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ABSTRACTS OF THE 3rd SWEDISH-HELLENIC LIFE SCIENCES RESEARCH CONFERENCE

Athens, March 25-27, 2010

Organizers

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Preface

The Swedish-Hellenic Life Sciences Research Conference of Athens, 25 -27 March 2010, is the 3d in the series of Conferences organized by the National Hellenic Research Foundation (NHRF) and the Örebro University (OU), proving that it is very near to become a tradition among the community of the people of both organizations involved in the fields of life sciences, namely, the Institute of Biological Research and Biotechnology, the Institute of Organic and Pharmaceutical Chemistry and the Theoretical and Physical Chemistry Institute from the part of NHRF and the School of Health and Medical Sciences, Division of Clinical Medicine as well as the School of Science and Technology, Örebro Life Science Centre from the part of the OU. This year, the conference was dedicated to the Biomedical research activities of both organizations.

The Organizers

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AN *IN VITRO* INFLAMMATION MODEL OF HUMAN EPITHELIAL CELL AND MACROPHAGE CO-CULTURES

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Objective: Urothelial cells are the first line of defence against infections in the urinary bladder. During an infection, the epithelial cells secrete inflammatory mediators such as cytokines and chemokines to recruit underlying immune cells, including macrophages, to the site of infection. It is therefore important when evaluating bacterial infections or treatment in an *in vitro* system that both cell types are present. An *in vitro* inflammatory model consisting of a co-culture of bladder epithelial cells together with macrophage cells was developed in order to evaluate the effects of uropathogens during bladder infections and treatment.

Materials and Methods: Urinary bladder epithelial cells and macrophage-like cells were cultivated in separate chambers of a cell culture insert system. The urinary bladder carcinoma cell line (5637 cell line) was cultured in the top chamber, while the macrophage-like cells prepared from monocyte THP-1 treated with phorobral 12-myrisate 13-acetate (PMA) were cultured in the bottom chamber. The cells were incubated for 24h at 37˚C and 5% CO_2 and the cytokine/chemokine profiles of each cell type were evaluated by ELISA.

Results: Co-culturing epithelial cells together with macrophage-like cells at a ratio of 1:1 in chambers separated by a 0.4 μm porous membrane did not influence the growth or viability of either cell type. Furthermore, the cell cultures did not show any altered levels of interleukin (IL)-6, CXCL-8 or TNF expression in the presence of the other cell type. This demonstrates that in a cell culture insert system, mixed cell types are tolerated.

Conclusion: An *in vitro* co-culture of epithelial cells and immune cells did not affect the growth or elicit an inflammatory response due to the presence of the other cell culture.

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REGULATION OF *PS2, CCND1* & *IEX-1* GENES IN HER2-OVEREXPRESSING BREAST CANCER CELLS: CORRELATION WITH TAMOXIFEN RESISTANCE

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Background: The majority of human breast tumors display estrogen-dependent growth, mediated by the estrogen receptor (ER). Tamoxifen is an ERα antagonist known to inhibit cell growth by down-regulating positive (*e.g. CCND1*) and up-regulating negative (*e.g. IEX-1*) regulators of cell proliferation and/or viability. However, approximately 30% of ER-positive breast cancers display de novo resistance to tamoxifen. Almost all patients with metastatic disease and approximately 40% of patients receiving adjuvant tamoxifen eventually relapse and die (*acquired resistance*). The mechanisms conferring resistance to tamoxifen remain unclear and clinicopathological indicators that could reliably identify patients likely to enjoy long term clinical benefits from the drug are presently lacking. Overexpression of HER2 receptor tyrosine kinase is observed in about 20-30% of breast tumors and is often associated with reduced response to tamoxifen. We sought to identify reliable and dynamic genomic markers of tamoxifen response in HER2 overexpressing breast cancer cells.

Materials and Methods: MCF7 breast cancer cells and two clones engineered to overexpress HER2 in different laboratories were used along with BT474 breast cancer cells, natively expressing high levels of HER2, and tested in the presence or absence of Herceptin (a HER2 inhibitory antibody). 4OH-tamoxifen-mediated differences in gene expression were evaluated using real time quantitative PCR. Cell growth and viability were determined using MTT and LDH release assays.

Results: CCND1 and *pS2* expression was up-regulated and IEX-1 expression was down-regulated in HER2-overexpressing cells compared to parental cells. In addition, HER2-overexpressing cells displayed reduced reversal of *pS2* and *CCND1* up-regulation and *IEX-1* down-regulation by 4OH-tamoxifen. The changes in *IEX-1* expression were more consistent with 4OH-tamoxifen effects on cell growth than those in *CCND1* and *pS2* expression. Treatment of BT-474 cells with tamoxifen and Herceptin, individually and in combination, affected cell growth in a manner that could be more accurately predicted by changes in IEX-1 expression.

Conclusion: Our findings unravel *IEX-1* gene expression as a dynamic marker of the responsiveness of the ER and HER2-positive breast cancer cells to inhibitors of hormone and growth factor signaling. The potential of *IEX-1* in optimizing design of breast cancer therapies in the neoadjuvant setting is underlined.

3

CLONING AND FUNCTIONAL STUDIES OF A SPLICE VARIANT OF CYP26B1: A CELLULAR STORAGE PROTEIN FOR ALL-*TRANS* RETINOIC ACID?

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Introduction: Retinoids, especially all-trans-retinoic acid (atRA), play an important role in regulating gene expression, cell proliferation and differentiation. The levels of atRA in cells and tissues are strictly regulated, and one of the important mechanisms is catabolism of atRA into inactive metabolites. There are several cytochrome P450 isoforms (CYPs) involved in retinoid metabolism, but CYP26 is the most dedicated enzyme responsible for atRA catabolism. Three isoforms of CYP26 have been identified in mammalian cells: CYP26A1, CYP26B1 and CYP26C1. In this study, the molecular cloning and characterization of atRA-induced spliced variant of CYP26B1 gene was described.

Materials and Methods: The CYP26B1 spliced variant was amplified from cDNA synthesized from atRA-treated human aortic smooth muscle cells (AOSMCs) and human umbilical cord endothelial cells (HUVECs). COS-1 cells were transiently transfected to investigate the metabolic activity of the CYP26B1 spliced variant. HPLC was used for studying the metabolism of atRA. Western blotting was used for detecting the expression of the CYP26B1 full length and splice variant in HUVECs and COS-1.

Results: Our data suggest that in the CYP26B1 spliced variant, exon 2 was missing. It was also evident that CYP26B1 spliced variant gene expression was induced by atRA in human endothelial and smooth muscle cells. The cellular levels of atRA were increased in cells transfected with the spliced variant compared to the control. It could be speculated that the CYP26B1 spliced variant has an affinity for atRA and therefore prevents the CYP26-mediated degradation. Western blotting showed that both CYP26B1 full length and splice variant forms were expressed in HUVECs.

Conclusion: The present study described the cloning and characterization of a spliced variant of the CYP26B1 gene that is expressed and induced by atRA in human vascular cells. Our findings suggested that a CYP26B1 splice variant lacking exon2 prevents degradation of atRA and may function as a cellular storage protein, inhibiting a complete depletion of atRA in the cell after induction.

