-test). In contrast, Karpas 299 (high IRF4/low VDR) was resistant to these effects of VD3 alone; however, VD3 reduced proliferation by 48% (p=0.002) in the presence of doxorubicin (2 ng/mL). *IRF4* siRNAs increased VDR expression in ALCL cells; IRF4-negative TCL tissues also showed increased VDR expression by immunohistochemistry. These findings suggest VD3 and VDR decrease IRF4-driven proliferation in ALCL, and help explain recent data that vitamin D insufficiency is a poor prognostic factor in TCL. The effects of VD3 in ALCL cells were greatest when combined with doxorubicin. Therefore, ensuring vitamin D sufficiency in ALCL patients may be particularly critical during administration of doxorubicin-based chemotherapy.

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STRUCTURAL BASIS AND SPECIFICITY OF ACETYLATED TRANSCRIPTION FACTOR GATA1 RECOGNITION BY BET-FAMILY BROMODOMAIN PROTEIN BRD3

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Recent data have demonstrated that small synthetic compounds, specifically targeting bromodomain proteins, can modulate the expression of cancer-related or inflammatory genes. Although these studies have focused on the ability of bromodomains to recognize acetylated histones, it is increasingly becoming clear that histone-like modifications exist on other important proteins, such as transcription factors. However, our understanding of the molecular mechanisms through which these modifications modulate protein function is far from complete. The transcription factor GATA1 can be acetylated at lysine residues adjacent to the zinc finger domains and this acetylation is essential for normal chromatin occupancy of GATA1. We have recently identified the bromodomain-containing protein Brd3 as a co-factor that interacts with acetylated GATA1 and shown that this interaction is essential for the targeting of GATA1 to chromatin. Here, we describe the structural basis for this interaction. These data reveal for the first time the specificity of and molecular basis for an interaction between a transcription factor bearing a histone-like post-translational modification and its cognate recognition module. We also show that this interaction can be inhibited by an acetyllysine mimic, highlighting the importance of further increasing the specificity of compounds that target BET bromodomains in order to fully realize their therapeutic potential.

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ENHANCED ULTRASOUND IMAGING AND TARGET-DELIVERY OF BONE MARROW STEM CELLS TO ATHEROMA WITH IMMUNOGENIC LIPOSOMES

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Background: Imaging and delivery of stem cells to vascular lesions remain a major challenge. Targeted molecular imaging offers a novel technology for better identification and treatment of vulnerable plaques. Targeted stem cell delivery with echogenic immunoliposomes (ELIPs) may possess the ability to function as both an imaging and a therapeutic agent. *Methods and Results:* Bone marrow stem cells isolated from transgenic mice and engineered to express green fluorescent protein were mixed with ELIPs conjugated to anti-CD34 and anti-ICAM-1 antibodies; the mixture was intravenously transfused into C57BL/6J mice and atheroma-prone apolipoprotein-E (apoE) knockout mice. Vascular ultrasound imaging showed little or no plaque signals in wild-type C57BL/6J mice but detected atheroma enhancement in apoE-null mice prior to injection of ELIP-targeted stem cells. Following injection of anti-CD34/ICAM-1 ELIPs into the apoE-null mice, marked acoustic enhancement was observed in the region with intimal thickness, but not in other areas. Histological examination of aortas one month after treatment revealed reduced lesion sizes and atheroma lipid content in animals receiving ELIP-stem cell injections. *Conclusion:* We have demonstrated that ELIP-targeted delivery of bone marrow stem cells provides a novel, minimally invasive strategy to image and treat atheroma. The data indicates a greater efficacy of the directed stem cell therapy and points to its potential outside of the vascular field.

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RADIATION TREATMENT: A MODEL FOR THE STUDY OF FREE RADICAL-MEDIATED DAMAGE

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Modern medicine is becoming increasingly aware of the significance of free radicals in both physiological and pathological processes. Nowadays, understanding and monitoring oxidative stress represents an extremely challenging task. Oxidative stress is regarded as an imbalance between free radical production and existing antioxidant capacity. Since there is no clinical presentation of this imbalance and no test is available, in order to assess whether an individual is under attack by free radicals or has a depleted antioxidant capacity, the study of predefined patients or diseases as models of free radicals effects is important. Radiotherapy exerts its antitumor effects through increased formation of free radicals, provoking cell death as a result of massive cellular damage. Free radicals, among other factors, induce or facilitate cell apoptosis and are counteracted by defense mechanisms designated to reduce their levels. An understanding of the mechanisms activated during and after tissue irradiation might be important for the elucidation of the events sequence after oxidant status imbalance. Having selected a study population of breast cancer patients under radiotherapy, we are interested in exploring pathways participating in oxidant status imbalance after radiation exposure. The significant amount of data, documenting the effects of combining radiotherapy with antioxidants, and the broad administration of antioxidant supplements for prevention and/or treatment of disorders raise crucial aspects to be considered.

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AUTOPHAGY, CELL DEATH AND DISEASE

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Autophagy is characterized by sequestration of bulk cytoplasm and organelles in double- or multi-membrane autophagic vesicles and their delivery to and subsequent degradation by the cell's own lysosomal system. This cellular phenomenon has multiple physiological functions including protein degradation and organelle turnover. Increasing lines of evidence indicate that the autophagy machinery may be recruited by an alternative, caspase-independent and non-apoptotic form of programmed cell death named "autophagic cell death". In some settings, autophagy and apoptosis seem to be interconnected positively or negatively, introducing the concept of "molecular switches" or "integration points" between them. Consequently, autophagy abnormalities are frequent in various human diseases, including cancer. Therefore, understanding the molecular mechanisms regulating human autophagy is crucial for the development of new approaches to diagnose and treat major health problems. In our lab in Sabanci University, Istanbul, we performed several unbiased screens to identify new regulators of human autophagy. Several new proteins, molecules and pathways were discovered and analyzed functionally. The consequences of autophagy abnormalities in human disease and the potential of drug-mediated autophagy modulation in disease treatment will be discussed from a basic and clinical scientific point of view.

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CLINICAL IMPORTANCE OF MICRORNAS IN EARLY DETECTION OF CANCER

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MicroRNAs (miRNAs) are a class of about 19-24 nt non-coding single-stranded small RNA molecules. miRNAs have recently emerged as key post-transcriptional regulators of gene expression. miRNAs are predicted to control the activity of more than 60% of all protein-coding genes. Recent studies have revealed that a single miRNA might bind to as many as 200 gene targets. The levels and types of miRNA expression in normal tissues are significantly different from those in tumor tissues and miRNAs in different tumors have their own specific expression pattern. These features of miRNA expression have been confirmed in the liver, lung, colorectal and ovarian cancer, leukemia and other malignancies. miRNAs have been demonstrated to play an important role in the multistep processes of carcinogenesis either by oncogenic or tumor suppressor function. Study of miRNA has been extended into many kinds of tumors. miRNA expression panels may be used to classify cancer to identify tissue origin for cancer of unknown primaries and for early detection of cancer. miRNAs may be used as potential diagnostic and prognostic tools or in the follow-up treatment for cancer.

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GENE EXPRESSION PATTERN CHANGES IN CANCER CELLS DEPENDING ON RESISTANCE LEVELS TO DOCETAXEL

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Background: Docetaxel is used in the treatment of breast cancer. Tumor cells acquire multi-drug resistance which is a serious limitation in cancer chemotherapy. In this study, gene expression patterns of MCF7 sublines, which have different levels of resistance to docetaxel, were investigated. *Methods:* Docetaxel-resistant sublines (MCF-7/30nMDoc and MCF-7120nMDoc) were stepwise selected by concentration increments. Cell proliferation assay was used to quantify the level of resistance. cDNA microarray analysis was performed in order to reveal the gene expression patterns. *Results:* MCF-7 sublines resistant to 30 nM and 120 nM docetaxel were subjected to cDNA microarray analysis and it was shown that more than 2,800 and 4,000 genes were significantly altered in these sublines, respectively. Approximately 850 genes were found to change in common in both sublines. *Dıscussıon:* Expression profiles in resistant sublines were significantly different, although some of the altered genes were common in both sublines. However, it was clear that the molecular mechanisms of resistance were apparently different. Stepwise alteration in gene expression levels seems to be the basis for the development of increasing degrees of drug resistance.

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GENETICS OF MALE INFERTILITY

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Infertility can be defined as the inability to become pregnant after one year of unprotected sexual intercourse. It is one of the most significant health problems today, affecting up to 15% of couples of reproductive age and an increasing number of couples require assisted reproductive technologies to achieve pregnancy. About 50% of infertility is caused by a male factor and it can be diagnosed by a decrease in certain male biological parameters such as ejaculate volume and spermatozoa number. Genetic factors, problems in sperm production, delivery and motility, hormone and/or receptor deficiencies compromise the common causes for male infertility. Although 30% of severe male infertility cases can be attributed to genetic defects, for a majority of patients the cause of the dysfunction remains unknown. Several different genetic factors have been described to affect male fertility. Only two genes can so far be undoubtedly linked to human male infertility: *SPATA16* and *AURKC*. It is believed that a better understanding of the spermatogenesis including the effects of all the related genes will allow us to identify a genotype-phenotype correlation in human infertility.

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ULTRA-SHORT SINGLE-WALLED CARBON NANOTUBES (US-TUBES) AS "SMART" ACTIVATABLE DRUG DELIVERY PLATFORM

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Chemotherapy is one of the major approaches for the treatment of cancer. To carry a sufficient amount of drug molecules to the tumor cells is essential for efficient cancer therapy. However, the use of many existing anticancer drugs in cancer therapy is often limited by complications in administration, such as limited solubility, rapid elimination, inefficient distribution, inability to cross cellular barriers and inability to differentiate between healthy and cancer cells, leading to systemic toxicity and adverse side effects that greatly limit the dosage level. Current research in the area of drug delivery focuses on the development of efficient drug delivery systems that selectively increase the concentration of anticancer drugs in tumor cells alone, thereby eliminating side effects while improving efficacy. Carbon nanotubes (CNTs) are well-known nanostructures with unique physical and chemical properties that make them desirable for many applications. Their use as a carrier of drug molecules offers several advantages over current drug delivery systems. Herein, we present the synthesis, characterization, and study of a new drug delivery system for the treatment of cancer, based on ultra-short singled-walled CNTs (US-tubes). The system (CDDP@US-tubes) is comprised of the chemotherapeutic drug, cisplatin (CDDP), loaded inside US-tubes, which will selectively unload solely in cancer cells by activation of a specific peptide sequence with a cancer-specific antigen which is wrapped around the CDDP@US-tubes agent (Figure 1). The CDDP@US-tubes were characterized by high-resolution transmission electron microscopy (Figure 2a, b), energy-dispersive spectroscopy (Figure 2c), X-ray powder diffraction, X-ray photoelectron spectroscopy and inductively-coupled optical emission spectroscopy. Dialysis studies performed in phosphate-buffered saline (PBS) at 37˚C demonstrated that CDDP@US-tubes leak CDDP much more slowly when CDDP@US-tubes are wrapped with pluronic-F108 as a surfactant (Figure 3).

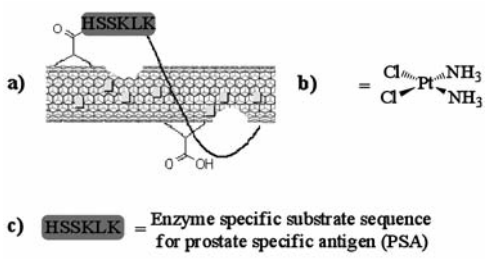

Figure 1. *Illustrations of: (a) the proposed US-tube/CDDP drug carrier system, (b) the CDDP chemotherapeutic drug and (c) the enzyme-specific peptide sequence for PSA.*

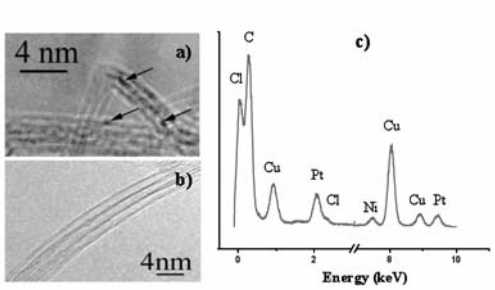

Figure 2. *High-resolution transmission electron microscopy images of: (a) CDDP@US-tubes and (b) empty US-tubes. In (c) the energy-dispersive spectrum of CDDP@US-tubes clusters is shown.*

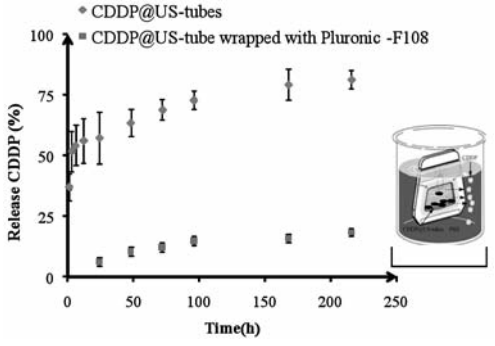

Figure 3. *Release performance of CDDP from US-tubes in PBS at 37°C.*

Studies are underway to determine the cytotoxicity of CDDP released from the US-tubes against different cancer cell lines. Furthermore, the desired peptide chain, which will include a substrate activation sequence for prostate-specific antigen (PSA), will be synthesized and externally attached to the US-tubes in order to cover the US-tube side-wall defects to prevent early drug release before PSA activation in cancer cells. Once the activation and drug release is confirmed *in vitro* in cell culture studies, *in vivo* experiments will be performed using a mouse model to investigate efficacy.

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CLINICAL/COMPUTATIONAL INVESTIGATION ON POST-TRAUMATIC HETEROTROPIC OSIFICATION IN HEAD INJURY PATIENTS: THE POSITIVE AND NEGATIVE ROLE OF TEMPRATURE IN PHYSICAL TREATMENT

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Background: Bone morphogenetic proteins (BMPs) induce bone formation and healing. Bone can form in extraskeletal tissue in response to trauma and elevating BMPs. Several BMPs are stimulated to increase due to neuroprotective effects. To maintain the injured zone of fraction warm is one of the suggested methods to prevent heterotropic ossification (HO). However, about half of patients present worse clinical situation. *Methods:* In a three-year clinical follow-up, a number of head-injured patients were analyzed after X-ray or CT scans. Monte-Carlo simulations and Ramachandran plots were performed using Hyperchem8 and VMD 1.8.2, respectively. PDB files of both 1tfg and 2tgi protein were selected from Protein Data Bank. The parameters ϕ and ψ of all 110 amino acid were calculated in both proteins after Monte-Carlo simulations at 290, 310 and 315K for 200 ps, surrounded by water. *Results:* A total of 21 patients manifested HO. In contrast to the expectation, nine of those patients revealed the worst situation after physical treatment below the IR thermogenerator. *Conclusion:* 1tfg is affected by increasing the temperature. The favored and allowed regions were decreased in a population of dihedral angles. Increasing the temperature above room temperature can cause more allowed dihedrals for 2tgi. It is suggested that the use of thermogenerator lamps advances HO in these patients.

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BIOINFORMATICS, METABOLOMICS AND PROTEOMICS IN MOLECULAR MEDICINE

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We employ a multidisciplinary approach that combines bioinformatics, biochemistry and molecular biology to address diverse problems in molecular medicine. For example, to counter the ever increasing problem of antibiotic resistance,

we have developed a novel antimicrobial strategy. This utilises synthetic peptides to block bacteria from reaching their target site and exerting their harmful effects rather than killing them. We are characterising the structure of these peptides and other medically important proteins, with a role in diverse disease processes such as dental caries, prion, amyloid disease and others. Physical techniques being used in these studies include surface plasmon resonance (SPR), Fourier transform infrared (FTIR) spectroscopy, circular dichroism (CD) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. Artificial intelligence-based bioinformatics methods are also being used for advancing our proteomics research, achieving rapid elucidation of protein secondary structure for high-throughput analysis and, thereby, obtaining a better picture of their role in health and disease and accelerating drug discovery. NMR spectroscopy and matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF) are being utilised for metabolomics and proteomics research, respectively. NMR spectroscopy is being used to identify alterations in the urinary metabolomic profile associated with disease processes such as multiple sclerosis and diabetes. MALDI-TOF technique is simultaneously used to monitor changes in the proteomic profile of these urine samples in order to identify protein-based disease biomarkers. Examples of results from our highly multidisciplinary project, combining bioinformatics, proteomics and metabolomics for advancing molecular medicine will be presented.

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GENE DOPING IN SPORTS

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The World Anti- Doping Agency (WADA) defines gene doping as "the non-therapeutic use of cells, genes, genetic elements, or of the modulation of gene expression, having the capacity to improve athletic performance". The principle of gene therapy is the delivery of a therapeutic gene to a cell in which the gene may compensate an absent or abnormal gene. In general, gene doping involves the use of gene therapies for increasing muscle growth, blood cell production, resistance to fatigue, endurance, oxygen accumulation and pain perception. There are several risks involved in gene doping concerning the accuracy of the transfer, the various techniques being used under the right guidance and the skills of the scientists who have therapeutic proficiency. Detection of gene doping is difficult. Gene therapy vectors may be measurable only shortly after administration and in many cases would require tissue sampling. The International Sports Federations and WADA have accepted performance-enhancing substances and methods as being doping and have forbidden them from protecting the athletes' health in order to ensure equal competitive conditions. Moreover, a WADA funded research project reported, for the first time, that the direct and long-term detection of gene doping by the abuse of gene transfer techniques is possible in blood samples.

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TARGETING MMP-3 GENE BY siRNA TRANSFECTION IN GASTRIC CANCER AGS CELLS

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**Previous address*

Background: The gastric epithelium is continuously exposed to toxic reactive oxygen species and matrix metalloproteinases (MMPs) are the enzymes known for their role in the invasion of tumor cells. Here, we report the suppression of MMP-3 in the gastric cancer cell line AGS by small interfering RNA transfection and its potential role in gastric carcinomas. *Methods:* Oxidative stress in the cells was assessed following H2O2 exposure *via* the oxidative stress marker 2,7-dichlorofluorescein diacetate. Transfection of cells with small interfering RNA specific to MMP-3, invasion assay, quantitative reverse transcriptase polymerase chain reaction analysis and overexpression of MMP-3 were used to determine the potential role of MMP-3 gene in gastric carcinomas. *Results:* The silencing of the MMP-3 gene resulted in a decrease of invading cells, while its overexpression caused an increase in the invading cells, compared to the untreated control cells. Moreover, it caused a 4.1-fold increase in MMP-10 and a 7.4-fold decrease in MMP-15 mRNA expression levels. *Conclusion:* The silencing of the MMP-3 gene decreased invading AGS cells and affected the expressions of MMP-10 and MMP-15, suggesting that targeting the MMP-3 gene in gastric carcinomas may be a therapeutic approach.

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ASSOCIATION OF 1128T>C NEUROPEPTIDE Y GENE POLYMORPHISM WITH DYSLIPIDEMIA IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background: Neuropeptide Y (NPY) is a neurotransmitter that is widely expressed in the central and peripheral nervous systems. It is known to regulate food intake in the hypothalamus, but it also favours energy storage through increased lipoprotein lipase activity in white adipose. The T1128C polymorphism in the NPY gene has been associated with elevated serum total cholesterol, LDL-C concentrations and TAG among adults. However, there is little data about NPY gene polymorphism and dyslipidemia in Iran. Therefore, we wished to investigate the relationship between NPY gene 1128T>C polymorphism and the outbreak of dyslipidemia in Iran. *Methods:* A total of 485 subjects (270 men and 215 women) who were candidates for angiography were recruited; 380 subjects with dyslipidemia and 105 subjects without dyslipidemia. DNA samples were extracted using the salting out method and the 1128T>C polymorphism was determined by PCR-RFLP for all subjects. *Results:* At baseline, a significant increase in TC genotype frequency was observed in all patients compared with the healthy group ($p<0.05$). There was significantly increased TC genotype in women compared to the men in the population studied ($p<0.05$). Also, there was significant difference between dyslipidemia and the lack of dyslipidemia in women, while no significant difference was observed in men. *Conclusion:* The 1128T>C polymorphism was associated with dyslipidemia in Iranian population. Therefore, this polymorphism may be associated with the dietary response to the lipid profile in a dyslipidemia group particularly in a female population.

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HER2 ILE655VAL POLYMORPHISM AND RISK OF BREAST CANCER IN TURKISH WOMEN

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Background: The aim of this study was to investigate a possible association of the HER2 Ile655Val polymorphism with the risk of developing breast cancer in Turkish women. *Patients and Methods:* The study was performed in 118 patients with breast cancer and 128 healthy individuals. HER2 Ile655Val gene polymorphism was performed by polymerase chain reaction and restriction fragment length polymorphism. The results were subject to statistical analyses. *Results:* In the present study, HER2 Val/Val genotype was not observed in any of the study groups. Moreover, the frequency of HER2 Ile/Val genotype was significantly higher in the patient group than in the control group ($p=0.009$, $\chi^2=6.785$, OR=1.983 95% CI: 1.181-3.328). When the clinicopathological parameters and frequencies of the HER2 genotypes were compared between the study groups, there were no significant differences between the patient and control groups ($p>0.05$). *Conclusion:* This study suggests that HER2 Ile/Val genotype may contribute to the development of breast cancer in Turkish women.

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DEVELOPMENT AND INVESTIGATION OF ETOPOSIDE RESISTANCE IN MCF7 BREAST CANCER CELL LINE

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Background: One of the major problems in cancer treatment is the failure of chemotherapy due to drug resistance. Etoposide is a DNA damaging agent used in the treatment of metastatic breast cancer. It directs cancer cells into apoptosis *via* inhibiting topoisomerase II alpha (TOP2A). Breast cancer cells develop resistance against etoposide which may involve alterations in the expression levels of TOP2A and mismatch repair genes. *Methods:* An etoposide-resistant subline was established from MCF7 cells by stepwise selection in dose increments. Expression analyses of TOP2A, MSH2 and MLH1 were performed by quantitative real time polymerase chain reaction. Statistical significance of relative differences in gene expression levels were estimated by one-way ANOVA. *Results:* The established resistant subline was found to be 2.3-fold more resistant to etoposide relative to the parental MCF7 cells. Expression levels of TOP2A, MSH2 and MLH1 in the resistant subline were significantly decreased 90%, 82% and 82% respectively, relative to the parental MCF7 cells. *Conclusion:* Decrease in the expression levels of TOP2A, MSH2 and MLH1 is important in the development of

chemotherapeutic resistance to etoposide in breast cancer. These genes may be considered for the development of new strategies to overcome resistance against topoisomerase II inhibitors.

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PROMOTER ANALYSIS OF THE P80-KATANIN GENE

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Background: The microtubule-severing protein katanin is a heterodimer of 60 kDa (p60) and 80 kDa (p80) subunits. The p60 subunit can break microtubules (MTs) whereas the p80 subunit targets p60 to the centrosomes. The ratio of the two subunits varies markedly in different tissues and at different stages of development, suggesting that the activity of the p60 subunit might be influenced by the levels of the p80. Promoter analysis of the p80-katanin gene which is crucial to understand the temporal gene expression pattern of katanin has not been studied yet. Therefore, we aimed to identify the critical promoter regions of KATNB1 (p80) gene required for its expression. *Methods:* For the characterization of the promoter of *KATNB1* gene, polymerase chain reaction (PCR) was used to amplify 1000 bp upstream nucleotides of *KATNB1* gene. By using nested PCR, shorter fragments were obtained. These promoter deletion constructs were cloned into a luciferase reporter plasmid vector which lacked eukaryotic promoter and enhancer sequences. Obtained deletion constructs were transfected to SHSY-5Y neuroblastoma cells and the luminometrical measurement was performed. *Results:* We identified 518 bp TATA-less promoter including a critical CpG island as an optimal promoter and 160 bp minimal promoter required for *KATNB1* gene expression. *Conclusion:* 518 bp TATA-less promoter is sufficient for KATNB1 gene expression.

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SPEEDY/RINGO INHIBITS CALPAIN-DIRECTED APOPTOSIS IN NEURONS

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Background: While according to the classic view neurons are post-mitotic and cannot reenter the cell cycle, recent studies showed mitotic reentry due to neurotoxic agents. Especially amyloid-beta aggregation destabilizes calcium homeostasis. Elevation of intracellular calcium levels overactivates the calpain and causes mitotic reentry of neurons. However, neurons cannot complete their cycle and undergo apoptosis. Recent studies indicated the ability of a novel cell-cycle protein, Speedy/RINGO, to inhibit apoptosis. Since calpain overactivation is one of the important factors causing apoptosis, our aim was to identify the protective effect of Speedy/RINGO on calpain-induced neurodegeneration. *Methods:* Speedy/RINGO was transfected into neurons. Calpain activation was then performed and cell cycle markers were analyzed by Q-PCR and immunocytochemistry. Caspase-3 activity was analyzed by Western-blotting to determine if apoptotic pathway was inhibited by Speedy/RINGO. *Results:* Q-PCR analysis showed that in none of the cell-cycle markers mRNA level was changed. Immunostaining results confirmed the Q-PCR analysis that there is no difference in expression levels. Western-blotting results determined no change in protein level of cleaved-caspase-3 in the presence of Speedy/RINGO. *Conclusion:* The lack of change in mRNA/protein levels implied that Speedy/RINGO keeps neurons under stable conditions. Furthermore, no change in cleaved caspase-3 level indicated that Speedy/RINGO may protect neurons against calpain's apoptotic effect.

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INVESTIGATION OF THE CYTOTOXIC EFFECTS OF EXTRACTS FROM ENDEMIC *BUPLEURUM* SPECIES IN RESISTANT BREAST CANCER CELL LINES

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The resistance developed to a broad spectrum of chemotherapeutic agents during cancer chemotherapy is called multiple drug resistance (MDR). The MDR phenotype acquired by drug-unresponsive patients remains a major hindrance to chemotherapy. P-gp overexpression is one form of MDR phenotype. Paclitaxel and vincristine are antimitotic chemotherapeutic agents. MCF-7 is a mammary epithelium cell line used as a model for breast cancer. The aim of this study was to investigate the cytotoxic effects of plant extracts in P-gp overexpressing paclitaxel-resistant (MCF-7/Pac) and vincristine-resistant (MCF-7/Vinc) MCF-

7 mammary carcinoma cell lines and their parental line (MCF-7/S). In this study, the roots of five endemic Bupleurum species (B. turcicum, *B. sulphureum, B. lycaonicum, B. pauciradiatum* and *B. heldrechii*) were collected and grinded. The plant powder was extracted in 70% ethanol by sonication and lyophilized. The effect of the total extracts on the viability of paclitaxel- and vincristine-resistant MCF-7 cells were determined by XTT cytotoxicity tests after incubation of cells in different doses of plant extracts for 72 h. IC_{50} values of total extracts for sensitive and resistant cell lines were calculated. The degree of toxicity of the total extracts to the cell lines was determined according to these values.

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DETERMINATION OF PHENOLIC COMPOUND, SAIKOSAPONIN AND PODOPHYLLOTOXIN CONTENTS IN THE ROOTS OF ENDEMIC *BUPLEURUM* SPECIES

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Multiple drug resistance (MDR) acquired against chemotherapy by cancer cells causes most of the chemotherapeutic agents not to show the expected impact on the patients and leads to the progression of the disease. Therefore, making the drug-resistant cells re-sensitive to the chemotherapeutic agents is extremely important for the success of the treatment. While podophyllotoxin is known to be an effective antitumor agent, types of phenolics and saponins derived from plants were shown to reverse the resistance by modulating the cell membrane protein P-glycoprotein (P-gp). The aim of this study was to determine the phenolic compound, saikosaponin and podohyllotoxin contents of total extracts from *Bupleurum* species by HPLC. The total phenolic content and DPPH antioxidant activity of the extracts were also investigated. In this study, the roots of five endemic *Bupleurum* species (*B. turcicum, B. sulphureum, B. lycaonicum, B. pauciradiatum* and *B. heldrechii*) were collected and grinded. The plant powder was extracted in 70% ethanol by sonication and the extracts were lyophilized. After dissolving the lyophilized extracts in methanol, the extracts were subjected to gradient elution by HPLC and the contents were determined. Total phenolic content and the DPPH antioxidant activity of extracts were also investigated. The MDR reversal activities of the compounds collected by HPLC will be further determined by fluorescent microscopy.

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INVOLVEMENT OF mTOR SIGNALLING PATHWAYS IN HEALTH AND DISEASE IN REPRODUCTIVE TISSUES

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The mammalian target of rapamycin (mTOR) is a Ser/Thr protein kinase that functions as energy, stress, amino acid, and nutrient sensor to balance metabolism and cell growth. mTOR forms two complexes (mTORC1 and mTORC2) which differ in their composition and regulation and exert distinct biological effects. Recently, a modulator of mTOR that binds to mTOR complexes, termed DEPTOR was described. However, the precise functions of DEPTOR are not fully elucidated. We have mapped in detail mTOR components in reproductive tissues. At placental level where mTOR appears to act as a nutrient sensor, DEPTOR is the predominant transcript compared with mTOR, rictor and raptor. Interestingly, a significant inverse correlation between DEPTOR and maternal stress was noted. Moreover, syncytialization of placental cells also exerts an effect in the expression of mTOR-related genes. Finally, a limitation to successful ovarian cancer chemotherapy treatments is the acquisition of drug-resistance. We have shown that in paclitaxel resistant ovarian cancer cell lines the activity of mTOR signalling pathway is altered. Collectively our data suggest that there is a higher order of complexity of mTOR signalling in the human placenta that can affect pregnancy outcome, and mTOR pathway may also mediate paclitaxel resistance in ovarian cancer.

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SAMPLE SIZE AND POWER CALCULATION IN MOLECULAR MEDICINE: A SIMULATION STUDY

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Background: Sample size and power calculation is a critical procedure when designing new studies, including those in the field of molecular medicine. Power analysis involves determining the statistical power of a design, given a significance level, sample size and effect size and it can be used to calculate the minimum sample size required to accept the outcome of a statistical test with a particular level of confidence. Sample size and power of a study can be calculated using statistical formulas. However, these formulas

have their own limitations and to judge about the accuracy of these calculations, knowledge of statistics is required. *Methods:* Sample size and power calculation methods were shown with the illustrated data sets. *Results:* The statistical power calculated from the small sample size and that calculated from the sufficient sample size of the same study were compared. It was found that the sufficient sample size provides higher power than the small sample size. *Conclusion:* Researchers can use these calculations as a tool to increase the strength of their inferences, and editors and reviewers of scientific publications may demand that statistical power be reported in all cases where a non-significant result is obtained.

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TWO HOMOLOGOUS PROTEINS CHI3L1 AND CHI3L2 OVEREXPRESSED IN GLIAL TUMORS ACTIVATE THE MAPK SIGNALING PATHWAY BUT LEAD TO DIFFERENT CELL FATE

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Background: Serial analysis of gene expression, Northern and Western analyses revealed the closely related chitinase-like genes *CHI3L1* and *CHI3L2* among genes with the most increased expression in glioblastoma. *Methods:* Recombinant proteins, cell line stably producing CHI3L1, soft agar colony formation, Erk1/2 phosphorylation and nuclear translocation, mitogenic, proliferation and apoptosis assays were used in this study. *Results:* It was found that *Chi3l1* possesses oncogenic properties. The influence of CHI3L2 protein on cell proliferation was opposite to the CHI3L1 effect. Evaluated parameters were diminished in cells treated by CHI3L2, in contrast to those stimulated by CHI3L1 application. Both proteins activated Erk1/2, but the activation duration was different: in cells treated by CHI3L2, sustained Erk1/2 activation was associated with their nuclear accumulation, while activation triggered by CHI3L1 led to the transient Erk1/2 phosphorylation with only brief nuclear translocation. Nuclear accumulation of active Erk1/2 resulted in phosphorylation of certain transcription factors. *Conclusion:* The consequences of events in Erk-mediated cascade depend on the duration of Erk phosphorylation as well as on the cellular context, namely the transcription factors activated by Erk1/2. Thus, the cellular response determines which factors are present in the cell. Activation of the phosphatidylinositol 3-kinase (PI3K) pathway is currently under investigation.

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USE OF MOLECULAR BIOLOGY IN MEDICAL DEFENSE AGAINST BIOTERRORISM AND RELATED EXPERIENCES OF THE MILITARY MEDICAL ACADEMY CBRN LABORATORY

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Bioterrorism is the intentional use of microorganisms and toxins, generally of microbial, plant or animal origin to produce disease and death in humans, livestock and crops. The deliberate use of biological agents including bacteria, viruses, rickettsia and toxins has emerged as significant threat in the last decade and after the anthrax cases that occurred following the terrorist attack in 11th September, 2001. Rapid and accurate detection and identification of bioterror agents is important not only to confirm that a bioterrorism event has occurred, but also to determine the required proper protective measures. Molecular analysis of samples such as powder-containing envelopes upon their arrival to the laboratory should be carried out for instant confirmation. Molecular assays should be sensitive and specific to detect low concentrations of agents. In general, nucleic acid-based systems are more sensitive. Improvement of rapid and advanced diagnostic systems and facilities such as battlefield detection systems, biosensors and fully automated biodetectors for real time sample collection, detection and identification in the field have been developed. On the other hand, a miniature flow cytometer (known as miniFlo) using an immunoassay system and a portable PCR identifying the DNA inside the cell are also available for the detection and identification of biological warfare agents. This lecture aims to give brief information about detection technologies for biothreat agents. In addition, the studies about biothreat detection in our medical CBRN Department which is unique in Turkey will be briefly mentioned herein.

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INVESTIGATION AND COMPARISON OF THE EXISTENCE OF MULTI-DRUG RESISTANCE (MDR1) GENE MUTATIONS AT GENOTYPE 1B AND DIFFERENT GENOTYPES OF HCV

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Background: P-glycoprotein (Pgp) is encoded by the human multidrug-resistance (*MDR1, ABCB1*) gene. Hepatitis C virus

species is classified into six genotypes that are clinically important in determining the potential response to interferon-based therapy. *Methods:* HCV-RNA assay was performed using real-time PCR. HCV genotyping and MDR1 mutations were both performed by using the reverse hybridization method. *Results:* The study included 85 HCV-RNA positive patients; among them 81 (95.2%) were identified as HCV-1b, 2 (2.4%) were HCV-2a and 2 (2.4%) were HCV-3a subtypes. Of the 81 HCV-1b cases, 51 (62.9%) were found to be positive with MDR1 mutations; 47 (58%) were heterozygous and 4 (4.9%) were homozygous. No MDR1 mutations were found in patients with HCV-2a and HCV-3a subtypes. Statistically significant differences were found between genotypes of HCV-1b and HCV-2a ($p<0.001$), HCV-1b and HCV-3a ($p<0.001$) for the occurrence of MDR1 mutations. Of all 81 cases with HCV-1b, 45 (58%) were female and 36 (44%) were male. Statistically significant differences were found between male and female with genotypes of HCV-1b for the occurrence of heterozygous ($p<0.05$) and homozygous ($p<0.05$) MDR1 mutations. The two–sample proportion test was used for all statistical analyses. *Conclusion:* The results of this study indicated a significant difference between the two groups HCV-1b/2a and HCV-1b/3a for the occurrence of MDR1 mutations. These results may be related with the tendency of patients infected with HCV-1b to exhibit poor IFN responsiveness compared with those infected with HCV-2a/3a.

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COMPARISON OF HPV PREVALENCE AND TYPES OF HPV IN WOMEN AT HIGH RISK OF CERVICAL CANCER WITH RISK FACTORS AND CERVICAL SMEAR

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Background: Human papilloma virus (HPV) is considered to be involved in cervical cancer and precancerous lesions. This study aimed to determine the prevalence and the types of HPV in women at high risk of cervical cancer and the correlation between HPV positivity and risk factors for developing cervical cancer. *Methods:* A total of 404 healthy women at risk were investigated in terms of risk factors. The existence of HPV was determined and 16 types were identified by using DNA-reverse hybridization. Cervical smear samples were obtained and biopsies were performed with colposcopic examination. Chi-square and multiple logistic regression analyses were used for statistical processing of the data. *Results:* HPV frequency rate was found to be 32.5% (n=131). HPV18 was found in 60 cases (45.8%) and HPV16 in 22 cases (16.8%) as the most frequently types. According to HPV types, high-risk rate for cervical cancer was found to be 78.7% (n=103). In cervical smear evaluations, epithelial cell abnormalities were found in 91 cases (22.5%), atypical squamous cells of undetermined significance in 82 cases (20.3%), low-grade squamous intraepithelial lesions in 7 cases (1.7%) and high-grade squamous intraepithelial lesions in 2 cases (0.5%). *Conclusion:* Women at high positivity in oncogenic HPV types should be followed and necessary precautions should be taken.

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HEREGULIN INDUCES HEAT SHOCK PROTEINS IN AN HSF1 DEPENDENT MANNER IN CANCER CELLS AND LEADS TO PROTECTION FROM APOPTOSIS

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Elevated HSP levels are observed in many cancer types and signal a poor prognosis in terms of survival and response to therapy. HSP expression protects cells from spontaneous apoptosis during transformation, in addition to apoptosis induced by therapy. These mechanisms may underlie the role of HSP in tumor progression and resistance to treatment. HSP transcription requires the heat shock transcription factor (*hsf1*), which is itself overexpressed in cancer and plays a role in invasion and metastasis. We show that HSP elevation is linked to the highly malignant factor heregulin β1 (HRGβ1), which induces HSP expression through HSF1. HSP expression is induced through a cascade response initiated by HRGβ1 binding to *c-erb*B receptors and leads to the inhibition of HSF1 antagonist glycogen synthase kinase3. Activated HSF1 plays an essential role in the protection of cells from apoptosis by HRGβ1, indicating the role for HSF1 in tumor progression pathway. *Methods:* Mammalian cell culture, immunoblot analysis, luciferase and β-galactosidase activity assays,

apoptosis assay and anchorage independent growth assay were used in this study. *Results:* HRGβ1 induced HSP expression through hsf1-dependent transcription. Signal transduction steps led to hsf1-dependent activation of HSP expression by HRGβ1. HRGβ1-induced, c-erbB-2-dependent and anchorage-independent growth involved significant participation of hsf1. The cytoprotective effects of HRGβ1 protection by heat shock led to HSP expression. *Conclusion:* Heregulin induces Hsp70 in an HSF1-dependent manner. Heregulin activates HSF1 through the ErbB/Pi3k/Akt pathway. Heregulin/ErbB/HSF1-mediated hsp synthesis inhibits apoptosis and prevents tumor progression.

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CYP21A2 ANALYSIS OF CONGENITAL ADRENAL HYPERPLASIA PATIENTS DUE TO 21-HYDROXYLASE DEFICIENCY

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Introduction: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder mainly caused by 21-hydroxylase deficiency. Females with severe 21-hydroxylase deficiency are exposed to excess androgens prenatally and are born with ambiguous genitalia. Moreover, most patients cannot synthesize sufficient aldosterone to maintain sodium balance and may develop potentially fatal "salt-wasting" crises, if not treated. *Methods:* A total of 100 patients and 80 controls were recruited to the study. CYP21 gene regions starting from promoter to the end of exon 4 were amplified by PCR. Mutations were detected by direct sequencing. The results were evaluated statistically. *Results:* A total of 10 novel mutations were detected. In addition to these mutations, 16 different polymorphisms were also found. G→A mutation at position 711 (13%) (novel mutation) and A/C→G mutation at position 777 (38%) were found to be statistically significant when compared to controls. *Conclusion:* Prenatal diagnosis has been utilized for over 20 years, especially by screening for nine known mutations. In order to establish the presence of G→A mutation at position 711 in different populations, the test for this mutation may be included in the screening protocol of prenatal diagnosis. By using prenatal treatment techniques in the prenatal stage, genital ambiguity, subsequent problems of sex misassignment, gender confusion and "salt-wasting" can be reduced in CAH patients.

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MOLECULAR REGULATION OF LYMPHATIC VASCULAR DEVELOPMENT: IMPLICATIONS FOR HUMAN DISEASE AND CANCER THERAPY

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Background: Aberrant formation of lymphatic vessels (lymphangiogenesis) is associated with a wide range of pathological conditions such as inflammatory disease, obesity and lymphedema. Further, most tumours depend on lymphangiogenesis for metastasis. Understanding the molecular control of lymphangiogenesis will suggest new ways to manage it pharmaceutically. *Results:* We discovered that Sox18 encodes a transcription factor that acts as the master regulator of lymphangiogenesis in the embryo. We now find that *Sox18* is reactivated during tumour lymphangiogenesis, and that mice mutant for *Sox18* show reduced metastasis. We have therefore developed a screening strategy to identify small inhibitory molecules able to modulate SOX18 protein activity, and have discovered a novel class of small molecules that disrupt SOX18 function *in vitro* and *in vivo*. *Conclusion:* Our findings provide a basis for innovative pharmacological SOX18-based strategies to modulate lymphangiogenesis, raising the prospect of complementing anti-angiogenic treatment with an anti-lymphangiogenic approach to cancer therapy.

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ANTIHYPERPLASTIC EFFECT OF LYSOZYME GENE IN A TESTOSTERONE-INDUCED RAT MODEL OF BENIGN PROSTATİC HYPERPLASIA (BPH)

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Background: The effects of lysozyme gene treatments on prostatic tissue were studied in an experimental model of

BPH. *Methods:* Adult Wistar rats weighing 200 to 400 g were used. They were completely randomized into 3 groups as control, testosterone, and testosterone+ lysozyme (Lys) gene. After mRNA isolation, reverse transcriptase polymerase chain reaction, restriction digestions and ligations, recombinant pHM6 vector harboring mouse lysozyme gene (pHM6mLys) was constructed. Single dose testosterone was administered intramuscularly, except group 1. 40 days after the beginning of experiment in group 3, Lys was injected into rat prostates tissue. A week later (day 47), all rats were sacrificed. *Results:* Testosterone treatment increased by 39% the prostate weight in rats. Lys therapy decreased by 38.7% the prostate weight in rats. Testosterone application caused an irregular acinar growth pattern in the prostate tissue with variable amout of stroma. İsolated or multiple stacked cell populations (pilling up formation) was identified in the epithelial layer and it was lined by cuboidal or cylindrical cells. *Conclusion:* We suggest that Lys may be useful in the treatment of BPH.

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THE EXPERIMENTAL MODEL OF TESTES TORSION AND APOPTOSIS

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Background: Apoptosis response was investigated in mild ischemic reperfusion damage in germ cells due to testicular torsion. *Methods:* A total of 44 Wistar albino rats were divided into control, sham, and 1, 2 and 4 hour torsion groups. Left testes were subjected to 720˚ of torsion followed by 4 hours of reperfusion. The ischemic reperfusion damage and the apoptosis level in left and right testes were evaluated histopathologically. *Results:* JTBS was normal in the controls and the sham group, accompanied by very low levels of apoptosis. In the 1 hour torsion group, the JTBS was lower compared to the controls and the sham group, and the average apoptosis level was higher. However, these levels were not drastically high and comparable to the right testes levels of the 2 and 4 hour torsion groups. In 2 and 4 hour torsion groups, the JTBS was also lower and the apoptosis levels higher in comparison to the controls as well as the sham and 1 hour torsion groups. *Conclusion:* The apoptotic examination was more sensitive to determine the testicular damage in mild ischemia and the opposing testicular damage compared to the JTBS.

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THE EFFECTS OF RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (RAGE) -374 T/A AND GLY82 SER AND PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPAR-γ) PRO12ALA POLYMORPHISMS ON CORONARY ARTERY DISEASE

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Background: The present study aimed to investigate the individual and combined effects of RAGE -374T/A, RAGE Gly82Ser and PPAR-γ Pro12Ala polymorphisms on the development of coronary artery disease (CAD). *Methods:* Polymerase chain reaction, restriction fragment length polymorphism and agarose gel electrophoresis techniques were used to determine RAGE -374T/A, RAGE Gly82Ser and PPAR-γ Pro12Ala. *Results:* Individual allele and genotype frequencies of RAGE -374T/A, RAGE Gly82Ser and PPAR-γ Pro12Ala polymorphisms were not significantly different between the study groups. However, when compared to the control group, wild-type T allele frequency was found higher in the patients with diabetes. To investigate the combined effects of RAGE and PPAR-γ polymorphisms, haplotype analysis was performed and there was no statistical difference between the haplotypes of RAGE Gly82Ser with RAGE -374T/A or PPAR-γ Pro12Ala. However, the frequency of RAGE-374T/PPAR12Ala haplotype was found to be higher in both patient groups with and without diabetes compared with the control group. *Conclusion:* The results of the present study demonstrated that possessing the A allele of *RAGE* -374T/A polymorphism for diabetic CAD patients and possessing the -374T/Ala12 haplotype of *RAGE* -374T/A and *PPAR-γ* Pro12Ala polymorphisms for the patient groups are the most important risk factors for CAD.