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MICRORNAS AND THEIR ROLE IN THE REGULATION OF GENE EXPRESSION IN CANCER

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MicroRNAs (miRNAs) are small (19-22 bases) single-stranded RNAs that negatively regulate the expression of protein-coding genes. miRNAs act by binding to the genes' 3'-UTR through partial complementarity, resulting mainly in translational inhibition (and mRNA degradation to a lesser extent). Over 700 human miRNAs have been identified to date and they are thought to control the expression of at least 1/3 of all protein coding genes. They have been heavily implicated in the regulation of (amongst other cellular processes) the cell cycle and differentiation and many have been shown to play a fundamental role in cancer. miRNAs participate in signaling pathways leading to carcinogenesis and metastasis by inhibiting the expression of proteins along these pathways. Importantly, a single miRNA may control more than one protein along a particular pathway or even two or more pathways simultaneously, thus coordinating expression of seemingly independent sets of genes. Expression of miRNAs is often controlled by transcription factors known to be involved in cancer. It is also not uncommon for miRNAs to participate in feedback and feedforward loops within signaling pathways, therefore fine-tuning the expression of key genes. Here, an overview of the multiple roles that miRNAs play in controlling the process of carcinogenesis and metastasis is given as well as examples of how they are regulated themselves in the context of cancer.

5

DEREGULATED EXPRESSION OF HNRNP A/B PROTEINS IN HUMAN NON-SMALL CELL LUNG CANCER: CORRELATION OF PROTEIN AND mRNA LEVELS IN PAIRED TUMOR /NON-TUMOR TISSUES

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Background: The heterogeneous nuclear ribonucleoproteins (hnRNP) of the A/B type (hnRNP A1, A2/B1 and A3) are highly related multifunctional proteins participating in alternative splicing by antagonizing other splicing factors, notably ASF/SF2. The altered expression pattern of hnRNP

A2/B1, and/or splicing variant B1 alone, in human lung cancer and the potential to serve as molecular markers for early diagnosis remains an issue of intense investigation. The main objective of the present study was the collection of paired tumor/non-tumor biopsies from patients with non-small cell lung cancer (NSCLC) to investigate the expression profiles of hnRNP A1, A2/B1, A3, in conjunction with ASF/SF2.

Materials and Methods: Semi-quantitative estimates of hnRNP A/B and ASF/SF2 protein levels in the tumor and the adjacent normal-looking area of the lung were obtained by Western blotting of tissue homogenates, combined with parallel immunohistochemical examination of hnRNP A/B on fixed tissue sections from the same patients. Direct comparison of both protein and mRNA expression levels followed the quantification of mRNA steady-state levels by real-time PCR.

Results: Our study unraveled hnRNP A1 as the most highly deregulated hnRNP A/B protein with a high frequency of over-expression (76%), followed by A3 (52%) and A2/B1 (43%). It also provided clear evidence for the mainly uncoupled deregulation of the hnRNP A/B protein group. Moreover, direct comparison of protein and corresponding mRNA levels showed a lack of correlation in the case of hnRNP A1, but not of A2/B1, suggesting the operation of different mechanisms for their deregulation. With respect to ASF/SF2, an overall similar to hnRNP A1 pattern of over-expression was evident.

Conclusion: Our results support the existence of distinct mechanisms responsible for the deregulated expression of hnRNP A/B in NSCLC and suggest hnRNP A1 as a potential novel biomarker for lung cancer diagnosis.

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THE ROLE OF ESTROGEN RECEPTOR (ER) B1 AND ERB2 IN ERA-NEGATIVE BREAST CARCINOMA

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Background: Estrogen receptor (ERα) is of primary importance in breast cancer prognosis and treatment. The recently discovered ERβ1 and its isoforms may also be important in this respect and their role could vary depending on ERα expression. To assess the role of ERβ1 and ERβ2 in ERα-negative breast carcinoma, their level of expression was correlated to clinico-pathological parameters, patient prognosis and response to treatment.

Materials and Methods: Routinely-processed sections of 97 ERα-negative breast carcinomas from 97 female patients (27-77 years, mean=54) followed-up for 7-152 (mean=60) months, were subjected to immunohistochemistry using ERβ1- and ERβ2-specific antibodies. Immunostaining was semi-quantitatively evaluated using Histoscore (range 0-7).

Results: ERβ1- and ERβ2-positivity (Histoscore ≥5; 5=median value) was detected in 66 of 97 (68%) and 65 of 96 (68%) cases, respectively. ERβ1 and ERβ2 were co-expressed in 51 (53%) cases. ERβ1- and ERβ2-expression were correlated (Spearman r=0.393, p=0.01). ERβ1-positivity was negatively correlated with tumour grade (p=0.039). ERβ2-positivity was positively correlated with lymph node metastasis (p<0.001). Tumour grade (p=0.013) and size (p=0.052), lymph node metastasis (p=0.01) and ERβ2-expression (p=0.007) were significantly higher in patients that relapsed. In multivariate analysis, grade and ERβ2-status were the most important variables to predict relapse (p=0.004 and 0.014, respectively). ERβ1- and ERβ2-status were not related to overall survival. In patients receiving chemotherapy (+/-radiotherapy), ERβ2-positivity was correlated with lower relapse-free survival (p=0.023).

Conclusion: In ERα-negative breast carcinoma: a) ERβ1 and ERβ2 are frequently and concurrently expressed, b) ERβ1-positivity is not related to patient survival and, c) ERβ2-positivity indicates poor prognosis and response to therapy.

7
PROTEASOME AS A TARGET IN ANTI-AGING

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Background: Aging is a universal phenomenon represented *in vitro* by replicative senescence. The proteasome is the main cellular proteolytic machinery that is down-regulated during replicative senescence of human fibroblasts. By contrast, its genetic activation through overexpression of proteasome subunits confers lifespan extension and maintenance of youthful morphological features for longer.

Results: Several natural compounds have been shown to exert antioxidant properties *via* proteasome activation

through different molecular pathways. Oleuropein, the major constituent of olives and olive oil, has been shown to promote proteasome activation through induction of conformational changes of the proteasome. These changes result in better survival against oxidative stress and a lifespan extension of primary human fibroblasts. Several compounds have also been shown to act as antioxidants through the activation of the transcription factor Nrf2, a key molecule involved in cellular protection against chemically induced oxidative stress. Nrf2 binding sites, known as Antioxidant Response Elements (AREs), have been identified in the promoters of several proteasome subunits. We have identified a natural compound, 18α-glycyrrhetinic acid that activates the Nrf2 pathway and promotes proteasome activation through this pathway. Proteasome induction results in lifespan extension and delayed establishment of senescent morphology, consistent with the effects of proteasomal genetic activation. Finally, we have also identified quercetin and its derivative, namely quercetin caprylate as a proteasome activator with antioxidant properties that consequently influence cellular lifespan, survival and viability of HFL-1 primary human fibroblasts. Moreover, when these compounds are supplemented to already senescent fibroblasts, a rejuvenating effect is observed.

Conclusion: Overall, our work shows that proteasome activation is possible in terms either of genetic manipulation or through the use of natural substances in human primary fibroblasts. We are currently investigating whether proteasome activation is possible at the whole organism level, taking advantage of the *C. elegans* model. Initial results confirm the beneficial effects of some natural compounds on the lifespan of the worm and further strengthen the importance of proteasome function in organismal aging.