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THERAPEUTIC APPLICATIONS OF ANTI-ANGIOGENIC STRATEGIES

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Angiogenesis, the formation of new blood vessels, is essential

for tumour growth and metastasis. Angiogenesis also has a crucial role in a variety of physiological conditions and 'angiogenic diseases' such as embryogenesis, wound healing, psoriasis, myocardial infarction, scleroderma, low back pain, rheumatic arthritis, stroke *etc*. Over the past 30 years or so an enormous amount of work has been undertaken to find specific markers for angiogenic endothelial cells. We have raised a panel of monoclonal antibodies to activation/ proliferation associated antigens such as CD105 which present on the luminal surface of vascular endothelial cells. When reactivity of our anti CD105 mab in normal and tumour tissue was compared with a number of routinely employed pan – EC markers, blood vessels within and in the immediate vicinity of the tumour mass strongly stained with our CD105 antibody (E9), whereas the same blood vessels were either weakly positive or not stained by pan-EC antibodies. Similarly this antibody was highly specific for angiogenic EC in human stroke tissues and myocardial infarcts. *In vivo* animal studies showed that CD105 antibody E9 localised to tumour vascular endothelial cells and CD105 based immunoconjugats inhibited the growth of the human breast tumours. An *ex vivo* study that utilized freshly excised kidneys from patients with renal cancer resulted in specific labelling of tumour vasculature by radio labelled anti-CD105 mab E9. Unpublished data of a phase 1 clinical trial revealed evidence of localisation of anti CD105 antibody in tumours and in some cases even had beneficial therapeutic effects. Similar experimental work is underway in other 'angiogenic diseases' in man.

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ETS DOMAIN TRANSCRIPTION FACTORS IN NEURODEGENERATIVE DISORDERS

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Background: Elk-1 is a transcription protein that belongs to the ETS domain superfamily. Elk-1 is traditionally studied as a mitogenic transcription factor that gets phosphorylated in response to the activation of the mitogenic MAPK signaling pathway, translocates to the nucleus and induces transcription from immediate-early genes, such as c-fos. However, recently our lab and others have shown the survival-related functions of Elk-1, while one group has shown cell death response initiated by Elk-1 in neurons. These findings, thus, put Elk-1 in a peculiar position. *Methods:* We have employed molecular techniques such as promoter cloning, site-directed mutagenesis, luciferase reporter assays and chromatin IP (ChIP) assays among many other techniques to study the effect of Elk-1 on the transcriptional activation from various neurodegeneration-related promoters. *Results:* In this study we analyzed the transcriptional regulation of various neuro-degeneration-related genes that contain Elk-1-binding motifs in their promoter sequences. We have shown that Elk-1 regulates the survival of motor neuron gene (SMN), which is commonly mutated in spinomuscular atrophy, and we have provided some evidence that Elk-1 can also regulate the transcription of SOD1 gene that is associated with familiar amyotrophic lateral sclerosis. *Conclusion:* We believe that Elk-1 is at the center stage of many neurodegenerative disorders, and our conclusion (combined with our previous data) is that this helps protect neurons against apoptotic cell death.

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GENETIC FACTORS AND HUMAN PHYSICAL CAPABILITY

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Human athletic performance is essentially multifactorial and determined by a wide range of factors, including training, nutrition, technological aid and genetic factors. Hereditary influences on endurance phenotypes such as maximal oxygen uptake and ventilatory thresholds are estimated to be around 50% in sedentary individuals. Understanding the genetics of endurance performance may provide valuable information to determine "advantageous" polygenic profiles. Although over 150 DNA polymorphisms have been found to be associated with some form of human physical performance, the probability of an individual possessing an optimal polygenic profile seems very small. Furthermore, the genetic basis of psychological phenotypes that would characterize the physiological trait is equally important in determining the athletic elite performance and this topic requires further attention. Overall, this brief presentation tries to summarize some notable polymorphisms in the light of evidence that complex genetic interactions are yet to be identified.

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ROLE OF APOPTOSIS IN THE RESPONSE OF SOLID TUMOR CELLS TO THERAPY

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Apoptosis has been widely proposed as a major mechanism of the anti-proliferative effects of various clinically used therapeutic agents, including DNA damaging agents and

mitotic inhibitors. A problem with these studies is that most available evidence is based on *in vitro* studies using high drug concentrations. The mechanisms of cell death induction by anticancer drugs *in vivo* are less clear. Apoptosis has been reported, but may be a secondary phenomenon to mitotic catastrophe. Therefore, whereas apoptosis is a relevant pharmacodynamic read-out for the efficacy of many drugs, apoptosis signaling may be of secondary importance as a determinant of drug sensitivity. The cellular response to cisplatin will here be described and used to illustrate the complex cellular response to clinically used chemotherapeutic agents. I will also discuss the therapy response of quiescent and hypoxic cells in the deep parenchyma of solid tumors. Drugs may not reach these cells at sufficient concentrations and the cells respond poorly to drugs that target proliferating cells. Our laboratory has found that hypoxic cells in the deep tumor parenchyma may not respond to therapy by induction of apoptosis. Strategies to target these cells will be discussed.

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LIPID PEROXIDATION PRODUCTS AND ANTIOXIDANT ACTIVITIES IN PREGNANT ANEMIC WOMEN BEFORE AND AFTER ORAL IRON SUPPLEMENTATION

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Background: Iron deficiency anemia (IDA) is one of the most common nutritional disorders worldwide, affecting all ages. Oral iron supplementation is a commonly-used strategy to meet the increased iron requirements of the risk groups. However, if provided in excess, iron supplementation may induce peroxidative damage through the production of reactive oxygen species. The present study was designed to evaluate the markers of oxidative stress and the antioxidative enzymes along with serum ferritin and transferrin receptor levels in iron-deficient pregnant anemic women and their response to oral iron supplementation. *Methods:* Iron (100 mg) and folic acid (500 μg) supplementation were given to all 133 anemic women (mild anemia n=50, moderate anemia n=50 and severe anemia n=33) along with 100 age-matched non-anemic pregnant women, taken as controls, daily for 100 days. Blood index, oxidative stress parameters, antioxidant enzymes, vitamins, serum ferritin and transferrin receptors were estimated as per standard protocols. *Results:* Haemoglobin (Hb) levels along with antioxidant enzymes, namely catalase, superoxide dismutase, glutathione reductase, reduced glutathione, total antioxidant capacity and ferritin levels were found significantly increased ($p<0.01$) in all treated groups. However, glutathione peroxidase and antioxidant vitamins, namely A, C and E and transferrin receptor levels were found significantly decreased in all treated subjects. Lipid peroxide levels, protein carbonyl contents, conjugated dienes, lipid hydroperoxide and oxidized glutathione levels were found significantly increased ($p<0.01$) after oral iron supplementation. *Conclusion:* On the basis of these results, it may be safely concluded that, although the recommended oral iron dose is effective for improving blood indices, this happens at the cost of increased oxidative stress. Therefore, it may be suggested that the doses of oral iron may be given according to individual requirements.

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INCIDENCE AND PROGNOSTIC SIGNIFICANCE OF C-KIT EXON 9 MUTATIONS IN NEOPLASIA IN NORTHERN INDIA

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Background: The proto-oncogene c-kit encodes a tyrosine kinase receptor. Abnormal activation of tyrosine kinases or the signaling pathways they control is thought to play a critical role in the neoplastic process of many human malignancies. Exon 9 is the frequent site for mutations in different kinds of neoplasia. *Materials and Methods:* PCR-SSCP and sequencing. *Results:* Of 147 neoplasia cases, 67 were male and 80 were female with ages ranging from 2 to 70 years (mean±SD, 41.6±6.47 years). We have detected a total of 20 mutations (16 point mutations and 4 deletions) in 14 cases. Mutations Ser451Cyst, Ala452Ser, Ser453Leu, Pro456Gln Asp458Tyr, Gln460Trp, Pro467His, Asp496Val, Lys499Gln were found in acute myeloid leukemia, prostate carcinoma, acute lymphoblastic leukemia and chronic myeloid leukemia. Mutations Pro467His and Lys499Gln were found in two cases. Mutation Asp496Val was found in six independent cases. Deletion at codons 480, 481, 482 and 483 were detected in two cases. *Conclusion:* This is the first report of c-kit gene mutations in neoplasia cases in Northern India. These observations suggest that mutations in exon 9 of the c-kit gene may be useful to determine the prognostic implications and how these mutations are related with progression and pathogenesis of malignancy.

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TURKISH POPULATION STRUCTURE AND GENETIC ANCESTRY: IMPACT OF MIDDLE EASTERN AND ASIAN RELATEDNESS AFFECTS PLASMA LIPIDS AND HEART DISEASE RISK

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Background: Turkey connects the Middle East, Europe and Asia and has experienced major population movements, especially admixture from Asia between the 11th and 13th centuries. *Methods:* The study population included 40 three-generation families and unrelated cohorts from several regions of Turkey as part of the Turkish Heart Study. Fine genetic mapping and detailed single nucleotide polymorphism (SNP) analyses (500,000 SNP genotyped; Illumina) were conducted. *Results:* Supervised STRUCTURE analyses (K=4) demonstrated a parental ancestry coefficient for Turks to be 38% European, 35% Middle Eastern, 18% South Asian, and 9% Central Asian. Unique lipid and lipoprotein profiles among the different populations reflect the diversity of genetic ancestry among individuals in the Middle East and Central Asia. Plasma high-density lipoprotein cholesterol (HDL-C) levels, low in Turks, were mapped to chromosome 15q21-23 in atherogenic dyslipidemic Turkish families. In addition to hepatic lipase, fine mapping revealed the importance of glucuronic acid epimerase (*GLCE*), a heparan sulfate proteoglycan biosynthesis enzyme, at this locus. SNP analyses demonstrated significant affects on both HDL-C and triglyceride levels. Interestingly, at the *GLCE* locus, bounded by recombination hotspots, Turks had a minor allele frequency of SNPs resembling Asian more than European ancestry. The importance of *GLCE* in lipoprotein metabolism was further supported by transgenic mouse studies. *Conclusion:* Genetic heterogeneity across population groups can provide insights into new metabolic pathways and indicates the need for detailed population-based studies to define risk factors unique to subgroups of people.

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THE NOVEL QUINUCLIDINONE DERIVATIVE 8A INDUCED APOPTOSIS IN THE HUMAN MCF-7 BREAST CANCER CELL LINES *VIA* THE SPHINGOMYELINASE PATHWAY

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Previously, we reported novel quinuclidinone derivatives that cause cytotoxicity in human non-small lung carcinoma epithelial cells, null for p53 (H1299). The goal of recent investigations is to develop target-based therapeutics and the present study aimed to investigate the effect of these derivatives on the cytotoxicity of human MCF-7 cells and normal lung epithelial cells (MCF-12 a). Here, we report that the quinuclidinone derivatives 8a and 8b induced growth inhibition mainly through apoptosis of breast cancer cells (MCF-7) with less cytotoxic effect in normal lung epithelial cells (MCF-12a) for derivative 8a, while 8b induced similar cytotoxicity for both breast cancer cells and normal breast epithelial cells. Accordingly, we chose 8a for further investigation; 8a induced G_1 phase arrest presumably to sensitize the breast cancer cells to apoptosis by increasing the expression levels of p21 and cyclin E. Moreover, 8a increased the expression levels of ERK1, p53 and Bax and it reduced the expression levels of Akt and Bcl-2. By investigating the sphingomyelinase apoptosis pathway, we observed that 8a significantly increased sphingomyelinase activity and increased the formation of ceramide, as well as it increased the expression levels of JNK phosphorylation, caspase 8 and caspase 9. Based on previous results, we propose that the quinuclidinone derivative 8a provokes apoptosis in human breast cancer cells (MCF-7) *via* the sphingomyelinase pathway.

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IN VIVO INTERACTIONS OF *S. CEREVISIAE* LINKER HISTONE WITH CHROMATIN REMODELING COMPLEXES

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Background: Chromatin structure is an ambiguous player in the processes of cellular transformation and cancer propagation. Complex interactions exist among histones and chromatin-modifying complexes. Any aberrations in these functional networks would potentially lead to chromatin rearrangements and changes in gene expression. An excellent model for chromatin structure and dynamics studies has always been the yeast (*Saccharomyces cerevisiae*). This study was focused on Arp4 – an abundant yeast nuclear protein which is an essential part of several chromatin-remodeling complexes and Hho1p, the yeast linker histone. *Methods:* Gene cloning techniques led to the development of double *arp4 deltahho1* mutant yeast cells. Studies of cell growth were performed under different stress conditions. The chromatin structure of the double mutant cells was studied biochemically. The hybrid system of the double mutant yeast

cells revealed the physical interactions among both proteins of interest. *Results:* The obtained double *arp4 deltahho1* mutant cells revealed defects in the ability to overcome replicative and temperature stress when compared to wild-type. Interestingly, the comparison with the single *arp4* mutants showed that the deletion of the gene for the linker histone allows the double mutant cells to better survive stress. *Conclusion:* Here, we presented *in vivo* data that the yeast linker histone, Hho1p and chromatin-remodeling complexes interact physically, thus potentiating the processes of gene expression.

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THE INVESTIGATION OF GENETIC POLYMORPHISM IN THE CARBONIC ANHYDRASE VI GENE EXON 2 IN TYPE II DIABETIC PATIENTS AND HEALTHY ADULTS: A PRELIMINARY STUDY

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Background: Salivary carbonic anhydrase (CA) VI has been implicated in taste and gastrointestinal dysfunctions, tooth erosion, and dental caries. Diabetes mellitus is a chronic metabolic disease with serious oral health problems. The genetic polymorphism of the CA gene exon 2 was determined in diabetic patients compared to healthy adults. *Methods:* A total of 23 Type II diabetic patients and 20 healthy aged matched adults were recruited in this study. Unstimulated whole saliva and blood samples were taken. Gene polymorphism of CA gene exon 2 was determined by PCR and DNA sequencing. Salivary CA activity was determined by the method of Verpoorte. *Results:* Salivary CA activity did not differ between groups ($p>0.05$). Three SNPs were detected as T55M, G70A, T71S with corresponding amino acid changes and also the change from G to C in codon 76 without corresponding amino acid change. These alterations were not significantly different between groups ($p>0.05$). *Conclusion:* This is the first study investigating the gene polymorphism of CA gene exon 2 in diabetic patients. These genetic alterations may be specific polymorphisms affecting the Turkish population and may contribute to oral problems seen in diabetic patients. Further investigations are necessary.

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APOPTOSIS IN TYPE-2 DIABETES AND DIABETES COMPLICATIONS

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Dysregulation of apoptotic signaling can play a primary or secondary role in various diseases. Type-2 diabetes is characterized by a significant deficit in beta cell mass. Beta-cell mass is regulated by proliferation, neogenesis and apoptosis. Excessive apoptosis in beta-cell plays an important role in diabetes. Various therapeutic strategies are being developed that target beta cells to restore their function by promoting neogenesis and regeneration or by preventing their apoptosis. Exendin-4 enhances the expression of the PDX-1 gene in islets and duct cells, increases beta-cell replication, neogenesis and beta-cell mass. The kidney is an important target organ in diabetes. Apoptosis contributes to progressive renal-cell depletion in diabetic nephropathy. Increased expression of nuclear-clusterin and TGF-β1 in tubuli and glomeruli cells, podocyte injury and microalbuminuria are seen in diabetic nephropathy. ACE inhibitors and angiotensin receptor blockers suppressing RAS provide renal hemodynamic control by blocking apoptosis, proteinuria and TGF-β1 expression by preventing podocyte loss, which leads to a decrease of bax and caspase-3 expressions. Further detailed analysis of targets and regulators of apoptosis may reveal novel therapeutic options for the management and treatment of diabetes and its complications.

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YERBA MATÉ (ILEX PARAGUARIENSIS) SUPPRESSES OXIDATIVE STRESS AND ENHANCES THE ANTIOXIDANT DEFENSE SYSTEM IN EXPERIMENTAL MODELS OF CARCINOGENESIS

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Background: Yerba maté (MT, *Ilex paraguariensis*) is a popular South American beverage that has been reported to have antioxidant properties. The aim of the present study was to evaluate the chemopreventive potential of MT on liver and

colon carcinogenesis initiated by dietylnitrosamine and azoxymethane, respectively. *Methods:* Hepatocarcinogenesis was induced with dietylnitrosamine in thirty 6-week-old male Wistar rats. Colon carcinogenesis was induced with azoxymethane in thirty 6-week-old male Fisher 344 rats. After the initiation period, the animals received MT extract (0.5 mg/kg) or vehicle for ten weeks. Liver and colon biopsies were collected for histological and DNA damage analysis. Tissues were also examined to determine the mRNA levels and the enzymatic activities of catalase, GPx and MnSOD and the nuclear translocation of Nrf2 was evaluated by an electrophoretic mobility shift assay. *Results:* MT significantly decreased the development of preneoplastic lesions as well as the levels of DNA damage in liver and colon. The induction of liver and colon carcinogenesis caused a down-regulation of catalase, GPx and MnSOD. After the intervention, the levels of those enzymes returned to baseline through the Nrf2 pathway. *Conclusion:* These data suggested that the chemopreventive effects of MT in the liver and colon are mediated by Nrf2, which induces the expression of antioxidant enzymes, increasing the cellular capacity to survive the oxidative stress.

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EFFECTS OF *HELICOBACTER PYLORI* INFECTION ON DNA DAMAGE SIGNALING PATHWAY

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Background: Helicobacter pylori is a major risk factor for gastric cancer development. Its oncogenic role is mediated by the chronic active inflammation which increases the levels of reactive oxygen and nitrogen species, whose role has been reported to be linked with carcinogenesis. Thus, the aim of the present study was to evaluate the effects of *H. pylori* on the pattern of DNA repair signaling pathways. *Methods:* To investigate the pathways involved with DNA repair induced by *H. pylori* infection, bacterial strain SS1 was cultured and, for co-culture, the gastric cell line (PG100) was cultured with *H. pylori* (2×10^6 CFU) for 24 and 48 h followed by real-time PCR array. *Results:* The real-time PCR array data revealed that 32% of genes were modulated by *H. pylori*. Among them, 18% related with DNA damage, cell growing and differentiation and apoptosis were up-regulated while 14% involved with DNA repair were down-regulated. *Conclusion:* These data from co-culture suggested that the infection by *H. pylori* regulates the expression of genes related with the DNA repair pathway, being a possible risk for gastric carcinogenesis.

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GENETICALLY MODIFIED UMBILICAL CORD BLOOD MONONUCLEAR CELLS FOR GENE-CELL THERAPY OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Current promising gene-cell therapy strategies for neuroprotection and stimulation of neuroregeneration after neurotrauma, stroke, and in neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), utilize adult stem cells as vectors for therapeutic gene delivery. *Methods:* We used hSOD1 (B6SJL-TG(SOD1-G93A)dl1Gur/J) transgenic mice as a model for ALS. Human umbilical cord blood mononuclear cells (hUCB-MC) were transfected with plasmids, expressing combinations of neurotrophic and neuroprotective growth factors, pBud-VEGF-FGF2, pBud-VEGF-L1CAM and injected into the retro-orbital space of ALS transgenic mice. *Results:* Genetically modified hUCB-MC migrated into the spinal cord of ALS transgenic mice and differentiated into cells expressing markers of endothelial cells and microglia, depending on the genetic modification. *Conclusion:* Genetically modified hUCB-MC is a promising approach for gene-cell therapy of ALS.

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MITOCHONDRIAL DNA POLYMORPHISMS/ MUTATION AND RISK OF ORAL CANCER

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Background: Though Otto Warburg proposed the involvement of mitochondria in the process of carcinogenesis more than 70 years ago, it is only in recent years that studies are being done to elucidate the role of mitochondrial DNA (mtDNA) in various cancers. We studied association between polymorphisms/mutation at mitochondrial and mitochondria associated genes and risk of oral cancer in Indian patients. *Methods:* Genotypes at more than 100 genes were determined

using Illumina platform for 399 cancer and 567 control individuals and somatic mutations in mtDNA were screened in a subset of patients. Data were analyzed to check the association between the risk of cancer and polymorphisms/mutations in these genes. *Results:* Polymorphisms at mtDNA and *POLG2* gene were found to be associated with the risk of cancer when compared with controls. Somatic mutations in D-loop of mtDNA were significantly more in cancer samples compared to control samples (Z-scores=5.6). However, the common 4,977bp deletion was almost absent in cancer mtDNA. *Conclusion:* So, both polymorphisms and mutation at mitochondrial and mitochondria associated genes enhance the risk of oral cancer. But functional work is needed to explain the roles of these genes in carcinogenesis.

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ENHANCEMENT OF RISK OF ORAL LEUKOPLAKIA AND CANCER BY COMBINATIONS OF POLYMORPHISMS AT *NAT1*, *NAT2* AND *XRCC1*

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Background: N-acetyl transferases (*NAT1* and *NAT2*) are important enzymes for the metabolism of tobacco carcinogens. Altered activity of these enzymes may lead to the formation of DNA adducts that may be repaired by *XRCC1*. Here, polymorphisms at *NAT1, NAT2* and *XRCC1* were studied to estimate the risk of oral leukoplakia and cancer. *Methods:* Genotypes at four SNPs on *NAT1* were determined in 389 controls, 224 leukoplakia and 310 cancer patients. Disease risk was estimated using polymorphic data at NAT1 and, also, combining previously published data at *NAT2* and *XRCC1*. *Results:* Polymorphisms at *NAT1* were not associated with the risk of oral leukoplakia and cancer. However, among the NAT1 rapid acetylators who had mixed tobacco habit, *XRCC1*-variant haplotypes enhanced the risk of leukoplakia and cancer compared to the risks attributed by *XRCC1* alone. Interestingly, the combination of *NAT1* rapid, *NAT2* slow and XRCC1 variant haplotypes increased the risk of leukoplakia and cancer with enhancement of more risk in the same tobacco habit group. *Conclusion:* The genotypes at *NAT1* could not modify the disease risk independently but could increase the risk significantly in subsets of patients in combination with *NAT2* and *XRCC1*.

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THE FUNDAMENTAL ROLE OF MOLECULAR BIOLOGY AND BIOMARKERS IN TREATMENT DECISION MAKING IN COLORECTAL CANCER (CRC)

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Despite progress made in the management of CRC in recent years, it remains a major public health problem in the Western world with an estimated 142,570 new cases and 51,370 deaths occurring in 2010 in the United States. Since clinical parameters seem to be inadequate for selection, a major challenge is the identification of specific biomarkers that are likely to predict response in the era of molecular targeted therapies. In the metastatic setting the impact of *KRAS, BRAF* and *PIK3CA* mutational status has been evaluated regarding patients response to anti-EGFR treatment. *KRAS* mutational status represents a paradigm for biomarker development in the era of molecular targeted therapies; anti-EGFR treatment is not indicated for patients with *KRAS* mutant tumors. In addition, patients carrying a *BRAF* mutation do not respond to anti-EGFR treatment. *PIK3CA* mutations appear to be predictors for response to anti-EGFR moAbs but definitive conclusions cannot been drawn yet. Furthermore, *AREG* and *EREG* RTqPCR expression has been consistently associated with outcome to anti-EGFR combination chemotherapy. In the adjuvant setting, the predictive and prognostic impact of microsatelite instability, 18q loss of heterozygosity and SMAD4 expression have been studied but none has been implemented in clinical practice yet.

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RECENT ADVANCES AND CHALLENGES IN THE COMPUTATIONAL DISCOVERY OF BIOMARKERS FOR GENOMICS, PROTEOMICS AND METABOLOMICS

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Biomarkers used in molecular medicine are indicators that generally help diagnose diseases and conditions, identify new treatments and predict clinical responses to the treatments. In some cases, they can also form the basis of drug targets. Computational intelligence and statistical methods and

strategies have widely been used to identify a small number of, but robust set of, the biomarkers that are likely to be associated with the diseases and conditions. However, in line with the development of the "omic" technologies, as the number of the biomarkers generated is increased, the complexity behind the analysis of such massive data has become more challenging. In this presentation, current computational techniques and their limitations will, therefore, be discussed by providing various practical applications in genomics, metabolomics and proteomics.The emphasis will be given to variations over the biomarkers identified when different groups of the patients are analysed. This will be further discussed to show the disparities of their confusing outcomes as well as to demonstrate how their confusing results can be made useful. The presentation will be concluded by addressing the problems, discussing the recent advances in this area, revealing forthcoming challenges and providing, hopefully, some useful recommendations.

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DHPM REVERSES T CELL DYSFUNCTION IN TUMOR MICROENVIRONMENT BY REGULATING SERCA

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Accumulating evidence in humans and experimental animals indicate that immune system malfunction occurs in the advanced stages of cancer. T-lymphocyte activation by antigen, leading to proliferation, is mediated by a complex series of intracellular signaling events that are initiated by a transient rise of a calcium. Of the two major types of Ca^{2+} pumps, the sarco (endo) plasmic reticulum calcium ATPase (SERCA) and the plasma membrane calcium ATPase (PMCA) pumps, the ormer represents a highly conserved family of Ca^{2+} pumps which actively sequester Ca^{2+} from the cytosol into the endoplasmic reticulum lumen against a large concentration gradient. It is evident that by maneuvering the SERCA pump expression status in the T cells of cancer patients, one may facilitate activation and potentiation of the immune system. An ideal anticancer drug should have the capacity to kill the malignant cells with minimum toxicity towards the normal cells. The present work reports the synthesis of a novel synthetic compound, ethyl-4-(3-nitro)-phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate, commonly known as 3,4-dihydropyrimidone (DHPM) and a potent inhibitor of the SERCA pump. DHPM down regulates SERCA3 gene expression and activity producing a rise in the cytosolic calcium concentration which leads to lymphocyte activation. It has been observed that when the lymphocytes were treated with a cell free MCF-7 cell supernatant it resulted in an up-regulation in the SERCA3 expression level and a fall in the cytosolic calcium levels that was associated with T cell apoptosis. Interestingly, DHPM treatment down-modulated SERCA3 expression and the cells were efficiently protected from the tumor-induced apoptosis. The effort was to dissect the molecular mechanisms underlying DHPM-mediated immunorestoration revealed the cross-talk between SERCA and other signaling molecules, *e.g.*, PKC, NFκB *etc.*, in deciding the fate of T cells. The data strongly suggest that inbalance in cellular calcium homeostasis is an important factor leading to T cell death during cancer and holds promise that DHPM may act as a potential immunorestoring agent in cancer patients.

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EPHRINBS ARE ESSENTIAL COMPONENTS OF THE REELIN PATHWAY TO REGULATE NEURONAL MIGRATION

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Coordinated migration of neurons in developing and adult brain is essential for its proper function. The secreted Reelin glycoprotein guides the migration of neurons and the loss of Reelin function in humans results in lissencephaly, a severe disorder, as well as to neurological disorders such as epilepsy, schizophrenia and Alzheimer's disease. The molecular mechanisms by which Reelin activates its receptors and controls cellular functions are largely unknown. In this study, we showed that the neuronal guidance cues ephrinBs are essential for Reelin signaling during brain development. EphrinBs genetically interacted with Reelin. Notably, compound mouse mutants (*rl+/–; ephrinB3–/–* or *rl+/–; ephrinB2–/–*) and triple ephrinB1B2B3 knockouts showed neuronal migration defects that recapitulated those observed in the neocortex, hippocampus and cerebellum of *reeler* mouse. Mechanistically, we showed that Reelin binds to ephrinBs. Clustering of ephrinBs led to recruitment and phosphorylation of Dab1, which is necessary for Reelin signaling. Conversely, the loss of function of ephrinBs severely impaired Reelin-induced Dab1 phosphorylation. Importantly, activation of ephrinBs rescued *reeler* neuronal migration defects in the absence of Reelin protein. Together,

these results identified ephrinBs as necessary co-receptors for the Reelin signaling pathway and, therefore, as putative therapeutic targets on the neurological disorders associated with loss of Reelin protein.

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ELECTROCHEMOTHERAPY AS AN APPROACH TO ENHANCE THE EFFICACY OF CHEMOTHERAPEUTICS

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Electrochemotherapy is a therapeutic approach providing delivery of non-permeant cytotoxic drugs into the cells by the electroporation of tumors. Two drugs that have shown potentiation of their cytotoxicity by electroporation in preclinical and clinical studies are bleomycin and cisplatin. Exposure of tumors to electric pulses provides destabilization of the cell membrane and thus diffusion of chemotherapeutics into the cells. Cytotoxicity of bleomycin is increased by several 1,000 folds and cisplatin by up to 70 folds. Therefore, with low chemotherapeutic doses, good local antitumor effect can be obtained, regardless of the tumor type treated. The antitumor effectiveness of electrochemotherapy either with bleomycin or cisplatin in patients with recurrent cutaneous and subcutaneous tumors was shown to be in the range of 70-80% local tumor control rate. Currently, electrochemotherapy is used predominantly in the treatment of skin metastases of melanoma, in palliative intent. Recently this technology is being developed also for the treatment of deep-seated tumors, such as liver metastases in ongoing clinical trials. The technology has now disseminated and, as a result, electrochemotherapy is currently being used in more than 60 European cancer centers. Its advantages, such as its high efficacy and the outpatient-based approach, provide the basis for its even broader application in cancer treatment.

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CARCINOGENICITY AND MUTAGENICITY OF AFLATOXIN B1: A STUDY OF A RAT MODEL FOR HUMAN HEALTH

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It is well known that aflatoxin B1 (AFB1) is a potent carcinogen in some animals and there is interest in the effect of long-term exposure to low levels of AFB1 on humans. Epidemiological studies in Asia and Africa have demonstrated a positive association between AFB1 and liver cell cancer (LCC). In present study, rats received weekly different concentrations of AFB1 through gavage for 12 consecutive weeks and they were monitored for the development of carcinomas. Samples from various organs, including liver, were collected for both mutation studies and protein expression using microarray techniques. The results indicated a close association of AFB1 and liver cancer in the rat model.

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EVALUATION OF THE NUMBER OF CARRIERS OF MEFV COMMON MUTATION IN FAMILIAL MEDITERRANEAN FEVER IN NORTHWEST IRAN

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Background: Familial Mediterranean Fever (FMF), one of the most common and periodic fever syndromes, is a recessive autosomal disorder. This disease is prominently present in the non-Ashkenazi Jews, Arabs, Turks and Armenian people. Considering the geographic location of northwest Iran and its neighborhood with two high-risk regions, *i.e.* Armenia and Turkey, a high prevalence of FMF in this area is expected. *Aim:* The purpose of this study was to evaluate the number of carriers of FMF common mutations in healthy controls. The result may be of help in estimating the prevalence of FMF, raising awareness to avoid potential amyloidosis. *Methods:* A total of 200 healthy controls who were not suffering from FMF were selected randomly from northwest Iran and gave their consent for the study. DNA was extracted from blood samples and the mutation was studied using PCR-RFLP and PCR-ARMS techniques. *Results:* Out of 400 studied alleles, 44 mutant alleles for E148Q mutation and 7 mutant alleles for V726A mutation were found. In the other three mutations, no mutant allele was found. The frequency of general alleles for these five common mutations was 0.1325, while the carrier rate was 23.4%. *Conclusion:* The carrier rate of 23.4% indicates that FMF is significantly prevalent in northwest Iran, suggesting that northwest Iran, like other high-risk areas, is susceptible to FMF.

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THE FEMALE AND THE MALE HEART: WHAT MAKES THE DIFFERENCE?

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Elevated cardiovascular risk in postmenopausal women and beneficial actions of estrogen replacement in animal models has been related to protective effects of estrogens. However, randomized trials of hormone replacement therapy in humans failed to confirm this and phytoestrogens may represent potential alternatives. This study utilised a 2-DE/ESI-LC-MS approach in order to identify variation of protein species with genistein receipt and gender in their relative abundance in the healthy murine heart. Increased levels of enzyme species involved in the oxidative phosphorylation and generation of ROS were accompanied by decreased amounts of antioxidants in male mice receiving genistein compared to control males, which have been previously associated with various pathological conditions. Exposure of female animals to genistein provoked an increased abundance of two species of LIM domain binding protein and one species of desmin. These proteins have been associated with cardiac hypertrophy and the use of these data as molecular markers warrants caution, since the animals did not exhibit any histological signs of cardiac hypertrophy. Moreover oral genistein treatment revealed a substantial effect on the relative abundance of both estrogen receptors. This may be relevant in gender based investigations in animal models, since standard rodent chow exhibits variable levels of phytoestrogens and should be taken into consideration for the design of appropriate studies.

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POLYMORPHISMS OF VITAMIN D RECEPTOR AND SUSCEPTIBILITY TO MENINGIOMA FORMATION

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Background: Vitamin D can induce differentiation and inhibit proliferation of normal and tumor cells. It binds to the vitamin D receptor (VDR) to regulate its gene expression. VDR has been shown to be present in many tissue types, such as bone, kidney, intestine, muscles, and brain. Gliomas and meningiomas are the most common types of brain cancer. *Patients and Methods:* VDR gene polymorphisms were investigated for potential correlations with gliomas and meningiomas. A normal control group of 122 healthy subjects and a patient group of 44 patients with meningioma were evaluated. Two polymorphic sites of VDR gene, Fok-I and Taq-I, were evaluated using the polymerase chain reaction-restriction fragment length polymorphism analysis. *Results:* There were significant differences in the distribution of VDR Fok-I genotypes between in the patients with meningioma and the healthy controls (p=0.05, χ^2=10.527). Patients with the VDR ff genotype were found to be at significantly higher risk for brain cancer than those with other genotypes (OR=6.47, 95% CI: 1.749-23.926, p=0.04). *Conclusion:* These results suggest that the VDR Fok-1 polymorphism may be associated with increased susceptibility to meningioma.

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PROTEOMICS: CURRENT TECHNOLOGIES AND APPLICATIONS TO MOLECULAR MEDICINE

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Proteomics' aim is to characterize, discriminate and identify the proteins in biological materials such as plasma and serum, cell lines, tumors, biopsies *etc*. in order to identify novel pathogenic pathways, diagnostic biomarkers and therapeutic targets. The major proteomic technologies (including 2D-PAGE, MALDI ToF/MS, SELDI ToF/MS, LC-MS/MS, ICAT, iTRAQ *etc*.) applied in the analysis of biological samples is presented during the first part of the presentation. The second part of the presentation refers to the most important aspects of proteomics regarding molecular medicine, mainly the prevention, diagnosis and treatment of human diseases. In this regard, the application of high throughput proteomic technologies on cancer is presented, with emphasis on the elucidation of carcinogenesis pathways and the characterization of specific biological markers for cancer diagnosis, as well as the identification of new therapeutic targets. Additionally, recent results from the application of proteomics to the analysis of human brain will be introduced. Finally, the extensive application of proteomics on human reproduction (*e.g*. study of the normal pregnancies, pregnancies caring embryos with abnormal phenotypes) and on the identification of potential biomarkers for non-invasive prenatal diagnosis are discussed. Despite the technological limitations of these technologies, there is little doubt that the

proteomic approach has the potential to identify novel diagnostic biomarkers and therapeutic targets in human diseases.

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RAPID STEROID ACTIVATION OF mTOR SIGNALING IN A HUMAN MYOMETRIAL CELL LINE

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Background: The mammalian target of rapamycin (mTOR) is a kinase that functions as a sensor to balance cell proliferation, nutrient availability and actin reorganisation. mTOR can form two distinct complexes termed mTORC1 and mTORC2 that can exert specific biological effects. Recently, it has been shown that estradiol can activate the mTOR pathway that subsequently regulates myometrial hyperplasia. In this study, we investigated the rapid effects of 17-β estradiol and progesterone on the activity of mTORC complexes, using a myometrial cell line as an *in vitro* model. *Methods:* RT-PCR, ImageStream analysis and Western blotting were used. *Results:* RT-PCR analysis confirmed expression of DEPTOR, mTOR, rictor and raptor as well as progesterone and estrogen receptors. ImageStream analysis elucidated the cellular distribution of these components. mTOR and DEPTOR were predominantly localised in the cytoplasm, although some nuclear expression was also evident. Treatment of an immortalised myometrial cell line with progesterone and 17-β estradiol (100 nM) induced phosphorylation of Akt, reaching maximum at 20 and 10 minutes, respectively. *Conclusion:* Collectively, these data indicate that sex steroids can rapidly induce mTORC2 activity in human myometrial cells *in vitro*. It is attractive to hypothesise that these effects can be mediated in a non-genomic manner.

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APPLICATION OF PEPTAMEN AND MODULEN FOR ENTERAL NUTRITION IN PATIENTS WITH CHRONIC HEART FAILURE III-IV NYHA CLASS

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Background: The syndrome of cardiac cachexia is a severe sequelae leading to a deterioration in prognosis of the CHF. The patients with signs of cardiac cachexia are characterized by higher rates of the inflammatory markers than patients with normal body weight. *Methods:* 180 patients with CHF were included in the study. The first group of patients received Modulen with a standard therapy. The second group received Peptamen with a standard therapy. And the third group received only standard therapy and the necessary amount of nutrients. *Results:* An increased levels of fat (from 16.6±3.1 kg to 18.5±3.3 kg) and lean body mass (from 47.2±5.8 kg to 44.4±5.9 kg), and a reduced levels of total fluid were detected in the patients receiving Modulen. There was also a reduction of proinflammatory cytokines, CRP (from 8.9±1.7 mg/l to 4.7±1.1 mg/l), TNFα (from 6.8±1.3 U/l to 3.4±1.4 U/l) and adiponectin (from 24.4±1.9 mcg/ml to 15.8±2.1 mcg/ml). *Conclusion:* The use of Modulen as enteral nutrition in patients with CHF corrects the body composition, improves the life quality and the prognosis.

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GENES BEYOND HUMAN PERFORMANCE, BECOMING AN ELITE SPORTSMAN

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Information gained by the human genome project represented a magnificent approach not only to genetic diseases but to phenotypic properties of human kind as well. By the improvements in molecular technology, nearly most of the genes responsible of our characteristics are clarified. One of the applications of this progression is in sports science. Becoming an elite sportsman is a complex attribute affected by gene-gene and gene-environment interactions. Other contributors such as motivation, skill development and taking an effective and enough training guidance, are difficult to quantify. But the main source of skills sufficient to become a sports champion still lies on the athlete's genetic endowment. This genetic endowment consists of many polymorphisms, each of which decides how to react to training and skill development. To date, over 200 performance-enhancing polymorphisms have been reported, some of which are only single genes, while the rest concern gene-gene interactions or combined polymorphism status. There are approaches aiming to determine a score for an individual who carries the suitable polymorphisms in order to attain elite sportsmanship. However, under the right guidance, an individual with the suitable genetic endowment will have a greater chance of being an elite sportsman.

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CAN WE USE THE CELL DEATH BIOMARKERS OF M30 AND M65 FOR BETTER MANAGEMENT OF CANCER PATIENTS?

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It is thought that the mode of cell death induced by chemotherapy may be apoptosis, which is also called programmed cell death, although other cell death modes (*e.g.* necrosis) may also be possible. One of the characteristics of apoptosis is the disruption of cytoskeleton where cytokeratin 18 (CK18) constitutes the major part. Once cells have undergone apoptosis, CK18 is cleaved at certain positions by apoptosis-specific proteases, also known as caspases. After the cleavage, the caspase-cleaved CK18 (M30 antigen) is released into the blood stream, following apoptosis. This neo-epitope is recognized by the so-called M30 antibody. Using an ELISA assay for M30 antigen, it is possible to detect the soluble M30 antigen in the serum of patients prior to and after chemotherapy, which allows predicting the efficacy of the drugs *in vivo*. In our study involving lung cancer patients (Ulukaya *et al*., Lung Cancer, *56*: 399-407, 2007), we found that the M30 antigen levels was statistically significantly increased after the application of chemotherapy. Likewise, it is elevated in breast cancer patients as well as gastrointestinal cancer patients following chemotherapy. M65 represents the full length of CK18 and it is released from the necrotic cells into the extracellular space. By employing the M65 ELISA assay, it is possible to measure the total cell death (apoptosis and necrosis), as this assay recognizes both the cleaved part and the full length of CK18. M30 antigen seems to be a favorable novel serum biomarker allowing better management of cancer patients, although more clinical studies are required. The pros and cons of these cell-death biomarkers will be discussed.

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RESEARCH ON EMBRYOS IN TURKEY ACCORDING TO MEDICAL ETHICS AND MEDICAL LAW

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In Turkish Law, *in vitro* embryos are regulated by the Regulation on Health Care for Assisted Reproduction Treatment (RHCART) (Üremeye Yardımcı Tedavi (ÜYTE) Merkezleri Yönetmeliği). The Regulation was issued in 1987 and it has been amended several times afterwards. Within this regulation framework, sperm and ova obtained in assisted reproduction treatment centers in Turkey can only be used for married couples to whom those reproductive cells belong. Therefore, sperm and ova taken from such couples cannot be used to initiate pregnancy in other individuals and selling these cells to other persons or centers is also prohibited. In addition, using sperm and ova for research purposes or embryos formed from such reproductive cells is also forbidden. At the same time, article 90 of the Turkish Penal Code covers some aspects of research on embryos. Moreover, the Oviedo Convention, ratified by Turkey and entered into force in 2003, has binding regulations about this issue. These three different legal regulations and some ethical guidelines about this issue are in conflict amongst each other, creating much confusion for the researchers. The aim of this paper was to discuss these conflicts and give some practical proposals.

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METABOLIC SYNDROME: NOVEL APPROACH USING A SMALL MOLECULE, FATOSTATIN, THAT REGULATES FAT SYNTHESIS

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Background: Metabolic syndrome refers to a confluence of inflammatory conditions occurring along with obesity and diabetes. Fatostatin, a synthetic drug-like diarylthiazole derivative, inhibited the insulin-induced adipogenesis of 3T3-L1 mouse and repressed the serum-independent growth of DU145 human prostate cancer cells. Here we identified the target of fatostatin and report the physiological impact of this small molecule. *Methods:* Microarray screening and real time PCR methods were used to identify changes in gene expression in cells treated with fatostatin. Deletion analyses, binding assays, Western blot, biochemical studies and confocal microscopy were used to identify and confirm the target of fatostatin. Male ob/ob mice were treated with fatostatin and biochemical and enzymatic activities were determined and compared to untreated control mice. *Results:* Fatostatin inhibits the ER-Golgi translocation of the sterol regulatory element binding proteins (SREBPs) through binding to SREBP-cleavage activating protein (SCAP). Fatostatin reduced body weight, blood glucose and hepatic fat accumulation in treated obese ob/ob mice. These positive outcomes are partly due to down-regulation of lipogenic

pathways controlled by SREBPs. *Conclusion:* Fatostatin or its analogs, may serve as a lead candidate for pharmacological intervention against metabolic syndrome and as a tool for gaining further insights into the roles of lipid metabolism in animals, including humans.

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ACTIVATION OF G-PROTEIN-COUPLED RECEPTORS FOR PEPTIDE HORMONES

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G-protein-coupled receptors (GPCRs) form a large superfamily of structurally-related proteins which mediate their effects by coupling to G-proteins. They are one of the largest gene families in the human genome and are a major target for drug discovery. Defining the binding site of agonists and antagonists to GPCRs, at the molecular level, is of fundamental importance to understanding their activation by hormones and to rational drug design. For GPCRs in general, the location of the ligand binding site and the molecular mechanisms underlying receptor activation are poorly defined. We systematically investigated the role of residues which are highly conserved throughout a sub-family of peptide-GPCRs belonging to Family A, using a combination of mutagenesis and molecular modelling. In a complementary approach, we synthesised analogues of the peptide hormone which incorporated residue changes predicted to recover activity at specific mutant receptors. Overall, these studies have identified specific residues that are required for ligand binding, intracellular signalling and cell-surface expression.

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CURRENT RESEARCH PROTOCOLS REGARDING THE USE OF TNFα FOR ISOLATED LIMB PERFUSION IN THE UNIVERSITY OF CRETE

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[2]Medical Department, University of Crete, Greece

Background: Isolated limb perfusion under mild hyperthermia (HILP) is a surgical method of regionally administering high doses of chemotherapeutic agents avoiding systemic toxicity. The introduction of tumor necrosis factor alpha (TNFα) in HILP increased significantly response rates and limb salvage in patients with inoperable recurrent melanoma of the limbs. The mechanism of synergism of TNFα with cytostatic agents is not fully understood even though targeting of neovascular endothelium by TNFα has been implicated. Moreover, no reliable indicators of postoperative tumor response exist in order to select patients more likely to benefit from this complicated surgical operation. *Methods and Results:* Using serial tissue and blood specimens from patients undergoing HILP with TNFα and melfalan (HILP-TM) for locally recurrent inoperable melanoma of the limbs, we aimed to assess whether: (i) administering TNFα affects, signaling implicated in melanoma chemoresistance such as PI3K/Raf kinase signaling (ii) the presence of different members of TNF receptors in vascular endothelium of patients is associated with complete and durable response and (iii) HILP-TM increases perioperatively the number of circulating melanoma cells. *Conclusion:* In this project, significant improvement of an established surgical operation might be expected by better patient selection and understanding of the mechanisms underlying chemoresistance in advanced melanoma.