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TOM1L1 IS INVOLVED IN IL-1 SIGNALLING IN THP-1 CELLS

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Background: Tom1 (target of myb) and its related proteins (Tom1L1/Sarcasm and Tom1L2) constitute a protein family that share an N-terminal VHS (Vps27p/Hrs/Stam) domain and a GAT (GGA and Tom1) domain, both of which are conserved in the GGA (Golgi-localizing, γ-adaptine ear domain homology, ADP-ribosylation factor (Arf)-binding protein and tom) gene family. Tom1L1 interacts with the Toll interacting protein (Tollip)through the GAT domain to form a complex and regulate endosomal trafficking of ubiquitinated proteins. The adaptor protein Tollip was identified initially as an intermediate in interleukin-1 (IL-1) signaling (1). IL-1 is an important proinflammatory cytokine that elicits its pleiotropic effects through activation of transcription factors such as NF-kB and AP-1 (2).

Methods: As Tollip and TOM1L1 form a complex that is involved in endosomal trafficking, the influence of TOM1L1 on IL-1 signalling was investigated.

Results: Exposure of THP-1 cells to LPS and IL-1β following Tom1L1 knock-down resulted in significant reduction of TNFα production.

Conclusion: TOM1-L1 plays an essential role in IL-1 signaling.

1 Zhang G and Ghosh S: Negative regulation of toll-like receptor-mediated signaling by Tollip. J Biol Chem *277*: 7059-7065, 2002.

2 Burns K, Clatworthy J, Martin L *et al*: Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. Nat Cell Biol *2*: 346-351, 2000.

9
REPAIR OF MELPHALAN ADDUCTS SHOWS A GENERAL 5' TO 3'-END GRADIENT EFFECT IN TRANSCRIBED GENES. CLINICAL IMPLICATIONS

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Background: Melphalan is a nitrogen mustard employed in the treatment of multiple myeloma (MM). In this report, the mechanistic basis of melphalan adduct repair, as well as its implication for clinical outcome in MM, were investigated.

Patients and Methods: Human primary fibroblasts with different repair backgrounds, as well as peripheral blood lymphocytes (PBMC) from 5 human volunteers and MM patients (9 responders, 7 non-responders) prior to therapeutic treatment were exposed to melphalan. The kinetics of DNA adduct formation/repair were evaluated in four gene loci with varying transcriptional activity and local chromatin condensation (b-actin, p53, N-ras, d-globin).

Results: In repair-active fibroblasts, the operation of very rapid repair was evident leading to the removal of ~60-75% of the adducts formed. A gradient of repair efficiency of the transcribed strands of the genes was found, with faster repair

at the 5'-end. A similar 5' to 3'-end gradient effect was seen in PBMC from healthy volunteers and in all MM patients. In the N-ras gene, non-responders to chemotherapy showed greater repair activity than responders. Interestingly, this difference appeared to be greatest at the 5'-end of the gene and to decrease towards the 3'-end. No difference between responders and non-responders was evident in regions outside the N-ras as well as at the overall genome repair.

Conclusion: Melphalan adducts showed a gradient of repair efficiency with faster repair at the 5'-end of transcribed genes, while the clinical outcome in MM patients may be related to differential removal of adducts located in specific domains of the genome.

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GLOBAL AND GENE-SPECIFIC HISTONE METHYLATION PATTERN IN COLORECTAL CARCINOMA CELLS

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Background: Cancer cells are the result of a progressive accumulation of genetic and epigenetic alterations in normal cells. During cancer progression epigenetic events, like alteration of histone modification markers, co-exist with genetic events, like point mutations in proto-oncogenes or in tumor-suppressor genes. We analysed the role of global histone modifications and how the modifications may be affected by pathways activated by oncogenes.

Results: Using Caco-2 cell lines stably over-expressing the oncogenic form of Harvey-RAS (H-RAS), Kirsten-RAS (K-RAS) and BRAF we generated oncogene-transformed cells that present a completely different phenotype compared to the parental benign cells. Notably, the phenotype of the H-RASV12 oncoprotein-transformed cells (Caco-H) is associated with Epithelial-Mesenchymal Transition aggresive characteristics, K-RASV12 oncoprotein-transformed cells (Caco-K) undergo to partial senescence programme and BRAF600E oncoprotein-transformed cells (Caco-BR) present microsatellite instability (MSI). A global histone modification analysis revealed a general de-regulation of histone modification markers, in particular H3K27me3 was particularly reduced by H-RAS. The expression analysis of histone modifier enzymes showed that variations of methyl- and acetyl-transferase enzymes as EZH2, JMJD3, PCAF GNC5 and HDACs are associated with the appearance of aggressive tumour properties. Chromatin- Immunoprecipitation (ChIP) analysis was used to follow at the local level, histone markers on the promoter of two selected genes, Cyclin D1 a cell cycle-related gene, and the EMT marker gene E-cadherin, in oncogene-transformed cells. Interestingly, Cyclin D1 and E-cadherin genes demonstrate inverse histone repression patterns on their promoters which were associated with their inverse protein and mRNA expressions. Furthermore, the dependence of histone modification markers on MER-ERK signaling pathways was verified.

Conclusion: In conclusion, Cyclin D1 and E-cadherin showed inverse mRNA and protein expression levels after stable overexpression of the activated forms of the three different ongogenes. Our findings suggest that Cyclin D1 and E-cadherin are reversely regulated by histone modifications in a RAS-dependent manner.

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O^6-METHYLGUANINE LEVELS IN MATERNAL AND CORD BLOOD LEUKOCYTE DNA AND THEIR RELATIONSHIP WITH MATERNAL DIETARY INTAKE OF N-NITROSO COMPOUNDS AND RELATED PRECURSORS

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Background: Epidemiological studies attempting to associate dietary exposure to n-nitroso compounds (NNOC's) and various human cancers have been inconclusive. However, exposure to NNOCs can occur not only *via* dietary intake but also *via* endogenous formation from precursor molecules such as nitrite and nitrogenous compounds. The difficulty in quantifying endogenous exposure to NNOC or related agents could be eased by the use of DNA adducts as biomarkers of exposure. O^6-methylguanine is considered to be the most important pre-carcinogenic modification caused by methylating NNOC's. However it is extremely difficult to be detected in human DNA due to its trace quantities and the difficulty to obtain large quantities of DNA for analysis. The need for sensitive and high-throughput methods to measure DNA damage is therefore widely recognized, especially in view of the prospect of large-scale molecular epidemiological studies.

Materials and Methods: We have recently developed a new immunochemical methodology satisfying these criteria. A limit of detection of <1 adduct/10^9 nucleotides, using 10 μg DNA per well, was achieved for the assay of

O^6-methylguanine. The new method is being applied on a large scale in the context of the NewGeneris project, looking at O^6-meG levels in maternal and cord blood DNA.

Results: A total of 1500 DNA samples will be analyzed from all the participating cohorts. Preliminary results showed that in the Rhea (CRETE) cohort, maternal adduct levels were higher than the respective cord levels (N=75 pairs; 2-tailed Wilcoxon test, $p<0.001$). There was no difference between the adduct levels in cord blood DNAs from the Rhea and INMA (Barcelona) cohorts (Mann-Whitney *U*-test, $p=0.30$). There was a statistical significant association between the O^6-meG levels in the maternal and the respective cord blood DNAs in Rhea's samples (N=75; Spearman correlation r=0.27, $p=0.016$). Comparison of adduct levels observed in the cord blood DNA and the estimated maternal exposure [based on food-frequency-questionnaires (FFQ)] to the above agents did not reveal any statistically significant correlations. This absence of a correlation may reflect weaknesses in the FFQ-based estimation of exposure to dietary or endogenous NOCs.

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mRNA EXPRESSION LEVELS OF ENAC SUBUNITS IN AIRWAY EPITHELIAL CELL LINES AND NASAL EPITHELIA

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Objective: The epithelial sodium channel (ENaC) gene encodes sodium channels that mediate Na^+ transport in epithelia and are essential for sodium and water homeostasis. An imbalance in the epithelial transport of Na^+ in the airways leads to disturbances in airway surface liquid volume (ASL). Both ASL depletion and airway flooding have serious consequences.

Materials and Methods: To assess the occurrence, and to analyse the mRNA levels of α- β-, and γ-ENaC subunits in cystic fibrosis (CF) and non-CF airway epithelial cells, a relative quantitative analysis of these subunits was a real time-polymerase chain reaction assay specific for each subunit, on two human respiratory epithelial-cell lines (CF and non-CF), and on nasal epithelial cells (non-CF).

Results: We found a slight increase in the expression levels of α-subunits, a significant increase in the expression of β-subunits, and a decrease in the mRNA levels of γ-subunit in CF cells when compared with normal cells. The expression levels of ENaC subunits in nasal biopsies were α>β>γ. We failed to detect the δ-ENaC subunit in nasal biopsies.

Conclusion: The up-regulation of ENaC α and β subunits might be associated with the Na+ hyper-absorption in CF tissue (1). The increased expression of β-subunits in CF-cells observed is of interest in view of an animal model for CF based on overexpression of the β-subunit in transgenic mice (2).

1 Bangel N, Dahlhoff C, Sobczak K, Weber WM and Kusche-Vihrog K: Upregulated expression of ENaC in human CF nasal epithelium. J Cyst Fibros *7*: 197-205, 2007.

2 Mall M, Grubb B, Harkema JR, O'Neal WK and Boucher R: Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. Nat Med *10*: 487-493, 2004.

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ABERRANT AMINO ACID TRANSPORT IN FIBROBLASTS FROM CHILDREN WITH ATTENTION-DEFICIT/ HYPERACTIVITY DISORDER

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Objective: Attention-deficit/hyperactivity disorder (ADHD) is a common and disabling neurodevelopmental disorder. The catecholaminergic system has been implicated in the pathophysiology of ADHD. The amino acid tyrosine is the precursor for the synthesis of the catecholamines dopamine and norepinephrine. An aberrant transport of tyrosine, as well as other amino acids, has been found in a number of other psychiatric disorders, such as schizophrenia, bipolar disorder and autism (1). Moreover, a competition for transport between tyrosine and alanine exists at the LAT1 isoform of the amino acid transport system L (2). Hence, the aim of this study was to investigate whether changes in tyrosine and/or alanine transport mechanismsare evident in children with ADHD.

Materials and Methods: Fibroblast cells were cultured from skin biopsies from 14 boys with ADHD (combined type), fulfilling the DSM-IV diagnostic criteria, and from 13 matching boys as controls without a diagnosis of a developmental disorder. Transport of the amino acids

tyrosine and alanine across the cell membrane was measured by the cluster tray method. The kinetic variables, maximal transport velocity (V_{max}) and affinity constant (K_m) were determined by use of the Lineweaver-Burke plot equation.

Results: A significantly increased V_{max} for alanine transport (p=0.031) was found in boys with ADHD in comparison to controls, but the Km of alanine transport did not differ significantly between the two groups. There were no significant differences regarding tyrosine transport in either V_{max} or K_m between children with ADHD and the comparison group.

Conclusion: The elevated transport capacity of alanine found in the present study implies that less tyrosine is transported across the blood brain barrier, due to a competition between alanine and tyrosine to get transported across cell membranes. This indicates less access of dopamine to the brain, resulting in disturbances of the catecholaminergic system, which is evidentin children with ADHD.

1 Fernell E, Karagiannakis A, Edman G, Bjerkenstedt L, Wiesel FA and Venizelos N: Aberrant amino acid transport in fibroblasts from children with autism. Neurosci Lett *418*: 82-86, 2007.

2 Vumma R, Wiesel FA, Flyckt L, Bjerkenstedt L and Venizelos N: Functional characterization of tyrosine transport in fibroblast cells from healthy controls. Neurosci Lett *434*: 56-60, 2008.

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TAF4B AND AP-1 IN CO-REGULATION OF CANCER CELL MIGRATION PROPERTIES AND EXPRESSION OF INTEGRIN A6 DURING EPITHELIAL TO MESENCHYMAL TRANSITION

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Background: The binding of the transcription factor IID (TFIID) complex, composed of the TATA box binding protein (TBP) and 14 TBP associated factors (TAFs), to promoter DNA is responsive to cellular signals and constitutes the first step in transcription. Its subunit TAF4b is present only in a limited number of complexes and is involved in promoter recognition by selective recruitment of activators. The Activating Protein (AP-1) family members participate in the oncogenic transformation *via* gene regulation.

Results: The immunoprecipitation of endogenous protein complexes led us to the documentation of specific interactions between Jun family members and TAFs, in a cell model of colon cancer and in cells which have acquired Epithelial-to-Mesenchymal Transition (EMT) characteristics. TAF4b was found to specifically regulate a number of Integrins, thus altering related cellular properties such as migration potential. Employing a chromatin immunoprecipitation approach in colon adenocarcinoma cell-lines, we further identified a synergistic role of TAF4b and c-Jun and other AP-1 family members on the promoter of Integrin α6, underlining the existence of a specific mechanism related to gene expression control.

Conclusion: We present evidence of an interdependence of TAF4b and AP-1 family members in cell-type specific promoter recognition and initiation of transcription in the context of cancer progression and EMT.

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NUCLEAR ERYTHROID FACTOR 2-MEDIATED PROTEASOME ACTIVATION DELAYS SENESCENCE IN HUMAN FIBROBLASTS

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Beckground: Replicative senescence in human fibroblasts is accompanied with alterations of various biological processes, including the impaired function of the proteasome. The proteasome is responsible for the removal of both normal and damaged proteins. Due to its latter function, proteasome is also considered a representative secondary antioxidant cellular mechanism. Nrf2 is a basic transcription factor responsible for the regulation of the cellular antioxidant response that has also been shown to regulate several proteasome subunits in mice.

Results: We have established in this study the proteasome-related function of Nrf2 in human fibroblasts undergoing replicative senescence. We demonstrate that Nrf2 has a declined function in senescence, whereas its silencing leads to premature senescence. However, upon its activation by a novel Nrf2 inducer, elevated levels of proteasome activity and content are recorded only in cell lines possessing a functional Nrf2. Moreover, treatment by the Nrf2 inducer

results in the enhanced survival of cells following oxidative stress, whereas continuous treatment leads to lifespan extension of human fibroblasts. Importantly the Nrf2-proteasome axis is functional in terminally senescent cultures as these cells retain their responsiveness to the Nrf2 stimuli.

Conclusion: In conclusion, these findings open up new directions for future manipulation of the senescence phenotype.