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ANTIOXIDANT AND ANTI-TYROSINASE ACTIVITIES OF PIPER CANINUM EXTRACT AND PURE COMPOUNDS

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Malaysia is rich in biodiversity and there are many potential medicinal plants yet to be explored. The piper family is widely used by the locals to treat a variety of illnesses. The aim of this study was to evaluate the biological activities of *Piper caninum* extract and purified compound. *Piper caninum* was extracted using methanol (extract: PCM), ethyl acetate (extract, PCEA) and hexane (extract, PCH). Three compounds were purified, identified and named: pc1_UTM, pc2_UTM and pc3_UTM. Both compounds and extracts were tested for antioxidant activities using DPPH scavenging assay, ferric-reducing antioxidant power (FRAP) and total phenolic content using Folin-Ciocalteu reagent. Anti-tyrosinase activities were also evaluated. The IC_{50} values revealed that pc1_UTM has the highest activities followed by pc2_UTM, pc3_UTM, PCEA and PCM mean while PCH was negative. PCEA exerted the highest percentage of tyrosinase inhibitor followed by PCM and PCH with values of 68.47%, 64.15% and 61.08% respectively. Meanwhile, pc3_UTM had the highest anti-tyrosinase activity (44.15%) followed by pc2_UTM (42.96%) and pc_utm1 (32.95%) for compounds. The study suggested that *Piper caninum* provides a source of natural antioxidants

and anti-tyrosinase agents and may be beneficial to human health. Presently, further studies on the molecular basis of the anti-inflammatory properties are carried out for the compounds.

Poster Presentations

(Alphabetically by presenter's surname)

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GENE EXPRESSION IN THORACIC AORTIC ANEURYSM TISSUES

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Background: Aortic aneurysms are localized dilatations in the aorta due to changes in the vessel wall. Thoracic and abdominal aneurysms have different aetiopathologies. The alterations in the vessel wall leading to this weakening are reported as medial degeneration, increased expression of matrix metalloproteinases (MMPs), leading to loss of extracellular matrix proteins such as collagen and elastin. *Aim:* To understand the expression of collagen I, III and elastin in thoracic aortic aneurysms (TAA), the involvement and relative roles of different MMPs in a large series of (n=60) TAA. *Patients and Methods:* TAA samples were provided by Kartal Koşuyolu, Advanced Training and Research Hospital. Total RNA was isolated from aneurysmal tissue. The expressions of genes (MMP-2, MMP-9, Collagen I, III and elastin) were determined by real time PCR. Samples were normalized to three housekeeping genes. *Results:* The expressions of collagen I, III and elastin were increased. Correlations betwen these genes and MMP-2, and 9 gene expressions were observed in patients with large aneurysms (>7 cm). *Conclusion:* Changes collagen and elastin gene expression correlate with protease expression in TAA.

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SYNTHESIS AND STUDY OF THE ANALGESIC EFFECTS OF NEW ANALOGUES OF KETAMINE ON FEMALE WISTAR RATS

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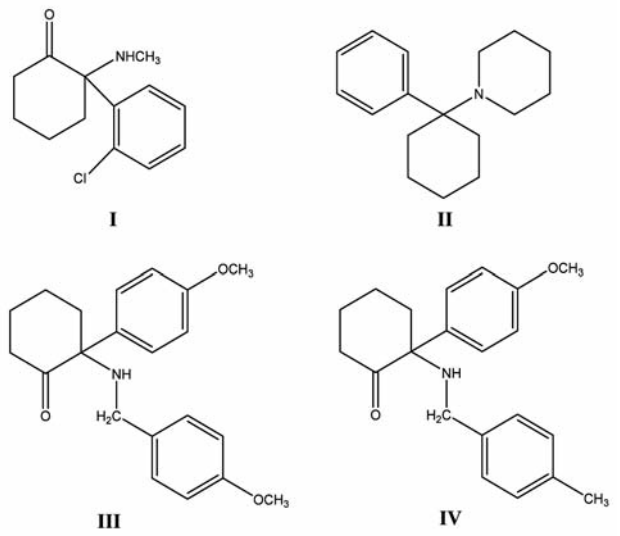
Background: Ketamine (2-o-chlorophenyl-2-methylamino-cyclohexan, CI-581, Ketalar, I) a potent derivative of phencyclidine (1-[1-phenylcyclohexyl]piperidine, PCP, II) and many of its analogues have shown anesthetic and analgesic effects. *Methods:* In this study, new derivatives of compound I, (2-[*p*-methoxybenzylamino]-2-[*p*-methoxyphenyl] cyclohexanone, ket-OCH_3, III) and (2-[*p*-methylbenzylamino]-2-[*p*-methoxyphenyl] cyclohexanone, ket-CH_3, IV) and their intermediates (V-VIII) were synthesized. Acute and chronic pain was evaluated in rats treated with compounds III and IV using tail immersion and formalin pain tests as models of acute thermal pain and acute and chronic chemical pain, respectively. The results were compared with ketamine and control (saline) groups, undergone the same pain tests. *Results:* The results indicated that, in tail immersion and formalin pain tests the new compounds (III, IV) were effective for decreasing pain most frequently compared to the control group but they could not potentiate as strong analgesic effects compared to ketamine. *Conclusion:* It is concluded that, adding the methoxyl group with the high electron donating and dipole moment on the phenyl ring and also substituting methylamine with methyl- or methoxyl-benzylamines could generate low analgesic effects in tail immersion and formalin pain tests compared to ketamine and control groups on rats at a dose of 6 mg/kg body weight.



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SYNTHESIS AND ANALGESIC EFFECTS OF NEW PYRROLE DERIVATIVES OF PHENCYCLIDINE IN MICE

Abbas Ahmadi[1], Jalal Solati[2], Sara Pakzad[1], Mojdeh Javadi[1], Nazereh Mansour-Rezaee[1], Horiesadat Hosseini[1] and Nasrin Afshin[1]

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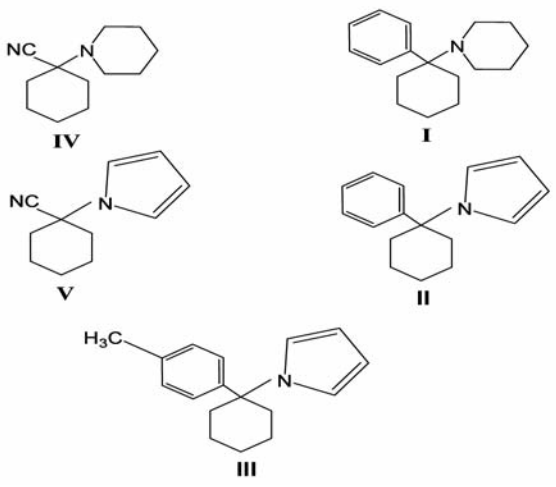
Background: Phencyclidine (1-[1-phenylcyclohexyl] piperidine, PCP, I) and many of its analogues have shown some pharmacological effects. *Methods:* In this study, new pyrrole derivatives of I, 1-[1-phenylcyclohexyl] pyrrole (II) and 1-[1-[4-methylphenyl][cyclohexyl]] pyrrole (III) and their intermediates were synthesized. Acute and chronic pain was examined on mice treated with compounds II and III using tail immersion and formalin pain tests, as models of acute thermal pain and acute and chronic chemical pain, respectively. The results were compared with pain estimations in PCP and control (DMSO) groups, undergone the same pain tests. *Results:* The results indicated that compound III generates higher analgesic effects in the tail immersion test compared to the PCP and control groups, demonstrating a marked and significant increase in tail immersion latency, but this effect was not observed for compound II at a dose of 1 mg/kg body weight. The formalin test showed that compound III was effective in acute chemical pain (phase I, 0-5 min after injection), while compound II was not effective at the same dosage compared to PCP and control groups. Also, chronic pain in the compound III group was significantly attenuated, while compound II was not effective as compared to other groups. *Conclusion:* It is concluded that, substitution of piperidine with the aromatic pyrrole ring in the PCP molecule alone will not be effective in tail immersion and formalin pain tests but the combination of this substitution with the addition of the methyl group (with high electron donating and dipole moments) on the phenyl group are effective in these types of pain compared to the PCP and control groups.



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ROSIGLITAZONE INHIBITS OSTEOCLASTOGENESIS BY REDUCING A PHYSICAL INTERACTION BETWEEN PPARγ AND NFATc1

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Background: NFATc1 transcription factor plays a key role in the signaling pathways of RANKL (receptor activator of nuclear factor-κB ligand)-induced osteoclastogenesis. Furthermore, NFATc1 auto-regulates its own gene. Activation of PPARγ (peroxisome proliferator-activated receptor gamma) is known to suppress NFATc1 expression. We therefore investigated the molecular mechanisms through which a PPARγ ligand rosiglitazone suppressed NFATc1 expression. *Methods:* RAW264.7 cells were cultured and osteoclast differentiation was assessed by tartrate-resistant acid phosphatase assay. NFATc1 expression was examined using RT-PCR and Western blot. Co-immunoprecipitation was carried out to assess any protein-protein interaction between PPARγ and NFATc1. siRNA strategy and ChIP assay were used to explore the interaction. *Results:* Rosiglitazone inhibited RANKL-mediated osteoclastogenesis in a dose-dependent manner. At transcriptional and protein levels, rosiglitazone markedly attenuated the increased expression of NFATc1 induced by RANKL. Unexpectedly, rosiglitazone also attenuated the enhanced physical interaction by RANKL between PPARγ and NFATc1. PPARγ knockdown abolished induction of NFATc1 gene by RANKL. RANKL-induced NFATc1 binding to the NFATc1 promoter was dependent on the presence of PPARγ. *Conclusion:* Rosiglitazone inhibits RANKL-induced osteoclastogenesis *via* down-regulation of NFATc1. This effect may result from failure of NFATc1 binding to its own promoter due to a decrease in the physical interaction between PPARγ and NFATc1.

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URINE CYTOLOGY, URINARY SURVIVIN mRNA EXPRESSION AND NUCLEAR MATRIX PROTEIN 22 IN THE DETECTION OF TRANSITIONAL CELL CARCINOMA OF THE BLADDER

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Background: The search for an objective and sensitive marker for the detection of bladder cancer is an active field of translational research. Thus, we aimed to assess the sensitivity and specificity of survivin mRNA expression and NMP22 BladderCheck (BC) test in comparison to urine cytology (UC) for the detection of transitional cell carcinoma (TCC) of the bladder. *Methods:* Voided urine samples collected from 41 healthy controls and 80 patients diagnosed with TCC of the bladder were subjected to UC, NMP22BC test and reverse transcription-quantitative real time PCR for survivin mRNA expression. *Results:* Survivin mRNA expression in healthy controls was significantly different from patients with TCC of the bladder ($p<0.0001$) with a positive correlation with G_2 and G_3 grade tumors, but not with low grade G_1 tumors. A cut-off value of 2 for survivin expression yielded 92% sensitivity and 100% specificity. NMP22BC had 61.3% sensitivity and 100% specificity, while UC had 40% sensitivity and 76% specificity. *Conclusion:* Quantitative urinary survivin mRNA expression enhances the sensitivity and specificity of UC alone or in combination with the NMP22BC test for the detection of TCC of the bladder.

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GENETIC DETERMINANTS OF L-THYROXINE DOSE REQUIREMENT IN ATHYROTIC PATIENTS WITH DIFFERENTIATED THYROID CANCER AND THEIR POTENTIAL ROLE IN ITS ETIOLOGY

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Background: Patients with differentiated thyroid cancer (DTC) are usually managed with total thyroidectomy and subsequent radioiodine ablation of the remnant thyroid tissue. L-thyroxine (L-T4) therapy (about 2 µg/kg) is necessary with a wide variation in patient dose requirements. Therefore, the aims of this study were to identify sodium iodide symporter (NIS) single nucleotide polymorphisms (SNPs) involved in thyroid hormone metabolism and to evaluate their association with DTC and L-T4 dose requirement in the Saudi population. *Methods:* Detection of SNPs was accomplished in 100 DTC patients and genotyping for 3 selected SNPs (rs4808708, rs4808709 and rs7250346) was performed in 409 DTC cases and 406 controls. *Results:* A total of 22 SNPs were identified in the *NIS* gene; 7 of them were novel. There was significant association of the G allele in rs4808708 and rs7250346 variants [(Odds ratio (95% CI), 1.30 (1.05-1.60); $p=0.016$ and 2.30 (1.20-4.39); $p=0.012$), respectively] and for the AA genotype of the rs4808708 variant [(Odds ratio (95% CI), 1.38(1.05-1.82)] with DTC, independent of age and sex. No significant association was found for these SNPs with L-T4 dose requirement. *Conclusion:* The study identified two NIS variants associated with an increased risk of DTC. In DTC patients, the variability in the L-T4 dose requirement does not appear to be related with NIS polymorphisms.

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TRANSCRIPTIONAL ACTIVATION OF THE COPPER/ZINC SUPEROXIDE DISMUTASE GENE BY ELK-1

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Background: Elk-1 is activated by the MAPK pathway and it is a member of the ETS domain transcription factor. Copper/Zinc superoxide dismutase SOD1 participates in the control of the intracellular concentration of reactive oxygen intermediates and catalyzes the dismutation of superoxide radicals. According to recent studies, Elk-1 has a binding domain in the SOD1 positive regulatory element in the promoter region and enhances SOD1 transcription. In this study, we aimed to explore the relationship between Elk-1 and SOD1. *Methods:* We determined the binding motifs of Elk-1 in SOD1 human and rat promoter regions. Primers were designed according to these regions and these regions were cloned into pgl-3 luciferase vector. In further analyses, we will make a luciferase assay to find out the effect of Elk-1 on SOD1 transcription. We will also use mutated Elk-1 expression plasmids to analyze the functional role of Elk-1. *Results:* Rat and human promoter regions were successfully cloned to pgl-3 vector. *Conclusion:* Since it has been shown that Elk-1 has a positive effect on SOD1 transcription we expect to see a decrease in activation of SOD1 when Elk-1 is absent or mutated.

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EFFECTS OF ROSIGLITAZONE OR METFORMIN TREATMENT ON LEPTIN AND OXIDATIVE STRESS PARAMETERS IN TYPE 2 DIABETIC PATIENTS

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Background: It is generally accepted that oxidative stress is responsible for the etiology and complications of diabetes. *Methods:* Leptin malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), paraoxonase and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured in 30 healthy subjects and 50 patients with diabetes mellitus. MDA and TAC were assayed with spectrophotometric methods. Leptin, SOD, CAT, paraoxonase and 8-OHdG were analyzed using appropriate commercially available kits. *Results:* Before treatment, SOD and TAC levels were decreased in all patients compared to controls (p=0.005, p=0.01, respectively), while MDA levels were increased (p=0.024). The patients with diabetes mellitus were divided into two groups: the rosiglitazone group (n=25) and the metformin group (n=25). Treatment was carried out for three months. Before treatment, in the rosiglitazone group, TAC levels were diminished (p=0.015), while MDA levels were increased (p=0.019) compared to controls. After treatment, decreased MDA and 8-OHdG levels were found (p=0.008, p=0.000, respectively) in the rosiglitazone group compared to the pretreatment values of this group. Before treatment in the metformin group, SOD levels were decreased compared to controls (p=0.000). After treatment, SOD and TAC levels were increased (p=0.029, p=0.000) in the metformin group compared to the pretreatment values of this group. *Conclusion:* Our results suggest that oxidative stress seems to decrease with rosiglitazone or metformin treatment.

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SERUM PARAOXONASE-1 ACTIVITIES AND OXIDATIVE STATUS IN PATIENTS WITH PLAQUE-TYPE PSORIASIS WITH/WITHOUT METABOLIC SYNDROME

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Background: Psoriasis is a chronic immune-mediated inflammatory skin disease associated with metabolic syndrome, which is made up of a cluster of disorders including obesity, diabetes mellitus, dyslipidaemia, and cardiovascular disease. The aim of the present study was to investigate serum paraoxonase-1 activities and oxidative status parameters in patients with plaque-type psoriasis with or without metabolic syndrome. *Methods:* In this study, psoriatic patients with (n=25) or without (n=27) metabolic syndrome according to the criteria of the International Diabetes Federation (IDF) were matched for age and sex to an equally sized control group (n=25). *Results:* In patients without metabolic syndrome, serum paraoxonase and arylesterase activities showed mean decreases of 29% and 6%, respectively, whereas in patients with metabolic syndrome, the mean decreases in the enzymes' activities were 35% and 11%, respectively, compared with those in the control group. Serum total antioxidant capacity and total oxidant status were not statistically significant in any of the three groups. *Conclusion:* The significant decrease observed in serum paraoxonase activity was independent of the metabolic syndrome in patients with mild-to-moderate plaque-type psoriasis, whereas the significant decrease in serum arylesterase activity was associated with the metabolic syndrome.

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TURNAROUND TIME OF CARDIAC MARKERS IN EMERGENCY DEPARTMENT

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Background: We aimed at determining the turn-around-time (TAT) of MB isoenzyme of creatine-kinase (CK-MB) and troponin I (TnI) in the host Emergency Department Laboratory. *Methods:* Serum CK-MB levels were assayed using in Advia 1800 instrument using the immuno-inhibition method, and TnI levels on Centaur CP using the chemiluminescence method (Siemens Healthcare Diagnostics, Germany). TAT values of TnI (n=11,532) and of CK-MB (n=17,399) from June to December 2010 were included in this study. In data analysis, the time outcomes were successfully clustered (two clusters; 0.86, 0.14) separately by Simple Expectation Maximisation (EM) class (weka). EM assigns a probability distribution to each instance which indicates the probability of it belonging to each of the clusters. EM allows one to decide how many clusters to create by cross validation, or it is possible to specify *a priori* how many clusters to

generate. *Results:* The time when the specimen was ordered to when the result was available was significantly shorter in 86 % of CK-MB (55.84±16.52 minutes, log likelihood: -27.77249) compared with 86 % of TnI (61.10±16.63 minutes, log likelihood: -25.27239). *Conclusion:* Local factors including clinical (physicians and nurses) and laboratory staff, sample transport, diagnostic equipment should be revised periodically in emergency departments in order to improve performance.

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THE STUDY OF SER892GLY AND GLY972ARG POLYMORPHISMS OF IRS-1 GENE IN TYPE 2 DIABETIC PATIENTS IN KONYA REGION

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[1]Department of Medical Biology, Selcuklu Medical Faculty and Departments of [2]Medical Biology and [3]Endocrinology, Meram Medical Faculty, Selcuk University, Konya, Turkey; [4]Biometry-Genetics Unit, Faculty of Agriculture, Selcuk University, Konya, Turkey

Background: Type 2 diabetes mellitus (T2DM) is characterized by a combination of peripheral insulin resistance and impaired insulin secretory capacity of pancreatic β-cell. Insulin receptor substrate-1 (IRS-1) is one of multiple proteins that mediate signal transduction of the activated insulin reseptor. We aimed at determining the allele profiles and the risk alleles of the SNPs Ser892Gly and Gly972Arg in the IRS-1 gene and to identify the associations with the disease in patients in the Konya region of Turkey, in which type 2 diabetes is very common. *Methods:* Our study included 169 type 2 diabetic patients and 69 healthy controls. Fasting plasma glucose, fasting insulin, HbA1c and c-peptide values were measured for all patients and controls. Oral glucose tolerance test was done in healthy controls. Target SNPs in IRS-1 gene were screened by polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP) technique. Chi-Square test was employed in examination of SNP genotypes relationship in patients and controls. Single point regression analysis and Kruskal-Wallis test were employed to obtain SNP genotype - biochemical phenotypes relationship for normally and non-normally distributed phenotypes, respectively. In all tests p-value <0.05 was considered statistically significant. *Results:* Ser892Gly and Gly972Arg polymorphisms in IRS-1 gene that were screened in our study were not associated with the disease (p>0.05). *Conclusion:* We found no evidence for an association of Ser892Gly and Gly972Arg polymorphisms of IRS-1 gene with type 2 diabetes (p>0.05).

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GENETIC CHARACTERIZATION OF FLUOROQUINOLONE-RESISTANT *KLEBSIELLA PNEUMONIAE* ISOLATES IN RUSSIA

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Background: Klebsiella pneumoniae is a frequent cause of nosocomial pneumonia and fluoroquinolones are potential antibiotics used for its treatment. In this study, the contribution of genetic factors in the development of *K. pneumoniae* resistance to fluoroquinolones was examined. *Methods:* Susceptibility testing of *K. pneumoniae* was performed by the disc-diffusion method. Genomic DNA was purified using DNA Express kit (Lytech Ltd, Russia). Detection of single nuclear polymorphisms in Ser83 and Asp87 codons of *gyrA* and in Ser80 and Glu84 codons of *parC* was performed by PCR-primer extension/reaction followed by mass spectrometry. *Results:* In total 96 isolates of *K. pneumoniae* were tested. The most susceptible to fluoroquinolones isolates (12/13; 92.3%) carried neither *gyrA* nor *parC* mutations. All moderately resistant isolates (7/7, 100%) exhibited mutations only in *gyrA*, while the majority of resistant isolates (74/79, 93.7%) revealed mutations both in *gyrA* and *parC* genes. Additionally, the capability of direct identification of such mutations in *K. pneumoniae* genomic DNA purified from urine samples was shown. *Conclusion:* Identification of fluoroquinolone resistance-associated mutations may be useful to bacteriologists; however, only the mutations in *gyrA* and *parC* genes led to clinically relevant resistance of *K. pneumoniae* to fluoroquinolones.

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THE NF-κB INHIBITOR IκBα NEGATES COLON CANCER CELL MIGRATION, INVASION, PROLIFERATION AND TUMOR GROWTH

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It is well accepted that the NF-κB pathways are involved in inflammatory diseases, cancer development and progression in human solid tumors. The NF-κB signaling element IκBα was shown to inactivate NF-κB activity through sequestration of this transcription factor in the cytoplasm. In the present study, we investigated the impact of the IκBα on the invasive growth of human colon cancer cells HCT8/S11 stably transfected by this endogenous NF-κB inhibitor. We report that IκBα ectopic expression inhibited NF-κB promoter activity induced by the Y527Fsrc oncogene and reduced HCT8/S11 cell migration in wound healing assays. Our data show that IκBα abrogated collagen type I invasion induced by the trefoil factors TFF1 and TFF3 but was ineffective on the invasive phenotype determined by leptin. Moreover, IκBα reduced HCT8/S11 cell proliferation *in vitro* and the growth of their corresponding tumor xenografts established in the athymic mice. Taken together our data demonstrated that the intrinsic NF-κB inhibitor IκBα negates several transforming functions in human colon cancer cells. Our data provide the rationale for further preclinical and clinical studies based on therapeutic interventions targeting NF-κB pathway.

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IDENTIFICATION OF ALLOSTERIC RESIDUES IN THE ATPase DOMAIN OF HSP70 MOLECULAR CHAPERONES UPON SUBSTRATE BINDING

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Background: Hsp70 chaperones play important roles in cells including protein folding, trafficking, degradation and enabling survival under stress conditions. DnaK is an Echerichia coli Hsp70 homolog comprising an ATPase and a substrate-binding domain. Communication between the domains is essential for chaperone function. Previous studies showed that DnaK(1-392), containing the ATPase domain and the entire linker region, can mimic the substrate-stimulated form of full-lengh DnaK showing an ATPase rate similar to that of the substrate-present state for the full-length protein. Using this knowledge, we aimed to understand the allosteric mechanism underlying the substrate binding effects to the ATPase domain by pinpointing the putative residues that are present in this path using DnaK(1-392). *Methods:* We identified sites that can be critical for allostery and applied protein engineering methods to make replacement mutants. We purified the mutants, measured their ATPase levels and studied their effects on stability using circular dichroism. *Results and Conclusion:* We found that a particular mutation site is critical for the activity and allostery of the ATPase domain of DnaK. This mutation caused an enhancement in the activity of the ATPase domain and also altered its stability by lowering the first melting transition from 50˚C to 48˚C, suggesting that mutation to that site may have a role in the regulation of the ATPase dynamics of the domain.

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EFFECT OF F68 PLURONIC BLOCK COPOLYMER ON ELECTROPORATION OF HeLa CELLS

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Background: Electroporation is an efficient non-viral gene delivery method having some adverse effects on cells; for example, the phospholipid bilayer of the cell is temporarily disturbed by electrical pulses and the cell pores may become too sizeable or collapse after membrane discharge, causing cell damage. Pluronic F68 is a non-ionic and small foaming surfactant consisting of a central polypropylene oxide and two polyethylene-oxide groups. F68 was displayed to interact with membrane lipid bilayer to stabilize it. In this study, we aimed to increase the transfection efficiency in HeLa cells by adding F68 during and after electroporation. *Methods:* HeLa cells were electroporated at various voltages in 0.2-mm cuvvettes containing RPMI-1640 medium without serum, with or without F68 and with 5μg of pEGFP-N2 plasmid DNA. After transfection, the cell viability and transfection efficiency were measured by using MTS assay and flow cytometry, respectively. *Results:* The optimum conditions for electroporation of HeLa cells were determined to be 140V at 500μF capacitance, which resulted in 36 % transfection efficiency. When the cells were electroporated in the presence of F68, the efficiency increased by 30%, lowering the cell death during electroporation. *Discussion:* The major drawback of electroporation is that some cells are highly sensitive to the

electrical stress occurring during electroporation. Our data suggest that F68 may enable the transfection of genes into sensitive cell lines by lowering cell death and increasing the transfection efficiency.

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DETECTION OF GENE POLYMORPHISMS ASSOCIATED WITH THE LOSS OF BONE MINERAL DENSITY (CASE REPORT)

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Background: Osteoporosis is a common disease which is characterised by low bone mass and an increased risk of fracture. Candidate genes which have been studied in relation to BMD (bone mineral density) and osteoporotic fractures include Vitamin D receptor, estrogen receptor, Col1 A1 gene, calcitonin receptor. We present here a case of a 39 year old man whose BMD result was: Z score, -4.2; T score, -4.3. *Methods:* We used clinical array systems to detect polymorphisms. This method is based on a low density chip at the bottom of a classical 2 ml tube. DNA was extracted from blood using EDTA. DNA amplification,denaturation, hybridization and the other steps were carried out. Biochemical analyses, complete blood analysis were recorded. *Results:* We analysed Col1A1-SP1, CTR-ALU1, ESR1X-XBAI, ESR1P-PVUII, VDRF-FOKI and VDRB-BSMI polymorphisms for collagen type1 gene, calcitonin receptor gene, estrogen receptor gene and vitamin D receptor gene respectively. The analysis showed that his genotype was Ss, Aa, ,pp, Xx, bb, Ff (normal genes are indicated by capital letters). *Conclusion:* Positive associations between these polymorphisms and bone density have previously been reported by several studies. These type of studies will assist in clinical desicion making and support certain therapies, especially for early age fractures and early treatment for bone prevention.

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SOME RARE COMPLEX MUTATIONS OF MEFV GENE IN DIYARBAKIR REGION OF TURKEY

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Background: FMF is an autosomal recessive inflammatory disorder that predominantly affects Jews, Armenians, Turks and Arabs. It is characterised by recurrent fevers, abdominal, chest, joint pains and erysipelas-like skin disease. *Methods:* In this study, 1,428 patients who attended to Dicle University, Medical Faculty with complaints of fever and joint and abdominal pains were studied for FMF mutation detection by Vienna lab. strip assay method. *Results:* Mutations were detected in 689 patients, eight of which were interesting since they combined complex mutations. The mutations were as follows: triple heterozygous, double homozygous, homozygous + heterozygous cases. Triple mutations: *E148Q/P369S/M694V (2 cases), E148Q/P369S/M694I (1 case)*. Double homosygous: *E148Q/E148Q/P369S/P369S (2 cases)*. Homozygous + heterozygous mutations: *E148Q/E148Q R761H/R761H M694V/M694V M6 94V* heterozygous (1 case), *E148Q* heterozygous (1 case) and *E148Q* heterozygous (1 case). According to our literature search, we considered the influence of these mutations on disease prognosis. Double homozygous case types were the most common reported in the literature. *Conclusion:* A high prevalence of FMF mutations was found in this region. Genetic analysis must be performed in all patients with suspected of FMF. Due to the complexity and interference of these mutations, genetic counseling and new-born screening must be performed in this region.

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EFFECTS OF THE PPAR-GAMMA AND APOLIPOPROTEIN E GENE POLYMORPHISMS ON CLINICAL AND LIPID CHARACTERISTICS IN PATIENTS WITH DIABETIC AND NON-DIABETIC CORONARY HEART DISEASE

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We investigated whether Pro12Ala and C161T variants in the PPARgamma2 gene are associated with the occurrence of type-2 coronary heart disease (CHD) in patients prospectively characterized for the presence or absence of diabetes mellitus. PPAR-gammaPro12Ala, PPAR-gamma C161T and apolipoprotein E gene polymorphisms were determined using PCR-RFLP in 262 patients with CHD (103 T2DM, 159 non-diabetic) and 105 healthy people. Statistical analysis revealed no significant difference both in the PPAR gamma Pro12Ala

and ApoE genotypes and allele frequencies between the study groups ($p>0.05$). However, PPARgamma ProAla heterozygote genotype and 12Ala allele seemed to be protective against hypertension and left ventricular hypertrophy in males. Statistically significant differences were observed in genotype frequencies between the healthy and diabetic/total patient groups in the distribution of PPARgamma C161T ($p<0.05$). The frequency of CC genotype of PPAR-gamma C161T was higher in the diabetic patients than in the controls. We can infer from these data that diabetic patients with CC genotype may be susceptible to the development of CHD and that T allele may be protective against this disease. The frequency of T161allele in diabetic and in total CHD patients was significantly lower in males than in the females. PPARgamma 161T allele was associated with increased total cholesterol levels in total patient group. We observed that 161T allele strengthened the effect of ApoE4 allele on cholesterol levels. Furthermore, male patients carrying T161 allele had a lower prevalence of left ventricular hypertrophy than female patients. Our findings suggest that PPARgamma C161T polymorphism may contribute to the development of CHD, especially in male diabetic patients.

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PROTEIN CARBONYLATION AS A BIOMARKER OF OXIDATIVE STRESS

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Background: Reactive oxygen species (ROS) can damage all biomolecules. Oxidative modifications of enzymes and structural proteins plays a significant role in the aetiology and/or progression of several human diseases. Protein carbonyl content is the most widely used marker of oxidative modification of proteins. Besides, it is known that TNF-α contributes to formation of ROS. We aimed to investigate changes of TNF-α and protein carbonyl levels in gastrointestinal cancer patients compared to healthy control individuals. *Methods:* Serum TNF-α and protein carbonyl levels in gastrointestinal cancer patients (n=108) and healthy controls (n=35) were measured. Patients were divided into three groups: the first group had gastric cancer, the second group had colorectal cancer and the third group had pancreas, liver and esophagus cancer. TNF-α and protein carbonyl levels were analyzed by using kits. *Results:* The levels of TNF-α and protein carbonyl were statistically higher in cancer patients compared with the healthy controls ($p<0.01$). The mean values for protein carbonyl were found to be significantly higher in the first and second groups than in controls or the third group ($p<0.01$). There was a significant difference between the third group and controls for TNF-α ($p<0.05$). *Conclusion:* Enhanced protein carbonyl levels may be a diagnostic biomarker for oxidative damage in cancer.

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ROLE OF C3435T AND G2677T/A POLYMORPHISMS IN THE DRUG-TRANSPORTER GENE MDR1 IN MULTIDRUG-RESISTANT EPILEPSY

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Background: Multiple drug resistance is a common problem in the treatment of epilepsy, and approximately 30% of patients continue to have seizures despite all therapeutic interventions. Among various classes of drug transporters, genetic variants of P-glycoprotein (P-gp) encoded by the multidrug resistance 1 (MDR1) gene have been associated with drug-resistant epilepsy. Our aim was to investigate the effect of the C3435T and G2677T/A polymorphisms of MDR1 on drug resistance in Turkish patients with epilepsy. *Methods:* MDR1 C3435T and G2677T/A were genotyped in 103 patients with epilepsy, classified as drug-resistant in 46 and drug-responsive in 57, and 150 control subjects without epilepsy. Genotypes of the C3435T and G2677T/A polymorphisms were determined by polymerase chain reaction followed by restriction fragment length polymorphism. *Results:* There was no statistically significant difference between genotype and allele frequencies of drug-resistant and drug-responsive epilepsy patients. The frequencies in the drug-resistant and drug-responsive groups did not differ significantly from control subjects. *Conclusion:* MDR1 polymorphisms investigated in this study are not associated with drug resistance in Turkish epileptic patients.

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ASSOCIATION BETWEEN GENETIC VARIATION ON CHROMOSOME 9p21 AND HYPERTENSION

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Background: Hypertension is a complex multifactorial disease caused by interactions between genetic and environmental factors. It is an independent determinant of cardiovascular risk. Recent genome-wide association studies (GWAS) revealed that some 9p21 variants are associated with cardiovascular diseases (CVD). The aim of this study was to investigate the possible influence of the single nucleotide polymorphism (SNP), rs10757274, on chromosome 9p21 on a sample of Turkish patients with hypertension. *Methods:* In this study, rs10757274 A/G polymorphism was studied in 170 patients with hypertension and 109 healthy subjects. Genomic DNA was extracted from peripheral blood leukocytes. 9p21 rs10757274 A/G polymorphism was genotyped by real time polymerase chain reaction. *Results:* For rs10757274 A/G polymorphism the frequencies of the AA, AG and GG genotypes were 12.4%, 46.4% and 41.2% in the hypertension patients and 24.8%, 53.2% and 22% in the healthy controls, respectively. An extremely significant relationship was observed in genotype distribution between the hypertension patients and the healthy controls for rs10757274 polymorphism (p=0.001). No significant relationship was observed when genotype frequencies were compared with body mass index values in the hypertension patients (F=2.365, p=0.097). *Conclusion:* We suggest that 9p21 rs10757274 A/G polymorphism may play an important role in the pathogenesis of hypertension.

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ASSOCIATION OF A COMMON VARIANT ON CHROMOSOME 9p21 IN TURKISH PATIENTS WITH METABOLIC SYNDROME

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Background: The metabolic syndrome is a complicated syndrome causing insulin resistance, hyperinsulinemia, central obesity, dyslipidemia, atherosclerosis and inflammation. It is often accompanied by different types of cardiovascular disease (CVD). Recent genome-wide association studies (GWAS) revealed that some 9p21 variants are associated with type-2 diabetes and CVD. In this study, we examined whether the single nucleotide polymorphism (SNP), rs1333049, on chromosome 9p21 is associated with the metabolic syndrome. *Methods:* A sample of 66 unrelated Turkish patients with metabolic syndrome and 122 healthy controls were investigated in this study. Genomic DNA extraction was performed from peripheral blood leukocytes. 9p21 rs1333049 C/G polymorphism was genotyped by RT-PCR. *Results:* The frequencies of the CC, CG and GG genotypes were 25.7%, 47% and 27.3% in the metabolic syndrome patients and 41%, 43.4% and 15.6% in the healthy controls, respectively. A statistically borderline significance was observed in the genotype distribution between the metabolic syndrome patients and the control group for the rs1333049 polymorphism (p=0.053). No significant relationship was observed when genotype frequencies were compared with body mass index values in metabolic syndrome patients (F=0.66, p=0.51). *Conclusion:* We suggest that the 9p21 rs1333049 polymorphism may play an important role in the pathogenesis of the metabolic syndrome. However, further studies are necessary to confirm these findings.

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THE POLYMORPHISM OF CODON D727E OF TSHR (THYROID-STIMULATING HORMONE RECEPTOR) GENE IN A TURKISH POPULATION WITH MULTINODULAR GOITER

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Background: Multinodular goiter involves more than one nodules, is the most common endocrine pathology in the world and is still a problem in the clinic. Genetic variation is an important factor in the development of the disease. This study was performed to determine the allele frequencies and distribution of genotypes of codon D727E polymorphism of the TSHR (thyroid-stimulating hormone receptor) gene in an endemic Turkish multinodular goiter population. *Methods:* Genomic DNA was extracted from 172 persons (118 patients with multinodular goiter and 54 healthy controls). The polymorphism of codon D727E of the TSHR gene was identified by polymerase chain reaction-based restriction analysis. *Results:* There was a statistically significant difference between the groups with respect to genotype distribution (p<0.05). The C and G allele frequencies were 92% and 8% in the patients, respectively, while the respective frequencies in the control group were 100% and 0%.

Conclusion: This study suggests that the polymorphism of codon D727E of the TSHR gene may be a marker for identifying multinodular goiter. However, there is no direct relationship between this gene polymorphism and the disease.

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THE ASSOCIATION OF PARAOXONASE I PHENOTYPES WITH ASYMMETRIC DIMETHYLARGININE IN MYOCARDIAL INFARCTION

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A genetic change affecting the primary sequence at glutamine 192 on paraoxonase-1 (PON-1) protein gives rise to two allozymes in human serum. The polymorphic BB type of PON-1 is believed to be associated with cardiovascular diseases because it is being less effective at protecting LDL from oxidation. Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase (NOS) inhibitor, is a major determinant of NO bioavailability in the vascular system and there is evidence for a causal relationship between increased ADMA levels and several cardiovascular risk factors. In this study, we investigated whether serum PON-1 activity and ADMA concentration were altered in myocardial infarction (MI). We also investigated the relationship between the allozymes of PON-1 and the arginine/ADMA ratio. Serum PON-1 activity was measured in 46 MI patients and the PON-1 allozyme phenotypes were determined. ADMA and arginine levels were measured using HPLC. No change in PON-1 activity was observed within 6 h of MI. In the study group, HDL levels were correlated with PON-1 activity. Control subjects with less effective BB phenotype had higher arginine and arginine/ADMA ratio than those bearing AA phenotype; the difference in the latter was significant (209 *vs*. 114, $p<0.05$). This phenotype-related difference was not observed in MI patients. It is concluded that the less effective BB phenotype of PON-1 may not be associated with cardiovascular heart disease, if the bioavailability of NO is sufficient to protect the vascular system from oxidative damage.

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AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE GENE *'PKD1'* SEQUENCE VARIATIONS IN TURKISH PATIENTS AND HEALTHY INDIVIDUALS

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Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common form of inherited kidney diseases which results in renal failure. One of the genes associated with the disease is PKD1 located on chromosome 16p13.3 located (the other gene is *PKD2*), which produces polycystin-1 protein, interacting polycystin-2, whose normal function is not well understood. The aim of the present study was to document the sequence variations found in the *PKD1* gene. *Methods:* Bidirectional DNA cycle sequencing analysis of entire coding exons and adjacent intronic segments of PKD1 gene was performed for ten patients with ADPKD clinical diagnosis and six healthy individuals. *Results:* Distributed between exons 5 and 15, a total of 25 different likely pathogenic germline sequence changes (1 nonsense, 24 missense mutations), 15 different SNPs and 2 intronic changes were identified in the patient group. Twenty one out of 25 likely pathogenic sequence changes are unique for the Turkish population. In the control group, 14 different intronic changes, 81 different missense mutations; 2 different nonsense mutations and 43 different SNPs were detected. *Conclusion:* The establishment of mutations and genotype - phenotype correlation in ADPKD patients will improve diagnosis and clinical prognosis of ADPKD patients.

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DNA SEQUENCE ANALYSIS OF *MEFV* GENE IN 3101 TURKISH PATIENTS CLINICALLY DIAGNOSED WITH FAMILIAL MEDITERRANEAN FEVER

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Background: Familial Mediterranean Fever (FMF, MIM249100) is a Mendelian periodic fever syndrome without evidence of high-titer autoantibodies or antigen-specific T cells. The causative disease gene is 16p13.3 chromosomally located *MEFV* (Mediterranean Fever) gene encoding the 781 amino acid protein, pyrin. Here we aimed to document the sequence variants found along the MEFV gene exons, intronic and promoter segments in Turkish patients with FMF clinical diagnosis. *Methods:* Detailed DNA sequencing analysis was performed for 3101 patients and families. *Results:* 11 novel mutations and 1 novel SNP in exons 2, 3, 5, 9, and 10 including R151S, S166L, G340R, P350R, G456A, Y471X, S503C, I506V, A511V, K695N, L709R, and P588P, G219G,

were characterized and registered in *INFEVERS* (database of hereditary autoinflammatory disorders mutations). Through the missense mutations; 115 were homozygous, 258 were compound and complex heterozygous, and 775 were heterozygous for one mutation. Allelic frequencies of M694V, E148Q, V726A, and M680I accounted for 38.1; 21; 11; and 10.8%, respectively; followed by P369S, R408Q, K695R, M694I, R761H accounting for 4,3; 3,5; 2,8; and 1,6%. *Conclusion:* DNA sequencing method is recommended, in particular for finding the hidden sequence variants along the entire MEFV gene in asymptomatic patients and individuals.

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STUDIES ON INTERLEUKIN 10 GENE -2849 PROMOTER AND ANGIOTENSIN CONVERTING ENZYME INSERTION/ DELETION POLYMORPHISMS IN TURKISH PREECLAMPSIA PATIENTS

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Background: The aim of the study was to investigate whether there is an association between interleukin (IL-10) -2849 and angiotensin converting enzyme (*ACE*) insertion/deletion (I/D) polymorphisms and PE. *Methods:* A total of 126 preeclamptic and 116 normotensive pregnant women were genotyped for IL-10 -2849 promoter and *ACE* I/D polymorphisms and the distribution of genotype and allele frequencies belonging to these polymorphisms in preeclampsia and controls was evaluated. Codominant, dominant and recessive models were applied in *ACE* gene I/D polymorphism. *Results:* For the *IL-10* gene, the AA genotype was found significantly more frequently in preeclampsia patients than normotensive controls (p=0.030). The A allele frequency was 14.3% in preeclampsia while it was 12.5% in normotensive controls (p=0.564). For *ACE* gene I/D polymorphism, in the codominant model the DD genotype was found to be significantly higher in preeclampsia patients than in normotensive controls (p=0.016). In the dominant model (DD frequency *versus* DI+II frequency) there was a significant relationship between DD genotype and preeclampsia (p=0.006). D allele frequency was 64.6% in preeclampsia patients while it was 56.1% in normotensive controls (p=0.062). *Conclusion:* There was a significant difference in terms of genotype distribution between preeclampsia patients and normotensive controls, while no difference was found in allele frequency both for IL-10 -2849 and *ACE* I/D polymorphisms.

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ANALYSES OF TUMOR NECROSIS FACTOR ALPHA -G308A GENOTYPES IN SCHIZOPHRENIA PATIENTS

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Background: Schizophrenia is a genetically complex neuropsychiatric disorder characterized by profound disturbances of perception, thought, cognition, emotion and social functioning and affects approximately 1% of the population worldwide. The pathogenesis of schizophrenia is unclear but various cytokines seem to be particularly involved. Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine with functions in nerve cell growth, differentiation, and apoptosis. The level of TNF-α production is thought to be influenced by a -308G/A promoter polymorphism. *Methods:* In this study, our aim was to investigate the association between TNF-308G/A polymorphism and schizophrenia patients. DNA was extracted from 100 patients with schizophrenia and 298 controls. TNF-308G/A polymorphism was analyzed using amplification refractory mutation system-polymerase chain reaction (ARMS–PCR). *Results:* The -308G/A genotypic and -308A allelic frequency was higher in patients with schizophrenia than healthy controls and a significant association was found between TNF-308G/A polymorphism and schizophrenia in Turkish patients (p<0.020, OR: 1.78, 95% CI: 1.07–2.91). *Conclusion:* Our study suggests that TNF-α -308G/A polymorphism may play a role in determining susceptibility to schizophrenia.

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PHOX2B GENE MUTATIONS IN NEUROBLASTOMAS

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Background: Neuroblastoma (NB) is characterized by genomic imbalances in tumor cells. Germline mutations of *PHOX2B*

were detected in Ondine syndrome and some of these cases developed NB. Acquired mutations of *PHOX2B* have previously been detected in rare cases of sporadic NB. In this study, we aimed to detect somatic *PHOX2B* mutations in 128 additional neuroblastic tumors. *Methods:* DNA sequence analyses of all *PHOX2B* exons were performed in 114 NB, 7 ganglioneuroblastoma and 7 ganglioneuroma samples. Sequence data were analyzed by "Mutation Surveyor" software. *Results:* Mutation analyses revealed a novel mutation of c.96G>A (D32N) in exon 1 in a NB case. In addition, c.1101_1118het-del18 and c.1098_1136het-del39 deletions in exon 3 that encodes the polyalanine tract were detected in a NB and a ganglioneuroma, respectively. Both of these deletions were previously undefined in terms of their size and effect on reading frame. Two different single nucleotide polymorphisms, c.552C>T and c.762A>C, were present in five NB samples. *Conclusion: PHOX2B* mutations are rare in NBs and can be observed in fully differentiated histological subtypes. The presence of *PHOX2B* mutation in a tumor with maturation suggests that it is prognostically insignificant. Since deletions of polyadenine tract changes the three-dimensional structure of PHOX2B and, thus, affects its function, inactivating *PHOX2B* mutations may contribute to the development of NB.

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THE EFFECTS OF RESUSCITATION WITH HYDROXYETHYL STARCH ON LIVER TISSUE AFTER ACUTE HEMORRHAGE IN RATS

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Background: An important aspect in the resuscitation treatment of hypovolemia is the re-establishment of normal tissue hemodynamics. This study was designed to investigate the histo-hemodynamical effects of resuscitation infusion of hydroxyethyl starch (HES) solutions on liver tissue, in an acute hemorrhage rat model. *Methods:* Male Wistar albino rats (n=12) weighing 270-340 g were randomly divided into three groups, namely the control group, the hypovolemia group and the isovolemic resuscitation group with 6% HES (130/0.4). The tissue samples were fixed in 10% formalin and prepared using a routine paraffin procedure. Sections were stained with hematoxylin and eosin. Anti-vascular endothelial growth factor (VEGF), anti-endothelial nitric oxide synthase (eNOS) and anti-inducible nitric oxide synthase (iNOS) primary antibodies were used for immunohistochemical examination. *Results:* Hypovolemia decreased both the mean arterial pressure and heart rate, and induced strong immunoreactivities of VEGF, eNOS and iNOS. In contrast, mild/moderate and moderate immunoreactivities were seen in the control and isovolemic resuscitation groups, respectively. *Conclusion:* Ischemia and angiogenesis caused by hypovolemia increased iNOS, eNOS, and VEGF immunoreactivities. The use of HES solutions, even in the acute period of hypovolemia, seems to be effective in the prevention of pathophysiologic changes of liver tissue.

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IDENTIFICATION OF PHOSPHORYLATION SITES ON PEA3 TRANSCRIPTION FACTOR AND THEIR EFFECTS ON CANCER CELLS

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Background: The PEA3 group of transcription factors (Pea3, Erm and Er81) possess an ETS-DNA binding region and are known to play an important role in oncogenic mechanisms. Overexpression of the Pea3 gene was noted to increase metastasis in various cancer types, including breast, prostate and colon cancer. Although phosphorylation-dependent activation mechanisms were demonstrated for Er81 and Erm, such mechanisms for Pea3 are unknown. In this study, we are predicting the phosphorylation sites on Pea3 and identifying the effects of these sites on cancer cells. *Methods:* Phosphorylation motifs of Pea3 protein were predicted using online bioinformatics tools. Silencing and enhancing mutants were constructed from wild-type Pea3 using PCR-based site-directed mutagenesis. In order to identify whether these motifs were the correct sites, luciferase reporter assays were designed. *Results:* From the results obtained in bioinformatics studies, we identified the sites having a strong possibility of phosphorylation by different kinases, including p38, MAPK, CDC2 or CDK5. *Conclusion:* Pea3 protein is associated with tumorigenesis and aggressiveness of cancer cells. We suggest that the identification of phosphorylation-dependent activation or repression mechanisms of Pea3 may be crucial in finding an appropriate therapy.

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OPTIMISATION AND VALIDATION OF DNA SEQUENCING FOR KIT MUTATION TESTING IN GASTROINTESTINAL STROMAL TUMOURS

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Background: Mutations of KIT gene exons 9, 11, 13 and 17 are associated with prognosis and benefit from targeted therapy in gastrointestinal stromal tumours. DNA sequencing is the gold standard in determination of mutations but it is a difficult labour- and time-consuming process. For these reasons, there is an urgent need for standardized sequencing procedures and validated results to provide accurate testing of *KIT* mutation status. *Methods:* Our pharmacogenetic laboratory *KIT* gene exon 9 (κ_1), 11 (κ_2), 13(κ_3) and 17 (κ_4) dideoxy-Sanger sequencing results were compared with those from an expert sequencing center which applies dideoxy sequencing and has a large sample volume (accepted as gold standard). *Results:* The inter-rate reliabilities were found to be outstanding (κ_1=1.00, Mc Nemar *p*: 1.00), outstanding (κ_2=1.00, Mc Nemar p: 1.00), moderate (κ_3=0.588, Mc Nemar *p*: 1.00; 95%CI, -0.092 – 1.268) and outstanding (κ_4=1.00, Mc Nemar *p*: 1.00), respectively. *Conclusion:* The reliability of our method is outstanding (exon9, 11 and 17) and moderate (exon 13), according to expert sequencing. Accurate detection of KIT gene mutation status, the validity of standardized testing procedures and methods are required. Our results clearly show that this method may give a reliable clinical outcome, compared with expert laboratory results.

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PYROSEQUENCING ANALYSIS FOR DETECTION OF KRAS MUTATIONS IN COLORECTAL CANCER

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Background: According to the NCCN and ASCO's colorectal cancer guidelines, *KRAS* oncogene mutation status has been reported to be a predictive marker of tumour response to epidermal growth factor receptor inhibitors (such as cetuximab and panitumumab). In this evaluation we compared the pyrosequencing method with the conventional dideoxy DNA sequencing (as a gold standard), microarray based genotyping technology and allele-specific polymerase chain reaction (ARMS-PCR). The usefulness of the methods was investigated for KRAS mutation analysis. *Methods:* The DNA was extracted from 10 µm thick sections of 37 colorectal tumour tissues taken from formalin fixed paraffin embedded (FFPE) tissue blocks (QIAamp DNA FFPE Tissue Kit, Zymo Research). *KRAS* mutation was analyzed by pyrosequencing, dideoxy sequencing, microarray technology and ARMS-PCR. *Results:* When pyrosequencing was compared with dideoxy DNA sequencing (κ_1), ARMS-PCR (κ_2) and microarray technology (κ_3), the inter-rate reliabilities were found to be substantial (κ_1=0.706 (p<0.001); 95% CI, 0.490-0.922), substantial (κ_2=0.625 (p<0.001); 95% CI, 0.355-0.895) and outstanding (κ_3=0.889 (p<0.001); 95% CI, 0.679-1,099), respectively. *Conclusion:* Pyrosequencing reliability is substantial according to DNA sequencing. It can also detect more mutations than ARMS-PCR and microarray technology.

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THE ROLE OF DNA SEQUENCING IN PHARMACOGENETIC APPLICATIONS

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Background: Inter-individual genetic differences are observed in both drug response and toxicity associated with anticancer therapy. Pharmacogenetic/genomic studies have successfully identified these genetic differences which are significant in the management of cancer. In particular, gene amplification and somatic mutations in the tumour genome may be predictive and prognostic for chemotherapy outcome. Therefore, tumour genetic material based mutation analysis may be a first step in personalised therapy selection. *Methods:* Classical dideoxy DNA sequencing is the gold standard in mutation analysis because it allows determination of all mutation possibilities. Genomic DNA was extracted from 10 µm thick sections and amplified with specifically designed primers of KIT gene exons 9, 11, 13 and 17. After post-PCR purification, pure amplicons were subject to sequencing PCR and DNA fragments were obtained for mutation analysis. *Results:* The DNA sequence was analysed from DNA fragments. Generated DNA sequences were compared with a reference sequence and mutations were determined. *Conclusion:* With this methodology all possible mutations and variants can be determined and resequencing studies elucidate new population variants. Therefore new variant and new drug combinations can be developed.

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UNUSUAL HIGH FREQUENCY OF *KRAS* MUTATIONS IN FEMALE COLORECTAL CANCER PATIENTS

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Background: KRAS mutations frequently occur in different types of cancers. Recent studies have shown that the presence

of activating *KRAS* mutations has been identified as a potent predictor of resistance to EGFR-directed antibodies such as cetuximab or panitumumab. *Methods:* Genomic DNA was extracted from formalin fixed paraffin embedded tissue sections and mutation analysis was performed by real-time polymerase chain reaction (PCR). *Results:* In this study, KRAS gene codon 12 and 13 mutation frequency was 38% and, conversely, 50% were wild-type. The most frequent mutations were glycine to valine on codon 12 (p.G12V, 27%), glycine to aspartic acid on codon 12 (p.G12D, 22%) and glycine to cysteine on codon 12 (p.G12C, 17%). Mutation frequency in females was higher (49%) than in males (30%). Conversely to this difference, the most common mutation types were similar in males and females (p.Gly12Val, p.Gly12Asp). *Conclusion:* In our experience from predictive testing for KRAS mutations, the mutation rate (44%) is higher than that published in the literature (35-40%) and Cosmic Database (33%, n=28,884). Our study suggests that KRAS mutations reveal a high mutation frequency (49%) in colorectal cancers from females and this group is the least likely to respond to anti-EGFR therapies.

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CORRELATION BETWEEN HETEROGENEOUS EXPRESSION OF SIALYLTRANSFERASES AND MUC16 IN OVARIAN TUMOR TISSUES

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Background: A number of glycoproteins, such as CA125, are abnormally glycosylated in ovarian cancer. Most aberrant glycosylations are associated altered sialyltransferase (ST) expression. The aim of this study was to evaluate the expression of six sialyltransferases and MUC16, as well as correlations in ovarian benign and malignant tissues. *Methods:* mRNA expression of six sialyltransferases and MUC16 was assessed in 16 human ovarian tumors (7 benign and 9 malignant tumors) by real time PCR. *Results:* mRNA of ST6GAL I and ST3GAL I were not significantly up-regulated in ovarian cancer tissues, while ST6GAL II and ST3GAL IV were not significantly increased in benign tumors. There was no change between ST3GAL III, ST3GALVI expression in different tumour sub-types. MUC16 was significantly increased in carcinoma tissue. A significant correlation was found between ST3GAL III and ST3GAL IV. MUC16 correlated with ST3GALVI and ST6GALI. ST6GAL I was well correlated with ST3GALVI. ST6GAL II was significantly correlated with ST3GALIII and ST3GALIV. *Conclusion:* The expression level of the examined sialyltransferases and MUC16 may be heterogeneous as a consequence of oncogenic transformation of the ovary.

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COMBINATION OF FUSION GENES, CYTOSINE DEAMINASE AND INTERFERON-BETA, IN THERAPEUTIC STEM CELLS RESULTED IN AN INHIBITION OF HUMAN HEPATOCARCINOMA CELL GROWTH *VIA* THEIR MIGRATORY ABILITY

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Background: Stem cells have received a great deal of attention for their clinical and therapeutic potential in treating human diseases and disorders. Recent studies have shown that it is possible for genetically engineered stem cells (GESTECs) to produce suicide enzymes that convert non-toxic prodrugs to toxic metabolites, selectively migrate toward tumor sites and to reduce tumor growth. In the present study, we evaluated whether these GESTECs are capable of migrating to hepatocarcinoma cells and examined the potential therapeutic efficacy of gene-directed enzyme pro-drug therapy against liver cancer cells *in vitro*. *Methods:* The expression of *cytosine deaminase (CD)* and *human interferon-beta (IFN-b)* genes in engineered stem cells was examined in GESTECs following gene introduction. In addition, chemo-attractant molecules, such as SCF, CXCR4, c-kit, VEGF, and VEGFR2, were confirmed in liver hepatocarcinoma cells, Hep3B, for migratory capability. To determine the migratory ability of engineered stem cells, we performed a modified transwell assay. When treated with 5-fluorocytosine (5-FC) in the presence of engineered stem cells, the viability of liver cancer cells was measured by MTT assay. *Results:* GESTECs, *i.e.*, HB1.F3.CD or HB1.F3.CD.interferon-β (IFN-β) cells, engineered to express a suicide gene, CD, selectively migrated toward liver cancer cells. Treatment of Hep3B human liver cancer cells with the prodrug 5-FC in the presence of HB1.F3.CD or HB1.F3.CD.IFN-β cells resulted in the inhibition of Hep3B cell growth. A high inhibitory effect on Hep3B cell growth was induced by HB1.F3.CD.IFN-β in the presence of 5-FC than by HB1.F3.CD alone. *Conclusion:* Based on the data presented herein, we suggest that GESTECs expressing CD may have a potent advantage for selectively treating human hepatocarcinoma. Furthermore, GESTECs expressing a fusion gene encoding CD and IFN-β may exert a synergic antitumor effect on this type of tumor.

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THERAPEUTIC ANTITUMOR EFFECTS OF GENETICALLY ENGINEERED STEM CELLS EXPRESSING CYTOSINE DEAMINASE AND HUMAN INTERFERON-BETA TO SELECTIVELY TARGET HUMAN NON-SMALL CELL LUNG CARCINOMA CELLS

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Background: Recent studies have shown that genetically engineered stem cells (GESTECs) produce suicide enzymes that convert non-toxic pro-drugs to toxic metabolites which selectively migrate toward tumor sites and reduce tumor growth. GESTECs were engineered to express bacterial *cytosine deaminase (CD)* and human *interferon-beta (IFN-b)* genes [*i.e.*, HB1.F3.CD or HB1.F3.CD.IFN-b]. The CD gene plays a role in converting non-toxic prodrug 5-fluorocytosin (5-FC) to the toxic agent, 5-fluorouracil (5-FU) and additionally the *IFN-b* gene has an antitumor effect. In the present study, we evaluated whether these GESTECs are capable of migrating to human non-small cell lung carcinoma cells and examined the potential therapeutic efficacy of gene-directed enzyme pro-drug therapy against lung cancer cells *in vitro*. *Methods:* Using RT-PCR, we examined the expression of the *CD* and *IFN-b* genes in engineered stem cells. Also, chemo-attractant molecules, such as SCF, CXCR4, c-kit, VEGF, and VEGFR2, were confirmed in lung cancer cells. To determine the migratory ability of engineered stem cells, we performed a modified transwell assay. When treated with 5-FC in the presence of engineered stem cells, the viability of lung cancer cells was measured by MTT assay. *Results:* In this study, we confirmed the expressions of *CD* and *IFN-b* genes in GESTECs and several chemo-attractant molecules in lung adenocarcinoma cells. A modified transwell migration assay was performed to determine the migratory capacity of GESTECs to lung cancer cells. GESTECs cells engineered to express a suicide gene, CD or/and IFN-b, selectively migrated toward lung cancer cells. Treatment of a human non-small cell lung carcinoma cell line (A549, a lung carcinoma derived from human lung epithelial cells) with the pro-drug 5-FC in the presence of HB1.F3.CD or HB1.F3.CD.IFN-b cells resulted in the inhibition of lung cancer cell growth. *Conclusion:* The results of this study show that GESTECs expressing CD or CD.IFN-b genes may selectively migrate toward lung cancer cells. Moreover, this GEPT system resulted in an anti-proliferative effect on lung cancer cells, suggesting that GESTECs expressing a suicide gene combined with the application of a pro-drug may have therapeutic potential for selectively targeting lung cancers. Furthermore, GESTECs expressing the fusion of *CD* and *IFN-b* genes may have a synergic anti-tumor effect compared to GESTECs expressing CD alone.

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INVESTIGATION OF FETUIN-A SINGLE NUCLEOTIDE GENE POLYMORPHISM IN URINARY STONE DISEASE PATIENTS

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Background: Urinary stone disease is a complex and multifactorial disorder which has high incidence ratio in the Turkish population. Various molecules and proteins are likely involved in the progression of calcium oxalate saturation and formation of crystals. One of the proteins suspected to be linked with the calcification mechanism is Fetuin-a. Single nucleotide gene polymorphisms at 766 C/G of the Fetuin-a gene were shown to be linked with disease progression. In this study our aim was to investigate the association between 766 C/G polymorhisms and urinary stone disease progression. *Results:* We found that there were no significant differences in genotype distribution of 766 alleles compared to control subjects (p=0.328). Moreover, we did not find any statistically significant difference in Fetuin A 766 G/T allele frequencies in urinary stone disease patients compared to healthy individuals (p=0.299). *Conclusion:* In preliminary results, 766 C/G gene polymorphism for Fetuin-a gene was not a significant factor for urinary stone disease.

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APOPTOTIC ACTIVITY OF QUERCETIN AND TAMOXIFEN IN CACO-2 CELLS

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Background: Polyphenolic compounds such as quercetin may be used in the treatment of cancer. Tamoxifen is a non-steroidal anti-estrogen drug widely used in the treatment of patients with estrogen receptor-positive breast cancer. Additionally, the efficacy of tamoxifen in CaCo-2 cells

suggests its potential effectiveness in colon carcinoma as well as non-estrogen sensitive tumors. The aim of the study was to investigate the apoptotic effects of quercetin and tamoxifen in CaCo-2 cells. *Methods:* CaCo-2 cells were cultured with quercetin and tamoxifen at concentrations of 25, 50 and 100 μM. The effects of quercetin and tamoxifen on apoptotic index was determined at 24th, 48 th and 72nd hours. *Results:* Apoptotic index was increased in cells exposed to quercetin and tamoxifen when compared to control cells in a dose-dependent manner. *Conclusion:* The induction of apoptosis by quercetin and tamoxifen in colon adenocarcioma may play an important role in cancer treatment.

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THE FREQUENCY AND SUBTYPES OF *BLASTOCYSTIS* IN PATIENTS WITH GASTROINTESTINAL SYMPTOMS

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Background: Blastocystis is a common parasite of humans and there are controversial reports about its pathogenicity. It has many subtypes; among them subtype 3 is both common and reported to be pathogenic for patients. The aim of this study was to identify the *Blastocystis* subtypes in a cohort of symptomatic patients from İzmir and Manisa provinces. *Methods:* A total of 617 stool samples from patients with gastrointestinal symptoms were examined with routine O&P examination and inoculated in Jones medium. Positive samples were further assessed to identify coinfections with other parasites (*Entamoeba sp., Cryptosporidium sp.*), bacteria (*Salmonella, Shigella* and *Eschericia coli*) and viruses (Adenovirus, Rotavirus) by culture and enzyme immunoassay (EIA) tests. DNA was isolated from each stool and culture sample, followed by PCR and sequencing. *Results:* Eighty-three (13.5%) stool and 81 culture samples were included in the study; 68 (11.0%) of them were only *Blastocystis*-positive. Subtype 3 was by far the most common subtype (n=38; 45.8%). Subtype 6 and 7 were firstly isolated from symptomatic patients from Izmir and Manisa. *Conclusion:* Subtype 3, which was reported to have pathogenic potential, was the most common subtype in our cohort. Patients with subtypes 6 and 7 need further assessment concerning the zoonotic potential of *Blastocystis*.

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EFFECT OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) GENE VARIANTS ON SCHIZOPHRENIA: RESULTS FROM A CASE-CONTROL STUDY IN TURKISH POPULATION

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Background: Many infectious agents have been linked to an increased risk of schizophrenia. However, it has never been clear which infectious agents are the most important, or how they might cause psychosis and other symptoms of schizophrenia. The CC chemokine monocyte-chemoattractant protein-1 (MCP-1) represents a potent stimulus for monocyte recruitment into inflammatory sites. Since there is a correlation between inflammation and schizophrenia, MCP-1 may be associated with an increased risk of schizophrenia. We investigated the relationship between MCP-1 A2518G polymorphism and schizophrenia in the Turkish population. *Methods:* The present analyses are based on 50 case subjects (29 men, 21 women) with schizophrenia and 129 non-case subjects (55 men, 74 women). Genotyping of the MCP-1 A2518G polymorphism was accomplished by PCR-RFLP method. *Results:* We detected a positive correlation between MCP-1 A2518G polymorphism and schizophrenia in this case-control study (p=0.041 X^2=6.40). Frequencies of MCP-1 A2518G genotypes were 69.8% AA, 2.3% GG and 27.9% AG in the control group and 52% AA, 8% GG and 40% AG in the patient group. *Conclusion:* It is likely that MCP-1 variants may affect the susceptibility to schizophrenia, however, a larger patient group should be studied to draw a firm conclusion.

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EFFECTS OF CAFFEINE ON OXIDANT–ANTIOXIDANT MECHANISMS IN THE RAT LIVER

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Background: Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid which exists in a variety of foods and drinks. The main aim of our study was to compare the potential antioxidant effects of caffeine on rat liver at two different doses of oral caffeine intake. We measured MDA levels, SOD and catalase activity in the liver. *Methods:* Thirty female Wister rats (mean weight, 250 g) were used. Rats were equally divided into three groups. Group 1 was the control group. Group 2 received 30 mg/kg of caffeine and group 3 received 100 mg/kg caffeine (non-toxic high dose) for 14 days (short time period) orally. *Results and Conclusion:* The 14-day low-dose (30 mg/kg) and non-toxic high-dose (100 mg/kg) caffeine usage decreased lipid peroxidation in the liver. Antioxidant enzyme (SOD and catalase) activities in the rat liver showed statistically significant increase with caffeine intake. Tissue catalase activity showed a strong positive correlation. Decreased lipid peroxidation and increased antioxidant enzyme activities which improve oxidative stress showed that the tested doses of caffeine may have an antioxidant activity. Determining the antioxidant mechanisms and the antioxidant effect of caffeine at a suitable dose requires advanced animal and human studies.

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PROTECTIVE EFFECT OF HUMAN TOOTH GERM CELLS ON H_2O_2-INDUCED TOXICITY IN MOUSE LEYDIG CELLS

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Background: Human tooth germ stem cells (HTGSCs) isolated from human tooth germs have the characteristics of mesenchymal stem cells (MSCs) with high differentiation capacity. Leydig cells produce testosterone in mammalian testis maintaining steroidogenic functions under the control of the luteinizing hormone (LH). Reactive oxygen species, produced as a byproduct of steroidogenesis, were reported to be toxic on steroidogenic pathways in correlation with aging. In this study we tested the protective effect of MSCs on Leydig cells, exposed to H_2O_2-induced oxidative stress. *Methods:* Fully-characterized MSCs were used in this study by collecting their conditioned medium and applying them on TM3 Leydig cells which were treated with 200 μM of H_2O_2 for 24h. After treatment, cell viability was measured by the MTS assay. P53 gene expression was determined with real time PCR. *Results:* 30% and 40% conditioned medium exerted protective effects on TM3 cells by lowering the levels of p53 expression. *Conclusion:* HTGSCs have a protective effect on TM3 cells exposed to H_2O_2. This effect may be due to factors secreted by HTGSCs to the culture medium. The data support the evidence that MSCs may provide a potential treatment against reactive oxygen species-related problems in the reproductive system.

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CYTOKINE PROFILE IN INDUSTRIAL WORKERS

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Background: Cytokines regulate inflammatory and immune reactions. This study was designed to investigate changes of the cytokine profile in workers of the chemical industry. *Methods:* A total of 111 individuals were examined. The study group comprised 72 workers exposed to hydroxylbenzene, as the main occupational hazard factor, while the control group comprised 39 non-exposed individuals. Cytokines levels (IL1β, IL4, IL6, IL8, IL10, IFNγ and TNFα) were measured by ELISA and expressed in pg/ml. *Results:* The study group demonstrated a statistically significant ($p<0.05$) decrease in IL1β (1.14±0.21), TNFα (1.03±0.09), IFNγ (4.03±0.34), IL10 (2.58±0.21) levels compared to the control group levels (1.66±0.25; 1.56±0.15; 5.64±0.56; 4.49±0.39; respectively). IL1β, IFNγ and TNFα increased cell sensitivity to apoptosis. IL6 concentrations (2.79±0.35) in the workers were reliably ($p<0.05$) higher than the control levels (1.75±0.14). Decreased IFNγ levels, IL4 (0.85±0.06) levels corresponding to the control levels (0.80±0.06), elevated IL6 levels and reduced expression of IL10 indicated a predominant Th2 immune response indirectly suggesting immunopathological changes determining a shift in T-helper cell differentiation. *Conclusion:* The modification of T-lymphocyte apoptosis caused by the impact of technogenic factors led to the shift of the cytokine balance in favor of Th2 response. The deficiency of proapoptotic IL1β, IFNγ, TNFα cytokines induced a decrease in apoptosis in the workers.

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MOLECULAR MARKERS OF APOPTOSIS IN INDUSTRIAL WORKERS

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Background: Apoptosis, a genetically regulated process involved into the differentiation, morphogenesis and

maintenance of cell homeostasis, is important for human mechanisms of adaptation to the occupational environment. This study aimed to identify the features of molecular activation markers in workers of the chemical industry exposed to hydroxylbenzene. *Methods:* A total of 111 individuals, including 72 workers in the study group and 39 non-exposed individuals in the control group, were examined. Both groups were comparable in age, sex and somatic diseases. $CD25^+$ and $CD95^+$ lymphocyte levels were measured by membrane immunofluorescence using a FACSCalibur flow cytometer (Becton Dickinson). Lymphocyte apoptosis levels were determined by staining with annexin V-FITC and propidium iodide (PI). *Results:* The study group demonstrated a statistically significant ($p<0.05$) increase in $CD25^+$ (13.84±0.68%) and $CD95^+$ (42.21±1.21%) levels compared to the control group (9.21±0.63%; 0.69±0.03, respectively). Reliably ($p<0.05$) decreased annexin V-$FITC^+PI^-$ cell levels (2.72±0.45%) were observed in the workers compared to the control group (4.77±0.42%). However, annexin V-$FITC^+PI^+$ levels (necrotic cells) in the study group (13.06±1.17) were within the range of the control levels (11.12±1.44). *Conclusion:* The presence of industrial chemical components in the occupational environment leads to the inhibition of apoptosis. Decreased apoptosis of effector cells together with hapten loading causes autoimmune, allergic and blastomogenic processes.

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INVESTIGATION OF CELLULAR LOCALIZATION OF THE R25W BEST1 MUTANT

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Background: Bestrophin-1 (Best1) is a transmembrane protein, predominantly expressed in retinal pigment epithelial (RPE) cells and localized in their basolateral plasma membrane. Best1 is thought to be a Ca^{2+}-activated Cl^- channel or a regulator of ion transport or both. Mutations in Best1 lead to retinal pathologies including the Best vitelliform macular dystrophy (BVMD). In this study, we investigated the cellular localization of the R25W Best1 mutant, transiently expressed in polarized Madin-Darby canine kidney (MDCK) cells. *Methods:* R25W Best1 mutant constructs were generated using site-directed mutagenesis and transfected in MDCK cells. For protein sorting experiments, confocal microscopy studies and statistical methods for quantification of mislocalization were used. *Results:* R25W mutants showed altered basolateral expression compared to wild-type Best1 in polarized MDCK cells. They were partly relocalized to the apical surface of the cells. *Conclusion:* The R25W mutation has been found in several BVMD patients. Alteration of its cellular localization may affect the ion equilibrium and overall function of RPE cells. These data represent an interesting insight into the underlying pathogenic mechanism of BVMD.

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HEMODYNAMICAL AND IMMUNOHISTOCHEMICAL EFFECTS OF DEXAMETHASONE TREATMENT ON ACUTE PARAQUAT INTOXICATION IN RAT BRAIN

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Background: Paraquat (PQ) toxicity leads to inflammation, oxidative stress and damage in tissues. Dexamethasone (Dexa) has been successfully used in PQ poisoning due to its anti-inflammatory effects. The aim of this study was to investigate the hemodynamical and immunohistochemical effects of Dexa treatment in acute PQ poisoning on brain tissue. *Methods:* A total of 16 rats were divided into the following four groups with four animals each: control, Dexa (100 mg/kg *i.p.*), PQ (25 mg/kg *i.p.*) and PQ+Dexa. Mean arterial pressure (MAP) and heart rate (HR) were recorded. Brain samples were fixed in formalin and prepared using a routine paraffin procedure. Anti-cyclooxygenase-1 (COX1), anti-cyclooxygenase-2 (COX2), anti-angiotensin converting enzyme (ACE), anti-aquaporin-1 (AQU-1), anti-vascular cell adhesion molecule (VCAM) primary antibodies were used for immunohistochemical examination. *Results:* MAP values were different between Dexa, PQ and PQ+Dexa groups at 60 min. HR increased in all groups compared with the control group at 120 min. Immunoreactivities were observed as moderate in the control group, minimal in the Dexa group and moderate in the PQ group except for COX2. Immunoreactivities were

observed as mild in the PQ+Dexa group, whereas VCAM immunoreactivity of this group was closer to that of the control group. *Conclusion:* Hemodynamical and tissue effects of PQ were reversed by Dexa in early stage and high-dose Dexa suppressed immunoreactivity in the brain. Dexa treatment may be useful in the acute process of PQ intoxication.

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ADMINISTRATION OF LIPOIC ACID AND ZINC PROTECTS AGAINST IONIZING IRRADIATION-INDUCED INTESTINAL TOXICITY

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Background: Radiation therapy of carcinomas of abdominal organs is accompanied by enteritis, a significant side-effect of radiation. Radiotherapy-induced enteritis also contributes to the morbidity of chemoradiotherapy of metastatic malignancies and also limits dose escalation protocols due to dehydration, intestinal ulceration and the requirement for treatment breaks. This study evaluated intestinal radioprotection by the lipoic acid (LA) and zinc (Zn) antioxidant agents and their combined use. *Methods:* Thirty five Sprague-Dawley rats were randomized into five groups: control; irradiation alone (IR); LA alone; Zn alone; and LA-Zn plus IR. Rats received oral LA (100 mg/kg/day) and/or Zn (10 mg/kg/day) five days before 10 Gy lower-body irradiation. *Results:* Microscopic examinations of irradiated intestinal tissues revealed the presence of decreased villus height and mucosal thickness. LA, Zn and LA+Zn treatments significantly increased the villus/cript ratio and mucosal thickness compared to irradiated controls at 24 h ($p<0.05$ for both). Pretreatment with LA and Zn markedly prevented villus atrophy. Combined use of LA and ZN provided the best protection for the irradiated intestinal mucosa. *Conclusion:* This study suggests that the combined pretreatment with LA and Zn may contribute to the prevention of radiation-induced acute intestinal toxicity in cancer treatment.

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COMBINED USE OF ZINC AND LIPOIC ACID DECREASES APOPTOSIS IN IRRADIATED LIVER TISSUE

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Background: Radiotherapy is an important modality in the treatment of cancer. One of the major limiting factors in cancer therapy is normal tissue toxicity. For this reason, chemical protection with antioxidant agents is an important area of interest for radioprotection. In the present study, we investigated the effects of zinc (Zn) and lipoic acid (LA) in rats using a radiation-induced liver injury model. *Methods:* A total of 35 adult Sprague Downey rats were randomly divided into 5 groups of 7 animals each. Group 1 was the control group. Group 2 received 10 Gy of lower-body irradiation as a single dose (RT). Groups 3 and 4 received the same irradiation as Group 2 plus 10 mg/kg/day Zn (RT+ Zn) or 100 mg/kg/day LA (RT+ LA) orally, respectively. Group 5 received the same irradiation and Zn plus LA orally as groups 3 and 4. Rats received oral LA or Zn five days before irradiation. Apoptosis was determined by the TUNEL assay at 168 h. *Results:* Irradiation increased apoptosis when compared with the control group ($p<0.05$). The rates of apoptosis of the rats in groups 3 and 4 were lower than in the RT group. We have clearly showed that the combined usage of LA and Zn significantly reduced radiation-induced apoptosis in liver tissue ($p<0.05$). *Conclusion:* The combined use of LA and Zn may prevent the liver from radiation-induced damage.

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EXAMINATION OF PLASMA MYELOPEROXIDASE LEVELS IN MYOCARDIAL INFARCTION

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Background: Free radicals and, as a result of them, primarily, LDL-cholesterol and the damages of the endothelial wall cause to atherosclerotic formations. Myeloperoxidase (MPO), a lysosomal enzyme in polymorphonuclear leukocytes is one of the factors involved in the inflammatory response. Previous studies have shown that MPO is synthesized in atherosclerotic lesions and joins proatherogenic lipoprotein oxidation. This study aimed at investigating the levels of MPO polymorphism in patients with myocardial infarction in the Turkish population. *Methods:* A total of 100 myocardial infarction patients and 100 healthy controls were included in the study. Plasma MPO levels were determined by ELISA. *Results:* Increased levels of MPO in patients with myocardial infarction were not statistically different from those in the controls.

However, elevated LDL-cholesterol and total plasma cholesterol levels and smoking were among the factors which contributed to increased levels of MPO. *Conclusion:* Follow-up studies are needed to achieve a better understanding of the role of MPO in the development of myocardial infarction.

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DIFFERENT ANTIOXIDANT PATHWAYS OF HOSPITALIZED PEDIATRIC PATIENTS WITH PANDEMIC INFLUENZA A (H1N1)

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Background: This study evaluated the relationship of total antioxidant capacity (TAC) and Coenzyme Q (Q10) levels with clinical outcome of hospitalized children with pandemic influenza. Serum copper (Cu) and zinc (Zn) levels were also determined to evaluate the antioxidative enzyme activity changes depending on their cofactor concentrations. *Methods:* A total of 66 patients, age range 48 days to 14 years, with suspected H1N1 virus infection were hospitalized and nasal swabs were sent to the National Influenza Reference Laboratory for confirmation of pandemic influenza A virus infection (H1N1) by rRT-PCR assay. *Results:* A total of 28 patients had H1N1 and 38 patients had seasonal influenza. Clinical and/or radiological pneumonia were detected in 24 of H1N1 patients and 4 patients were exitus. TAC, Q10 and Zn levels were decreased significantly in H1N1 patients compared to those with seasonal influenza. There was no relationship between mortality and biochemical parameters. Serum Cu levels, WBC and the other routine laboratory tests were not changed significantly in patients with H1N1. *Conclusion:* Our data showed that pathogenesis of pandemic influenza infection had additional different mechanisms of oxidative stress from that of seasonal influenza. Antioxidants which use the lipid pathway or Zn may be the main oxidative factors that take part in the pathophysiology of H1N1.

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ANGIOTENSIN-CONVERTING ENZYME (*ACE*) GENE POLYMORPHISM AND RISK OF HYPERTONIC DISEASE IN THE UKRAINIAN POPULATION

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Background: Angiotensin-converting enzyme (ACE) is a key component of the renin-angiotensin system, the most important humoral regulator of blood pressure. The aim of this study was to evaluate the contribution of the *ACE* gene polymorphism in the risk of developing hypertension disease in the Ukrainian population. *Methods:* Patients with hypertonic disease (n=229) and healthy controls (n=284) were included in the study. The two groups consisted of age-matched individuals of both sexes. Polymerase chain reaction was used to detect the I/D alleles of the *ACE* gene. *Results:* The frequencies of II, ID and DD genotypes among the hypertensive group were 27.1%, 50.2% and 22.7%, respectively, whereas, in the control group, they were found to be 38.0%, 44.0% and 18.0%, respectively. The II genotype was significantly lower in the hypertensive group than in the control group (odds ratio: 0.61, 95% CI: 0.41-0.88, χ^2=6.87, p<0.05). The D allele was also more frequent in the hypertensive group than in the control group (odds ratio: 1.38, 95% CI: 1.07-1.76, χ^2=6.36, p<0.05). *Conclusion:* The D allele of *ACE* I/D polymorphism may be a potential risk allele for hypertonic disease in the Ukrainian population.

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***SACCHAROMYCES CEREVISIAE* – A PROMISING TOOL FOR STUDYING HUMAN BRAIN TUMORS**

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Background: Glioblastoma multiforme is the most aggressive among the human gliomas with exclusively bad prognosis. Recently, we have started researching some epigenetic characteristics of these tumor cells. Our attention is focused on a very specific linker histone subtype - H1 zero, characteristic in highly differentiated cells. We have noted that its quantity is reduced to completely missing in human glial cells. Therefore, we started developing *Saccharomyces cerevisiae* as a model for studying this cancer type. *Methods:* The biochemical methods for the isolation of specific linker histones allowed quantification of H1 zero in normal and tumor brain cells. Gene cloning techniques were applied for yeast linker histone knock-out and subsequent cloning of human H1 zero in yeast cells. Standard methods for monitoring cell growth were further used. Chromatin structure was assessed by Chromatin Comet Assay and AFM. *Results:*

Successful cloning of human H1 zero in *S. cerevisiae* cells was proved unambiguously. The growth potential of cells transformed with H1 zero conveyed interesting characteristics that will be discussed in detail. *Conclusion: Saccharomyces cerevisiae* may be used in studying linker histones and chromatin structure as important epigenetic phenomena in tumor development.

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THE RELATION BETWEEN APE1 ASP148GLU POLYMORPHISM AND BIPOLAR DISORDER: RESULTS FROM A CASE-CONTROL STUDY IN TURKISH POPULATION

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Background: Bipolar disorder (BD) is one of the most severe forms of mental illness. Studies have consistently reported increased lipid peroxidation and changes in the major antioxidant enzymes in individuals with BD, suggesting that oxidative stress may play a role in its pathophysiology. The excessive generation of reactive oxygen species can lead to lipid oxidation and DNA damage. The polymorphisms on DNA repair genes may have important effects on the increased risk of BD. We investigated the relationship between APE1 Asp148Glu polymorphism and BP in this study. *Methods:* The present analyses are based on 50 case subjects with BD and 92 non-case subjects. Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism. *Results:* There was no correlation between APE1 Asp148Glu polymorphism and BD (p=0.182). Frequencies of APE1 Asp148Glu genotypes were 54.3% AA, 15.2% GG and 30.4% AG in the control group and 42% AA, 12% GG and 46% AG in the patient group. *Conclusion:* It is unlikely that APE1 Asp148Glu polymorphism is associated with BD. However, the study group size should be increased to support a final conclusion.

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ULTRASTRUCTURE OF THE SEMINIFEROUS EPITHELIUM AND INTERTUBULAR TISSUE OF THE INFERTILE HUMAN WITH AZOOSPERMIA

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Background: The aim of the present study was to investigate abnormal morphology in the testes of azoospermic patients. *Methods:* Testicular biopsy specimens taken from patients with azoospermia were studied by electron microscopy. Biopsies were performed in azoospermic patients in order to distinguish between sperm positive and sperm negative group. *Results:* In the sperm positive group we observed Sertoli cells which were supported by several successive generations of germ cells. The tails of spermotozoan were clearly seen. However, in the sperm negative group, complete germ cell loss was characterized in the tubulus seminiferus. Large and irregular Sertoli cells contained abundant intracytoplasmic organelles and a prominent nucleus. Peritubular wall presented with increased collagen fibers. The intertubular tissue of the human testis is composed of loose connective tissue containing blood vessels, macrophages, mast cells, and Leydig cells which occur either as single cells or form small clusters. *Conclusion:* From the testicular biopsies obtained from azospermic patients in the ICSI procedure it is observed that sperm negative patients show significant morphological damage to testicular germ cells compared to sperm positive patients. Therefore it is concluded that this low spermatogenic efficiency in sperm negative patients significantly impairs their fertility.

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RELATIONSHIP BETWEEN OXIDATIVE STRESS AND ESSENTIAL HYPERTENSION

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Background: Recent evidence suggests that increased vascular oxidative stress contributes to the pathophysiology of hypertension. *Methods:* In the present study, the levels of MDA, coenzyme Q10 and TAC were measured to compare the extent of oxidative stress in 38 patients recently diagnosed with hypertension and 22 healthy controls. MDA was measured as thiobarbituric acid (TAC) reactive substances. TAC was assayed with a spectrophotometric method. Coenyzme Q10 was quantitated by HPLC technique. *Results:* In the total patient group, significant differences were found in MDA and TAC compared with the control group (p<0.05, p<0.01, respectively). There was no significant difference between the two groups according to

coenzyme Q10. The hypertensive patients and healthy subjects were divided into two groups; dipper or non-dipper. Decreased TAC levels were found in the normotensive non-dipper group compared with the hypertensive non-dipper group ($p<0.01$). MDA levels were decreased in the normotensive dipper group compared with the hypertensive dipper group ($p<0.05$). The MDA and CoQ10 levels in the normotensive non-dipper group were lower than in the hypertensive dipper group ($p<0.01$, $p<0.05$, respectively). *Conclusion:* This study revealed that there is a consistent statistically significant difference between essential hypertensives and controls with respect to the measured parameters. This study showed that oxidative stress is increased in patients with hypertension.

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MGMT PROMOTER METHYLATION IN BULGARIAN PATIENTS WITH GLIAL TUMORS

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Background: MGMT is a repair protein that removes alkyl groups from the O6 position of guanine in DNA, thus protecting the cell from alkylating agents. Promoter hypermethylation of the gene *MGMT* has been extensively studied in glioma patients because of its importance for prognostic and treatment purposes. Methylation of *MGMT* has been associated with prolonged survival and might be suitable for use as a predictor of response to treatment with alkylating drugs. *Methods:* To determine the prognostic value of *MGMT* in Bulgarian patients with glial tumors, we assessed its promoter methylation in glial tumor tissues from 50 patients by methyl-specific PCR. *Results: MGMT* was found to be methylated in 12 patients (24%). No statistically significant correlation was observed with mutations in *IDH1* and no prognostic significance was found for *MGMT* in the examined group. Although *MGMT* was not shown to be a prognostic factor in patients who underwent only surgery, in the group of patients treated with radio- and/ or chemotherapy *MGMT* methylation status was associated with the overall survival ($p=0.031$). *Conclusion:* Promoter hypermethylation of the gene *MGMT* may be applied as a prognostic factor in Bulgarian patients with gliomas.

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MMP2 GENE POLYMORPHISMS AND *MMP2* MRNA LEVELS IN PATIENTS WITH SUPERFICIAL VARICES OF LOWER EXTREMITIES

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Background: Although superficial varices of the lower extremities are common and with high morbidity, their etiology has not been elucidated yet. Previously, it was thought that venous hypertension was responsible for such cases by causing valvular insufficiency. However, recent findings indicate that the changes in the venous wall structure may be the main initiating factors of this condition. Matrix metalloproteinase enzyme-2 (MMP2) is one of the enzymes known to have a role in remodelling the extracellular matrix, mainly in vascular structures. *Materials and Methods:* We studied two functional gene polymorphisms in -735 and -1306 regions of the MMP2 gene and their effects on mRNA expression of MMP2. We used a previously defined method for polymorphism analyses, namely PCR-RFLP. *Results:* CC genotype and C allele for MMP2 -735 gene region were more common in the control group, while there was no significant difference between the groups for MMP2 -1306 gene polymorphisms. MMP2 mRNA levels were higher in the group that had both varices and coronary artery disease (CAD). *Conclusion:* There was no significant effect of MMP2 polymorphisms on mRNA expression. As MMP2 mRNA levels were higher in CAD patients with varices compared to the CAD only and varices only groups, it is necessary to perform further studies to elucidate the relationship between CAD and varices.

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LIPID PEROXIDATION AND PARATHYROID HORMONE ARE THE STATISTICAL DETERMINANTS OF CALCIUM IN THE ERYTHROCYTES OF PERITONEAL DIALYSIS PATIENTS

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Background: The aim of this study was to examine the alterations in calcium and lipid peroxidation in red blood cells (RBCs) and serum samples of continuous ambulatory peritoneal dialysis (CAPD) patients. We also investigated the relationship between parathyroid hormone (PTH) and calcium homeostasis. *Methods:* Routine blood counts and blood analysis were performed using standard laboratory procedures in serum samples. The concentration of thiobarbituric acid-reactive substances (TBARS) was measured in erythrocytes and serum samples. RBC calcium was measured by Fura-2AM in a spectrofluorometer. *Results:* In CAPD patients, hemoglobin, albumin and high-density lipoprotein cholesterol levels were lower, while glucose, very-low density lipoprotein cholesterol, triglyceride, magnesium, PTH, sensitive C-reactive protein and uric acid levels were higher than in the controls. TBARS levels in RBCs and serum samples and cytosolic calcium in RBCs were all found to be significantly increased in CAPD patients compared to the control subjects. Multiple regression analysis showed that RBC TBARS and serum PTH were the independent predictors of RBC calcium in our study. *Conclusion:* Our results confirmed that oxidative stress is an important risk factor for CAPD. The results of multiple regression analysis suggest that RBC calcium is affected by the increased levels of both TBARS and PTH.