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INVOLVEMENT OF ZEBRAFISH FUSHI TARAZU FACTOR1A IN THE DEVELOPMENT OF PHARYNGEAL ARCHES

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Background: The zebrafish fushi tarazu factor 1a (Ff1a) gene codes for a nuclear transcription factor belonging to the NR5A super family. Ff1a is involved in sterol and steroid metabolism in tissues such as gonads, adrenals, and liver. The *ff1a* gene contains putative Sox9 enhancer motifs with a possibility to directly regulate gene expression. Sox9a and Sox 9b have been shown to be involved in chondrogenesis and the development of pharyngeal arches in zebrafish 1. We have shown that the zebrafish ff1a gene is expressed during chondrogenesis in the pharyngeal arches 2 and are now studying the involvement of Ff1a in cartilage development as well as its regulation by Sox9 genes in zebrafish.

Materials and Methods: To determine the role of Ff1a in chondrogenesis we have performed morpholino knock-down experiments of *sox9a, sox9b* and *ff1a*. Whole mount *in situ* hybridization were performed to localize the gene expression of *ff1a* following *sox9a* and *sox9b* knock-down. Analysis of Ff1a protein expression was performed following knock-down of *ff1a*.

Results: The *ff1a* gene expression was reduced or absent following knock down with Sox9a and Sox9b morpholinos, while Ff1a protein levels were reduced following knock down of Ff1a. Rescue experiments demonstrated that the effects were specific to the three studied genes. Cartilage development was analysed using Alcian blue staining and showed that knock-down of any of the three genes resulted in reduction of cranio-facial cartilage formation.

Conclusion: Our results suggest that Ff1a is involved in cartilage development in the craniofacial region of developing zebrafish. However, more research is needed in order to clarify the mechanism by which Ff1a regulates chondrocyte development.

1 Yan Y-L *et al*: A zebrafish sox9 gene required for cartilage morphogenesis. Development *129*: 5065-5079, 2002.

2 Koskinen J, Karlsson J and Olsson P-E: Sox9a regulation of ff1a in zebrafish (Danio rerio) suggests an involvement of ff1a in cartilage development. Comparative Biochemistry and Physiology, Part A, pp. 1-5, 2008.

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ONCOGENIC MUTATIONS IN KRAS AND BRAF AND THEIR IMPLICATION IN TRAIL RECEPTOR EXPRESSION IN COLORECTAL TUMOURS

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Background: Colorectal cancer (CRC) is a multi-step process based on the accumulation of mutations in different cancer-related tumour suppressor genes and oncogenes, including *KRAS* and *BRAF*, components of the MAPK/ERK pathway. MAPKs play a central role in the transduction of signals for growth and differentiation and also act as important modulators of various apoptosis-inducing signals. Available studies vary significantly in the reported frequencies of mutations in these two genes in colon cancer.

Results: We investigated 101 CRC adenocarcinomas from Greek patients undergoing surgery for colon cancer in order to determine the frequency of *KRAS* and *BRAF* mutations by PCR and direct sequencing. In 51 of these tumours and their respective normal samples the levels of expression of receptors DR4 and DR5 of TRAIL, a molecule initiating apoptosis, were studied by RT-PCR, in order to investigate whether the presence of mutations contributes to the regulation of the death receptors (DRs). In a subset of tumours (11/51) immunohistochemical analysis for protein expression was also performed. Findings showed that 10/101 (9.9%) samples were positive for *KRAS* mutation, while for *BRAF* the frequency was 2.97% (3/101). DR5 was found to be the most frequently up-regulated receptor (47%), while DR4 was overexpressed in 37% of the samples. Both DRs were up-regulated in the same sample in 31% of the tumours. Protein expression analysis yielded results similar to the transcriptional analysis. Increased DR expression was recorded as early as Duke's A stage and the presence of

mutations was found to significantly amplify the up-regulated expression of TRAIL receptors that already existed in the tumour samples, potentially sensitizing them to TRAIL-based therapies.

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PHYCOCYANIN AND PHYCOCYANOBILIN FROM *SPIRULINA* AS POTENTIAL PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY. INTERACTIONS WITH BIOLOGICAL PHOSPHATES STUDIED BY UV/VIS SPECTROPHOTOMETRY

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Background: Spirulina, now named Arthrospira, is a microscopic and filamentous alkalophilic cyanobacterium, belonging to blue-green algae. It has a long history of use as a safe food lacking toxicity. Recent interest on potential health effects of *Spirulina* is mainly due to its chemical composition. In this respect, some major bioactive components of *Spirulina* seem to play significant role in these beneficial effects: the biliprotein phycocyanin (PC), γ-linolenic acid and various polysaccharide preparations. Research activities of our Institute have been recently focused on biochemical actions of PC and phycocyanobilin (PCB), the open chain tetrapyrrole chromophore of PC, in order to evaluate their potential use in photodynamic therapy (PDT). PDT is a promising new treatment modality for several diseases, most notably cancer. In PDT, light, O_2, and a photosensitizing drug are combined to produce a selective therapeutic effect where the mode of photosensitizer drug delivery and its absorption spectrum are of paramount importance1.

Results: By using UV/Vis difference and derivative spectrophotometry we found that the absorption spectra of purified PCB and crude PC from *Spirulina* extracts drastically change following interaction with a variety of inorganic and biologically important phosphates. In this respect: (i) PCB spectrum is drastically modified (blue-shifted) in a time-, pH- and concentration-dependent manner, in presence of inorganic phosphates (Pi), organic phosphate esters as well as the highly phosphorylated protein phosvitin and DNA. Modification of PCB spectrum by Pi is faster at alkaline pH values. Non-dialyzable PCB-complexes are produced in the presence of macromolecules. Some of the absorption spectral changes of PCB observed in the above experiments may be due to a modification of a pyrrole PCB ring analogous to that observed with the formation of imidazole or mercaptoethanol-PCB adducts2. (ii) PC spectrum changes in a time-, pH- and concentration-dependent manner, in presence of Pi and organic phosphate esters. (iii) Alkaline phosphatase alters the absorption spectrum of PC suggesting the presence of endogenous phosphate esters critical for the spectral properties of this biliprotein. These results may help in the development of specific PCB or PC derivatives for use in PDT.

1 Nyman S and Hynninen PV: J Photochem Photobiol B, *73*: 1, 2004.

2 Tu J-M *et al*: JACS *131*: 5399, 2009.

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VALIDATION OF A NEW IMMUNOCHEMICAL ASSAY WITH HIGH-THROUGHPUT POTENTIAL FOR PAH- DNA ADDUCT DETECTION

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Background: Highly sensitive, high-throughput methods to measure human exposure to polycyclic aromatic hydrocarbons (PAHs) are required for large scale molecular epidemiology studies. A highly sensitive benzo[a]pyrene diol-epoxide (BPDE)-DNA sandwich enzyme-linked immunosorbent assay (ELISA) using a rabbit anti-BPDE-DNA antiserum has been developed in NHRF, Athens to detect PAH-DNA adducts.

Results: The aim of the present study was to further validate the new assay. The test DNA samples were from MCF 7 cells treated with benzo[a]pyrene (BaP) in culture, from liver of mice treated *in vivo* with several doses of BaP, benzo[b]fluoranthene (BbF) and dibenzo[a,h]anthracene (DBahA), and from lung tissue from smoking lung cancer patients. DNA isolations were achieved by phenol extraction, salting- out and a Qiagen kit. DNA adduct measurements were performed by the new immunoassay and by the 32P-postlabelling method. BaPDNA adduct levels from the MCF 7 cells were in the order of 1 in 105 normal nucleotides. The ratio between the adduct values measured by ELISA and 32P-postlabelling was about 0.5. There was a strong, highly significant positive correlation between the DNA adduct measurements of the PAH dose-response curves by ELISA and 32P-postlabelling in the animal samples (r between 0.87 and 0.99). However, adduct levels were significantly lower when measured by ELISA than by 32P-postlabelling, *i.e.* 1:5

for BaP, 1:30 for BbF and 1:5 for DBahA, which also indicates the different affinity of the anti-BPDE-DNA antiserum to the different PAH-DNA structures. Quality of the DNA substantially affected the performance of the immunoassay. Further comparative DNA adduct measurements are underway for additional validation of the new immunoassay.

Treated MCF 7 cells were obtained from Dr. D.H. Phillips (ICR, Sutton). The anti-BPDE-DNA antiserum was provided by Dr. M.C. Poirier (NCI, Bethesda, USA). K. Kovacs was recipient of an ECNIS exchange fellowship to NHRF in 2008. This work has been supported by ECNIS NoE No 513943.

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SP1 BINDS THE EXTERNAL PROMOTER OF P73 GENE AND INDUCES THE EXPRESSION OF TAP73 γ ISOFORM IN LUNG CANCER

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p73 possesses an extrinsic P1 promoter and an intrinsic P2 promoter, giving rise to TAp73 and ΔNp73 isoforms, respectively. The ultimate effect of *p73* in oncogenesis is thought to depend on the ratio of apoptotic TA to antiapoptotic ΔN isoforms. In this study, we aimed to identify novel transcription factors that affect the synthesis of TA isoforms. Using bioinformatics tools, *in vitro* binding assays and ChIP analysis we characterized a region extending -233 to -204 bps upstream to the transcription start site of human P1 *p73* promoter containing conserved Sp1 binding sites. Treatment of cells with Sp1 RNAi and Sp1 inhibitors functionally suppresses TAp73 expression, indicating positive regulation of P1 by Sp1 protein. Thus, Sp1 has the potential to alter the TA/ΔN ratio in lung cancer *via* regulation of TAp73 transcription. We also demonstrated that TAp73 γ is the only TA isoform overexpressed in several lung cancer cell lines and in 26 NSCLCs, consistent with Sp1 overexpression, therefore questioning the apoptotic role of this specific p73 isoform in lung cancer.

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COVALENT BINDING OF AGT TO DNA THROUGH A BIS-FUNCTIONAL AGENT AND ITS POSSIBLE IMPLICATION TO HUMAN CARCINOGENESIS

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1, 2 dibromoethane (DBE) belongs to a large class of potentially hazardous compounds, the haloalkanes and is known to be both mutagenic and carcinogenic in experimental animals and probably carcinogenic to humans. The cytotoxicity of DBE was increased in bacteria overexpressing the human or bacterial O^6-methylguanine-DNA-methyltransferase(AGT). AGT repairs the DNA damage produced by n-nitroso compounds by removing the alkyl groups, mainly from the O^6 position of guanine in a stoichiometric way and transfers it to a Cys-residue in its active site. In vitro studies showed that DBE is a substrate for AGT but it can also react with the DNA *via* its second nucleophilic site. Thus, a DNA-DBE-AGT complex is formed which might be toxic for the cells. We have used a Hela tet-on system that leads to a 15-fold overexpression of AGT in the presence of doxycycline in order to verify that the AGT context in isogenic cell lines is a major determinant of the cytotoxicity of DBE. Cell viability methods were used to test the growth retardation that DBE provoked to cells and showed that the LD20 of cells overexpressing the AGT was 60% lower than those that did not express it at all. To study the formation of the DNA-DBE- AGT complex three methodologies were used: a) Dot blot analysis where DNA was extracted and hydrolyzed and the AGT protein was detected by an anti-AGT antibody (ab), b) Immunoprecipitation of AGT followed by DNA detection using an anti-DNA ab and c) DNA isolation followed by western blot analysis for AGT detection.

Coincubation of DNA, purified AGT and DBE gave a strong positive signal for the formation of the complex utilizing all the above mentioned methods. Dibromopropane also mediated DNA-AGT complex formation though to a lesser extent while dibromopentane did not show any measurable interaction. Detection of the DNA-DBE-AGT complex after treatment of HeLa cells has so far not been achieved but efforts to do so are continuing. However repetitive administration of DBE in A549, MCF7, and Caco2 cell lines showed a remarkable decrease of the amount of MGMT protein as well as its mRNA transcript. Recently we have acquired the technology and know how for epigenomic analysis of various genes including MGMT and we believe that this technological advantage will permit us to explore the biological implications of the formation of AGT-DNA crosslinks.

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DIFFERENTIAL EFFECT OF ONCOGENES BRAF AND RAS ON RHO-MEDIATED INDUCTION OF EMT PROPERTIES IN HUMAN COLON CANCER CELLS

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During colorectal cancer progression BRAF and KRAS oncogenes are often mutated, about 15% and 35% respectively, but the two mutants rarely coexist in the same tumour. Unlike *BRAF* and *KRAS*, mutations in RHO genes are extremely rare in tumours, but they contribute with distinct roles in invasion and metastasis. The aim of this study was to compare and define the effect of $BRAF^{V600E}$, $KRAS^{G12V}$ and $HRAS^{G12V}$ oncoproteins in cell migration and invasion pathways mediated by specific members of the Rho GTPase family. For that, human epithelial colon adenoma cells were stably transfected with the 3 oncogenes separately and analyses were performed. Results presented here show that $BRAF^{V600E}$ induces a partial EMT process in colon cancer cells and provides them with a more aggressive phenotype resulting in high migration and invasion ability through activation of RhoA. KRASG12V does not change the epithelial morphology of the cells, but also enhances their ability to migrate and invade and Cdc42 is the key molecule for that. On the other hand, HRASG12V induces a very aggressive EMT morphology resulting in highly enhanced migration and invasion ability that are associated with Rac1 activity. Thus, the methodology used in this study discriminates oncogene-specific cell migration and invasion pathways and may contribute to the design of colon cancer therapeutics targeting specific Rho pathways according to the oncogenic mutations carried by the patient.

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ESTROGEN RECEPTOR BETA ISOFORMS 1 AND 2 DIFFERENTIALLY MODULATE GROWTH AND INVASIVENESS OF BREAST CANCER CELLS IN RESPONSE TO ESTROGEN AND ANTIESTROGENS

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Background: Estrogen receptor (ERα) is a key player in breast cancer prognosis and treatment. ERα is engaged in extensive cross-talk with the second ER subtype, ERβ1, and the major non-hormone-binding isoform of the latter, ERβ2. Both ERβ isoforms are thought to exert a dominant opposing action on ERα. The role of ERβ isoforms on breast cancer prognosis and treatment remains elusive.

Materials and Methods: MCF-7 breast cancer cells were stably transfected with ERβ1 or ERβ2. Clones were analysed for their potential to bind ER-subtype-selective estrogens and antiestrogens (whole Cell Binding and Scatcard assays) and proliferate in their presence/absence (MTT assay, LDH assay, FACS analysis). The capacity of the cells for anchorage-independent growth and invasion was determined by Soft Agar Colony Formation and Matrigel Invasion assays. Estrogen and anti-estrogen regulation of genes and proteins with a key role in cell growth and viability was analyzed using quantitative real-time PCR and western blotting, respectively. Statistical significance was assessed using the *t*-test.