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POLYMORPHISMS OF THE -765 G→C AND -1195 A→G ON CYCLOOXYGENASE-2 GENE AFFECT THE RISK OF PREECLAMPSIA

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Cyclooxygenase-2 (COX-2, prostaglandin synthase 2), an inducible enzyme in the generation of prostaglandins, plays an important role in inflammatory processes. Several single nucleotide polymorphisms (SNPs) in the promoter region of *COX-2* gene, which are associated with altered transcriptional activity, have been described. Enhanced production of vasoconstrictor metabolites leading to hypertension is the main characteristic of preeclampsia. It has been shown that neutrophils from preeclamptic women express significantly more COX-2 than those from healthy pregnant women. This study aimed to investigate the frequency of two sSNPs in the promoter region of the *COX-2* gene (-765 G→C and -1195A→G) in women with preeclampsia. *COX-2* gene polymorphisms were performed by polymerase chain reaction and restriction fragment length polymorphism in 128 controls and 74 patients. Genotype distribution and allelic frequencies for -765G→C polymorphism of the *COX-2* gene were significantly different between patients and controls (p=0.000 and p=0.042, respectively). The odds ratio for preeclampsia risk associated to the -765G allelic variant was 4.07 (95% CI: 0.89-18.56). The AA genotype of the -1195 SNP was present at a significantly higher frequency among all pre-eclamptic subjects (p=0.000, χ^2: 13.4, OR: 3.44, 95% CI: 1.74-6.77). Over 80% of the preeclamptic individuals were -1195A homozygotes compared with 55% of the control subjects. These findings suggest that SNPs on the promoter region of the COX-2 gene may reduce the risk for preeclampsia.

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ALTERATION IN SERUM OF TOTAL ANTIOXIDANT CAPACITY AND LIPID HYDROPEROXIDE IN RENAL FAILURE PATIENTS

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Background: Free-radical production from a number of sources is likely to be increased in chronic renal failure (CRF). These highly reactive free radicals can cause damage through several pathways. End-stage renal disease is associated with numerous complications, which may partly result from excessive amounts of reactive oxygen species and/or decreased antioxidant activity. The natural antioxidant system consists of antioxidant enzymes and numerous antioxidant compounds and protects the functional and structural molecules against reactive oxygen species-mediated cytotoxicity and tissue damage. *Methods:* The aim of this study was to investigate the changes in the levels of total antioxidant capacity and lipid hydroperoxide in patients with renal failure. A total of 64 CRF patients and 22 healthy controls were enrolled in the study. Total antioxidant

capacity and lipid hydroperoxide levels were measured by spectrophotometric methods. *Results:* Serum levels of total antioxidant capacity were significantly increased in the controls compared to the CRF patients ($p<0.01$). Although the lipid hydroperoxide levels of the patient group seemed to be higher than those of the control group, no significant difference was observed between the two groups. *Conclusion:* The lowering of the total antioxidant activity in CRF patients on hemodialysis may contribute to the increased oxidative damage and the development of renal complications.

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Y CHROMOSOME MICRODELETION ANALYSIS, THROMBORISK FACTORS AND CYTOGENETIC ANALYSIS IN PATIENTS WITH UNDESCENDED TESTIS

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Background: Undescended testis is the failure of one or both testes to descend into the dartos layer of scrotum. Detecting AZF deletions in adult infertiles with undescended testis history is important in order to determine patient prognosis. Chromosomal abnormalities in patients with undescended testis were reported to indicate a prevalence of 3-4%. It is reported that the anti-mullerian hormone (AMH) plays a role in the first phase of testis descendence and MTHFRC677T polymorphism increases AMH concentration. *Methods:* The study included 40 patients with undescended testis and 40 healthy children. Y chromosome microdeletion analysis was performed and thromborisk factors were estimated by PCR-RFLP and real-time PCR, respectively. Also, chromosome analysis was performed for both study groups. *Results:* There were neither chromosomal abnormalities nor Y chromosome microdeletions in either study group. When the groups were evaluated for thromborisk factors, there was still no significant difference between them. *Conclusion:* MTHFRC677T heterozygous polymorphism was more frequent in the patient than in the control group although this difference was not statistically significant. Y chromosome microdeletions and chromosome abnormalities were not detected in either group. These results may be positive prognostic factors for the patients with undescended testis.

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THE INTRONIC POLYMORPHISM OF ANGIOTENSIN I CONVERTING ENZYME AMONG TUNISIAN PATIENTS WITH CORONARY HEART DISEASE

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Background: The acute coronary syndromes (ACS) are classified among the major causes of mortality in the industrialized countries. The increased angiotensin I converting enzyme (ACEI) activity characterizes a hereditary predisposition to ACS. The aim of this study was to evaluate the ACEI activity in Tunisian patients with coronary heart disease, and investigate the association between this activity and an intronic deletion (D) of 287pb on the intron 16 of the ACEI gene. *Methods:* The study population included 72 coronary patients (mean age 59.72±12.3 years) and 34 healthy control subjects (mean age 53.3±9.6 years). ACEI activity was measured by kinetic method. The intronic deletion (D) was identified by PCR technique. *Results:* An increased activity of ACEI was observed in patients compared to control subjects (84.38±33.83UI/L *vs*. 59.06±18.2UI/L, $p=10(-5)$). A raised relative frequency of the D/D genotype (51.4%) was observed, whereas among the controls, the I/I genotype prevailed (62%). D/D genotype was always associated with highest ACEI activity for the patients and the control subjects. *Conclusion:* This deletion (homozygous or heterozygous) predispose to cardiovascular diseases.

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FIBRONECTIN IMPROVES OSTEOBLAST-LIKE CELLS INTERACTION WITH NEW APATITE-NANODIAMOND COATINGS

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New apatite (AP)/nanodiamond (ND) coating has been developed to improve the physical and biological properties of stainless steel (SS) *versus* single AP coating. Homogeneously electrodeposited AP-ND layers demonstrate increased mechanical strength, interlayer cohesion and ductility.

Fibronectin (FN) is one of the earliest proteins to be laid down in the extracellular matrix and its accumulation in the areas of skeletogenesis suggests its involvement in the early stages of bone formation. FN is important for the initial cellular interaction with biomaterial. In the absence of serum, osteoblast-like MG63 cells attach well but spread poorly on either AP or AP-ND substrates. Pre-adsorption with serum or FN improves cellular interaction, an effect better pronounced on AP-ND coating. In a single protein adsorption study, FITC-labeled FN showed enhanced deposition on AP-ND layers, consistent with the significantly improved cell adhesion, spreading and focal adhesion formation (in comparison to SS and AP), particularly at low FN adsorption concentrations (1 μg/ml). Higher FN concentrations (20 μg/ml) abolished this difference, suggesting that the promoted cellular interaction of serum (where FN is low) is caused by the greater affinity for FN. Moreover, it was found that MG63 cells tend to rearrange both adsorbed and secreted FN on the AP-ND layer, suggesting a facilitated FN matrix formation.

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EVALUATING MEFV GENE MUTATIONS IN PATIENTS WITH BEHCET'S DISEASE

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Background: Behcet's disease is characterized by repetitive aphthous ulcers, genital ulcers, uveitis, skin lesions and articular, neurologic and vascular gastrointestinal involvement. Encouraging results have been reported in some MEFV gene studies for Behcet's disease. E148Q, M694I, M680I, M694V, V726A MEFV gene mutations are reported to cause some symptoms in Behcet's disease. This study investigated whether there is a relation between MEFV gene mutations and Behcet's disease. *Methods:* Forty Behcet's disease patients without FMF diagnosis and symptoms in themselves or their family and twenty healthy controls were included in the study. Following DNA isolation E148Q, M694I, M680I, M694V, V726A mutations were carried out by real time PCR. *Results:* According to MEFV mutation scanning, eight patients and one healthy control were heterozygous for M694V; two patients and one healthy control were heterozygous for M680I; two patients were heterozygous for V726A; four patients were heterozygous, one healthy control was homozygous, two healthy controls were heterozygous for E148Q. No M694I mutation was observed in either group. *Conclusion:* There was not a statistically significant difference in MEFV mutation frequencies between Behcet's disease patients and the healthy controls. Furthermore, total mutation frequency was found to be 35% and M694V showed the highest frequency compared to the other mutations.

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ANTI-ANGIOGENIC EFFECTS OF METOPROLOL AND α-LACTALBUMIN ON PRIMARY AND METASTATIC COLON CANCER CELL LINES

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Background: Angiogenic factors, such as vascular endothelial growth factor (VEGF), its receptors, matrix metalloproteases (MMPs) and nitric oxide synthase (NOS), are involved in the progression of many carcinomas. The aim of this study was to investigate the anti-angiogenic effects of metoprolol succinate, a selective β1 receptor blocker, and α-lactalbumin on primary and metastatic human colon cancer cell lines by using indirect immunohistochemical methods. *Methods:* Primary (Colo-320) and metastatic (Colo-741) human colon cells were cultured in RPMI-1640 medium, containing 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin solution in a humidity incubator at 37˚C, containing 5% CO_2. Cells were grown on coverslips and incubated with metoprolol and/or α-lactalbumin for 48 h. Cells were fixed and immunostained with anti-VEGF, anti-flk-1, anti-eNOS, anti-iNOS, anti-MMP-2 and anti-MMP-9 primary antibodies using the avidin-biotin-peroxidase method. Staining intensities were measured using a semi-quantitative method. ANOVA statistical tests were used to compare the measurements. *Results:* Primary and metastatic colon cancer cells had moderate/strong VEGF and iNOS immunoreactivities; moderate/mild flk-1 and eNOS immunoreactivities; mild MMP-2 and MMP-9 immunoreactivities, respectively. Decreased immunoreactivities were detected on colon cancer cells in metoprolol and α-lactalbumin treated groups ($p<0.05$). *Conclusion:* Strong/ moderate VEGF and NOS immunoreactivities on colon cancer cells may suggest an increase of cell invasion or metastasis. Due to their anti-angiogenic effects, metoprolol and α-lactalbumin may be used as additional drugs for cancer therapy.

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RELATIONSHIP BETWEEN PON1 GENE Q192R POLYMORPHISM AND OXIDATIVE STRESS OF REPERFUSION

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Background: The HDL-associated enzyme paraoxonase 1 (PON1) plays a preventive role against oxidative stress. The purpose of this study was to investigate a possible association of the PON1 Q192R polymorphism with the risk of developing oxidative stress after reperfusion. *Patients and Methods:* Knee replacement surgery is an ischemia/reperfusion model by usage of tourniquet applied on the knee area to restrict the blood flow during the operation. The study constituted of 51 patients undergoing elective arthroscopic knee surgery and 50 healthy individuals. PON 1 gene Q192R polymorphism was performed by polymerase chain reaction and restriction fragment length polymorphism. Statistical analyses were performed using SPSS for Windows version 11.0. *Results:* Distribution of genotypes of the PON1 Q192R polymorphism was approximately: 80.4% (QQ), 15.7% (QR) and 3.9% (RR) in the patient group and 30% (QQ), 4% (QR) and 66% (RR) in the healthy controls. The frequency of the QQ genotype was higher in patients compared to controls ($p<0.05$, $\chi^2=0.507$, OR=9.567 95% CI: 3,818-23,970). *Conclusion:* Our data suggest that the PON1 192 wild-type (QQ) genotype may be associated with the risk of oxidative stress after reperfusion in Turkish population.

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KRAS IMMUNOHISTOCHEMICAL ANALYSIS IN *HELICOBACTER PYLORI*-ASSOCIATED CHRONIC GASTRITIS AND GASTRIC CANCER

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Background: KRAS is a proto-oncogene and its protein upregulation is believed to play a role in promoting cancer. In this study, we investigated KRAS expression in gastric cancer and *Helicobacter pylori*-associated chronic gastritis (HPCG), a known precursor for gastric cancer. We aimed at determining a possible protein marker for early development of gastric cancer. *Methods:* The expression of KRAS in 62 cases of HPCG and 31 cases of gastric cancer was investigated immunohistochemically on archived formalin-fixed, paraffin-embedded specimens. Slides were scored using four-step scoring system (0, 1+, 2+, and 3+) and were analyzed with Wilcoxon Signed-rank test and Mann Whitney *U*-test, SPSS version 17.0. We considered $p<0.05$ to be significant. *Results:* The expression of KRAS in gastric cancer was found to be significantly higher than in HPCG ($p=0.02$). We found 20 (68%) of 31 gastric cancer cases with moderate to strong KRAS expression and 15 (24%) of the 62 HPCG cases had moderate immunoreactivity. In both conditions, the KRAS expression was significantly higher than in its adjacent normal areas ($p=0.00$). *Conclusion:* Overexpression of KRAS was significantly higher in gastric cancer compared to HPCG. Further studies are necessary to ascertain the role of KRAS in the development of HPCG to gastric cancer.

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DIFFERENTIAL EXPRESSION AND REGULATION OF CALCIUM ION TRANSPORT-RELATED GENES, TRPV6, PMCA1, NCKX3 AND CABP-28K, IN HUMAN PLACENTAL BEWO CELLS

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Background: Preeclampsia is a pregnancy-specific disease characterized by the *de novo* development of concurrent hypertension, proteinuria and oxidative stress in the placenta. During last trimester of gestation, the Ca^{2+} transport from mother to fetus increases in response to the accelerated demand for Ca^{2+} caused by bone mineralization in the fetus. The calcium transporters TRPV5 and 6 are cytosolic diffusion of Ca^{2+} bound to calcium binding proteins (CaBP-9k/-28k) and basolateral extrusion of Ca^{2+} through plasma membrane Ca^{2+}-ATPase 1(PMCA1) and to a lesser extent by $Na^+/K^+/Ca^{2+}$ exchanger (NCKX3). *Methods:* Cell membrane and cytosolic calcium transporters, *i.e.*, TRPV6, PMCA1, NCKX3 and CaBP-28k, were investigated by RT-PCR and Western blot analysis at induced oxidative stress in human placental BeWo cells. *Results:* In hypoxia, the expression of TRPV6 mRNA and protein level was not altered; however NCKX3 and CaBP-28k were induced by hypoxia in BeWo cells compared with a control (normoxia). In addition, the expression of PMCA1 mRNA and protein was decreased at hypoxic BeWo cells. *Conclusion:* These results indicate that calcium transporters, TRPV6, PMCA1, NCKX3 and CaBP-28k, are distinctly expressed by induced oxidative stress, suggesting that alterations of these calcium transporters may be a determinant factor affecting calcium transfer by hypoxic stress in BeWo cell model.

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DISTINCT EXPRESSION OF DIVERSE CALCIUM TRANSPORT GENES IN THE PLACENTA OF CALBINDIN-D9K AND -28K KNOCKOUT MICE

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Background: Calbindins (CaBPs) have a high affinity for Ca^{2+} ions. Many types of calcium channel, intracellular calcium binding proteins, NA^{+}/Ca^{2+} exchangers (NCX), transient receptor potential cation channels (TRPV) are found in the placenta. In this study, the calcium channel in maternal–fetal Ca^{2+} transport was investigated using the phenotypes of wild-type, CaBP-9k, CaBP-28k and CaBP-9k/28k knockout (KO) mouse models. *Methods:* Expression of calcium transport genes in three dissected sections of placenta (MP: maternal-placenta, CP: central-placenta, FP: fetal-placenta) were examined at gestational day 19 in these mice. *Results:* The expression of TRPV6 was highest in CaBP-28k KO mice at MP and CP, or CaBP-9k KO mice at FP. The level of CaBP-9k was significantly induced in CaBP-28k KO mice at MP and CP, but not altered at FP. In addition, the expression of CaBP-28k was more reduced in CaBP-9k KO mice than WT in all sections of placenta. *Conclusion:* These results indicate that TRPV6 participates in transferring calcium ions between maternal and fetal compartments and alteration of CaBP-9k/28k is involved in intracellular Ca^{2+} buffering system among KO mice. Taken together, the TRPV6 and CaBP-9k genes may play a role as a key element in controlling calcium transport in the placenta during pregnancy.

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DISTINCT EXPRESSION OF PLACENTAL CALCIUM TRANSPORT PROTEINS, TRPV6, PMCA1, NCXK3, AND CABP-28K, IN PREECLAMPTIC PREGNANT WOMEN

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Background: The ionic form of Ca^{2+} serves as a universal intracellular messenger to modulate many processes such as neurotransmission, hormone secretion, and programmed cell death. The calcium transporters TRPV5 and 6 are cytosolic diffusion of Ca^{2+} bound to calcium binding proteins (CaBP-9k/-28k) and basolateral extrusion of Ca^{2+} through plasma membrane Ca^{2+}-ATPase 1 (PMCA1) and to a lesser extent by $Na^{+}/K^{+}/Ca^{2+}$ exchanger (NCKX3). *Methods and Results:* We demonstrated the expression of cell membrane and cytosolic Ca^{2+} transporters in dissected three sections (fetal-, central-, maternal-) of healthy or pre-eclamptic human placenta. During human preterm labor, placental expression of calcium transporters (TRPV6, PMCA1, NCKX3) mRNA and protein were levels increased in pre-eclamptic placenta (PEP) in fetal and maternal sections, however, the expression of CaBP-28k mRNA and protein level decreased in PEP in fetal and maternal sections compared with normal placenta (NP). *Conclusion:* Taken together, these results indicate that placental calcium transporters have potential roles in different sections of placenta between NP and PEP, suggesting that induced calcium transporters of PEP may be involved and that pre-eclamptic stress in human placenta is a determinant factor affecting calcium transfer in pre-eclamptic placental tissues.

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ESTROGEN AND PROGESTERONE REGULATE APOPTOSIS- AND ENDOPLASMIC RETICULUM STRESS-RELATED GENES IN THE UTERI OF CALBINDIN-D9K AND -D28K KNOCKOUT MICE

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Background: Calcium (Ca^{2+}) is a regulator of apoptotic signaling. Calbindins (CaBPs) have a high affinity for Ca^{2+} ions. Uterine CaBPs appear to be involved in the regulation of myometrial activity by intracellular Ca^{2+}. The aim of the present study was to evaluate the regulation of apoptosis in the uteri of CaBP-9k, CaBP-28k, and CaBP-9k/28k knockout (KO) mice. *Methods:* The expressions of Bax, bcl-2, caspases and endoplasmic reticulum (ER) stress genes were examined by Western blot analysis in the uterus of KO mice. *Results:* Our findings indicated that Bax was enhanced in the uteri of CaBP-28k and CaBP-9k/28k KO mice. The expressions of caspase 3, 6, and 7 were higher in both CaBP-28k and CaBP-9k/28k KO mice. CHOP and Bip levels were elevated in CaBP-28k KO mice. When immature mice were treated with 17β-estradiol (E2) or progesterone (P4) for three days, the expressions of Bax and caspase 3 were increased by E2 treatment in WT and CaBP-9k KO mice, and by P4 treatment in CaBP-28k KO mice. *Conclusion:* These results indicate that CaBP-28k blocks the up-regulation of apoptosis-related genes and ER stress genes, implying that CaBP-28k may decrease the expression of genes involved in apoptosis and ER stress in murine uterine tissue.

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IN VITRO ANALYSES OF GASRAL ASPIRATES FROM PREMATURELY BORN INFANTS WITH NEONATAL RESPIRATORY DISTRESS SYNDROME

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Background: The absence of a "mature" alveolar surfactant in the lungs is the main reason for the development of neonatal respiratory distress syndrome (NRDS) that often has a lethal outcome. In order to determine the infants' lung maturity and the necessity of surfactant therapy, we investigated the surface properties of gastral aspirates from infants with NRDS and from normally-born infants. *Methods:* The pendant drop method allows the determination of the surface characteristics of small aliquots of gastral aspirate: equilibrium surface tension in static conditions and maximal and minimal surface tension during dynamic compression-decompression cycles. *Results:* The gastral aspirates of infants with NRDS had higher equilibrium and maximal and minimal surface tension values compared to the healthy infants. *Conclusion:* Our results showed significant differences in the surface behavior of gastral aspirates between prematurely- and normally-born infants. The method used may be useful for clinical diagnostic applications.

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INTERACTION OF VIPOXIN AND ITS COMPONENTS WITH LIPID MONOLAYERS

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Background: The neurotoxin vipoxin, isolated from the *Vipera ammodites meridionalis* venom, is a heterodimeric complex, composed of two structurally related subunits, a toxic phospholipase A_2 (PLA_2), and a non-toxic and catalytically inactive one. PLA_2 (EC 3.1.1.4) plays a crucial role in numerous physiological processes including remodeling of phospholipids, biosynthesis of prostaglandins, cell proliferation and signal transduction. Venom PLA_2 enzymes exhibit a variety of pharmacological effects such as neurotoxicity, myotoxicity, platelet aggregation, hemolytic effect and inflammatory processes. *Methods:* The Wilhelmy method was used, which allows the determination of the surface parameters of the formed monolayer, namely the equilibrium and the maximal and minimal surface tension and shape of the hysteresis curve. *Results:* We investigated PLA_2 activity against the following lipid monolayers: DPPC, DPPS, POPC, POPG, POPS and DOPE. PLA_2 displayed strong affinity to the phospholipids with unsaturated fatty acid at the sn-2 position. *Conclusion:* The obtained results provided information about the structure-function relationship of the investigated PLA_2 and its impact on the different pharmacological effects of PLA_2.

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EXPRESSION OF GENES AND PROTEINS IN SMOOTH MUSCLE CELLS ISOLATED FROM THORACIC AORTIC ANEURYSMS

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Aortic aneurysms are formed by an irreversible localized dilatation of the aorta. The alterations in the vessel wall are medial degeneration, loss of smooth muscle cells (SMC) and increased expression of matrix metalloproteinases, leading to loss of extracellular matrix proteins. *Aim:* To understand the changes in SMC specific genes and proteins in aneurysms of the thoracic aorta (TAA) and the effect of different culture conditions on SMC phenotype. *Patients and Methods:* Twenty five TAA samples were provided by Kartal Koşuyolu, Advanced Training and Research Hospital. SMC were isolated from the samples using the explant technique and immunohistochemistry was performed to characterize SMC marker proteins. Gene expression in SMC was quantified by real time PCR. *Results:* There are significant differences between the expression of SMC contractile genes and proteins which form the contractile apparatus, such as actin, myosin heavy chain and calponin and markers of a contractile-synthetic switch such as h-caldesmon and collagen I in cells of TAA patients. *Conclusion:* Our results indicate that SMC in TAA tissues present with a phenotypic diversity, which persists in SMC isolated from tissue.

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MATRIX METALLOPROTEINASE-2 AND -9 ACTIVITY IN THORACIC AORTIC ANEURYSMS

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Background: Aortic aneurysms are formed by remodeling of a certain part of the aorta, leading to localized dilatation and weakening of the vessel wall. The alterations in the vessel wall leading to this remodeling are: medial degeneration, increased expression of matrix metalloproteinases (MMPs), leading to degradation of extracellular matrix proteins such as collagen and elastin. *Aim:* To understand the role of MMP-2 and -9 activation in thoracic aortic aneurysms (TAA) and the correlation of ELISA, gel zymography and *in situ* zymography. *Patients and Methods:* Aortic aneurysmal samples and serum were obtained from 20 ascending TAA patients during surgery by Kartal Koşuyolu, Advanced Training and Research Hospital. MMP-2 and MMP-9 serum concentrations were measured by ELISA. Active and total MMP-2 and -9 levels were determined by semi-quantitative gel zymography. These data were correlated with *in situ* zymography for elastase activity following cryosectioning of the samples. The expression of certain genes in aneurysmal tissue were determined by real time PCR. *Results:* There were significant differences between the serum levels and expression of MMP-2 and MMP-9 genes and their activities measured by zymography in tissue of TAA patients.

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THE CYTOTOXICITY OF PACLITAXEL IN LONG-TERM CULTURED CELLS AND THE POSSIBLE THERAPEUTIC EFFECTS OF AMLODIPINE ON PACLITAXEL CYTOTOXICITY

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Background: We aimed at evaluating the toxic effects of paclitaxel, an antineoplastic drug, on cultured amnion cells and the recovering effects of amlodipine, a calcium canal blocker, on the cytotoxicity of paclitaxel. *Methods:* Surplus cell cultures of amniosynthesis samples were used. After the 14-day incubation, amlodipine in different concentrations (10^{-5}, 10^{-6}, 10^{-7} M) was added, one dose daily, for four days into the cultured cells within the microplates. Then, the 10^{-7}-M and 10^{-8}-M solutions of paclitaxel were applied only once to some of the amlodipine-added wells or to the wells not including amlodipine. There were 12 experimental groups: Group 1 (control), Group 2 (10^{-7} M paclitaxel), Group 3 (10^{-8} M paclitaxel), Group 4 (10^{-7} M amlodipine + 10^{-8} M paclitaxel), Group 5 (10^{-7} M amlodipine + 10^{-7} M paclitaxel), Group 6 (10^{-5} M amlodipine), Group 7 (10^{-6} M amlodipine), Group 8 (10^{-5} M amlodipine + 10^{-7} M paclitaxel), Group 9 (10^{-6} M amlodipine + 10^{-7} M paclitaxel), Group 10 (10^{-5} M amlodipine + 10^{-8} M paclitaxel), Group 11 (10^{-6} M amlodipine + 10^{-8} M paclitaxel), Group 12 (10^{-7} M amlodipine). After the experiment, the viabilities of the cells were measured using a mQuant spectrophotometer. The data were statistically analyzed. *Results:* Paclitaxel in both concentrations caused significant cytotoxicity ($p<0.05$). There were no statistically significant differences ($p>0.05$) between the control group and the groups receiving a drug combination (Groups 4, 5, 8-11). *Conclusion:* The cytotoxic effects of paclitaxel on healthy cells may be decreased by amlodipine.

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TESTING THE PRESENCE OF TICK-BORNE ENCEPHALITIS VIRUS (TBEV) IN IXODID TICKS (ACARI: IXODIDAE) FROM TOKAT AND ORDU VICINITY

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Background: Tick-borne encephalitis virus (TBEV) is a member of the genus Flavivirus in the family of Flaviviridae. Tick-borne encephalitis (TBE) is an endemic disease from Europe to Asia. TBE is an infectious disease involving the central nervous system, which may result in death. There are three major forms of the disease: Central European, Far Eastern and Siberian. TBEV is transmitted to humans by ixodid tick species such as *Ixodes ricinius, Ixodes persulcatus* and *Haemphysalis concinna*. *Methods:* In the present study, the presence of TBEV in hard ticks from the Tokat, Ordu and Fatsa provinces of Turkey were tested using reverse transcriptase-polymerase chain reaction (RT-PCR). *Results:* According to the RT-PCR results, no TBEV was detected in ticks collected in this study. *Conclusion:* These results indicated that hard ticks of the regions tested have not harbored TBEV and have no potential for transmission of TBEV.

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IDENTIFICATION OF BAG-1 INTERACTING PARTNERS FROM MCF-7 HUMAN BREAST CANCER CELL LINES

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Background: Bag-1 (Bcl-2 associated athonogene-1) belongs to an anti-apoptotic Bag family that acts as an adaptor protein to regulate a wide variety of cellular processes, including proliferation, cell survival, transcription, apoptosis and motility. Bag-1 has four functionally distinct isoforms that can interact with various molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/ proteasome machinery, Bcl-2, Raf-1 kinase, nuclear hormone receptors and DNA. In human tumors, the expression and interacting partners of Bag-1 isoforms are frequently altered, thus these may serve as powerful prognostic/predictive carcinogenic markers. This study aims at delineating the interacting partners of Bag-1 isoforms in Mcf-7 breast cancer cells. *Methods:* We first cloned the Bag-1L gene to a cloning vector carrying a tag for affinity purification of complexes, transfected Mcf-7 breast cancer cells with the designed vector and obtained stable cell lines overexpressing our clone. After extensive purification steps, we obtained complexes from human cell lines. SDS-PAGE, Western-blot analysis and tandem mass spectrometry were applied for the identification of the interacting partners of Bag-1. *Results and Conclusion:* Our stable cell lines expressing Bag-1 isoforms confirmed already known interacting partners. Current studies are underway to determine novel partners of Bag-1 isoforms. We believe that once the complete sets of Bag-1 complexes are obtained and the interacting partners are determined for Mcf-7 cell lines, the role of each Bag-1 isoform in the mentioned pathways can be understood better. This can further provide routes to study tumor development.

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BCL3 GENE POLYMORPHISMS AND NON-SYNDROMIC CLEFT LIP WITH OR WITHOUT CLEFT PALATE

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Background: Orofacial clefts form as a result of interaction of environmental and genetic factors. It has been reported that gene *BCL3* (B-cell leukemia/lymphoma-3) on chromosome 19q13.1-13.2 may play a role in the etiology of non-syndromic cleft lip with or without cleft palate (NSCLP) based on linkage and association studies in several populations. The aim of the study was to investigate the possible contribution of *BCL3* gene in the development of non-syndromic orofacial clefts. *Patients and Methods:* Five single nucleotide polymorphisms (SNPs), rs7257231, rs10401176, rs8103315, rs1979377 and rs2927456, in the *BCL3* gene were analyzed using MALDI-TOF in two distinct sample cohorts. Transmission distortion was performed in 102 trios and case-control analyses were performed in 132 patients and 335 healthy, unrelated and randomly selected individuals from Latvia. The data were analyzed with FBAT and PLINK software after data cleaning. Isolated cleft cases were excluded from the analysis because of their small sample size. *Results:* An association between the SNP rs7257231 in *BCL3* and the non-syndromic cleft lip with or without cleft palate was found (p=0.0098). Haplotype analyses in Latvian families and individuals also showed significant associations with the non-syndromic cleft lip with or without cleft palate. No associations were found in the case-control comparisons. *Conclusion:* Our results in the Latvian population continue to support a role for *BCL3* in the non-syndromic cleft lip with or without cleft palate in humans. These results showed evidence of the possible involvement of *BCL3* gene in the etiology of NSCLP; however, further studies are necessary to clarify this finding.

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A NOVEL MITF-TRANSCRIPTION INHIBITOR AND ITS EFFECT ON MELANOGENESIS IN MELAN-A CELLS

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Background: Microphthalmia associated transcription factor (Mitf) is a key regulatory transcriptional factor of pigmentation-related genes including tyrosinase. Inhibition of

tyrosinase transcription by blocking the binding of Mitf with its promoter E-box DNA can control the pigmentation. However, no such chemicals were reported so far. *Objective:* To discover and evaluate the small molecule inhibitors of Mitf-E-box DNA. *Methods:* Candidate chemicals were screened by virtual screening from pharmacophore data followed by Mitf E-box DNA protein chip. After selecting the best chemical, its inhibitory activity on binding interaction between Mitf and E-box DNA, electrophoretic mobility shift assay (EMSA) was performed. To evaluate the depigmenting activity of Compound #17 {1-[2-(4-Chloro-phenoxy)-ethyl]-1H-benzoimidazol-2-ylsulfanyl}-acetic acid, cellular melanin assay, and Western blot were performed in melan-a cells. *Results:* Among 27 chemicals selected from a pharmacophore data by virtual screening, Compound #17, which showed the most potent inhibitory activity against Mitf-E-box DNA binding in protein chip, was screened. EMSA results confirmed the specific inhibition of Compound#17 on Mitf-E-box DNA binding. In melan-a cells, Compound #17 reduced tyrosinase expression and melanin synthesis (62.5% with 25 μM). *Conclusion:* The results show that Compound #17 is the first small molecule inhibitor of Mitf-E-box DNA binding with depigmenting activity.

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COMPARISON OF MULTIPLEX REAL-TIME PCR WITH BLOOD CULTURE FOR IDENTIFICATION OF BLOODSTREAM PATHOGENS

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Background: Rapid identification of pathogens and early antibiotic treatment are critical for reducing mortality in patients with severe infection. We evaluated a recently-developed multiplex real-time PCR test that can detect 90 clinically important bacteria and fungi in whole blood and compared its results to those of a conventional blood culture (BC). *Methods:* Real-time PCR (Magicplex Sepsis Test, Seegene, Korea) was compared with blood culture for its ability to detect bacteria and fungi in blood samples from 181 patients in whom sepsis was suspected. *Results:* Magicplex gave a positive rate of 24.3% (44/181), whereas the positive rate with BC was 16.0% (29/181). The performance of Magicplex compared with BC was as follows: overall percent agreement 84.5% (153/181), positive percent agreement (sensitivity) 79.3% (23/29), and negative percent agreement (specificity) 86.1% (131/152). The total procedure from DNA extraction to identification of pathogens was finished within six hours. Analytical sensitivity of 30 CFU/ml was obtained for *C. albicans*, *S. aureus*, *S. pneumonia*, and *P. aeruginosa*. *Conclusion:* Magicplex Sepsis Test is a rapid and sensitive method for detection of pathogens compared with conventional blood culture. Our results suggest that this assay could be a valuable complementary tool in the diagnosis and management of sepsis.

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ANALYSIS OF DIFFERENT MEFV TRANSCRIPTS EXPRESSION IN FAMILIAL MEDITERRANEAN FEVER

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Introduction: Pyrin/Marenostrin, which are the products of MEFV, have a regulatory role in inflammation. Several alternatively spliced MEFV transcripts were previously identified. Some transcripts (FL, 2a, 2Δ, 8ext, 9ext) can produce different pyrin isoforms, although other transcripts are degraded by nonsense-mediated-decay (NMD). We aimed to compare different MEFV transcripts levels between familial Mediterranean fever (FMF)-patients and healthy-controls. *Methods:* Total RNA was isolated from venous blood of 42 FMF-patients and 20 healthy-controls. Expression of alternatively spliced MEFV transcripts was quantified using SYBR-Green based LightCycler 480 real-time PCR system. Three primers were used which span the junction of exon2-3, exon1-3 and exon4-5. The expression levels were quantified using the β2M gene as an internal control. *Results:* Two MEFV transcripts levels, which were analyzed using primers specific to exon2-3 and exon4-5, were found to be significantly decreased compared to controls (Exon2-3 p=0.017 and exon4-5 p=0.0052). However, the MEFV transcript (lacking the second exon) levels (analyzed by primers spanning exon1-3) were found to be significantly higher in FMF patients compared to healthy controls (p=0.026). *Conclusion:* As shown previously, FMF-patients had decreased MEFV expression levels compared to healthy-controls in total. Finding an increase in exon2 spliced form in FMF patients might indicate its role in disease pathology.

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BRADYKININ TYPE-2 RECEPTOR EXPRESSION AND BIOAVAILABILITY OF NO

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Background: It has been reported that endothelium-dependent coronary vasodilation induced by bradykinin is enhanced in $eNOS^{-/-}$-mice. *Methods:* BKR-2 protein levels were evaluated in aorta, heart and lung of two different $eNOS^{-/-}$ mouse strains (Shesely and Goedecke), mice with endothelial-specific overexpression of eNOS ($eNOS^{++}$), mice which express eNOS only in the endothelium ($eNOS^{-/-}/eNOS^{+}$) and in C57Bl/6 mice treated with different doses of NO-donor PETN (0, 6 and 60 mg PETN/kg BW/day) and with the NOS inhibitor L-NA (100 mg/kg/BW). Finally, experiments using endothelial and smooth muscle cells incubated with NO-donors and L-NA were performed. *Results:* Lung BKR-2 protein showed a significant 1.5-3 fold upregulation in both $eNOS^{-/-}$ strains, while myocardial and aortic BKR-2 were significantly up-regulated in the Shesely-strain only. In $eNOS^{-/-}/eNOS^{+}$, BKR-2 was similar to C57Bl/6, suggesting a causal role for eNOS-deficiency. In striking contrast, BKR-2 protein level was not different in organs of $eNOS^{++}$ mice as compared to $eNOS^{n}$. Neither treatment with different doses of PETN nor L-NA resulted in changes of BKR-2 in C57Bl/6. Likewise, endothelial and smooth muscle cells showed no BKR-2-response to NO- donors and L-NA. *Conclusion:* These data suggest that upregulation BKR-2 in $eNOS^{-/-}$ is largely compensatory and independent of NO.

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THERMODYNAMIC BEHAVIOUR OF BLOOD PLASMA FROM PATIENTS WITH COLORECTAL CANCER

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Background: Differential scanning calorimetry (DSC) was recently recognized as a novel approach for diagnosis of diseases. A typical blood plasma melting profile was found in healthy individuals, whereas strongly modified DSC profiles were observed in patients. Therefore, DSC is expected to prove useful for biomedical studies. *Methods:* DSC is a highly sensitive technique that measures precisely the thermally-induced conformational transitions of biomolecules and determines the thermodynamic parameters of protein denaturation. Since DSC monitors global heat changes upon denaturation, all interactions contributing to the stability of the protein conformational states can be probed. *Results:* DSC scans of plasma from patients with colorectal cancer (CRC), the majority at T1-4N0M0 and T3-4N1M0 stages, *i.e.* all without distal metastasis but some with lymph node involvement were recorded. Three groups of profiles can be distinguished based on the variations in the main thermal transitions of the DSC profiles. Typically CRC thermograms possess featureless high temperature region compared to that of healthy individuals, possibly a characteristic feature for malignancy.

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EFFECTS OF MANGANESE SUPEROXIDE DISMUTASE (MnSOD) ALA-9VAL POLYMORPHISM ON BLADDER CANCER

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Aim: The aim of this study was to investigate the association between Manganese superoxide dismutase (MnSOD) Ala-9Val polymorphism and bladder cancer. *Patients and Methods:* The study included 157 patients (mean age=63.2 years) with histopathologically confirmed transitional cell carcinoma of bladder and 224 controls (mean age=61.7 years). For the determination of MnSOD Ala-9Val gene polymorphism, PCR was used. Statistical analysis for genotype distribution was performed by using the chi-square test. *Results:* There were not significant differences in the age and body mass index between patients and controls. No statistically significant association between MnSOD Ala-9Val polymorphism and the risk of bladder cancer was found among the controls and the bladder cancer patients ($p>0.05$). *Conclusion:* According to our findings; the

distribution of Val/Ala genotype in MnSOD polymorphism is frequent in the Turkish population. However, no statistically significant association between MnSOD Ala-9Val polymorphism and the risk of bladder cancer was found among the controls and the bladder cancer patients. It is, therefore, suggested that MnSOD Ala-9Val polymorphism is not a risk factor for bladder cancer in the Turkish population.

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THE EFFECTS OF PPAR-γ PRO12ALA AND LOX-1 K167N VARIANTS ON TURKISH CORONARY ARTERY DISEASE PATIENTS

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Background: Peroxisome proliferator-activated receptor-γ (PPAR-γ) is expressed in activated monocytes and foam cells of atherosclerotic lesions. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is dramatically up-regulated in adipocytes upon treatment with PPAR-γ ligands. This is a direct effect of PPARγ and has identified the molecular basis for PPARγ repression and activation of this gene. The aim of this study was to investigate the effect of LOX–1 K167N and PPARγ Pro12Ala gene polymorphisms together in Turkish coronary artery disease (CAD) patients. *Methods:* LOX-1 K167N and PPAR-γ Pro12Ala polymorphisms were studied by the PCR-RFLP method in 76 patients with CAD and 55 healthy people. *Results:* The frequencies of KK genotype, K allele ($p<0.05$) and KK and ProPro genotypes together ($p=0.004$) were higher in the patients than in the controls, while the frequencies of NN genotype ($p<0.05$) and Ala and N alleles together ($p=0.004$) were higher in the controls than in the patients. Ala and K alleles together were higher in the patients than in the controls ($p=0.02$). It was observed that the decreasing CAD risk in patients who had Ala alleles increased with the K allele (OR: 0.865→3.483), smoking (OR: 0.865→3.242) and male gender (OR: 0.865→2.993). *Conclusion:* We suggest that male gender, smoking and K allele decrease the protective effects of Ala allele. Finally, LOX-1 K167N and PPAR Pro12Ala variants are effective on CAD by gene-environment and gene-gene interactions.

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ASSOCIATION BETWEEN BETA3-ADRENERGIC RECEPTOR GENE POLYMORPHISM WITH BODY MASS INDEX AND BONE MINERAL DENSITY IN TURKISH POSTMENOPAUSAL WOMEN

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Background: Previous studies have suggested that beta3-adrenergic receptor (*ADRB3*) gene is associated with the body mass index (BMI), which is an important predictor of bone mineral density (BMD). The present study investigated the relationship between *ADRB3* Trp64Arg polymorphism, BMI and BMD in Turkish postmenopausal women. *Methods:* A total of 53 healthy control, 112 osteopenic and 81 osteoporotic postmenopausal women were recruited. For the detection of *ADRB3* Trp64Arg polymorphism, polymerase chain reaction-restriction fragment length polymorphism techniques were used. BMD was measured at the lumbar spine and hip by dual-energy X-ray absorptiometry. *Results:* The distribution of *ADRB3* Trp64Arg genotypes was similar in the three groups ($p>0.05$). "Arg/Arg" genotype was not observed. In the osteoporotic group, "Trp/Trp" genotype was associated with low BMI values compared to "Trp64Arg" genotype ($p=0.052$) and "Arg" allele ($p=0.049$). Furthermore, subjects with "Trp/Trp" genotype had lower BMD values at the femoral neck ($p=0.044$) and total hip ($p=0.043$) than those with "Trp/Arg" genotype. No significant effects of the *ADRB3* Trp64Arg genotypes on BMI and BMD values were found at any site in the osteopenic and healthy groups. *Conclusion:* Our data suggest that the *ADRB3* Trp64Arg polymorphism may contribute to the determination of BMI and BMD in the Turkish postmenopausal women.

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OXIDATIVE STRESS INDUCED BY FREE RADICALS AND ITS CORRECTION BY USING ANTIOXIDANTS *IN VIVO*

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Background: The aim of our study was to investigate if additional therapy of antioxidants (*e.g.* Se, α-tocopherol) may affect the reduction-oxidation (red-ox) state of the Chernobyl clean-up workers (ChCW) from Latvia. ChCW in comparison to people of the same age, gender and other criteria, suffer more chronic and oncological diseases and they are subjected to many chronic inflammatory processes that, in their turn, are related to free radical production and accordingly the red-ox state fluctuations. *Methods:* Men of age 40 to 65 years (ChCW and healthy volunteers) were involved in the study. Each group received different combinations of antioxidants or placebo. Reduced glutathione (GSH), total antioxidant status, glutathione peroxidase (GSH-Px), Cu, Zn-superoxide dismutase (SOD) *etc*. and several lipid peroxidation (LPO) markers – malondialdehyde *etc*. were detected in blood, plasma and erythrocytes. *Results:* The results indicate that there is a tendency for LPO intensity to decrease during and after antioxidant therapy; in some patient groups, however, antioxidative defense improved by increase of Se and GSH, GSH-Px, SOD accordingly. *Conclusion:* We cannot confirm that additional antioxidant therapy guarantees an improvement in all cases related to higher free radical production, although our results demonstrate it may probably help to keep the red-ox state in balance.

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GENOTYPE AND ALLELE FREQUENCY OF *MDR1, CYP2D6* AND *CYP2C9* IN THE UKRAINIAN POPULATION

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Background: The frequency of functionally relevant alleles and polymorphisms of genes encoding xenobiotic metabolizing enzymes has wide ethnic variation. Pharmacogenetic studies of the frequency of genotypes and allelic variants of *CYP2D6*4, CYP2C9*2, *3* and *C3435T* polymorphism of *MDR1* have not yet been conducted in Ukraine. *Methods:* Polymerase chain reaction and restriction fragment length polymorphism were used to determine the genotypes of *CYP2D6, CYP2C9* and *MDR1* in 920 healthy men and women of Ukrainian origin. *Results:* The distribution of *C3435C, C3435T* and *T3435T* genotypes of *MDR1* gene were 26.75%, 50.88% and 22.37%, respectively. The frequency of the C and T alleles was 0.48 and 0.52, respectively. The frequency of the non-functional *CYP2D6*4* allele was 0.19, while that of the wild-type allele was 0.81. The distribution of **1*1, *1*4* and **4*4* genotypes of *CYP2D6* gene were 64.79%, 31.35% and 3.86%, respectively. *CYP2C9*2* and **3* alleles were found with frequencies of 0.86 and 0.12, respectively. *Conclusion:* The assessment of the distribution of alleles of important xenobiotic metabolizing enzymes among the Ukrainian population showed similarities to other Caucasians. Examination of the frequencies of these genes may be useful before prescribing pharmacotherapy.

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THROMBOPHILIC MUTATIONS IN CHILDHOOD CARDIAC AND GREAT VESSEL THROMBOSIS

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A dramatic increase in childhood venous thromboembolism has been reported in the last decade. Children with congenital heart disease (CHD) are the largest pediatric patient group, accounting for one third of children suffering from venous thromboembolism. Both the acquired and the inherited prothrombotic risk factors have a major impact in the development of thrombosis. This study was conducted to analyze the incidence of Factor V (G1691A, A1090G, and A1299G), prothrombin G20210A, methylenetetrahydrofolate reductase (C677T, A1298C, T1317C) mutations and PAI-1 4G/5G polymorphism in children who had cardiac thrombosis (CT) diagnosed in our institution from January 1997 to June 2009. The data strongly suggest that underlying disorders such as CHD, malignancy, and cardiomyopathy and clinical risk factors such as cardiac surgery, central venous catheters, and systemic infections are important contributors for the development of CT. The type of disorder usually determines the site of thrombosis. This study also shows that to ensure early diagnosis, routine screening for CT should be performed in patients who have undergone an invasive procedure. Our results strongly suggest that the FVG1691A and PTG20210A mutations may be predisposing molecular risk factors for CT in these patients and more aggressive prophylaxis is suggested in such a situation.

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HPV INFECTION AND *TP53* CODON 72 POLYMORPHISM IN CERVICAL CARCINOMA PATIENTS FROM SERBIA

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Background: Genetic susceptibility to HPV infection and its impact on the course of cervical carcinomas is unclear. The aim of this study was to examine the correlation of HPV infection with demographic, clinicopathological and patient characteristics, as well as *TP53* codon 72 polymorphism. *Methods:* DNA was isolated by the salting out method from the carcinoma tissue of 48 patients. Presence of HPV, HPV16 and HPV18 was detected through PCR amplification of L1, E7 and E1 viral genes respectively. TP53 codon 72 polymorphism was assessed by restriction fragment length polymorphism. *Results:* HPV was present in 62.5% carcinomas; 58.3% were HPV16; 16.7% HPV18 and 14.6% HPV16 *plus* HPV18. HPV infection was common in well and moderately differentiated (52.9% and 75.0%) tumors, in contrast to poorly differentiated tumors (40.0%). The median age of diagnosis of disease varied from 48 years for patients without HPV infection to 52 years for patients with multiple HPV infection. The frequencies of *TP53* genotypes Arg/Arg, Arg/Pro and Pro/Pro in HPV-positive tumors were 66.7%, 30.0% and 3.3% in comparison with 61.1%, 38.9% and 0% for HPV-negative tumors. Multiple infection was present only within the Arg/Arg genotype. *Conclusion:* Absence of HPV infection correlates with a more aggressive phenotype and an earlier occurrence of cervical carcinomas. Arg/Arg genotype of *TP53* gene is more prone to multiple infection.

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A COMPARATIVE STUDY OF CELL-FREE EPSTEIN-BARR VIRUS DNA AND MICRORNAS IN NASOPHARYNGEAL CARCINOMA SCREENING

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Background: Nasopharyngeal carcinoma (NPC) is a malignant squamous cell carcinoma in the head and neck region. Epstein-Barr virus (EBV) is associated with undifferentiated NPC. Cell-free EBV DNA is routinely used as a serological marker. Circulating nucleic acids were recently suggested as non-invasive diagnosis markers. This study aims to examine the use of microRNAs (miRNAs) as a supplement to existing screening methods. *Methods:* The expression levels of hsa-miR-21, hsa-miR-1301, ebv-miR-BART7 and ebv-miR-BART22 were investigated in ten paired tumor-normal tissues, plasmas of sixty other NPC patients and twenty-five cohorts. Plasmas from thirteen NPC patients were evaluated for both EBV and miRNAs levels between pre-operative and post-operative (three months after radiotherapy) patients. MiRNA and EBV DNA quantitation were performed by quantitative real-time PCR and EBV quant kit, respectively. *Results:* MiR-BART7 showed higher expression in tumor tissues (p=0.038) and in NPC plasma samples with sensitivity 71.15% and specificity 76.47%. Among patients who had already received radiotherapy, 12 of 13 patients showed elevated miR-BART7 expression whereas 4 of 13 patients showed elevated EBV DNA, 3 of 13 patients had increased and 5 of 13 undetermined. *Conclusion:* MiR-BART7 showed significantly higher expression in NPC with good sensitivity and specificity. Further studies will be undertaken on the functional role of miR-BART7.

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BIOLOGICAL ACTIVITY OF NOVEL PEPTIDES AS NOCICEPTIN-RECEPTOR LIGANDS

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Background: The aim of this study was the biological screening of newly synthesized analogs of G-protein-coupled nociceptin receptor. *Methods:* On electrically stimulated vas-deferens smooth-muscle preparations, cumulative dose-response curves were created for nociceptin and its derivatives, applied alone or after blockade of opioid or nociceptin receptors. *Results:* The novel analogs are based on nociceptin(1-13)NH_2 as a chemical template. In three of the derivatives, Phe[1] was substituted by 1-[(methoxyphosphono)methylamino]cyclo-alkanecarboxylic acid with 5,- 7-, or 8-membered rings. They inhibited the smooth muscle contractions only in the highest concentration used. In other three peptides, the substituent was linked to Phe[1]. These peptide analogs showed good agonist activity and specificity to nociceptin receptors. *Conclusion:* The removal of Phe[1] in the peptide molecule reduced the potency, efficacy and selectivity of the new compounds towards nociceptin receptors. The enlargement of the peptide carbon ring did not significantly modify the activity of the new analogs.

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PLURONIC F68 INCREASES THE EFFICIENCY OF GROWTH FACTORS IN REDUCING KETAMINE-INDUCED NEUROTOXICITY

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Background: Ketamine is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist which is used as an injectable anesthetic. It was shown that ketamine causes neurotoxicity and cell death. It was found that long-term ketamine administration increases hyperphosphorylated tau-positive cells, which is a hallmark of Alzheimer's disease. In this study, pluronic F68, a drug delivery reagent, combined with growth factors, fibroblast growth factor-2 (FGF-2), insulin-like growth factor (IGF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and vascular endothelial growth factor (VEGF), were used to eliminate ketamine-induced neurotoxicity. *Methods:* Firstly, F68 was applied on SH-SY5Y cells and cell viability was measured by using MTS test. Non-toxic concentration (80μM) of F68 was mixed with the studied growth factors and applied on SH-SY5Y cells exposed to neurotoxicity induced by ketamine. After 24 h, the cell viability was measured by using MTS test. *Results:* FGF-2, VEGF, IGF, NGF and BDNF reduced the ketamine-induced toxicity and F68 significantly increased the efficiency of all growth factors in reducing the neurotoxicity. *Conclusion:* These data suggest that F68 increases the efficiency of growth factors in ameliorating the side effects of ketamine. F68 may be used as a medicinal agent to reduce the adverse effects of ketamine.

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STUDY OF THE HIGHER ORDER CHROMATIN STRUCTURE IN FRAGILE X MENTAL RETARDATION GENE (FMR1) KNOCK-OUT *DROSOPHILA* MUTANTS

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Background: Mutations in the fragile X mental retardation gene (FMR1) are responsible for the most common form of hereditary mental retardation known as the fragile X syndrome. FMRP is a RNA binding protein, which regulates translation and mRNA trafficking in neurons. In *Drosophila*, dFmr1 has also been shown to participate in heterochromatin-mediated gene silencing. In order to understand the molecular mechanisms by which dFMRP fulfills its function we used *Drosophila melanogaster* as a model organism. Our aim was to study the possible involvement of dFMRP in changes of the chromatin structure and dynamics in *Drosophila*. *Methods:* The method of Comet assay is widely used for the detection of cuts in the DNA molecule. By combining this method with the activity of several nucleases we developed a method called chromatin Comet assay (ChCA). This method may reveal changes which appear in the chromatin structure of chromatin-dependent mutant cells. *Results:* Using the method of ChCA, we obtained results showing differences in the higher-level organization of chromatin structure in the *dfmr1* knock-out mutants. These differences involve the size and the way of organization of the chromatin loops. *Conclusion:* A hypothesis concerning the molecular mechanisms, which may lead to the observed phenotype differences in chromatin will be discussed in detail.

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GENETIC FACTORS IN VENOUS THROMBOSIS AND PULMONARY EMBOLISM

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Aim: To define the role of genetic infringements in venous thrombosis and thromboembolic complication occurrence after surgery. *Methods:* After local ethic committee approval and informed consent, 86 patients were studied who underwent general surgery. For venous thrombosis and pulmonary embolism verification we used ultrasound Doppler, echocardiography and angiography. All patients had molecular-genetic tests to reveal mutations of II and V clothing factors. *Results:* Twelve of 86 patients had deep vein thrombosis (13.9%). In five of them pulmonary embolism occurred in the post operative period. In 7% of all patients we revealed heterozygote mutation of II and V factors of coagulation. The frequency of such mutations in patients without thrombotic complication was 3.4%, but in patients with thrombotic complication it was 33.3% ($p<0.05$). In patients with thrombotic complications we observed mutation of both II and V factors in 16.6%, the frequency of single mutation of factor II or V was 8.3%. *Discussion:* Mutation of factor II results in a ten-fold increase in risk of thrombotic complication, mutation

of V factor results in a 2.4-fold increased risk and mutation of both factors results in a 16.3-fold increased risk of thrombotic complication. Anticoagulant therapy is required in patients with mutation of II and/or V factor of clothing.

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IMPORTANCE OF THE TNFα GENE POLYMORPHISM FOR INTENSIVE CARE STRATEGY IN PATIENTS WITH SEPTIC COMPLICATIONS AFTER SURGERY

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Aim: To study role of the polymorphism of the tumor necrosis factor (TNFα) gene for prognosis of septic complications in patients after urgent surgery. *Methods:* We studied the frequency of septic complications after urgent abdominal surgery in 152 patients. For all patients we used genotype examination to reveal polymorphism of gene (308G>A) TNFα. *Results:* A total of 49 patients had septic complications after surgery (group 1) and 103 patients had no such complications (group 2). The pathological genotype AA of TNFα gene was found in 68.6±6.62% patients of group 1 and in 9.9±3.36% patients of group 2 ($p<0.05$). AG genotype was present in 19.7±9.45% patients of group 1 and in 38.5±6.7% patients of group 2 ($p<0.05$). Genotype GG was present in 13.5±9.05% patients of group 1 and 53.9±6.93% patients of group 2 ($p<0.05$). *Discussion:* Prevalence of genotype AA of TNFα gene in patients of group 1 let us to suggest its role in development of septic complications after surgery. Genotype AA TNFα can be settled as early prognostic criteria of risk for development of septic complications in surgical patients and may substantiate early prevented forced treatment of septic complications to decrease morbidity and mortality.

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THE RELATIONSHIP BETWEEN TNF-ALPHA GENE POLYMORPHISMS AND SUSCEPTIBILITY TO BEHCET'S DISEASE IN NORTH-WEST IRAN

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Background: Behçet's disease (BD) is a multisystematic disease which includes recurring mouth and genital ulcers along with inflammation inside the eye and skin problems. BD patients have higher serum levels of tumor necrosis factor (TNF). The positive response of BD patients to antiTNF therapy suggests a pivotal role for TNF-alpha in this disease. *Aim:* The aim of this study was to investigate the possible relation between TNF-alpha-1031T/C and TNF-alpha-308G/A polymorphisms and the susceptibility to BD in the population of North-West Iran. *Methods:* The distribution of two polymorphisms within the TNF gene promoter region was compared in 53 BD patients and 79 healthy controls using PCR-RFLP. *Results:* A significant difference was observed with respect to the allele frequency of TNF-alpha-1031C which was higher in BD patients compared to the controls; while the allele frequency of TNF alpha-308A revealed no difference between the two groups. *Conclusion:* The frequency of CG haplotype was significantly high in BD patients while the frequency of TA haplotype was significantly low in these patients. This result reveals that in the population of North-West Iran, TNF alpha gene is linked to BD susceptibility.

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REARRANGEMENT OF THE MLL GENE IN THERAPY-RELATED MYELODYSPLASTIC SYNDROME (MDS) IN PATIENTS PREVIOUSLY TREATED WITH AGENTS TARGETING DNA- TOPOISOMERASE II

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Background: Therapy-related acute myeloid leukemias (t-AML), with balanced translocations affecting 11q23 point in *MLL* gene, are one of the most serious complications of treatments with topoisomerase II inhibitors. However, there are few reports of t-AML. We aimed at studing if these translocations are cumulative dose dependent, their frequency in therapy-related MDS and the relationship between their presence and the response criteria. *Methods:* The study included 60 patients with various malignancies (NHL 94.4% and 5.6% neuroblastoma) in remission treated by topoisomerase 2 inhibitors; 36 patients with and 24 patients without therapy related myelodysplasia features. All bone marrow samples of the patients were evaluated by

fluorescence *in situ* hybridization (FISH) for 11q23 point breakage in MLL gene. *Results:* MLL gene rearrangement frequency was 38.9 % in dysplastic *versus* 8.3% in non dysplastic groups; $p<0.001$. It was associated with bad course (r 0.5, $p<0.0001$). It was associated with a worse overall survival (mean 13±2 *versus* 39±3 months, log rank p value <0.0001). It was dose dependent with a cut-off value of 290 mg/kg of topoisomerase II inhibitors, as assessed by ROC curve (area under the curve 0.84±0.05, $p<0.0001$). *Conclusion:* MLL gene may be etiopathogenetically involved in hematological neoplasias and survival.

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MOLECULAR MECHANISMS OF HIGH RESISTANCE TO ANTITUMOR DRUGS IN HUMAN MELANOMA

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Background: Melanoma is a highly aggressive tumor characterized by a strong resistance to antitumor drugs. Several biochemical mechanisms of melanoma resistance were investigated in this study. *Methods:* Melanoma cell lines were established from melanoma patients. The tumor cell survival was measured 72 h after drug treatment. The expressions of melanoma antigen MAGE-3, proangiogenic vascular endothelial growth factor (VEGF) and apoptosis inhibitor survivin were measured by RT-PCR. The activity of a group of proteins called the ABC transporters which mediate the active efflux of chemotherapeutic drugs from cancer cells was measured on the velocity of the rhodamine-123 efflux by flow cytometry. *Results:* All investigated melanoma cell lines expressed MAGE-3 and splicing variants of survivin and VEGF. The levels of survivin expression correlated with the levels of VEGF, but no correlation with melanoma sensitivity to antitumor drugs was found. The rhodamine efflux was seen in all lines; the highest efflux velocity was seen in ABCB1$^+$ melanoma cell line. This line was the most resistant to doxorubicin. *Conclusion:* The intrinsic melanoma resistance involves multiple biochemical mechanisms which are important for the development of new therapeutic methods.

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TARGETED DELIVERY OF ANTICANCER DRUGS USING rhAFPfr-LINKED PLGA-NANOPARTICLES BY RECEPTOR-MEDIATED ENDOCYTOSIS

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Background: Polymeric-based delivery systems linked to tumor-specific ligands are a promising approach for targeting anticancer drugs into tumor. Our previous studies showed that α-fetoprotein- (AFP-) specific receptor is the universal tumor-specific antigen which is abundantly expressed on the surface of tumor cells and that AFP and a recombinant C-terminal human AFP fragment (rhAFPfr) effectively deliver anticancer drugs in the tumor cells. A biodegradable polymer, the copolymer of poly(lactic-co-glycolide) (PLGA), is being extensively used in biomedical applications because of its ability to encapsulate various drug molecules. *Methods:* rhAFPfr was bound to PLGA-nanoparticles (PLGA-NPs) with encapsulated cytotoxic drugs. The accumulation and antiproliferative activity of NPs was studied in human breast adenocarcinoma cell lines MCF-7 and MCF-7Adr. *Results:* rhAFPfr-linked PLGA-NPs bound specifically to the AFP receptor on the tumor cell surface. An enhanced cytotoxicity of these NPs was demonstrated. rhAFPfr-linked PLGA-NPs allows the effective reversal of multidrug resistance of tumor cells due to overexpression of the mdr1 gene. *Conclusion:* rhAFPfr can be used as a protein vector for the targeted delivery of PLGA-NPs with encapsulated cytotoxic drugs to tumor cells.

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GAMMA-GLUTAMYLTRANSPEPTIDASE ACTIVITY IN NORMAL AND TUMOR HUMAN LUNG CELL LINES

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Background: Gamma-glutamyl transpeptidase (EC 2.3.2.2, GGT) is a cell-surface protease, hydrolyzing γ-glutamyl residues from glutathione. Its activity is low in normal cells but increases with tumor progression. The dynamics of GGT surface activity in the normal human lung cell line P was compared with those in two tumor lung cell lines, A549 and SK-MES-1. *Methods:* The dynamics of GGT activity was determined by γ-glutamyl-*para*-nitroanilide as a substrate. The velocity of reaction was measured with and without pre-incubation with glycine, glycyl-glycine or alanine. *Results:* P and SK-MES-1 cell lines showed a linear reaction course, with a speed of 2.5 and 1.64 μM p-NA/min/10^6 cells, respectively. A549 cells showed a biphasic reaction pattern with the two phases having a speed of 2.56 and 4.19 μM p-NA/min/10^6. Pre-incubation decreased the relative velocity for tumor lines in comparison with cells pre-incubated only in buffer. *Conclusion:* Normal cells showed intermediate GGT-activity in comparison with tumor cells. Surfactant-producing A549 cells had higher activity, probably due to the aggressive nature of lung adenocarcinomas. Amino acids and peptides did not activate the enzyme in either of the tumor lines. GGT can be studied as a diagnostic marker for non-small cell lung carcinomas.

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INVESTIGATION OF EPHX1 GENE POLYMORPHISMS IN COLORECTAL CANCER PATIENTS

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Background: Metabolism of chemical carcinogens, including their activation and detoxification, plays a key role in carcinogenesis. Microsomal epoxide hydrolase (EPHX1) plays an important role in the metabolism of polyaromatic hydrocarbons (PAHs) and detoxification of procarcinogens. The aim of this study was to investigate the association between the development of colorectal cancer and EPHX1 gene polymorphisms. *Methods:* We investigated the polymorphisms in exon 3 (T>C, Tyr113His) and exon 4 (A>G, His139Arg) of the EPHX1 gene in 101 colorectal cancer patients and 118 controls by polymerase chain reaction-restriction fragment length polymorphism. *Results:* The frequencies of the TT, TC and CC for EPHX1 exon 3 were 38.1%, 55.1% and 6.8% in the controls and 38.6%, 43.6% and 17.8% in the patients, respectively. The frequencies of EPHX1 exon 4 genotypes were 79.2% AA, 18.8% AG and 2% GG in the control group and 62.2% AA, 37% AG and 0.8% GG in the patient group. Individuals carrying EPHX1 exon 3 CC genotype had a 2.6-fold increased risk (p=0.012), while those carrying the EPHX1 exon 4 AG genotype had decreased risk of colorectal cancer compared to the controls (p=0.003). *Conclusion:* Our results suggest that the exon 3 Tyr113His and exon 4 His139Arg polymorphisms of EPHXI may be associated with an increased risk of colorectal cancer.

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KINETIC ANALYSIS OF THE AMINO TERMINAL END OF ACTIVE SITE LOOP OF LACTATE DEHYDROGENASE FROM *PLASMODIUM VIVAX*

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Background: Malaria is one of the most deadly and widespread diseases caused by Plasmodium parasites. Development of resistance to common drugs leads to the determination of novel targets. Because *Plasmodial lactate* dehydrogenase is different from its mammalian counterparts, it has been determined as a drug target. In this study, kinetic parameters were analysed on the previously mutated *P. vivax* LDHs to mimick *Toxoplasma gondii* I LDH (LMITg), *Toxoplasma gondii* II LDH (LMIITg), *Eimeria acervulina* LDH (LMEa) and *Eimeria tenella* LDH(LMEt). *Methods:* Mutant LDH genes were amplified by PCR, subcloned into the plasmid vector and overproduced in *E. coli*. Enzymes were purified by using Ni-NTA agarose matrix and kinetic properties analyzed. *Results:* The K_{cat} value of LMITg, LMEa, LMEt and LMIITg mutant enzymes were similar to wild-type *P. vivax* LDH. However K_m value of mutant proteins was found to be higher indicating decreasing substrate binding affinity to the active site loop. *Conclusion:* Decrease in the enzymatic activity indicates that the amino terminal end of active site loop is sensitive to changes, supporting the suggestion that this site should be evaluated as an ideal target in drug design studies for both *Plasmodium* and *Apicomplexans*.

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APOPTOTIC SPECK LIKE PROTEIN mRNA QUANTITATION IN DIFFERENT MEFV MUTATION PROFILES OF FAMILIAL MEDITERRANEAN FEVER PATIENTS

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Background: Familial Mediterranean Fever (FMF) is a hereditary autoinflammatory disorder characterized by episodes of inflammation in the absence of high-titer autoantibodies or antigen-specific T cells. For the inflammatory pathways, apoptotic speck-like protein containing a caspase recruitment domain (ASC) is the essential adaptor protein for caspase 1 mediated interleukin (IL)-1β and IL-18 processing in inflammasomes. This study aimed to indicate a possible relation between ASC protein expression and different combinations of MEFV mutations in FMF patients. *Methods:* Reverse Transcriptase real time PCR analysis was performed to document the comperative ASC gene expression quantities in MEFV gene mutation (+) groups of compound heterozygous (20), heterozygous (20), homozygous (20), and SNP groups (20). *Results:* No significant fold-change was obtained between compound heterozygous and heterozygous; compound heterozygous and homozygous; compound heterozygous and SNP group; heterozygous and homozygous; heterozygous and SNP; homozygous and SNP groups ($p>0.05$). However, there was a slight trend toward higher ASC gene expression in compound heterozygous in comparison with the heterozygous, homozygous, and SNP groups. *Conclusion:* The slightly increased gene expression is thought to contribute to encompassing the typical FMF phenotypes; but it is not considered as a modifier gene in disease pathogenesis.

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MOLECULAR ANALYSIS IN THE IDENTIFICATION OF THE *NOTCH3* GENE SEQUENCE VARIANTS AND GENETIC DIAGNOSIS

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Background: Autosomal dominant disorder CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is an adult-onset neurologic disorder characterized by recurrent strokes and dementia. This study aimed to indicate CADASIL related mutations in the causative disease gene, *NOTCH3*, and perform genotype-phenotype correlations. *Methods:* Bidirectional DNA cycle sequencing analysis of entire coding exons, introns and promoter of *NOTCH3* gene was performed for 5 patients with CADASIL clinical diagnosis and 15 healthy individuals. *Results:* Genetic testing confirmed the following *NOTCH3* sequence variations: p.Gln151Glu; p. Arg169Cys; p.Ala2223Val; Ala202Ala, Cys846Cys, Pro914Pro, and Pro1521Pro; IVS7 +16A>G, IVS17 +23G>A, IVS21 +49C>T, IVS23 +21T>A, IVS26 +24G>C, IVS26 -21G>T, IVS31 +37G>A; IVS29 +4 T>C; IVS32 – 8 T>C; IVS32 +28 T>G; IVS32 +45T>C; IVS32 +55 A>G; 3'UTR+402 A>C. While Gln151Glu and Arg169Cys mutations are known CADASIL related pathogenic mutations, the pathogenic effect of Ala2223Val remains unknown. None of the sequence changes (except SNPs) of the *NOTCH3* gene were identified in any 30 chromosomes of healthy individuals. *Conclusion:* Since CADASIL disease is often misdiagnosed with early initial symptoms because of its frequency, clinical heterogeneity and mode of inheritance and clinical presentation, accurate diagnostic confirmation can be obtained only on a molecular genetic basis.

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CELL CYCLE MARKERS SHOW DIFFERENT EXPRESSION AND LOCALIZATION PATTERNS IN NEURON-LIKE PC12 CELLS AND PRIMARY HIPPOCAMPAL NEURONS

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Background: Neuron-like PC12 cells are extensively used in place of neurons in published studies. The aim of the present study was to compare mRNA and protein expressions of cell cycle proteins in terminally differentiated post-mitotic primary hippocampal neurons, mitotically active PC12 cells and NGF-differentiated post-mitotic PC12 cells. *Methods:* The mRNA expression levels of cell-cycle related genes were analyzed with Real-Time quantitative PCR, protein expression levels by Western-blotting and sub-cellular localization by immunocytochemistry. *Results:* In hippocampal neurons, the presence of cell cycle proteins was detected only at the mRNA level except for cyclinA, cyclinE and Cdk4, which were detectable also at the protein level. In NGF-treated PC12 cells, cyclinD and Cdk4 were localized in the nucleus, while in neurons cyclinD expression was not detectable, and Cdk4 localized in the cytoplasm. CyclinA and cyclinE were also

localized differently in both cell types. *Conclusion:* These results suggest that PC12 cells and primary neurons are different in terms of cell cycle protein expression and localization. Thus, it may not be very appropriate to use these cells as neuronal model system in order to understand neuronal physiological activities, upstream of where there may lie cell cycle activation triggered events.

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VITAMIN E IS ACTIVATED BY PHOSPHORYLATION TO α-TOCOPHERYL PHOSPHATE

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Background: α-Tocopherol, traditionally described as an antioxidant, appears to possess specific cellular functions independent of its radical scavenging properties. Recently, a derivative of α-tocopherol, tocopheryl phosphate (α-TP), phosphorylated at the OH group of the chromane ring, has been found to be present in biological tissues and foodstuffs, indicating that this form is a natural derivative of α-tocopherol. *Methods:* The effects of α-TP on cell proliferation and CD36 mRNA expression were investigated and compared with those produced by α-tocopherol *in vitro* and *in vivo*. *Results:* While it is similar to α-tocopherol, α-TP appears to be more potent than α-tocopherol in inhibiting cell proliferation, down regulating CD36 transcription, inhibiting atherosclerotic plaque formation. In cells and animals α-TP does not act by liberating α-tocopherol; rather, the intact molecule appears to be more potent than α-tocopherol itself. *Conclusion:* The finding that α-TP is more potent than α-tocopherol, that it can be synthesized in cells, tissues and animals supports the hypothesis that α-tocopherol produces a novel, more potent molecular species with cell signaling properties.

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GENOME-WIDE ANALYSIS OF FORMALIN-FIXED PARAFFIN-EMBEDDED PULMONARY METASTATIC TUMOR SAMPLES OF OSTEOSARCOMA PATIENTS

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Background: Osteosarcoma is the primary malignant tumor of bone with an incidence rate of 19% among all cancer types. The vast majority of patients have pulmonary metastases at the time they are diagnosed and about half of them develop lung disease later. Pulmonary metastatic tumors lead to poor prognosis and increased death rate. Therefore, it is essential to investigate the cellular and molecular mechanisms underlying the pulmonary metastasis of osteosarcoma. In this study, a genome-wide scan for loss of heterozygosity (LOH) and copy number changes was conducted using formalin-fixed paraffin-embedded (FFPE) samples of metastatic osteosarcoma patients in order to identify molecular markers in osteosarcoma metastasis. *Methods:* DNA isolation from paired normal and tumor FFPE samples was performed with a modified protocol of Qiagen DNA isolation kit. Affymetrix 250K Sty-Mapping SNP array, containing approximately 250,000 SNPs, was used to investigate genome-wide LOH and copy number changes of chromosomal regions. *Results:* Chromosomal regions 1q41, 2q14.3, 5q14.3, 8q23.3, 14q12 and 15q11.2 were identified as regions with significant copy number amplification, while 1q12, 2p11.2, 6q22.31 and 16p11.2 were identified as regions with significant copy number loss. LOH events were detected in all samples. *Conclusion:* Regions of LOH and copy number changes identified in this analysis may provide insights into the underlying genes and processes involved in osteosarcoma metastasis. The present study also demonstrated a feasible approach to use archival FFPE samples for the assessment of LOH and copy number changes.

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THE ANALYSIS OF POLYMORPHISM OF GENES ACE, ENOS3 AND TNFα IN PATIENT WITH RHEUMATOID ARTHRITIS OF KAZAKH POPULATION

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Aim: To assess the role of allele polymorphism of I/D 287 bp ACE, 4, 5 times repeating 27 bp in intron 4 (4b, 4a) eNOS3 and G308A TNFα genes in rheumatoid arthritis (RA) in the Kazakh population. *Patients and Methods:* A total of 66 patients of Kazakh nationality with RA were surveyed. The control group was comprised of 100 healthy Kazakhs of comparable gender and age. Genotyping was performed by the restriction fragment length polymorphism method.

Results: There was no significant difference in the frequency of homozygous for the mutant allele, genotype DD: 24% of the mutant allele D and 56% in the intervention group in contrast to the data of the control group 22.8, 48.6, χ^2=0.02 (p=0.88) and χ^2=0.58 (p=0.44), respectively. The mutant genotype 4aa was not defined in patients with RA. The AA genotype occurred more frequently in RA patients (53% and 4.0%, accordingly, p<0.00001, χ^2=50.5) and rarely GG genotype (7.6% and 37.0%, accordingly, p=0.00001, χ^2=16.7), than in control. The A allele of the TNFα gene significantly increased the risk of RA (OR=5.3). *Conclusion:* Polymorphism of the TNF gene is associated with a predisposition to the development of RA in the Kazakh population.

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DETECTION OF *PORPHYROMONAS ENDODONTALIS* IN ACUTE PERIRADICULAR ABSCESS BY REAL-TIME PCR

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Background: The escape of microorganisms and their products from the root canal system into the periradicular tissue can cause tissue damage and precipitate periradicular inflammation. An acute periradicular abscess can develop as a result of the excessive and sudden microbial invasion of periradicular tissues. The microbiota involved is mixed and dominated by anaerobic bacteria. *P. endodontalis*, a black-pigmented anaerobe, is a highly oxygen sensitive and fastidious microorganism. Therefore, it cannot be easily detected in periradicular abscess by conventional microbiological methods. Owing to difficulties in isolating and identifying *P. endodontalis* by using conventional methods the present study was undertaken to determine the presence of *P. endodontalis* in microbiology samples taken from patients with acute periradicular abscesses using a sensitive molecular method, real-time PCR. *Methods:* Microbial samples were collected by aspiration from 17 patients diagnosed as acute periradicular abscess. DNA was extracted from the samples by using a QIAamp DNA mini-kit and analyzed with real-time PCR. *Results:* Real-time PCR used in this study allowed the detection of *P. endodontalis* in 6 of 17 (35.3%) cases diagnosed as acute periradicular abscess. *Conclusion:* Our findings suggest that *P. endodontalis* may participate in the pathogenesis of acute periradicular abscesses.

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DETECTION OF *TANNERELLA FORSYTHIA* IN ACUTE APICAL PERIODONTITIS AND CHRONIC APICAL PERIODONTITIS BY REAL-TIME PCR

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Background: Apical periodontitis, an inflammatory disorder of periradicular tissues, can be classified as acute or chronic periodontitis. Acute apical periodontitis (AAP) presents with acute clinical symptoms but no periapical bone resorption. Chronic apical periodontitis (CAP) is characterized by the absence of clinical symptoms and the presence of a periapical radiolucency. *Tannerella forsythia* is a slow growing, fastidious microorganism that is difficult to culture. In recent years, molecular genetic methods have been used to detect microorganisms that are impossible or difficult to culture. The aim of the present study was to investigate the prevalence of *T. forsythia* in AAP and CAP by using a sensitive molecular method, real-time PCR. *Methods:* Microbial samples were collected from 16 single-rooted teeth with necrotic pulps. Teeth were categorized by diagnosis as having acute apical periodontitis (n=9) or chronic apical periodontitis (n=7). DNA was extracted from the samples by using a QIAamp DNA mini-kit and analyzed with real-time PCR. *Results: T. forsythia* was detected in five of nine samples diagnosed as AAP (55.5%), and three of seven samples with CAP (42.8%). In general *T. forsythia* was found in eight of sixteen cases (50%). *Conclusion:* Our findings suggest that *T. forsythia* may participate in the pathogenesis of different forms of periradicular lesions.

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AN INTERESTING STIMULATION OF ADRENAL MACROPHAGES IN CHRONIC MILD STRESS-EXPOSED RATS

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Background: Macrophages are supposed to play a role in the immune-adrenocortical interaction within adrenals. We aimed to examine changes of adrenal macrophages in the stress model. *Methods:* A total of 24 Sprague-Dawley rats (12 males, 12 females) were divided into four groups of 6 males or 6 females each. Two groups were controls of the females and

males. The other two groups, the stress groups, received a chronic-mild-stress procedure for two weeks. Histological slides of the removed adrenal glands were prepared, and stained with Haematoxylin-Eosin (HE) and Periodic acid-Schiff (PAS), and examined under a light microscope. For morphometrical evaluation, adreno-cortical thicknesses were measured using ocular-meter, and compared between experimental groups. *Results:* Adreno-cortical thickness was significantly increased in the stress groups ($p<0.05$), suggesting an augmentation of cortical function due to stress. Excessive adrenocortical macrophages with yellowish-brown cytoplasm containing lipofuscin granules were observed in HE-slides of stress groups. These abundant cells were also PAS-positive indicating richness in lysosomes. *Conclusion:* Chronic-mild stress can alter adreno-cortical structure to stimulate steroidogenesis. Macrophages not only express phagocytic activity but also secrete cytokines stimulating hypothalamic-pituitary-adrenal axis. Thus, secretion of adreno-cortical glucocorticoids can also be induced by adrenal macrophages during stress. Additionally, these macrophages keep digesting cellular debris resulting from increased adreno-cortical metabolism in the stress groups.

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DETECTION OF HUMAN CYTOMEGALOVIRUS (HCMV) AND EPSTEIN-BARR VIRUS (EBV) IN PERIAPICAL LESIONS

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Background: The recent use of molecular methods to detect Herpesviridae in periapical lesions has suggested that some Herpesviridae participate in the etiology of periapical lesions. Herpesviridae may cause disease as a direct result of viral infection and replication, or because of virally induced impairment of the host defence. As the type and prevalence of Herpesviridae and microorganisms in endodontic infections differ among geographic locations, the aim of this study was to investigate the presence of HCMV and EBV in Turkish patients with periapical lesions. *Methods:* Microbial samples were collected from nine symptomatic and seven asymptomatic patients with periapical lesions at the time of apicoectomy. To obtain the DNA from the samples, Qiagen® DNeasy Blood and Tissue Kit was used according to the manufacturer's instructions. To detect CMV and EBV-DNA, Fluorion® real-time PCR amplification kits for CMV and EBV were used. *Results:* HCMV-DNA was detected in four of the nine symptomatic and two of the seven asymptomatic periapical lesions. EBV-DNA was observed in two symptomatic and one asymptomatic periapical lesion. *Conclusion:* The present data suggest that HCMV or EBV infections may participate in the pathogenesis of periapical lesions.

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FREE OXYGEN RADICALS ASSOCIATED WITH GROWTH IN COELIAC DISEASE

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Background: Coeliac disease (CD) is an immune-mediated disease of gluten-sensitive individuals. Oxidative stress has been reported to play an important role in the pathogenesis of CD. The aim of this study was to investigate the frequency of the superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) polymorphisms and whether their possible association with the mutations DQA1*0501, DQB1*0201, DRB1*04 are frequent in CD. *Patients and Methods:* The study involved 56 CD patients, 35 female (62.5%) and 21 male (37.5%), with a mean age 6.66±4.18 years. Height and weight measurements of the patients were obtained to evaluate their growth and development. The correlation between the SOD and GSH-PX polymorphisms and the investigated mutations was also investigated. *Results:* SOD and GSH-PX polymorphisms were found in homozygote, heterozygote and wild-type patients. At least one of the mutations DQA1*0501, DQB1*0201 and DRB1*04 were found in 41 patients. *Conclusion:* Although the etiology of CD is not entirely clear, many mechanisms have been suggested. The retardation of growth and development in CD patients may be associated with oxidative stress and decreased antioxidant capacity.

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ASSOCIATION OF THE SNP-19 IN THE CALPAIN-10 GENE WITH TYPE 2 DIABETES PATIENTS IN TURKISH POPULATION

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Background: Calpain-10 (CAPN10) has been identified as a susceptibility gene in type 2 diabetes mellitus (T2DM) and may be implicated in insulin resistance and impaired insulin secretion. SNP-19, an insertion/deletion polymorphism consisting of two (2R) or three (3R) repeats of a 32-bp sequence, is a noncoding regions of CAPN10 and is believed to alter risk by affecting the transcriptional regulation of CAPN10. The aim of this study was to identify the effect of SNP-19 on the development of the T2DM in Turkish population. *Methods:* 169 patients with type 2 diabetes and 69 healthy controls were included. Fasting plasma glucose (FPG), fasting insulin, HbA1c and c-peptide values were obtained from patients and controls. Oral glucose tolerance test was done in healthy controls. Polymerase chain reaction-single stranded conformational polymorphism technique was used in genotyping. Chi-Square test was employed in examination of SNP genotypes relationship with patient and control and Hardy-Weinberg (HW) equilibrium within patients and control. One-Way ANOVA and Kruskal-Wallis tests were employed to obtain SNP genotype effect on biochemical phenotypes for normally and non-normally distributed phenotypes, respectively. *Results:* SNP-19 genotype frequencies for 2R/2R, 2R/3R, and 3R/3R were 27.5%, 43% and 29.5% in patients and 28.6%, 35.7% and 35.7% in controls, respectively. Both patients and control groups were in HW equilibrium. Genotype distribution was independent of patients and control groups. No genotype effect was observed on HbA1c, Homo-IR, FPG and prevalence of diabetes. *Conclusion:* No association was observed between T2DM and SNP-19 of CAPN10 in Turkish population.

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ASSOCIATION BETWEEN THE CCR5 32-BP DELETION ALLELE AND OBSESSIVE-COMPULSIVE DISORDER (OCD)

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Background: Obsessive-compulsive disorder (OCD) affects children and adults. As in most psychiatric disorders, genetic and environmental factors play an important role in the development of OCD. Chemokines and their receptors are involved in the regulation of a variety of normal functions in the brain, including those of neurodevelopment, intercellular communication and neuronal survival. The chemokine receptor 5 (CCR5) binds a number of chemokines and may play a role in the development of OCD. We prospectively investigated whether CCR5 delta 32 Insertion/deletion polymorphism account for an increased risk of obsessive-compulsive disorder. *Methods:* The present analyses are based on 48 case subjects with obsessive-compulsive disorder and 100 non-case subjects. Genotyping of the CCR5 32-nucleotide deletion polymorphism was accomplished by enzymatic amplification of genomic DNA and agarose gel electrophoresis of amplified fragments. *Results:* CCR5-delta32 genotype distribution of cases differed significantly from that of controls (p=0.043). Frequencies of CCR5 delta 32 insertion/deletion genotypes were 81% wt/wt, 7% deletion/deletion, 12% wt/deletion in the control group and 95.8% wt/wt, 0% deletion/deletion and 2% wt/deletion in the patient group. *Conclusion:* It is likely that CCR5 delta 32 insertion/deletion variants affect susceptibility to OCD.

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'NANOBACTERIA' IN THE CALCIFIED BREAST TUMOR

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Introduction: The formation of organized inorganic crystalline structures within macromolecular extracellular matrices is referred to as 'biomineralization'. The molecular basis of mineralization remains largely unknown. Recently, it has been shown that bacteria play a major role in the biogeochemical cycles for mineral formation. Biomineralizied calcifications that are formed by calcium deposits are also detected by mammogram examination. Some of the detected microcalcifications may be related to malignancy. Taken together, 'nanobacteria' or 'calcified nano-particles' are thought to be a source of malignant calcifications in breast cancers. The aim of this study was to investigate the presence of nanobacteria in breast tumor tissue. *Materials and Methods:* Initially, only medium and fetal bovine serum (FBS) were cultured to obtain and screen calcified nano-particles. Then cultures were prepared from calcified breast tumor tissue taken from 15 breast cancer patients and 15 cultures only with medium supplemented with γ-radiated FBS as a control. Presence of particles was investigated with transmission electron microscopy, microbiologic and spectrophotometric methods. *Results:* No nanobacteria-like particles were found in the samples.

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LIPID PROFILES, LIPOPROTEIN(a), OXIDIZED LDL AND ADIPONECTIN LEVELS IN PATIENTS USING CARBAMAZEPIN AND VALPROIC ACID

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Background: Epilepsy is a common chronic disorder requiring long-term therapy. Recent evidence indicates that prolonged use of antiepileptic drugs (AEDs) may modify vascular risk factors; however, the influence of AED therapy on atherosclerosis is not well-known. The aim of this study was to evaluate the effect of the use of carbamazepin or valproic acid on the risk for atherosclerosis. *Methods:* A total of 64 patients receiving valproic acid and 44 patients receiving carbamazepin were included in the study as case groups, while 48 healthy subjects were included as a control group. Cholesterol, triglyceride, LDL, HDL, lipoprotein(a), oxidized-LDL, and adiponectin levels were studied both in the case and control groups. Lipoprotein(a), oxidized-LDL and adiponectin levels were measured by ELISA. Other data including cholesterol, triglyceride, LDL and HDL levels were obtained from patient files. All statistical analyses were carried out using SPSS 11.0. *Results:* Cholesterol and LDL levels were significantly lower in the valproic acid group than in the other groups. HDL levels in the carbamazepin group were significantly higher than in the other groups. Adiponectin levels were significantly decreased in the valproic acid group. There was no significant difference between the three groups regarding triglyceride, oxidized-LDL and lipoprotein(a) levels. *Conclusion:* In this study no evidence indicating an increased risk for arteriosclerosis due to AED use was found.

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EFFECTS OF HUMAN TOOTH GERM DERIVED MESENCHYMAL STEM CELLS ON CANCER CELLS TREATED WITH ANTI-CANCER DRUGS

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Background: Tumor cells interact with their microenvironment during their development and metastasis. Mesenchymal stem cells (MSCs) are important components of the tumor microenvironment, modulating tumor growth and development in many ways. In this study, we investigated the effects of human tooth germ (HTG) derived MSC-conditioned medium (CM) on MCF-7 and SHSY5Y cells treated with anticancer drugs, doxorubicine and paclitaxel. *Methods:* Effective doses of doxorubucine and paclitaxel on MCF-7 and SHSY5Y cells were determined by MTS (cell viability assay). CM of HTG stem cells was collected when the cells reached 70% confluency. The effect of this CM on doxorubucin and paclitaxel treated MCF-7 cells was measured by MTS assay and real time PCR(analysis of apoptotic markers caspase3 and p53). *Results:* The results showed that CM of HTG stem cells increased the survival of doxorubicin and paclitaxel treated MCF-7 and SHSY5Y cells by around 30%. It was also shown that CM reduced doxorubicin and paclitaxel induced apoptosis. *Discussion:* Our findings demonsrate that CM of MSCs has a protective effect on MCF7 and SHSY5Y cells treated with doxorubucin and paclitaxel, reducing the effect of anticancer drugs. These results provide supporting evidence that MSCs play a role in the growth of tumor cells by secreting cytokines or chemokines, which increase the cell survival of tumors during anticancer therapy.

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INHIBITORY EFFECTS OF *PAEONIA SUFFRUTICOSA* ON ALLERGIC REACTIONS BY INHIBITING THE NF-KAPPAB/I KAPPAB-ALPHA SIGNALING PATHWAY AND BY SUPPRESSING THE PHOSPHORYLATION OF ERK IN AN ANIMAL MODEL AND HUMAN MAST CELLS

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Background: The root cortex of *Paeonia suffruticosa* Andrews (PSA), also known as Moutan Cortex, is known to have anti-allergic and anti-inflammatory properties. This study investigates the effect and mechanism of PSA by *in vivo* and *in vitro* methods. *Methods:* To confirm the effects of PSA extract on degranulation and histamine release, peritoneal mast cell were prepared from orally administered PSA from rat 1h prior to antigen challenge. Mast cells were isolated from peritoneal cells. The amount of histamine released, TNF-α and IL-6 were measured by ELISA assay

using mast cells. Also, an *in vivo* model of the anti-DNP IgE-mediated PCA reaction was investigated from dorsal skin sites. *Results:* Treatment with the root cortex of PSA (up to 0.4 mg/ml) showed no cytotoxicity in human mast cells. The ethanol extract of PSA (200 mg/kg) significantly inhibited the passive cutaneous anaphylaxis reaction *in vivo* and suppressed the release of histamine from rat peritoneal mast cells induced by compound 48/80. It was also found that PSA decreased the expressions of TNF-alpha and IL-6 in PMA- and A23187-stimulated HMC-1 cells. PSA also induced the inactivation of I kappaB-alpha and NF-kappaB, as well as the suppression of the phosphorylation of extracellular signal-regulated kinase (ERK). *Conclusion:* Our findings therefore suggest that PSA may be a promising compound for anti-allergic inflammation by inhibiting the NF-kappaB/I kappaB-alpha signaling pathway and by suppressing the phosphorylation of ERK.