Results: ERβ1 and ERβ2 expression decreased ERα protein levels and rendered cells more and less sensitive, respectively, to the mitogenic effect of estradiol, while also exerting inhibitory effects on cell growth. ERβ1 but not ERβ2-expressing cells displayed growth inhibition, reduction of the S phase fraction and cell death upon treatment with estradiol, tamoxifen and ERβ1-selective agonists; they also displayed decreased anchorage-independent and invasiveness in the presence and absence of estradiol, respectively. However, tamoxifen markedly increased the invasiveness of ERβ1-expressing cells. In addition, ERβ1-expressing cells displayed a higher induction of the *pS2* gene (a marker of canonical ER function) by estradiol and ERβ1-selective agonists, and of the *IEX-1* gene (a growth-inhibitory/pro-apoptotic gene in breast cancer cells) by tamoxifen.

Conclusion: Our findings propose an oncosuppressive role for ERβ1 but not ERβ2, since only ERβ1-expressing cells displayed markedly reduced anchorage independence and invasiveness in the presence and absence of estrogen, respectively, compared to MCF-7 cells. However, they also highlight that tamoxifen induces the invasiveness of ERβ1-expressing cells, offering an explanation for disease recurrence following treatment with the drug. In addition, they propose treatment of ERβ1-positive tumours with aromatase inhibitors rather than tamoxifen.

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ENZYMATIC ESTERIFICATIONS USING LIPASE FROM *RHIZOMUCOR MIEHEI* IMMOBILIZED ONTO SILICA MATRIX

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Industrial use of lipases is limited by several factors that can be overcome by immobilizing the enzyme onto solid supports. Immobilization presents significant advantages, such as mechanically robust and thermal stability, as well as easy separation of the immobilized catalyst from the reactant-product solution (1).

In this work, we have examined the ability of mesostructured silica matrices, prepared by using a nonionic fluorinated surfactant, CF3(CF2)7C2H4(OC2H4)9OH, to be used for the adsorption of *Rhizomucor miehei* lipase. Physical adsorption on a solid surface took place in this study, in which interaction between NH or C=O groups of the enzyme and the surface silanols of the mesostructured silica matrix occurs (2).

The quantity of adsorbed enzyme was determined by solid state UV spectroscopy. Moreover, the activity of the lipase was studied following the enzymatic synthesis of fatty as well as phenolic acids with aliphatic alcohols. The optimization of the system was the main purpose of this work, in which significant factors, such as stability and reusability were investigated.

1 Stamatis H, Xenakis A and Kolisis FN: Biorganic reactions in microemulsions: The case of lipase. Biotechnol Advances *17*: 293-318, 1999.

2 Michaux F, Zoumpanioti M, Papamentzelopoulou M, Stébé MJ, Blin JL and Xenakis A: Immobilization and activity of *Rhizomucor miehei* lipase. Effect of the matrix properties prepared from nonionic fluorinated surfactants. Proc Biochem *45*: 39-46, 2010.

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TRANSCRIPTOMIC COMPUTATIONAL ANALYSIS OF THE CELL DEATH INDUCED BY N-METHYL-N-NITROSOUREA, A MODEL SN1 METHYLATING AGENT, IN HUMAN LUNG CANCER CELLS

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Since lung cancer is the most frequent cause of cancer death, new therapeutic approaches are eagerly needed. Methylating agents constitute a widely used class of anticancer drugs, the effect of which on human non small-cell lung cancer (NSCLC) has not been adequately studied. N-methyl-N-nitrosourea (MNU) induces cell death through a distinct mechanism in two human NSCLC cell lines studied, A549($p53^{wt}$) and H157($p53^{null}$). In A549($p53^{wt}$), it induces a non-apoptotic cell death whereas in H157($p53^{null}$), MNU induced apoptotic cell death, confirmed by cytofluorometry of DNA content and immunodetection of apoptotic markers. Thus, the mechanism of the cell death induced by S_N1 methylating agents is cell type-dependent and must be assessed prior treatment. To further investigate the differential effect of MNU, a time course gene profiling study using DNA microarrays (24, 48 and 72h of treatment) was performed. The number of differentiated genes greatly varied between the two cell lines. In A549 cells, 3511 genes were found as significantly altered among the three treatment times, whereas in H157 cells the relevant number was only 835. In both cell lines the most pronounced alterations in gene expression between treated and non-treated cells, were observed at 48h. GO-based meta-analysis further associated modulation of several biological processes and functions with gene expression changes. It is noteworthy that the altered functions derived between the two cell lines differ. Regarding H157 cells, functions related to cholesterol and fatty acid biosynthesis, but also immune responses were those that were among significantly altered, while in A549 cells, the altered functions were mainly related to mitotic relative functions, cell cycle and proliferation.

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GENOME-WIDE ANALYSIS OF GLUCOCORTICOID RECEPTOR TARGETS: RELEVANCE TO INFLAMMATION

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Glucocorticoids (GCs) are essential adrenal steroids that regulate a variety of physiological processes including development, apoptosis, metabolism and homeostasis. The biological actions of GCs are mediated through the ubiquitously expressed glucocorticoid receptor (GR), a

ligand inducible transcription factor that binds a variety of promoter elements and regulates gene transcription in a cell- and promoter-specific manner. GCs exert essential immunosuppressive and anti-inflammatory actions and have been widely used as drugs to treat immune and inflammatory disorders. One of the mechanisms by which GR inhibits inflammation is repression of the nuclear factor-κB (NF-κB) signalling, however the molecular networks underlying the anti-inflammatory function of GR are not well established. To determine the interplay between glucocorticoid signalling and inflammation we mapped the GR, p65 (member of Rel/NF-κB transcription factors) and RNA polymerase II (RNAPII) binding sites in HeLa cells in the presence or the absence of GCs and the proinflammatory cytokine TNFα. The chromatin immunoprecipitation (ChIP) assay combined with massively parallel sequencing (ChIP-seq) was used to identify the binding sites on a genome-wide scale. Our data revealed the presence of known and novel GR and p65 binding sites across the human genome. Furthermore, the global profile of RNAPII binding sites identified genes differentially regulated by GR and/or NF-κB. This study sheds light on the GR and NF-κB cross-talk and substantially contributes to our understanding of the mechanisms and the molecular networks underlying the diverse biological effects of GCs and their relevance to inflammation.

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BIOINFORMATIC ANALYSIS OF MASTIC OIL TREATMENT IN DIFFERENT TUMOR CELL LINES REVEALS MOLECULAR MECHANISMS TARGETING CELL GROWTH AND SURVIVAL

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Mastic oil, the essential oil from Pistacia lentiscus variation chia, that contains a mixture of bioactive monoterpenes, has been shown to suppress tumor growth both *in vitro* and *in vivo*. Recent evidence based on microarray experiments to study the molecular basis of the inhibitory actions of mastic oil on Lewis Lung Carcinoma cell growth suggests mechanistic links underlying the anti-proliferative, pro-apoptotic and anti-inflammatory effects of mastic oil. The present study, further investigates the mastic oil induced response of 3 human tumor cell lines: lung adenocarcinoma (A549) erythromyeloblastoid leukemia (K562) and colon carcinoma (HCT116). Following the expression of selected genes by RT-PCR in various time points of mastic oil-treatment (3 to 48h) we present that at least some of them show a common pattern of expression with LLC cells. In addition, we sought out to identify genome-wide alterations by using DNA microarrays in two time points (12 and 24h). The number of differentiated genes varied between the different cell lines. In A549 cells, 546 genes were found as significantly altered among the 2 time points, whereas in HCT116 and K562 cells the relevant number was 2769 and 3328 respectively. GO-based meta-analysis further associated modulation of several biological functions with gene expression changes. Specifically, in all 3 cell lines, functions related to cell cycle and division as well as mitosis and regulation of spindle organization were among the significantly altered ones, while in HCT116 and K562 cells apoptosis was found to be induced in 12 and 24h respectively.