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EXOCRINOPATHY MEDIATED BY AUTOANTIBODIES ISOLATED FROM SJOGREN SYNDROME PATIENTS' SERA

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SS(Sjögren's syndrome) autoantibodies have been proposed as pathogenic. However, its inhibitory mechanism on the M3R(muscarinic type 3 receptor) function remains unknown. Purified IgGs were obtained from the SS patients' sera(SS IgG) and tested for their inhibitory effects on the M3R function. Preincubation of the cells with SS IgG significantly decreased CICT(carbachol-induced $[Ca^{2+}]_i$ transient). In contrast, normal IgG had no effect on the CICT. The inhibitory effects of SS IgG on the CICT were analogous to the SS IgG incubation periods. We then examined whether SS IgG induces internalization of M3R. Incubation of the cells with SS IgG significantly decreased M3R expression at the membrane with a simultaneous increase in M3R detected within the cytosol. The amount of membrane clathrin, but not Gβ, was also altered after SS IgG incubation. Immunofluorescence staining further demonstrated the co-localization and subsequent internalization of M3R with clathrin following SS IgG treatment, which was prevented by pretreatment with the lysosomal inhibitor. In conclusion, these result may provide a potential mechanism for the exocrinopathy commonly observed in SS patients.

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THE EFFECT OF LYSOPHOSPHATIDIC ACID ON THE TOXICITY OF CHEMOTHERAPEUTIC AGENTS IN PROSTATE CANCER PC-3 CELLS

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Background: Docetaxel, mitoxantrone and estramustine, which have apoptotic and cell killing activity, are commonly-used agents in prostate cancer treatment. Lysophosphatidic acid (LPA) is a bioactive phospholipid that is involved in various cellular events, including cell proliferation. The aim of this study was to investigate the effect of LPA on the toxicity of docetaxel-, mitoxantrone- and estramustine-treated PC-3 cells. *Methods:* PC-3 cells were treated with different concentrations of LPA (1-15μM) in order to find the optimum dose. After the determination of the optimum LPA dose, PC-3 cells were treated with docetaxel (10 mM), mitoxantrone (0.2 μM) and estramustine (10 μM) with or without 10 μM LPA. In each group cell proliferation was determined by a commercial cell proliferation assay kit. *Results:* It was found that 10 μM LPA was the optimum dose and LPA treatment increased cell proliferation in docetaxel-, estramustine- and mitoxantrone-treated cells. *Conclusion:* We found that the cell killing activity of docetaxel, mitoxantrone and estramustine was strongly antagonized by LPA. This study suggests that the blocking of LPA secretion, which is highly expressed in cancer cells, may enhance the cell killing activity of docetaxel, estramustine and mitoxantrone in prostate cancer treatment.

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ATYPICAL *VKORC1* HAPLOTYPES IN THE OMANI POPULATION: IMPLICATIONS FOR DOSE VARIABILITY OF VITAMIN K ANTAGONIST

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Purpose: To study *VKORC1* haplotypes in the Omani population and correlate it with warfarin dose response. *Methods:* In this case control study, blood was collected from 156 Omani healthy individuals (controls) and 212 patients taking warfarin, who were on stable anticoagulation with an international normalized ratio between two and three at three consecutive follow-ups, after an informed consent. The genetic polymorphism of warfarin dose influencing loci *VKORC1* (*1, *2, *3, and *4), were studied using a PCR-based targeted genomic DNA sequencing. *Results:* The observed frequencies for *VKORC1*1, *2,*3,*4* haplotypes were 0.08, 0.28, 0.29, 0.14 respectively. Additionally, four different novel haplotypes were also found, two of which were present at a frequency above 3% in the Omani subjects. The mean warfarin daily dose (mg) was 3.5, 2.25, 8.0, and 7.0 in *VKORC1*1/*1,* *2/*2, *3/*3, and *4/*4 respectively. The mean warfarin daily dose (mg) was 4.9 in atypical A homozygotes and varied between 5.1-5.3 and 3.0-5.25 for atypical A and B compound hetrozygotes respectively. *Conclusion:* The prevalence of warfarin sensitive *VKORC1*2/*2* genotype was 9.4%. The atypical haplotypes showed warfarin sensitivity that was intermediate between *1/*1 on one hand and *3 and *4 homozygotes on the other hand.

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FETAL HEMOGLOBIN EXPRESSION IN SICKLE CELL DISEASE PATIENTS FROM OMAN: CORRELATION WITH *BCL11A, HBSIL-MYB, HBG*$_2$ SNP'S

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Background: Although Sickle cell disease (SCD) is a monogenic disorder, other genetic modifiers are known to alter the clinical phenotype. *Aim:* This study investigated SNP's in chromosomes 2, 6 and 11 that are known to be involved in modulating the fetal hemoglobin (HbF) levels in SCD patients from Oman. *Methods:* After receiving ethics committee approval, 88 consecutive SCD patients participated in the study after giving informed consent. Molecular analysis included genotyping of *BCL11A, HBSIL-MYB, HBG2* SNP's by direct DNA sequencing using Tag SNP's rs11886868, rs4671393 (BCL11A-Ch.2), rs7776054, rs9399137, rs4895441 (HBSIL-MYB-Ch.6),& rs7482144 (HBG2-Ch.11) to correlate with the HbF expression in the SCD patients. *Results:* Stepwise regression analysis of the 6 SNP's demonstrated a significant association between the BCL11A, HBSIL-MYB, HBG2 SNP's and HbF levels.

SNP	Effect size	Standard error	*p*-value
rs74821144	4.161	0.689	0.000
rs9399137	5.563	1.266	0.000
rs4671393	–1.661	0.836	0.05

Conclusion: Together, these 6 SNP's accounted for ~46% variation in the HbF levels in our SCD study participants and in part explains the clinical heterogeneity seen in these patients. Three SNP's, one each in chromosomes 2, 6 and 11, namely rs4671393, rs9399137 and rs74821144 respectively, demonstrated the strongest effect on HbF levels in this SCD patient cohort.

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PHARMACOECONOMIC AND PHARMACOGENOMIC INFLUENCE OF BIOMARKERS IN CANCER THERAPY

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Background: The average yearly incidence for all cancers reported by the Oman National Cancer Registry is 882 new patients per year and has been steady over the past decade. Aims: This study aimed to investigate the pharmacogenomic influences of biomarker based cancer therapy and its cost implications. *Methods:* The study analyzed usage of trastuzumab, cetuximab and rituximab in breast cancer, colorectal cancer and non-Hodgkin's lymphoma, respectively. Patient numbers treated in each category were compared and correlated with the usage of respective monoclonal antibody and cost of therapy. *Results:* The average costs for trastuzumab targeting HER2 receptor, cefuximab targeting epithelial growth factor receptor (EGF] with unmutated KRAS and rituximab targeting CD20 ranged from 30000-70000 US dollars for breast cancer treatment, 2000-4000 US dollars for colorectal cancer treatment and 6000-9000 US dollars for non-Hodgkin's lymphoma treatment, respectively. There was a 100% increase in cetuximab and 57% increase in rituximab but no increase in trastuzumab usage in 2010. *Conclusion:* Increased use of cetuximab and rituximab is cost effective considering their role as standard of care. Pharmacoeconomics of newer monoclonal antibody based treatments for these biomarkers like panitumumab, for HER2 receptor and ofatumumab targeting CD20 needs further evaluation in comparison to the existing therapies.

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DETECTION OF TYPE 1 COLLAGEN ALPHA 1 GENE -1997 G/T POLYMORPHISM IN POSTMENOPAUSAL OSTEOPOROTIC TURKISH WOMEN

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Background: Osteoporosis is a disease characterised by reduced bone mass, which leads to microarchitectural deterioration of bone tissue and increased fracture risk. Genetic factors play an important role in the pathogenesis of osteoporosis. Type I collagen is the most abundant protein of the bone matrix and the collagen type I alpha 1(COLIA1) gene has been considered one of the most important candidate genes for osteoporosis. *Methods:* In this study we analyzed the relationship between -1997 G/T single nucleotide polymorphisms at the COL1A1 regulator region. We analyzed the DNA of 79 osteoporotic postmenopausal patients and 43 healthy postmenopausal Turkish women. All the subjects were genotyped by using polymerase chain reaction–restriction fragment length polymorphism. *Results:* Patient genotypes were: TT 26%; GT 61% and GG, 12%, respectively, whereas controls were TT 40%, GT 44% and GG 16%, respectively. The TT genotype of the -1997 G/T polymorphism in the COLIA1 gene was significantly associated with the risk of developing osteoporosis in our cases. *Conclusion:* Our results suggest an important role of the COLIA1 gene in osteoporosis. Mutations in the regulatory region within a gene may lead to different levels of gene expression and thus the -1997 G/T polymorphism may be a valuable genetic marker.

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GENETIC POLYMORPHISMS IN THE ESTROGEN RECEPTOR ALPHA GENE IN TURKISH PATIENTS WITH FAMILIAL PROSTATE CARCINOMA

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Background: Polymorphisms of estrogen receptor (ER) alpha gene in the estrogen metabolism pathway may be involved in the risk of developing prostate carcinoma. We evaluated the association between genetic polymorphisms in the ER alpha gene and the risk of developing familial prostate carcinoma. *Methods:* A total of 34 cases with prostate carcinoma whose first-degree relatives had prostate carcinoma and 30 healthy age-matched male controls were enrolled. The genotype of ER alpha gene was analyzed. *Results:* Among the controls, the ER alpha *Pvu*II genotypes of C/C, C/T and T/T were observed in 37%, 26%, and 37%, respectively, whereas the respective percentages among the patients were 18%, 41%, and 41%. Among the controls, the ER alpha *XbaI* genotypes of G/G, G/A and A/A were observed in 33%, 37%, and 33%, respectively, whereas the respective percentages among the patients were 12%, 47% and 41%. The C/C genotype of the PvuII site and G/G genotype of the *XbaI* site in the ER alpha gene were associated significantly with the risk of developing prostate carcinoma. *Conclusion:* Polymorphisms of the ER alpha gene in the estrogen metabolism pathway are associated significantly with the risk of familial prostate carcinoma.

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EVALUATION OF MOLECULAR ASPECTS OF MALE INFERTILITY IN THE LATVIAN POPULATION

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Background: Some aspects of male infertility may be explained analysing Y-chromosomal and autosomal genes that are involved in the process of spermatogenesis. The aim of this study was to investigate molecular-genetic aspects of idiopathic male infertility in the Latvian population. The study included 100 idiopathic infertile men (infertile group) and 153 healthy individuals (control group). *Methods:* Y-chromosome microdeletions were analyzed by two multiplex PCR. Y chromosome haplogroups (Y-Hg) were detected using appropriate biallelic markers. *CFTR* gene mutation, delF508,

R117H and IVS8 polymorphism analysis was performed by PCR and followed by RFLP and sequencing. *Results:* Y-chromosome microdeletions were detected in 5% (5 cases of 100: 3 cases AZFc; 2 cases AZFa+b+c). Y-Hg analysis showed that Hg N3a1 and Hg R1a1 were less frequent in the infertile group compared to the control group, however Hg K* was predominantly found in the infertile group ($p<0.001$). Analysis of *CFTR* gene mutation, delF508, R117H and IVS8 polymorphism did not confirm association with infertility. *Conclusion:* The frequency of Y-chromosome microdeletions in males with idiopathic infertility was 5%. Y chromosome Hg K* may be associated with male infertility. *CFTR* gene mutations, delF508, R117H and IVS8 polymorphisms do not affect the process of spermatogenesis directly.

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ROLE OF C-KIT EXON 11 MUTATIONS AND HLA-G POLYMORPHISM IN LEUKEMIA IN NORTHERN INDIA

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Background: C-Kit gene is a receptor tyrosine kinase class III that is expressed by early hematopoietic progenitor cells and plays an important role in hematopoietic stem cell proliferation, differentiation and survival. The 14bp insertion / deletion of the HLA-G gene has been shown to play an important role in various types of neoplasia. *Methods:* PCR-SSCP followed by direct DNA sequencing. *Results:* Of 31 leukemia patients, 18 were male and 13 were female with ages ranging from 2 to 65 years. The mean age of patients was 32.3 years and SD±1.03. A total of nineteen mutations were detected in six patients that include Lys550Asn, Tyr568Ser, Ile571Leu, Thr574Pro, Gln575His, Tyr578Pro, Asp579His, His580Gln, Trp582Ser, Arg586Thr, Asn587Asp and Arg588Met and novel point mutations at codons Ile563Lys, Val569Leu, Tyr570Ser, Ile571Thr and Pro577Ser. Ile571Leu and Trp582Ser substitution was found in two independent cases. The frequency of I/I, I/D and D/D genotype in patients was 19.37%, 48.38% and 32.25% and in controls 24%, 46% and 30% respectively. *Conclusion:* These observations suggest that mutations in exon 11 of the c-kit gene might represent useful molecular genetic markers for leukemia while there is no significant association of HLA-G polymorphism in leukemia.

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RESPONSE TO ATHEROGENIC DIET IN SYRIAN HAMSTERS

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Background: Cost-effective small animal models of atherosclerosis and data on the use of atherogenic diets in non-transgenic animals is important for testing anti-atherogenic therapies. *Methods:* We used Syrian hamsters (*Mesocricetus auratus*) on high cholesterol/high dietary fat atherogenic diet (the Paigen diets' modification) as a model for atherosclerosis. Animals were sacrificed after 14 weeks' diet and histological examination of their organs and tissues was performed. *Results:* The body mass was significantly lower in the test group compared with the control. A severe hepatomegaly was detected in all hamsters in the test group. The maximum liver mass was up to 25% of the whole body mass. Histological features of severe hepatosis were observed. Concentric myocardial hypertrophy was found in the test group. *Conclusion:* Syrian hamsters strongly responded to atherogenic diet and can be used as a model for testing anti-atherogenic therapies.

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PREPARATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO ADIPONECTIN FOR ITS MEASUREMENT IN HUMAN SERUM

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Background: Low levels of adiponectin are associated with increased prevalence of cardiovascular disorders, including ischemic heart disease. *Methods:* Monoclonal antibodies were prepared against purified recombinant human adiponectin. The reactivity of these monoclonal antibodies on Western blot analysis was restricted to a monomer and

trimer form of adiponectin. With these monoclonal antibodies, we developed a novel ELISA system. The specificity of this system was verified by analysis of serum fractions separated by gel-filtration chromatography. *Results:* The developed ELISA system was specific to high-molecular-weight (HMW) multimer adiponectin. The HMW adiponectin concentrations measured with this novel ELISA system were: 11.01±2.58 mkg/ml (mean±SD) in healthy women and 8.42±1.59 mkg/ml in healthy men. The serum HMW adiponectin concentration was lower in patients with ischemic heart disease than in healthy controls; 6.01±2.73 mkg/ml *vs*. 8.42±1.59 mkg/ml (p=0.015) in men and 5.79±2.98 mkg/ml *vs*. 11.01±2.58 mkg/ml (p=0.0003) in women, respectively. *Conclusion:* The developed, novel ELISA system, specific for the HMW form of adiponectin may be used for diagnostic clinical examinations in patients with ischemic heart disease.

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REGULATION OF ADIPOSE TISSUE DERIVED STEM CELLS DIFFERENTIATION BY RECOMBINANT ADIPONECTIN

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Background: Adipose-derived stromal/stem cells (ASCs) can be obtained from the lipoaspirate of individual donors for regenerative medicine applications. Previously we have shown that adiponectin levels are lowered in some diseases. The aim of this study was to investigate the ASC differentiation in the presence of the adipogenic medium with insulin and in the presence of human recombinant adiponectin. *Methods:* ASC were isolated from human subcutaneous lipoaspirate. Flow cytometry and immunocytochemical staining were used for ASC characterization. *Results:* The ASC population was $CD34^-$, $CD44^+$, $CD90^+$, $CD105^+$ and HLA-DR^{low}. In 1% of ASCs the alpha-fetoprotein receptor (embryonic cell marker) was found and 7% ASCs possessed SP phenotype. When ASCs were cultured in standard medium, 1% of ASCs were spontaneously differentiated into adipocytes. After three weeks culturing in adipogenic medium, 87.5% ASCs acquired the typical morphology of lipid-laden cells containing intracellular lipid droplets; however, in the presence of adiponectin, only 4.3% of ASCs differentiated in adipocytes. When ASCs were cultured in the presence of adiponectin without adipogenic medium, the cells did not differentiate into adipocytes. *Conclusion:* The lower levels of adiponectin in patients with some diseases may alter ASC differentiation after transplantation.

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IS THERE ANY CORRELATION BETWEEN IL-10 POLYMORPHISMS AND BIPOLAR DISORDER?

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Background: The neurobiology of bipolar disorder is not completely understood. Cytokines have received increasing attention as potential mediators of the interaction with immune and neuroendocrine system and specific pathways involved in mood, energy and activity control. Previous reports have suggested the association of mania and bipolar depression with a pro-inflammatory state. Genes encoding cytokines are highly polymorphic and single nucleotide polymorphisms, associated with increased or reduced cytokine production, have been described. The aim of this study was to define the genetic immunological scenario associated with bipolar disorder. *Methods:* Eighty-two bipolar Turkish outpatients affected by bipolar disorder, and 298 healthy controls were enrolled into the study. We analyzed allele and genotype distribution of -592C/A, -819C/T and -1082G/A IL-10 promoter polymorphisms by Polymerase Chain Reaction Sequence Specific Primers technique. *Results:* There were no significant differences in genotype/allele distribution of IL10 -1082G/A, -819C/T, -592C/A between patients with bipolar disorder and controls (p≥0.05). We observed different genotype frequencies of IL-10 polymorphisms in the bipolar patients and controls. In particular, bipolar patients were characterized by a high percentage of the GA genotype for -1082G/A locus (p=0.029), and the GCC/ACC genotype included all three polymorphic loci (p=0.006). On other hand, the ACC/ATA genotype was detected lower in bipolar patients than controls (p=0.028). *Conclusion:* Our results showed that IL-10 promoter polymorphisms might be associated with bipolar disorder in Turkish patients.

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INTERSECTINS AND DISEASES: CLUES FROM THEIR FUNCTIONAL CHARACTERISTICS

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Background: Adaptors of intersectin (ITSN) family serve as scaffolds for multiprotein complex assembly during clathrin-mediated endocytosis. Abnormalities of ITSN1 expression have been associated with endocytic brain anomalies reported in Down syndrome and at the early stages of Alzheimer's desease. ITSN2 was proposed to be a predictive marker for breast cancer. *Methods:* Immunoprecipitations, pull-down experiments, determination of subcellular localization by direct and indirect immunofluorescence, mass-spectrometry and RT-PCR were used in this study. *Results:* We identified 17 alternative splicing events affecting ITSN1 pre-mRNA and found an alternative transcription initiation site in the fifth intron of ITSN1 gene. ITSN1 isoforms differed in their domain organization, interaction with protein partners, localization in different tissues and stages of development. Using mass spectrometry analysis and *in silico* prediction, we identified 11 novel protein partners of ITSN1 and ITSN2. These proteins are involved in signal transduction, actin and tubulin cytoskeleton formation, endocytosis, cell adhesion and migration. Using a *Xenopus* animal model, we demonstrated the role of ITSN2 in the coordinated changes of actin cytoskeleton during early embryonic development. *Conclusion:* The results demonstrated the complex regulation of ITSN adaptor proteins that provide interfaces for the interaction between basic endocytic machinery and different cell processes such as signalling, sorting and cytoskeleton rearrangements.

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PRODUCTION OF MEASLES TRUNCATED HEMAGGLUTININ PROTEIN IN PROKARYOTIC CELLS FOR PREPARING RECOMBINANT VACCINE

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Background: The Measles virus, a member of Paramyxoviridae family with a non-segmented negative strand RNA, has two surface glycoproteins: H and F. H is responsible for the cell tropism, receptor binding, and hemagglutinating activity. It has several conformational epitopes for induction of immune system to produce neutralizing antibody. Recent studies confirmed the protective activity of some linear epitopes. In this respect, we chose a specific sequential hemagglutinin epitope for cloning and expression in prokaryotic cells *Methods:* Measles Virus genomic material was extracted from infected Vero cells. Two third of 5' end of the H gene was amplified by designed primers containing secreted signal for production of truncated protein during RT-PCR reaction and inserted into the cloning and expression vector respectively. Finally the BL21 strains of *E. coli* were transformed with expression vector in order to produce recombinant protein. *Results:* The cloned fragment of Measles virus H gene was confirmed by sequencing. SDS-PAGE and Western-blot using goat polyclonal antibody against Measles Virus indicated the presence of proper amount of recombinant H protein. *Conclusion:* According to our study, after neutralizing assay these clones may be further used in the development of a recombinant vaccine against the Measles virus.

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ANALYSIS OF THE INFLUENCE OF BRAF V600E MUTATION ON THE METHYLATION STATUS OF MLH1 AND MGMT AND MICROSATELLITE INSTABILITY IN PATIENTS WITH THYROID CANCER

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Background: Thyroid cancer is the most common cancer of the endocrine system and its incidence has increased very rapidly in recent years. The BRAF mutation is the most commonly occurring known genetic alteration in thyroid cancer. This mutation can cause hypermethylation of some repair genes, such as MLH1 and MGMT, and consequent microsatellite instability (MSI). The aim of the present study was to evaluate the effects of BRAF V600E mutation on methylation status of MGMT and MLH1 and MSI. *Methods:* Tumor tissue samples were taken from 85 patients harboring the BRAF V600E mutation. Genomic DNA was amplified by polymerase chain reaction (PCR) using seven markers.

Methylation analysis of MGMT and MLH1 was done by means of methylation specific (MSP)-PCR. *Results:* Amongst the patients, 70% showed MSI. We observed that MGMT and MLH1 promoter hypermethylation was a frequent occurrence in MSI (66%). *Conclusion:* These data suggest that this silencing mechanism plays a major role in the inactivation of these genes and thus contributes to the development of thyroid cancer. A significant correlation of MLH1 and MGMT hypermethylation with MSI was found in these tumors.

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EFFECTS OF CAFFEIC ACID PHENETHYL ESTER (CAPE) ON SOME BIOCHEMICAL AND HISTOPATHOLOGICAL PARAMETERS IN SELECTED TISSUES OF DIABETIC RATS

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Background: Phenolic compounds are commonly used as food additives and form the active ingredients of folk medicines due to their antioxidant, anti-inflammatory and anti-carcinogenic effects. In this study, the effects of CAPE on diabetes-damaged liver function and integrity of liver, pancreas, kidney, testis and heart were investigated. *Methods:* Diabetes was induced by administrating 45mg/kg streptozotocin (*i.p.*) to the rats. CAPE treatments were performed for 60 days and blood and various tissues were analysed. Histopathological changes were graded as absent (0), mild (1), medium (2), and severe (3), according to the severity of alterations. *Results:* CAPE treatments considerably lowered plasma glucose concentraion in CAPEI and CAPEII groups. Insulin levels of all CAPE treated groups were 2-fold higher than those in the diabetic group. CAPE administration significantly reduced the cholesterol and triglyceride concentrations elevated by diabetes. Diabetes-increased plasma ALT activity was decreased to the control level by all three CAPE treatments. In comparison with control, histopathological alterations in liver, pancreas, kidney, testis and heart tissues of diabetic rats were normalized in various degrees with CAPE treatments. *Conclusion:* CAPE treatment was able to significantly lower hyperglycemia and reverse some damage in tissues of diabetic rats. CAPE applications might be considered in treating hyperglycemia and tissue complications in diabetic patients.

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THE EFFECT OF ACETYLYSALICYLIC ACID (ASA) ON PLASMA ARGINO-VASOPRESSIN (AVP) LEVELS IN TYPE II DIABETICS

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Background: Acetylsalicylic acid (ASA) irreversibly blocks the platelet cyclo-oxygenase enzyme system, preventing formation of thromboxane A2 and inhibiting platelet aggregation for the life of the affected platelet. AVP is a pleiotropic peptide which affects a wide range of peripheral and centrally regulated functions in order to conserve water in the kidney. This study aimed at investigating the importance of ASA on osmoregulation and glycemic control in diabetic rats. *Methods:* Twenty four rats were randomly divided into four groups: Control(I), ASA Control(II), Diabetic(III) and ASA Diabetic(IV). Diabetes was induced by STZ treatment (30 mg/kg, two injections) in obese rats. Plasma AVP levels were analyzed by ELISA. Serum electrolytes (Na, K, Cl), creatinine, albumin and total protein levels were analyzed by an auto-analyzer. *Results:* At the end of the study ASA treatments increased the serum AVP levels (2-fold) in diabetic rats. Also, ASA treatments decreased the fasting blood glucose levels in diabetic rats. Nevertheless, sodium, creatinine, albumin and total protein levels in plasma were also decreased by ASA. *Conclusion:* The hypoglycemic effect of ASA can be attributed to increase in blood volume by AVP levels. This may be a new approach in the efficacy of ASA in reducing the fasting blood glucose levels in diabetics.

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ANTIBIOTIC RESISTANCE AND PHYLOGENETIC PATTERNS IN UROPATHOGENIC *ESCHERICHIA COLI*

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Background: Escherichia coli is the most frequent agent of urinary tract infections (UTIs). The aim of this study was to assess whether *Escherichia coli* phylogenetic groups were

associated with the level of antibiotic resistance in community-acquired bacteriuria. *Methods:* Between June and September 2010, urine specimens were collected from 1000 patients, all with clinically diagnosed UTIs, attending Mottahri and Pymanieh Hospitals in Jahrom, Iran. Specimens were examined for different uropathogens using standard microbiological procedures. A total of 60 uropathogenic *E. coli* were isolated and characterized. Triplex PCR was used to classify the phylogenetic groups, and susceptibility testing was performed by the disc diffusion method. Data were assessed by statistical analysis. *Results:* Overall, 70% of the sixty *E. coli* isolates belonged to phylogenic group D, 23.3% to A, and 6.7% to B1. The highest sensitivity and resistance to antibiotics were observed with nitrofurantoin (97.6%) and trimethoprim-sulfamethoxazole (48.3%), respectively. Antibiotic resistance to one and more than three antibiotics, respectively, was most frequent in group A (35.7%/42.9%), followed by D (35.7%/38.1%), and B1 (50.0%/0%). *Conclusion:* Phylogenetic group D was predominant in *E. coli* community-acquired bacteriuria and statistical analysis showed that phylogenetic groups were not associated with the level of antibiotic resistance ($p>0.05$).

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ASSOCIATION BETWEEN G/A455 FIBRINOGEN POLYMORPHISM AND PREMATURE CORONARY ARTERY DISEASE IN IRANIAN PATIENTS

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Introduction and Objective: Several studies have shown an association between plasma fibrinogen levels, coronary heart disease premature myocardial infarction (PMI) and stroke. Despite intensive investigations, there is no clear evidence for an effect of beta-fibrinogen gene polymorphism on plasma fibrinogen levels and myocardial infarction. The aim of this study was to determine the association of the G/A455 polymorphism of beta-fibrinogen gene with plasma fibrinogen levels and myocardial infarction. *Patients and Methods:* We conducted a case-control study consisted of 100 patients with premature ischemic heart disease (IHD) with age below 50 years and 100 volunteer subjects without evidence of IHD as control group. The two groups were matched according to age, gender and smoking status. Plasma fibrinogen levels were measured by Clauss clotting time method and G/A455 mutations were identified by PCR followed by HaeIII restriction enzyme digestion of amplified DNA and electrophoresis. *Results:* Plasma fibrinogen levels were higher in the patients with premature myocardial infarction than in control subjects ($p=0.001$, odds ratio=2.12). In the control group, the plasma fibrinogen levels were significantly higher in smokers than non-smokers ($p=0.032$). However, there was no association between 455G/A polymorphism, plasma fibrinogen levels ($p=0.65$) and incidence of PMI ($p=0.663$). *Conclusion:* Increased plasma fibrinogen level was associated with cigarette smoking and risk of PMI but no association was found with G/A455 polymorphism.

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PEPTODOGLYCAN FROM *STAPHYLOCOCCUS AUREUS* INCREASES *MUC5AC* GENE EXPRESSION *VIA* THE RSK1-CREB PATHWAY IN HUMAN AIRWAY EPITHELIAL CELLS

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Background: Respiratory tract exposure to viruses, air pollutants or bacteria pathogens can lead to pulmonary diseases. The knowledge on the molecular mechanisms of mucous overproduction caused by these pathogens is necessary for the development of new therapeutic strategies. It is well established from *in vitro* data that the overexpression of MUC5AC is induced by peptidoglycan (PGN) derived from *Staphylococcus aureus*. However, the mechanisms by which PGN activates *MUC5AC* gene expression in the airway remain unclear. The aim of this study was to identify the mechanisms of PGN-induced *MUC5AC* gene expression. *Methods and Results:* We found that PGN could induce *MUC5AC* and MUC8 gene expression in a time- and dose-dependent manner. Moreover, activation of ERK1/2 and JNK increased after treatment of cells with PGN, whereas phosporylation of p38 was undetected. Of these MAPKs, pharmacological inhibition of ERK1/2 decreased PGN-induced MUC5AC gene expression. In addition, we examined the activation of p90 ribosomal S6 kinase 1 (RSK1) as a downstream signaling target of ERK1/2 in PGN signaling. The activation of RSK1 was prevented by pretreatment with PD98059. We also found that RSK1 mediated the PGN-induced phosphorylation of cAMP response element-binding protein (CREB) and the transcription of *MUC5AC*. Furthermore, the cAMP-response element (CRE) in the *MUC5AC* promoter appeared to be important for PGN-induced *MUC5AC* gene expression in NCI-H292 cells. *Conclusion:* These results give additional information about the intracellular signal transduction pathway involved in mucin production during inflammation.

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PROMOTER HYPERMETHYLATION OF *CDKN2A* GENE IN BULGARIAN PATIENTS WITH LARYNGEAL SQUAMOUS CELL CARCINOMA

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Background: An important mechanism for gene inactivation is the *de novo* methylation of normally unmethylated CpG islands within the promoter region of carcinoma-associated genes. Such a gene involved in laryngeal carcinogenesis is *CDKN2A*, localized on chromosome 9p21. It is a tumor-suppressor gene which encodes a cyclin-dependent kinase inhibitor with an important role in the regulation of the G_1/S cell-cycle checkpoint. Hypermethylation of *CDKN2A* promoter is reported in 9-47% of laryngeal tumors. *Methods:* Genomic DNA was extracted from 50 laryngeal fresh-frozen tumor tissues and was bisulfate-converted. Promoter hypermethylation of *CDKN2A* gene was analyzed by methylation-specific polymerase chain reaction (MSP) using primer sets specific for unmethylated and methylated sequences. *Results:* MSP analysis demonstrated the hypermethylation of *CDKN2A* gene in 20 (40%) patients with laryngeal cancer. A significant increase of *CDKN2A* promoter hypermethylation was observed in patients with an early stage cancer (T1-T2) (p=0.017). Epigenetic changes were more frequent in specimens from younger patients (≤60 years of age) compared to older ones, but the differences were not statistically significant. *Conclusion:* Our results indicate that epigenetic modifications of *CDKN2A* gene are implicated in a significant proportion of cases with laryngeal carcinoma, especially at the early stages of its development.

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TRANSCRIPTION PROFILING OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Background: Head and neck squamous cell carcinoma (HNSCC) is one of the most prevalent tumour types characterized by a high mortality. The poor prognosis results from a lack of markers of early stages of the disease. Here we present results of a whole genome transcription profiling of 101 patients with HNSCC. *Methods:* Three snap-frozen samples were collected from each patient, a sample of tumour, peritumoural tissue and normal bucal tissue. The patient-matched RNA samples analyzed by microarray allowed us to extract individual related variations of the transcriptome. *Results:* Clustering analysis of the data revealed a strong contrast between normal and tumour tissues together with sub-classification of peritumoural tissues. We further distinguished keratinizing and non-keratinizing tumours as two separate clusters. We detected 1500 differentially expressed transcripts between tumours and normal tissues. We observed genes related to extracellular matrix or its remodelling, specific chemokines and cytokines. We detected deregulated KEGG pathways associated with DNA replication, cell cycle, cytokine interaction, immune response and several signalling pathways. The overall metabolism was down-regulated in the tumours. *Conclusion:* The presented data of large-cohort whole-genome transcription profiling reveal several marker genes distinguishing tumour and normal tissues. The results are interpreted in the broad scale of metabolic, regulatory and signalling pathways.

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THE MOLECULAR-GENETIC DETERMINANTS OF CARDIORENAL COMPLICATIONS IN PATIENTS WITH ARTERIAL HYPERTENSION IN THE KAZAKH POPULATION

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Aim: To define the involvement of polymorphisms C344T of gene CYP11B2, Leu10Pro of gene TGF-β1 and C677T of gene MTHFR in the development of cardiorenal complications in patients with arterial hypertension (AH) in the Kazakh population. *Patients and Methods:* A total of 142 patients with AH were surveyed, with mean age 51.8±4,7 years, blood pressure average 165.1±1.58/104.6±1.02 mm Hg, duration of

AH 10.4±3.5 years. The control group included 119 healthy Kazakhs of comparable age and gender. The genotyping was performed by the restriction fragment length polymorphism method. *Results:* It was established that the CC-genotype of the CYP11B2 gene is associated with concentric type remodeling and disturbance of diastolic function of the left ventricle (LV). The genotype Pro/Pro of the TGF-β1 gene was associated with the development of concentric hypertrophy of the LV and with augmentation of thickness, a complex of intim/media of the common carotid artery. An association of genotype Pro/Pro of gene TGF-β1 with depression of rate of a glomerular filtration less than 60 ml/min/1.73m^2 was found. The frequency of T677T genotypes of the MTHFR gene was significantly higher among AH patients with microalbuminuria. *Conclusion:* The essential influence of polymorphisms C344T of CYP11B2, Leu10Pro of TGF-β1 and C677T of MTHFR genes on remodeling of cardiovascular system and kidneys in patients with AH is established in the Kazakh population.

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CURCUMIN INHIBITS CANCER CELL MIGRATION AND INVASION IN SQUAMOUS CELL CARCINOMA OF THE TONGUE THROUGH DOWN-REGULATION OF MMP10

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Background: Curcumin is a natural polyphenol. Its anticancer effect has been σηοςν in various cancer types, including head and neck squamous cell carcinoma. Our study hypothesized that curcumin may suppress the invasiveness of tongue squamous cell carcinoma (TSCC) through the down-regulation of matrix metalloproteinase 10 (MMP10). *Methods:* mRNA-microarray analysis was performed on HN21B, in response to curcumin. Immunohistochemical staining of MMP10 was performed on a tissue array. MMP10 expression levels were verified by qRT-PCR, Western blotting and immunohisto-chemical staining on TSCC cell lines. The effect of curcumin on TSCC cell lines was assessed by wound healing, adhesion, migration and invasion assays. *Results:* Microarray analysis showed that MMP10 was down-regulated by 2.36-fold in HN21B in response to curcumin. In 13 out of 20 cases (65%), the TSCC tissue showed higher MMP10 expression than the adjacent normal tissue. Curcumin treatment of TSCC cell lines resulted in down-regulation of MMP10 gene and protein expression level in a dose-dependent manner. Curcumin also suppressed adhesion, migration and invasion of TSCC cell lines. *Conclusion:* Curcumin treatment reduced MMP10 expression level in TSCC cell lines. This correlated with the reduction of invasiveness of TSCC cells. Application of curcumin as a chemotherapeutic agent warrants further investigation.

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CHARACTERISTICS OF CARBONYL DERIVATES OF BLOOD PROTEINS IN PATIENTS WITH CHRONIC PYELONEPHRITIS

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Early studies have demonstrated the activation of lipid peroxidation in the erythrocytes and have suggested the development of carbonyl stress of proteins in the plasma and erythrocytes in chronic pyelonephritis patients. This study examined the carbonyl derivative content in blood plasma and erythrocytes in patients with chronic pyelonephritis. A total of 33 patients with verified acute chronic pyelonephritis and 20 healthy donors were included in the study. The carbonyl derivatives of proteins were examined by using a reaction test with 2,4-dinitrophenyl hydrazine. A significant accumulation of carbonyl derivatives of proteins was shown in blood plasma. There was an increase of ketone- and aldehyde-dinitrophenylhydrazones of basic classes by 4 and 4.8 times, respectively, in patients compared to controls (p<0.001). The content of ketone- and aldehyde-dinitrophenylhydrazones of neutral classes were higher by 3.86 and 6 times, respectively, in patients compared to controls (p<0.001). Persistency of oxidative modified proteins in the patients' blood induced a broad spectrum of molecular damage depending on the type of proteins involved in the carbonylation process. Carbonyl stress, developing at the same time with other metabolic disorders, aggravated the influence of alternative mechanisms in renal chronic inflammatory pathology.

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DETERMINATION OF EXTRACELLULAR NUCLEIC ACIDS CONTENT IN BLOOD AND URINE OF PATIENTS WITH TUBULOPATHY AND GLOMERULOPATHY

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Research during the last 10-15 years has established the interrelation of the amount and changes of the contents of

extracellular circulating nucleic acids (cirNA) with the development of some pathological processes. This study examined the content of cirNA in the blood plasma, erythrocytes and urine in 33 patients with chronic pyelonephritis, 17 patients with glomerulonephritis, 20 patients with interstitial nephritis and 32 healthy donors. Plasma RNA concentration in pyelonephritis patients was decreased 7.5-fold compared to the controls, whereas RNA in erythrocytes and urine was increased. DNA concentration in the erythrocytes and urine of pyelonephritis patients was decreased. The level of DNA decreased in the blood of glomerulonephritis patients. In these patients, DNA and RNA urine concentrations were higher 1.45- and 6.62-fold, respectively compared to the controls. The content of RNA and DNA in the erythrocytes, blood plasma and urine were increased in the patients with interstitial nephritis. Our results demonstrated the different pattern of cirNA content changes in blood and urine of patients with tubulopathy and glomerulopathy. This may be caused by the diverse range of cell sources of cirNA, the efficient elimination of acids and the nephron functional condition.

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DOES RESTRICTION OF AMINO ACIDS OTHER THAN METHIONINE AFFECT TELOMERE LENGTH AND TELOMERASE ACTIVITY IN AGING RATS?

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According to the replicative theory, aging has been attributed to the shortening of telomeres with each cell cycle. Calorie restriction is the most powerful experimental manner to slow down aging. Recently, it was suggested that instead of calorie restriction, variations in the proportions of some dietary components might also affect longevity. The present study was undertaken to determine the influence of amino acids other than methionine on aging. The telomere length and telomerase activity were evaluated in the liver of the aging male Wistar rats that were fed either with normal diet (ND) or a 40% protein restricted diet, except for methionine (PREMD), for 4 months. Although liver telomerase activities of both 8- and 16- month old rats receiving either ND or PREMD seemed to have some variations, the differences were not significant. Comparison of the telomere length of 8- and 16-month old rats receiving ND showed that 16-month old rats have 36% shorter telomeres ($p<0.05$). 16-month old rats receiving PREMD did not exhibit significantly different telomere length than the 8-month old rats receiving the same diet. Our results indicate that the restriction in dietary levels of the amino acids other than methionine may have some preventive contribution to the telomere length but, do not have any effect on telomerase activity.

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EFFECT OF MELATONIN AND LACTOFERRIN ON ODONTOGENIC DIFFERENTIATION OF HUMAN TOOTH GERM STEM CELLS

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Background: Melatonin is known to regulate a variety of physiological processes including the control of circadian rhythms, regulation of seasonal reproductive function and regulation of body temperature. Lactoferrin is an iron-binding glycoprotein endowed with multiple functions, including non-specific immune-defence against pathogens, immuno-modulatory activity and regulation of cell growth. It was suggested that melatonin and lactoferrin may have an influence on tooth formation and growth. In this study, we tested melatonin and Lactoferrin on odontogenic differentiation of human tooth germ stem cells (HTGSCs). Method: HTGSCs with mesenchymal stem cells were seeded in 6 well and 24 well plates followed by addition of odontogenic differentiation medium, with and without melatonin 50 nM and lactoferrin 50 μg/ml. Differentiation was evaluated by measuring the level of alkaline phosphatase (ALP) activity and dentin-sialophosphoprotein (DSPP) expression. *Results:* The data demonstrated that melatonin and lactoferrin increase odontogenic differentiation of HTGSCs significantly, which was confirmed by ALP assay and immunocytohemistry analysis. When lactoferrin and melatonin were added to the cells in combination, they increased the differentiation to a lesser extent than that if they were used alone. *Discussion:* Pulpa or dentin regeneration is a challenging task after root canal treatment. Many tooth filling materials show toxic effects on dental pulp cells, reducing the level of regeneration. Our results suggest that both lactoferrin and melatonin might have therapeutic potential in pulpa regeneration and recovery of dentin tissue.

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COMBINED EFFECTS OF APOLIPOPROTEIN E AND PCSK9 R496W VARIANTS IN CORONARY HEART DISEASE

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Background: Proprotein convertase subtilisin-like kexin type 9 (PCSK9) plays a key role in plasma cholesterol metabolism by mediating the expression of low-density lipoprotein (LDL) receptors. ApolipoproteinE encoded by apoE gene serves as a ligand for LDL receptor and functions in the removal of remnants of chylomicron and VLDL in plasma. *Methods:* We investigated the effects of the PCSK9 R496W and ApoE polymorphisms in 62 patients with coronary heart disease (CHD) and 51 healthy controls by PCR-RFLP method. *Results:* ApoE4 and ApoE2 allele frequencies were higher in the patients than in the controls, but not significantly. T496 frequency was higher in the controls (p=0.001, OR:0.464, 95% CI:0.279-0.772). ApoE2-C496 (p=0.010, OR:2.122, 95% CI:1.171-3.848) and ApoE4-C496 (p>0.05) alleles together were higher in the patients, while ApoE2-T496 (p=0.001, OR:0.441, 95% CI:0.258-0.754) and ApoE4-T496 (p=0.006, OR:0.465, 95% CI:0.259-0.834) alleles together were lower in the patients. *Conclusion:* These results suggest that the combined presence of the PCSK9 C and non-Apo E3 alleles (E2 and E4) present an increased risk for the development of CHD. The protective effect of PCSK9 T496 allele was conserved and the detrimental effect of PCSK9 C496 allele was strengthened even in the presence of mutant ApoE alleles in CHD. It can be speculated that there might be gene-gene interactions among PCSK9 and ApoE polymorphisms.

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EVALUATION OF THE RELATIONSHIP BETWEEN DEGREE OF VALVE DAMAGES CAUSED BY RHEUMATIC HEART DISEASE WITH INTERFERON GAMMA 874 T/A POLYMORPHISM

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Background: IFN-γ may be responsible for the increased valvular fibrosis and calcification in the pathogenesis of rheumatic heart disease (RHD). However the role of IFN-γ genetic variant in RHD has not been studied. The aim of this study was to investigate the possible relationship between the IFN-γ gene polymorphisms and RHD in the Turkish population. *Methods:* IFN-γ 874 polymorphisms were determined with Amplification Refractory Mutation System PCR in 152 RHD patients and 151 healthy controls. Patients were classified into two groups; the first group consisted of patients with severe valve damage (SVD) and were treated with valve replacement or balloon valvuloplasty and the second group consisted of mild valvular damage (MVL). *Results:* IFN-γ 874T allele and TT genotype were significantly increased in patients compared to healthy controls (for T allel p=0.002,OR=2.40 and for TT genotype p=0.018, OR=2.14). Patients with SVR showed increased frequencies of -874 TT (p=0.009, OR=3.66) and patients with a history of valvuloplasty showed increased frequencies of -874TT (p=0.012, OR=2.75). Moreover, decreased frequencies of INF gama 874 AA genotype (p=0.01, OR=3.06) were found in patients with a history of valve replacement. *Conclusion:* The data demonstrated that RHD, especially severe valve damage, is associated with IFN-γ 874TA polymorphism in the Turkish population.

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EFFECTS OF THAI MEDICINAL HERBAL EXTRACTS CONTAINING ANTI-PSORIATIC ACTIVITY ON THE EXPRESSION ON NF-KB SIGNALING BIOMARKERS IN HACAT KERATINOCYTES

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Background: Psoriasis is a chronic inflammatory skin disorder characterized by rapid proliferation of keratinocytes and incomplete keratinization. Discovery of safer and more effective anti-psoriatic drugs is an area of active research. Using a HaCaT keratinocyte cell line as an *in vitro* model, we previously found that ethanolic extracts from three Thai medicinal herbs, *Alpinia galanga, Curcuma longa* and *Annona squamosa*, contain anti-psoriatic activity. In the current study, we aimed at investigating if these Thai medicinal herbal extracts play a molecular role in suppressing psoriasis *via* regulation of NF-κB signaling biomarkers. *Methods:* Using semi-quantitative RT-PCR and a report gene assay, we analyzed the effects of these potential herbal extracts on ten different genes of the NF-κB signaling network in HaCaT cells. *Results:* In accordance with our hypothesis, we found that the extract derived from *Alpinia galanga* significantly increased the expression of TNFAIP3 and significantly reduced the expression of CSF-1 and NF-κB2. *Curcuma longa* extract significantly decreased the expression of CSF-1, IL-8,

NF-κB2, NF-κB1 and RelA. *Annona squamosa* extract significantly lowered the expression of CD40 and NF-κB1. *Conclusion:* These herbal extracts function against psoriasis *in vitro* by controlling the NF-κB signaling biomarkers, suggesting that further *in vivo* research should be conducted in studying their beneficial therapeutic effects on psoriasis.

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EVALUATION OF ANTI-HEAT SHOCK PROTEIN 60 (HSP60) ANTIBODIES AND INTERLEUKINS CONCENTRATIONS IN PERIPHERAL BLOOD OF NEONATES AFTER AUTOLOGOUS UMBILICAL CORD BLOOD TRANSFUSION (AUCBT) DURING OPEN HEART CARDIAC SURGERY

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Background: In this study we investigated the influence of autologous umbilical cord blood (AUCBT) on the inflammatory response and the level of anti-Hsp 60 antibodies in neonates after total repair of complex congenital heart defects (CHD), to assess its safety. *Methods:* Between September 2009 and December 2010, 39 neonates were studied. The first group consisted of 8 neonates who received AUCBT only during surgery and their stay in the intensive care unit. The second group included 11 neonates who underwent surgical repair using homologous donor blood. As a control group, we chose 20 healthy newborns without any cardiac malformation. We investigated serum levels of antibodies to heat shock protein 60 (Hsp 60) and concentrations of cytokines (IL8, TNF α) pre- and post-operatively. *Results:* The serum concentration of IL-8 preoperatively was significantly higher in both groups of patients compared to the control group. On the first post-op day, the concentrations of serum TNFα and IL-8 increased significantly in both groups when compared to the preoperative concentrations. On the eighth day after surgery, in 40% of patients from the second group, the levels of anti-Hsp 60 antibodies were increased significantly ($p<0.05$). Also on the eight day after surgery we found a correlation between the serum levels of anti-Hsp 60 antibodies and the concentration of TNFα in the second group ($r=-0.76$, $p<0.05$). In both groups, on the eighth day after surgery, the serum concentrations of IL-8 and TNFα were significantly higher in patients with lower levels of anti-Hsp60 antibodies. *Conclusion:* The use of AUCBT in neonatal cardiac surgery is safer in comparison to allogeneic donor blood transfusion.

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BEHAVIOR OF TWO MICROTUBULE-ASSOCIATED PROTEINS AFTER INFECTION OF HeLa CELLS WITH ENTEROHAEMORRHAGIC *E. COLI* (EHEC) O157:H-

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Background: Escherichia coli O157 (EHEC) is a foodborne pathogen causing both diarrheal and systemic disease. Bacterial invasion is characterized by rearrangement of actin cytoskeleton, known as 'attaching and effacing' histopathology and is caused by Type III secretion proteins. The aim of the present study was to determine alterations of microtubule-associated intracellular traffic in HeLa cells after infection with EHEC. *Methods: E. coli* O157 was cultivated for 18 h in TSB at 37˚C. HeLa cells were cultured on sterile coverslides in standard conditions. The HeLa monolayers were inoculated with bacterial cells in final concentration of 10^6 cells/ml and incubated for 1 h at 37˚C. Slides were fixed and double labeled with anti-VDP (FITC-conjugated secondary antibody) and anti-dynein (TRITC-conjugated secondary antibody). *Results:* We observed increased VDP clustering under the plasma membrane after EHEC inoculation. The VDP signal was stronger and covered a larger area in infected cells. Concomitantly, dynein decreased and concentrated in the central part of the cells. *Conclusion:* EHEC infection influences microtubule-associated motor proteins in HeLa cells. Bacteria most likely exploit signal transduction pathways related to the cytoskeletal components of the host cells to promote their invasion.

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STUDY OF THE INTERACTION BETWEEN SYNTHETIC METHIONINE-ENKEPHALINS AND MODEL LIPID MEMBRANES

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Background: Enkephalins are pentapeptides (Tyr-Gly-Gly-Phe-Met/Leu) with a proven antinociceptive action. It is believed that their interaction with the lipids composing the membranes is important their bioactivation. *Methods:* The interaction of the synthetic methionine-enkephalin (Met-enk) and its amidated derivative (Met-enk-NH_2) with dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) was evaluated using thin liquid films (TLF) and the method of Scheludko and Exerowa. *Results:* The study of the TLFs formed by DMPC and DMPG and by lipids-Met-enk/Met-enk-NH_2 mixtures showed differences in the leakage kinetics of the films, which was characterized by a different time for the TLF formation and its different thickness in equilibrium. *Conclusion:* The comparison of the parameters studied showed that the enkephalin-lipid interaction is predominantly due to hydrophobic interactions. However, the amidation of Met-enk resulted in converting the zwitterionic Met-enk into a cation, which led to a change in the mixed lipid-Met-enk-NH2 TLF behavior. Most probably, these effects were due to the electrostatic forces between the amidated enkephalin and the phospholipids heads.

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DETECTION OF MULTIPLE GENETIC CHANGES IN NEUROBLASTOMA BY MLPA

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Background: The biological hallmark of neuroblastoma is acquired genomic imbalances in tumor cells. MLPA is sensitive enough to detect a single copy number change at multiple loci effectively. In this study, we aimed at detecting genomic aberrations in a series of 146 sporadic neuroblastoma cases by using MLPA. *Methods:* MLPA analyses of multiple loci at chromosomes 1, 2, 3, 4, 7, 9, 12, 14 and 17 were performed and the associations between genetic parameters were evaluated. *Results:* Major anomalies were MYCN amplification (MNA) (17.7%), 1p (19.4%), 3p (15.3%) and 11q (21.0%) deletions and 17q (70.2%) gain. Significant associations were determined between MNA and 1p deletion and 11q and 3p deletions (p=0.000 for each pair). *Conclusion:* Genetic analysis of multiple loci by MLPA revealed the following three different tumor groups: (i) tumors with MNA and 1p deletion or at least one of them, (ii) tumors with 11q and 3p deletions or at least one of them and (iii) tumors with anomalies other than 1p, 3p, 11q deletions or MNA. In conclusion, our results showed that MLPA can confidently and effectively be utilized to detect multiple genomic imbalances at a time and these changes may be classified into genetic subtypes of neuroblastoma.

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INVESTIGATION OF THE EPITHELIAL GROWTH FACTOR RECEPTOR GENE VARIATIONS IN BLADDER CANCER PATIENTS BY INFINITI™ ANALYZER

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Background: The epidermal growth factor receptor (EGFR) is a tyrosine kinase transmembrane receptor, which activates signal transduction pathways upon ligand binding. It regulates essential processes in carcinogenesis and EGFR inhibitors were recently used in the treatment of epithelial cancers. Although the EGFR gene is frequently mutated in a subset of carcinomas, no genetic alterations have been reported in bladder tumors to date. Only two EGFR single nucleotide polymorphisms have been found to be associated with increased bladder cancer risk. *Methods:* DNA samples extracted from leukocytes of 48 bladder cancer patients were analyzed for the 50 known mutations in EGFR exons 18-21 using the INFINITI® EGFR Assay. The assay is automated by the INFINITI™ Analyzer, which uses a film-based microarray technology. *Results:* None of the samples was found to be positive for the mutations within the tyrosine kinase domain of EGFR. *Conclusion:* Mutations in the kinase domain of EGFR are rare events in bladder cancer and large-scale molecular analyses should be performed prior to considering tyrosine kinase inhibitors for the treatment of bladder tumors.

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ANALYSIS OF GENE ASSOCIATION OF THE ENDOTHELIN-1 SYSTEM WITH CARDIOCEREBRAL COMPLICATIONS AMONG PATIENTS WITH ARTERIAL HYPERTENSION IN THE KAZAKH POPULATION

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Aim: To estimate the genetic input of polymorphisms G5665T of ppEDN1 and C1363T of EDNRA genes in the development of cardiocerebral complications among patients with arterial hypertension (AH) in the Kazakh population. *Patients and Methods:* A total of 130 patients of Kazakh nationality with AH of the IInd-IIItd degrees (mean age 54.8±4.7) were surveyed. The control group was comprised of 74 healthy Kazakhs of comparable gender and age. Complex echocardiographic investigations, ultrasonic investigation of the main arteries of the brain and a computer tomography scan of the brain were performed. The genotyping was performed by the restriction fragment length polymorphism method. *Results:* The AH patients with T1363T genotypes of the EDNRA gene had a significantly higher level of endothelin-1- 10.42±0.56 pg/ml (χ^2=4.37). The carriage of T5665T genotype of the ppEDN1 gene increased the risk of neurovisual signs of 'leukoaraiosis' three-fold (χ^2=4.91). The frequency of the T1363T genotype of the EDNRA gene among AH patients with lacunar ischemic impairment was significantly higher (χ^2=4.5). The T1363T and C1363T genotypes of the EDNRA gene significantly increased the resistance index in the middle cerebral artery (OR=4.8) and decreased the pulsation index (OR=2.8). *Conclusion:* The carriage of T1363T genotype of the EDNRA gene and the T1363T genotype of the EDNRA gene significantly increased the risk of cardiocerebral complications among patients with AH in the Kazakh population.

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5-FLUOROURACIL AND GEMCITABINE EFFECTS ON MCF-7 *VIA* THE JAK/STAT PATHWAY

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Background: 5-Fluorouracil (5-FU) and gemcitabine are important agents for the treatment of several solid tumors, including breast carcinoma. The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway transmits a wide range of cytoplasmic signals from cytokines, growth factors and hormones that bind to specific cell-surface receptors. Thus, aberrant activation of the JAK/STAT pathway may predispose to malignancy due to deregulation of proliferation, differentiation or apoptosis. In this study we aimed to investigate the effects of 5-FU and gemcitabine on MCF-7 cells in the JAK/STAT pathway. *Methods:* MCF-7 breast cancer cells were cultured in RPMI containing 10% FCS, 1% L-glutamine and 1% penicillin/streptomycine. When the cells became confluent, they were incubated with gemcitabine and 5-FU for 24 h. Distribution of JAK1,2,3 and STAT2,3,4,5 were evaluated on treated and non-treated MCF-7 cells by indirect immunohistochemistry method. *Results:* After treatment with 5-FU, JAK1 and STAT2, expressions were decreased in MCF-7 cells, compared with gemcitabine-treated or non-treated groups. *Conclusion:* According to immunohistochemical analyses, the JAK/STAT pathway may be affected after 5-FU treatment rather than gemcitabine treatment.

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NAT2 GENE POLYMORPHISM DISTRIBUTIONS IN HEALTHY TURKISH INDIVIDUALS FROM THE MERSIN REGION

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Human arylamine N-acetyltransferase 2, phase-II drug-metabolizing enzyme (NAT2; EC 2.3.1.5) encoded by the *NAT2* gene plays a key role in the bioactivation of aromatic and heterocyclic amines. Its involvement in drug metabolism and disease susceptibility remains a central theme for pharmacogenetic research, because of its genetic variability among human populations and this variation may affect the rate of activation or detoxification of genotoxic compounds. Some single nucleotide polymorphisms in the *NAT2* gene may change protein structure and/or stability and segregate human phenotypes into rapid, intermediate and slow acetylation phenotypes. The knowledge of acetylator status may help to individualize selection and drug dosing, to predict toxicity and to improve the clinical outcome of therapy. In this study we aimed at determining the distribution of NAT2 genotypes in unrelated Turkish individuals from the Mersin region. Genomic DNA was extracted from blood samples of 104 healthy subjects using High Pure PCR Template Preparation Kit (Roche Applied Science, Germany). NAT2 polymorphisms G→A 191 NAT2*14A, C→T 481 NAT2*5A, G→A 590 NAT2*6A, G→A 857 NAT2*7A/B were analyzed with a NAT2 Mutation Detection Kit (Roche Applied Science) in Light Cycler Real Time PCR. The distributions of NAT2*5A, NAT2*6A, NAT2*7A/B and NAT2*14A wild-type were: 39.4%, 57.7%, 57.7% and 54.8%, respectively; heterozygous genotypes were 53.8%, 38.5%, 39.4% and 44.2%, respectively; mutants were: 6.7%, 3.8%, 2.9% and 1.0%, respectively.

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REVERSAL OF MULTIDRUG RESISTANCE IN ZOLEDRONIC ACID RESISTANT MCF7 CELL LINE

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Background: Multidrug resistance (MDR) is developed in tumor cells against various cytotoxic agents. Zoledronic acid is a bisphosphonate which interferes with cell-to-bone matrix attachment and prevents bone fractures in breast cancer treatment. Development of resistance to zoledronic acid was previously examined in our laboratory. Overexpression of breast cancer resistance protein could correlate with zoledronic acid resistance in MCF7 cell line. In the current study, the effect of isoflavonoid class inhibitor Biochanin-A was investigated in a zoledronic acid resistant MCF7 (MCF7/8000nMZol) cell line to reverse BCRP-mediated MDR. *Methods:* The MCF7/8000nMZol cell line was developed by stepwise selection in drug concentration increments and its resistance was confirmed by XTT cell proliferation assay. The expression of *BCRP, MDR1, MRP1* and *β-actin* were analyzed by real-time qPCR (RT-qPCR) after Biochanin-A treatment at different concentrations. The effect of Biochanin-A on proliferation of MCF-7/8000nMZol cells was investigated using XTT cell proliferation assay. *Results:* The MCF7/8000nMZol cell line showed approximately 3.5 fold resistance against zoledronic acid compared to MCF7/S. RT-qPCR results indicated increased *BCRP* expression level in MCF7/8000nMZol cells. *MDR1* and *MRP1* expressions were not detected in both cell lines. Biochanin-A did not show toxic effects at low concentrations. *Conclusion:* These results indicate that overexpression of *BCRP* correlates with zoledronic acid resistance. Low cytotoxicity of Biochanin-A may imply that Biochanin-A could be a potential candidate for reversal of BCRP-mediated MDR.

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WOUND HEALING PROPERTIES OF MODIFIED SILVER NANOPARTICLES AFTER TOPICAL APPLICATION

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Background: Silver has been used for centuries due to its antimicrobial and wound healing properties. In this study, citrate-reduced and oligonucleotide-modified silver nanoparticles (AgNPs) were tested for their wound healing property in an animal model of ulcer. *Methods:* A 12-base oligonucleotide was used to coat the citrate-reduced AgNPs. The healing process was monitored daily during five days by measuring the ulcer diameter. The tissue samples obtained from the healed ulcer regions were analyzed for epithelial damage, congestion, inflammatory cell infiltration, fibroblast proliferation and collagen re-construction. *Results:* The histological analysis revealed that the AgNPs and AgNP-oligonucleotide signaled congestion, inflammatory cell infiltration, fibroblast proliferation and new collagen synthesis. Although the fibroblast proliferation seemed to be the same for both AgNPs and AgNP-oligonucleotide, the collagen synthesis was further improved with the AgNP-oligonucleotide. *Conclusion:* The results of this study suggest that both citrate-reduced and oligonucleotide-coated AgNPs can be used to speed up the healing process.

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EFFECT OF SOME INHIBITORS ON MARIGOLD POLYPHENOL OXIDASE ACTIVITY

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Background: Polyphenoloxidase (PPO), [EC 1.14.18.1] is a copper containing enzyme that catalyzes o-hydroxylation of monophenols to o-diphenols and oxidation of the o-diphenols to o-quinones in the presence of molecular oxygen. The polymerization of these quinones leads to the formation of a heterogeneous group of melanins, and browning pigments that cause organoleptic and nutritional modifications in plants. PPO is widely distributed among plants, bacteria and animals. In this study, the effects of some compounds on PPO activity in marigold (*Calendula officinalis*) were investigated *Methods:* Marigolds were homogenized in 0.1 M of phosphate buffer (pH 7.0) containing 1 mM ascorbic acid and 0.5% polyvinylpyrrolidone. The homogenate was filtered and centrifuged and PPO activity was determined at 420 nm. Sodium azide, ascorbic acid, thiourea and L-cysteine were used as PPO inhibitors and PPO activity was determined by using 4-methylcatechol as a substrate. IC_{50} values were calculated from the plots of inhibitor concentration *versus* percentage inhibition of PPO. *Results:* The IC_{50} values were 1.5 , 20, 3.6 and 15 mM for thiourea, sodium azide, ascorbic acid and L-cysteine, respectively. *Conclusion:* Among these inhibitors, thiourea and ascorbic acid were the most effective inhibitors for marigold (*Callendula officinalis*) PPO.

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PROTECTIVE EFFECT OF NOOTROPIC DRUG NOOPEPT AGAINST Aβ-INDUCED NEUROTOXICITY IN DIFFERENTIATED PC12 CELLS

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Background: Noopept (N-phenyl-acetyl-L-prolylglycine ethyl ester) demonstrates wide spectrum cognition restoring activity in several animal models of Alzheimer's disease (AD). The neuron-like cultures exposed to fragments of β-Amyloid peptide (Aβ) are known to constitute a reliable experimental *in vitro* model of AD. This study was conducted to find out whether Noopept is able to protect PC12 cells from $A\beta_{25-35}$-induced cellular toxicity and to clarify the underlying mechanisms. *Methods:* To elucidate the ability of Noopept (10 μM, 72 h before Aβ treatment 5 μM, 24 h) to affects the sensitivity of cells to Aβ, cell viability, apoptotic rate, intracellular reactive oxidative species (ROS) accumulation and neurite outgrouth were measured. *Results:* It was shown that cultures treated with Noopept + Aβ had higher cell viability, a lower apoptosis rate and an attenuated overproduction of intracellular ROS compared with cells exposed to Aβ alone. Scoring of neurite morphological features revealed that Noopept treatment led to elongation of neurite length, an increase in total neurite numbers and neurit branching points at the same conditions. *Conclusion:* The present study demonstrates the ability of Noopept to ameliorate the cellular neurotoxicity features induced by Aβ and provide evidence for Noopept potency to prevent the progression of AD.

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CELL DEATH BY THE α-LACTALBUMIN AND METAPROLOL IN PRIMARY AND METASTATIC HUMAN COLON CANCER CELL LINES IS CONTROLED *VIA* MITOCHONDRIAL PATHWAY

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Background: The prevalence of colorectal cancer is common for both women and men. HAMLET (human α-lactalbumin) consists of Ca^{+2} binding protein α-lactalbumin which attaches on the surface of tumor cells. Adrenoceptors modulate diverse intracellular processes, such as DNA synthesis through activation of mitogen-activated protein kinases. This study aimed at investigating the effects of the α-lactalbumin and β1-adrenoceptor antagonist metaprolol in human colon cancer cell lines. *Methods:* Colon primary (Colo-320) and metastatic (Colo-741) cancer lines were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 1% L-glutamine and 1% penicillin-streptomycin at 37˚C and 5% CO_2 in air. Each cell line was divided in four groups. The first group was the control group and it was not treated with any drugs. The second group was treated with α-lactalbumin, the third with metoprolol and the fourth group with α-lactalbumin and metoprolol for 48 h. The distribution of apoptotic cells was determined using a TUNEL assay, while the distribution of cytochrome-c, Bax, Bcl-xL and caspase-3 was determined using indirect immunuperoxidase assays. *Results:* The TUNEL positive cells were more detectable in Colo-320 after the combination treatment (α-lactalbumin and metoprolol). While immunoreactivities were observed in all groups, cytochrome-c immunoreactivites were statistically increased in the group of combination treatment. *Conclusion:* The combination treatment (α-lactalbumin and metoprolol) affected Colo-320 rather than Colo741 induction of apoptosis and activation of cytochrome-c.

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GLY 71 ARG MUTATION OF UDP-GLUCURONOSYL TRANSFERASE IN CORD BLOOD

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Variant promoter polymorphism of A(TA)7TAA is rare in the Asian population. These mutations are missense mutations that are in the coding region of UGT1A1. The most common of these mutations is at nucleotide 211, which has guanine adenine change (G-A). Glycine at position is replaced by 71 arginine. Variations in the coding region of UGT1A1 result in dysfunction of the abnormal UGT1A1 enzyme. The aim of this study was to determine and investigate the genotype distributions of the GLY 71 ARG mutation in cord blood. Twenty newborn infants were included in this study. The distribution of GLY71 ARG mutation of the UDP-Glucuronosyl transferase I A1 gene was determined by polymerase chain-based restriction fragment length polymorphism. We found no significant difference in the distribution of the GG AG AA allelic and genotypic frequencies. Further candidate gene analysis can be expected to elucidate the genetic background of this mutation.

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PREVALENCE OF ANTI-HBC AND OCCULT HEPATITIS B VIRUS INFECTIONS IN KOREAN BLOOD DONORS

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Background: This study was performed to determine the prevalence of anti-hepatitis B core antibodies among Korean blood donors and the frequencies of Hepatitis B virus (HBV) DNA and anti-HBs in anti-HBc-positive donors. *Methods:* A total of 12,461 consenting blood donors were consecutively enrolled from the Korean Red Cross Blood Services from April to October 2008. All donors were screened for anti-HBc with an electrochemiluminescence immunoassay. Repeat reactive anti-HBc-positive donors were assayed for anti-HBs and for HBV-DNA using the Cobas TaqScreen multiplex test on individual donation. *Results:* Of the 12,461 donors, 1,682 (13.5%) were reactive for anti-HBc. Among the different age groups, there was a steady increase in the anti-HBc positive rate, ranging from 2.0% in the age group of <20 years to 80.0% in the age group of ≥60 years ($p<0.0001$). Of the anti-HBc-positive donors, 1,523 (90.5%) were anti-HBs-positive. HBV DNA was detected in two donors who were anti-HBc-positive and HBsAg-negative. The prevalence of occult HBV infection was 0.016% and the HBV nucleic acid test yield was 1 in 838 (0.12%). *Conclusion:* This study helps to determine the current status of hepatitis B infection and the prevalence of occult HBV infection in the blood donor population in Korea.

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OPTIMIZATION OF DNA EXTRACTION FROM FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE: AN ANALYSIS OF THYROID AND LIVER NEEDLE BIOPSY SAMPLES

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Background: Formalin-fixed paraffin-embedded (FFPE) tissues represent the largest source of archival biological material available for genomic studies associated with the pathogenesis of cancer and other diseases. However, DNA degradation induced by formalin and the obstruction of paraffin to DNA extraction make it difficult to recover high-quality DNA from formalin fixed and paraffin embedded tissues. The small size of specimen (such as in a liver biopsy) is another problem in PCR analyses. *Methods:* In this study, different methods for the extraction of genomic DNA from FFPE samples were compared: extraction with proteinase K-phenol/chloroform and commercial kits. The examined methods were optimized *via* several modifications in liver needle biopsy and thyroid samples. Then, the obtained DNA was used for the amplification of a 224 bp fragment of BRAF gene and a 303 bp fragment of interleukin-6 gene in PCR. Amplicons were analyzed by electrophoresis in a 2% agarose gel, visualized by ethidium bromide staining. *Results:* The high efficiency of commercial kits in the DNA extraction compared to the proteinase K-phenol/chloroform method were established as statistically significant ($p<0.05$).*Conclusion:* The determination of high quality DNA, especially from small-sized FFPE, samples can contribute worthwhile knowledge to future studies.

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ROLE OF INTERLEUKIN 6 -174G/C PROMOTER POLYMORPHISMS IN PATIENTS INFECTED BY HEPATITIS B VIRUS

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Background: Hepatitis B (HBV) is one of the most important health problems in the world. IL-6 has been shown to be a major inflammatory cytokine, inducing cell proliferation and expression of acute response genes, such as fibrinogen and C-reactive protein in hepatocytes. IL-6 levels are elevated in patients acutely infected with HBV and have been associated with progression of infection to chronic hepatitis. *Methods:* In the present study, the role of IL-6-174G/C polymorphism was investigated on individuals which are diagnosed as hepatitis B virus carrier (n=19), chronic hepatitis (n=10) and cirrhosis (n=13), and non-infected individuals with HBV (n=8). In order to determine IL-6 polymorphism, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied using paraffin embedded liver needle biopsy specimens. *Results:* The genotype frequencies in 50 samples were observed as 76% homozygote typical (GG), 22% heterozygote (GC) and 2% homozygote atypical (CC). In this study, a significant statistical association was established between IL-6 polymorphism (GG, GC and CC genotype) and chronic hepatitis and cirrhosis compared to

hepatitis B virus carriers and non-infected individuals with HBV (p=0.033). *Conclusion:* IL-6-174G/C polymorphism is an imperative risk factor for the progression of the infections related to chronic hepatitis B.

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HUMAN TOOTH GERM STEM CELLS PROTECT SH-SY5Y CELLS FROM AMYLOID-β (Aβ)1–42 -INDUCED NEUROTOXICITY

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Background: The deposition of extracellular amyloid-β(Aβ) peptide, which is generated from the cleavage of amyloid precursor protein, is considered to be the main hallmark of Alzheimer's disease. The accumulation of the main aggregate amyloid-β(Aβ)1-42 has been linked to progressive neuronal death observed in the hippocampal and cerebral cortexes. Stem cells are thought to be promising in terms of treating Alzheimer's disease, since they protect neuronal cells from neurotoxicity. Human tooth germ stem cells (HTGSCs) have the characteristics of mesenchymal stem cells and are easily obtained from waste dental materials. In this study, we tested the protective effect of HTGSC-conditioned medium (CM) against Aβ1-42-induced neurotoxicity in SH-SY5Y cells. *Methods:* CM was collected when the cells reached 70% confluency and applied on the SH-SY5Y cells, which were exposed to Aβ1-42 (5μM). After 24 h, the cell viability was measured by using the MTS test. *Results:* CM of HTGSCs significantly increased the cell viability of SH-SY5Y in the presence of Aβ1-42. *Conclusion:* There is evidence of a chemical crosstalk between stem cells and neurons affecting the survival of neurons in case of neurodegenerative diseases. These data suggest that HTGSCs may secrete soluble protective cytokines or chemokines which antagonize Aβ1-42 neurotoxicity in neurons.

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THE DISTRIBUTION OF CYP2C9 AND CYP2C19 GENE POLYMORPHISMS IN THE MERSIN POPULATION

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CYP2C9 and CYP2C19 are xenobiotic-metabolizing enzymes that metabolize foreign compounds, such as clinically-used drugs and other xenobiotics. More than 12 variants of CYP2C9 and CYP2C19 are known, some of which can be linked to altered drug metabolism. CYP2C9*2 (430C→T), CYP2C9*3 (1075A→C), CYP2C19*2 (681G→A), CYP2C19*3 (636G→A) are the most common alleles of CYP2C9 and CYP2C19. The nucleotide changes in the CYP2C9*2 and*3 alleles lead to changes in the amino acid sequence (R144C for CYP2C9*2 and I359L for CYP2C9*3) and, thus, to decreased enzyme activity. In the case of *CYP2C19*2, *3*, the nucleotide changes lead to a splicing defect, stop codon and GTG initiation codon. The aim of this study was to determine the distribution of CYP2C9 and CYP2C19 gene polymorphisms in the Mersin population. A total of 117 healthy subjects were selected among the people in the Mersin region. Blood was collected in EDTA-containing tubes and DNA was extracted from the leucocytes. CYP2C9*2, CYP2C9*3 CYP2C19*2 and CYP2C19*3 alleles were determined by CYP2C9 and CYP2C19 Mutation Detection Kits by Light Cycler Real Time PCR. No mutant genotype was observed. The distribution of CYP2C9*2, CYP2C9*3 and CYP2C19*2 heterozygous genotypes was found to be 16.2%, 28.2% and 17%, respectively. CYP2C19*3 was found to be wild genotype in all subjects.

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GROWTH OF ENDOMETRIAL CANCER CELLS WAS SYNERGISTICALLY INHIBITED BY THERAPEUTIC STEM CELLS WITH CYTOSINE DEAMINASE AND INTERFERON-BETA

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Background: Recently, much attention has been given to gene therapy, especially gene-directed enzyme/prodrug therapies (GEPT) which are advantageous over radiotherapy or/and chemotherapy. The cytosine deaminase (CD)/5-fluorocytosine (5-FC) system is well-known as one of the GEPT that induces metabolic suicide following administration of prodrug 5-FC. The present study used engineered human neural stem cell lines to confirm the therapeutic efficacy of this system. Parental stem cell HB1.F3 was modified by *E. coli* CD and human *interferon-beta* (IFN-b) gene to produce HB1.F3.CD and

HB1.F3.CD.IFN-b cells, respectively. *Methods:* Endometrial cancer and engineered stem cells were cultured in 10% FBS contained DMEM. After evaluation of CD and *IFN-b* gene expression in the stem cells using RT-PCR, we investigated the expressions of chemoattractant molecules, such as SCF, CXCR4, c-kit, VEGF, and VEGFR2, in endometrial cancer cells. To determine migration ability of these engineered stem cells compared to primary cells, we performed a modified transwell assay. Using co-culture system and MTT assay, we examined the herapeutic efficacy of engineered stem cells with a prodrug 5-FC to selectively target endometrial cancer cells *in vitro*. *Results:* In this study, we confirmed the migratory capacity of these engineered stem cells to endometrial Ishikawa cancer cells. Important chemoattractant factors of tumor tropic ability in these stem cells appear to be attributing to growth factors, their receptors and chemokines secreted by cancer cells. We confirmed that several factors such as SCF, VEGF/VEGFR2, CXCR4, and c-kit were expressed in endometrial cancer cells. Also using co-culture system and MTT assay, decreased viability of endometrial cancer cells was identified in the presence of HB1.F3.CD and HB1.F3.CD.IFN-b cells. Endometrial cancer cell viability was more decreased when co-culture with HB1.F3.CD.IFN-b rather than HB1.F3.CD. *Conclusion:* These results suggest that engineered stem cells expressing CD and/or IFN-b may have a therapeutic potential against endometrial cancer cells *in vitro,via* their tumor tropism. In addition, genetically modified stem cell-based gene therapy can be applied to potential therapy for endometrial cancer *via* their tumor-tropic capacity.

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MIGRATORY AND THERAPEUTIC EFFECTS OF ENGINEERED STEM CELLS EXPRESSING A SUICIDE ENZYME AND INTERFERON-BETA AGAINST BREAST CANCER CELLS

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Background: Engineered stem cells may be advantageous in gene therapy for potential of treatments to various human cancers including brain, hepatocarcinoma, ovary, and prostate. *E. coli cytosine deaminase* (*CD*) as a suicide gene that can convert 5-fluorocytosine (5-FC), a non-toxic prodrug, to its active form, 5-fluorouracil (5-FU), which induces tumor killing effect and apoptosis as inhibition of DNA synthesis. Also, human *interferon-beta* (*IFN-b*) is known as a cytokine with antitumor effects. In this study, human neural stem cells were engineered by *E. coli CD* and human *IFN-b* genes for cancer gene therapy, the selective antitumor effects of genetically engineered stem cells were confirmed. *Methods:* Breast cancer cells (MDA-MB-231 and MCF-7) and genetically engineered stem cells (HB1.F3, HB1.F3.CD, and HB1.F3.CD.IFN-b) were cultured in RPMI and DMEM containing 10% FBS. Expression of transducted *CD* and *IFN-b* genes, and chemoattractant ligands and receptors were confirmed by RT-PCR. To evaluate the migratory ability of these stem cells, we performed a modified migration assay *in vitro*. In addition, using MTT assay, we tested the therapeutic efficacy of engineered stem cells after treatment with 5-FC in the presence of breast cancer cells. *Results:* We confirmed that engineered stem cells expressed *CD* in HB1.F3.CD and *CD plus IFN-b* in HB1.F3.CD.IFN-b, and both HB1.F3.CD and HB1.F3.CD.IFN-b cells were selectively migrated toward these breast cancer cells, MCF-7 and MDA-MB-231. The viability of breast cancer cells was significantly reduced by co-culture with HB1.F3.CD and HB1.F3.CD.IFN-b in the presence of a prodrug, 5-FC. More strong inhibition of breast cancer cell growth was observed by HB1.F3.CD.IFN-b compared to HB1.F3.CD. In addition, chemoattractant molecules, such as SCF/c-kit, VEGF/VEGFR2, and SDF-1/CXCR4, were identified in these breast cancer cells, indicating that these molecules play a role in the tumor tropic effect. *Conclusion:* These results suggest that engineered stem cells expressing *CD* and/or *IFN-b* may have a therapeutic potential against breast cancer cells *in vitro via* their tumor tropism. A further study is warranted to prove *in vivo* efficacy by applying this in a xenograft animal model for gene therapy to human breast cancer therapy. In addition, immuno-deficient and transgenic or knockout mouse models should be employed to elucidate the therapeutic usefulness of these engineered stem cells expressing specific target genes.

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MATERNAL-FETAL PROINFLAMMATORY CYTOKINE GENE POLYMORPHISM AND PRETERM BIRTH

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The association between maternal-fetal proinflammatory cytokine genotype and preterm birth was studied. Isolated genomic DNA from maternal and cord blood samples of 100

preterm and 101 term labors were used for TNFα (-238G/A, -308G/A), IL-1α (4845G/T) and IL-1β (-511C/T) genotyping. TNFα -238GA genotype in term neonates was significantly higher than in the premature neonates ($p<0.05$). Maternal-fetal TNFα -238 heterozygocity was associated with term labor ($p<0.05$). TNFα -308GA and AA genotype was associated with term labor (mothers and neonates: $p<0.05$ and $p<0.001$, respectively). The incidence of term labor was significantly increased in patients with the TNFα -308GA genotype. When the -308GA carrier has a fetus with GG genotype the incidence of preterm labor increases ($p<0.01$). The 4845T allele was significantly more frequent in preterm mothers and neonates ($p<0.001$, $p<0.001$). The effect of maternal-fetal genotype for the pregnancy outcome reveals that the presence of maternal 4845GG and GT genotypes increase term labor incidence, while fetal 4845TT genotype was a significant independent risk factor for preterm birth ($p<0.01$). The IL-1β -511TT genotype was significantly more frequent in preterm neonates. The preterm labor risk was significantly increased in maternal -511TT genotype or fetal CT genotypes, while if maternal -511CT or TT genotypes had a -511TT fetus, the incidence of term pregnancy outcome increases ($p<0.01$).

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LIPID PROFILE, CENTRAL OBESITY, OBESITY AND GLN223ARG LEPTIN-RECEPTOR GENE POLYMORPHISM IN TURKISH MEN WITH OBSTRUCTIVE SLEEP APNEA SYNDROME

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Background: The relationship between Gln223Arg polymorphism of the leptin receptor gene (LEPR) and lipid profile, body mass index (BMI) and central obesity was investigated in Turkish men with obstructive sleep apnea syndrome (OSAS). *Methods:* DNA was obtained from leukocytes of 88 patients and 32 controls. All subjects underwent polysomnography to confirm the OSAS diagnosis. Polymerase chain reaction-restriction fragment length polymorphism was employed to obtain polymorphism of the LEPR gene. *Results:* There were no significant differences in allelic frequencies and genotype distributions of the examined polymorphism of the LEPR gene between OSAS patients and control subjects ($p>0.05$). The effects of OSAS, genotype and their combination onto continuous variables were analyzed using a linear method. There was no impact of LEPR gene polymorphisms on waist and neck circumference in men with OSAS. We observed a statistically significant impact of genotype ($p=0.038$) and genotype+OSAS ($p=0.019$) on serum LDL levels in a co-dominant model in lean patients. OSAS patients carrying the R allele had significantly lower BMI than those not carrying the R allele ($p=0.035$). *Conclusion:* Gln223Arg SNP in the LEPR gene is not associated with OSAS, whereas it is associated with BMI and serum LDL levels in Turkish men with OSAS.

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Q223R POLYMORPHISM AND LEPTIN LEVELS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME

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Background: Recent studies show that Gln223Arg polymorphism of the leptin receptor gene (LEPR) is associated with circulating leptin levels. The aim of this study was (i) to characterize Gln223Arg polymorphism of LEPR and the serum leptin levels, which may have implications on the cardiovascular and metabolic risk associated with obstructive sleep apnea-hypopnea (OSA) and (ii) to investigate the relationship between leptin level of serum and Gln223Arg polymorphism in Turkish men. *Methods:* A total of 28 untreated patients with OSAS (apnea-hypopnea index: AHI≥15) and 16 non-OSAS (AHI<5) were included in the study. To confirm the diagnosis, all patients underwent standard polysomnography (PSG). Serum samples were taken in the morning after overnight fasting. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was employed to obtain genotypes of the LEPR gene. Leptin levels were measured by a commercial kit. *Results:* Higher serum leptin levels were not found in OSAS patients compared to controls ($p>0.05$). There were no significant differences in allelic frequencies and genotype distributions of the examined polymorphism of the LEPR gene between OSAS patients and control subjects ($p>0.05$). There was no relationship between serum leptin levels and genotypes according to fitted models (co-dominant: QQ, QR, RR ($p>0.05$) and dominant model: QQ and QR/RR ($p>0.05$)). *Conclusion:* OSAS has no association with serum leptin levels and Gln223Arg polymorphism of the leptin receptor gene.

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CHARACTERIZATION OF FIBRINOLYTIC ENZYME FROM *BACILLUS CEREUS* 13BN

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There are more than twenty enzymes in the body that assist in clotting of the blood, but only one endogenously fibrinolytic enzyme, called plasmin, is able to break the clot down (Sumi *et al*., 1987). Recently, microbial fibrinolytic enzymes, such as nattokinase and streptokinase have been used commercially as alternatives to chemical therapy. Because of the medical importance, the study of fibrinolytic enzyme production and properties from locally isolated bacteria *Bacillus cereus* 13BN, which is found in Malaysian fermented food, *belacan*, has been undertaken. From the enzyme assay through fibrin plate methods and caseinolytic test, B.cereus 13BN has higher fibrinolytic enzyme activity, 343 $FUml^{-1}$, compared to reported *B. vallismortis* Ace02 (248 $FUml^{-1}$) and *B. subtilis* KCTC 1027 (188 $FUml^{-1}$). Various kinetics factors were studied including incubation times, initial enzyme concentration, pH and temperature to investigate the optimal properties of the enzyme. Additionally, the enzyme named subtilisin 13BN not only strongly hydrolyzes thrombi *in vivo*, but also converts plasminogen to plasmin. Though clinical results are necessary for thrombolytic application, this newly isolated subtilisin 13BN has a potential as a fibrinolytic enzyme.

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CLONING AND EXPRESSION OF A NOVEL FIBRINOLYTIC ENZYME FROM NEWLY ISOLATED *BACILLUS CEREUS* 13BN

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Since chemical thrombolytic agents are expensive and have undesirable side effects, natural sources of fibrinolytic enzyme have now attracted much more attention than more conventional agents. Thus, a potential fibrinolytic enzyme producer from locally isolated bacteria, *Bacillus cereus* 13BN and some of its properties were studied. The extracellular protease enzyme was isolated from Malaysian fermented food, belacan (shrimp paste) and cloned into pET22b(+) expression vector before been introduced into *Escherichia coli* BL21(DE3). The nucleotide sequence of cloned fibrinolytic enzyme gene revealed a single open reading frame of 4221 bp coding 1407 amino acids. The enzyme was actively expressed by transformant *E. coli*, containing constructed recombinant plasmid name as pET22b316. Its strong fibrinolytic activity can be seen through the fibrin clot degradation pattern on SDS-page gel. Meanwhile, the specific activity for recombinant enzyme is smaller (0.929U/ml) than endogenous sp. (0.934U/ml). Even though both the endogenous and recombinant enzyme have the same biochemical characteristics *i.e*. molecular weight (~70 kDa) and a serine protease, their optimal properties on temperature and pH are slightly different (pH 7.8, 50˚C and pH 7, 40˚C respectively). These two enzymes not only strongly hydrolyze thrombi *in vivo*, but also convert plasminogen to plasmin. It may thus be a potent effective natural oral thrombolytic agent replacing ordinary chemical therapy.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL HEXAPEPTIDES WITH AMINOPHOSPHONATES MOIETY AS NOCICEPTIN RECEPTOR LIGANDS

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Background: The study aimed at synthesis and biological screening of new analogs of Ac-RYYRWK-NH_2 (a hexapeptide with high affinity to the nociceptin receptor), modified at the N-terminal with 1[(methoxyphosphono) methylamino]cycloalkanecarboxylic acid. *Methods:* The compounds were tested for agonistic activity *in vitro* on electrically stimulated *vas deferens* smooth-muscle preparations. The selectivity was tested after blockade of the opioid and nociceptin receptors. *Results:* The replacement of the acetyl group by aminophosphonates moiety reduced the potency of the peptide, but not the specificity to the nociceptin receptor. When Arg at position 1 was substituted with cyclic aminophosphonates, the agonistic activity significantly decreased. The enlargement of the carbon cycle in the substituent (5-8 C-atoms) additionally diminished both the activity and the selectivity for the nociceptin-receptor. *Conclusion:* The incorporation of aminophosphonates in position 1 of hexapeptides decreased the nociceptin-like activity of the newly synthesized analogs.

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MUTATION SPECTRUM OF WILSON DISEASE IN LATVIA

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Introduction: Wilson disease (WD) is an inherited copper-metabolism disorder with an average prevalence of 1:30,000, caused by mutations in the gene *ATP7B*, which is expressed predominantly in the liver and the central nervous system. There are over 300 mutations, with the most prevalent mutation among individuals of Northern European origin being H1069Q. *Aim:* To investigate the frequency of H1069Q mutation in the *ATP7B* gene in Latvian WD patients and to perform mutation detection in seven exons of the gene *ATP7B* in WD patients. *Patients and Methods:* Mutation analysis was performed in the DNA of 58 WD patients whose symptoms and laboratory tests gathered three or more points by the WD scoring system. Mutation H1069Q testing was performed by PCR Bi-PASA. Seven exons of the gene *ATP7B* (2, 7, 8, 14, 16, 17 and 18) were sequenced. *Results:* Five mutations were identified: two previously described (H1069Q, 2298_2299insC), and three novel mutations (V1036I, V748M and D1267G), thus genetically confirming WD diagnosis in 26 patients; for two patients this was done pre-symptomatically. *Conclusion:* In Latvian WD patients five different mutations were found and the H1069Q mutation was present at 50% of the disease alleles.

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VITAMIN D RECEPTOR GENE POLYMORPHISMS AND ORAL CANCER RISK

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Background: A number of studies have suggested that genetic background may play an important role in the etiology of oral squamous cell carcinoma (OSCC). Vitamin D receptor gene (VDR) is involved in a wide range of cellular processes and may influence susceptibility toward OSCC. The aim of this study was to determine the role of VDR gene polymorphisms in the development and clinical parameters of OSCC. *Methods:* The study was conducted in 105 oral cancer patients and a control group of 120 individuals. Genetic polymorphisms in VDR gene (EcoRV, Apa, Taq) were determined using the PCR-RFLP method. *Results:* The homozygous variant genotype *EE* was significantly associated with decreased OSCC risk [odds ratio (OR)=0.351, 95% confidence interval (CI)=0.159-0.773, p=0.008] compared to the common *ee* genotype, as well as the *AA* genotype in comparison to the *aa* genotype [OR=0.519, CI=0.259-1.038, p=0.046]. No association with reduced oral cancer risk was observed for Taq polymorphism [OR=0.915, CI=0.364-2.302, p=0.522]. EcoRV polymorphism was significantly associated with nodal status (p=0.04). *Conclusion:* Our data suggest that EcoRV and Apa polymorphisms may be associated with a decreased oral cancer risk, while the observed association of EcoRV polymorphism with the presence of lymph node metastases may indicate a role of VDR gene in tumor dissemination.

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ANTIOXIDANT AND ANTI-TYROSINASE ACTIVITIES OF *PIPER OFFICINARIUM* LEAVE EXTRACTS

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Piper officinarium, which belongs to the Piperaceae family, is used in local traditional medicine in Indonesia and Malaysia. This is the first evaluation of antioxidant and anti-tyrosinase activities on *Piper officinarium*. Different extracting solvents, including methanol (POLM), ethyl acetate (POLE) and hexane (POLH), were used. Crude extracts were evaluated for their antioxidant activities by DPPH and FRAP assay. Total phenolic content (TPC) was determined according to the Folin-Ciocalteu method. *Piper officinarium* was also evaluated for anti-tyrosinase activities. POLH had the highest antioxidant activity follow by POLM, POLE and POLH with their IC_{50}, 1274 μg/ml, 99.83 μg/ml and 59.34 μg/ml respectively when compared to ascorbic acid with only 13.00 μg/ml. TPC showed close correlation with the DPPH radical scavenging activities, with POLE being the highest followed by POLM and with no activity from POLH. POLM is the only extract that is positive for anti-tyrosinase activity with 26.43% inhibition. Research is underway for the isolation of pure compounds.
POLE: *Piper officinarium* leaves Ethyl acetate
POLM: *Piper officinarium* leaves Methanol
POLH: *Piper officinarium* leaves Hexane

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