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DNA METHYLATION OF THE *P14^{ARF}*, *RASSF1A* AND *APC1A* GENES DEFINE A POOR PROGNOSIS SUBSET OF COLORECTAL CANCER PATIENTS INDEPENDENTLY OF TUMOR STAGE AND TUMOR DIFFERENTIATION

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Objective: To prospectively study the methylated fraction of CpG sites in the promoter regions of *O^6-MGMT, p14ARF, p16^{INK4a}, RASSF1A* and *APC1A* in tumor tissue from patients with colorectal cancer (CRC) to investigate whether promoter hypermethylation of any of these genes predicts the outcome (survival) of the patients.

Patients and Methods: Newly developed Pyrosequencing® assays were used to examine the methylation of the promoter regions of *O^6-MGMT, p14^{ARF}, p16^{INK4a}, RASSF1A* and *APC1A* in DNA samples from 111 primary CRC and 46 matched normal colorectal mucosa samples from the same patients. Follow-up time was around 20 years.

Results: Partial methylation was found in the following frequencies 34% for *O^6-MGMT*, 29% for *p14^{ARF}*, 28% for *p16^{INK4a}*, 14% for *RASSF1A* and 27% for *APC1A*, normal mucosa was always unmethylated. There was a significantly poorer prognosis in univariate analysis for CRC patients who

had methylated *p14^{ARF}* gene promoter (p=0.036), and for CRC patients who had methylated *O^{6}-MGMT* gene promoter, the prognosis was better through the first 120 months post-treatment. Concurrent methylation of one or more of the genes from the set *p14^{ARF}, RASSF1A* and *APC1A*, was significantly (p=0.021) associated with poorer prognosis even adjusting for Duke's stage and tumor differentiation. The other genes studied were not significantly related to outcome.

Conclusion: DNA methylation of the *p14^{ARF}, RASSF1A* and *APC1A* genes define a poor prognosis subset of colorectal cancer patients independently of tumor stage and tumor differentiation. *O^{6}-MGMT* methylation may play a protective role.

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GENE EXPRESSION ANALYSIS OF CHICKEN DURING DEVELOPMENT SUBSEQUENT TO PFOS AND PFOA EXPOSURE

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Perfluorinated compounds have been manufactured for over 50 years and have a wide range of applications due to their surfactant properties. They have been used as fabric protectors to repel water and dirt, fire-fighting foams, non-stick coatings, insecticides and all weather clothing. PFCs are widespread in the environment and are found globally in wildlife. Among the most abundant are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). The levels in common guillemot eggs from the Baltic Sea are among the highest found in the Scandinavian environment. Previous studies show effects on embryo survival of chicken at levels close to levels found in the Baltic guillemot. To investigate the possible mechanisms of action, microarray gene expression analysis was conducted. Chicken eggs were incubated at 37.5 degrees and 60% relative humidity. PFOS or PFOA was administered to eggs on day 4 of incubation by injection into the air cell of the egg. 1 µl injection solution per gram egg was used. The injection solutions contained PFOS or PFOA dissolved at different concentrations in sterile water with 5% or 2.5% dimethyl sulfoxide respectively. The doses were 10 and 3 mg/kg for PFOS and 1.6 and 0.5 mg/kg for PFOA. The vehicle solutions were used as control treatment. At day 19 the embryos were sacrificed and the liver was extracted. Four livers per dose and each vehicle control were used. RNA was extracted from the livers using Qiagen RNeasy Mini Kit. cDNA was synthesized from mRNA and used for microarray analysis on Agilent two-color chicken microarrays.

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THE UCP3 -55C>T POLYMORPHISM IS INDEPENDENTLY RELATED TO ACADEMIC ACHIEVEMENT IN 15-YEAR OLD ADOLESCENTS

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Objective: Studies on academic achievement of adolescents in school have focused on socioeconomic factors, but genetic effects have been implicated indirectly through strong association between academic achievement and the IQ of the parents. Uncoupling protein 3 (UCP3) is a mitochondrial anion carrier protein that is highly expressed in skeletal muscle. We showed recently that the UCP3 polymorphism rs1800849 (-55C>T) is associated with plasma homocysteine levels in youth. We previously found that tHcy levels were negatively related to academic achievement estimated by school grades, and therefore we hypothesized that UCP3 could be associated with academic achievement. The aim of this study was to analyze the association between polymorphisms in the UCP3 gene and academic achievement in a cohort of Swedish adolescents

Materials and Methods: The study group consisted of 356 Swedish adolescents (14-16 years of age), a subgroup in the European Youth Heart Study, a cross sectional study of risk factors for cardiovascular disease. Academic achievement was estimated from the sum of scores in 17 core subjects. Six polymorphisms in the UCP3 gene were genotyped by DNA sequencing using the pyrosequencing technique.

Results: After adjusting for age, gender, pubertal status, folate and vitamin B12 intake and MTHFR 677C>T polymorphism, we observed that participants who were homozygous or heterozygous for the T allele of the UCP3-55C>T (rs1800849) polymorphism had significantly lower academic achievement than those with the wildtype (CC) genotype (R2 of the model=0.257).

Conclusion: The negative effect on academic achievement, measured as sum of school grades was demonstrated for the UCP3-55C>T polymorphism. Possible mechanisms involve homocysteine neurotoxicity and muscular energy metabolism.

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INTERACTIVE PARTNERS AND MOLECULAR CHARACTERISTICS OF THE MAMMALIAN HNRNP A3 PROTEIN

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hnRNP proteins are considered important participants in almost every step of the mRNA maturation processes. In particular, the group of hnRNP A/B comprises abundant nuclear proteins, that have a major role in basic as well as alternative splicing and the ability for nuclear/cytoplasmic shuttling. Compared to the best known members (hnRNP A1 and A2/B1), hnRNP A3 is a relatively new protein, for which we have an interest in ascribing its biochemical and molecular properties. To this extent, the auxiliary domain of hnRNP A3 was expressed as a GST-fused product and applied, in parallel with a GST-fused hnRNP A2, in pull-down assays using nuclear and cytoplasmic extracts from human (HeLa, A549) cell lines. These studies revealed the unique ability of this domain of hnRNP A3 to form an extensive network of protein-protein interactions, not only with the other A/B, but also with hnRNP M and L proteins, in both cellular compartments. All interactions, with the exception of A1, were highly enhanced by previous RNase digestion of the extract. Furthermore, immunochemical studies, using a commercial and a homemade rabbit polyclonal antibody provided clear evidence for the existence of two discrete hnRNP A3 isoform types, one common to human and rodent and the second comprising a major isoform unique to rodent cells. Evidence is also provided for an increased expression of hnRNP A3 in human lung cancer, in common to other A/B type proteins. Our findings provide evidence for novel characteristics related to hnRNP A3 and give further support to its anticipated involvement, together with the other hnRNP A/B proteins, in mRNA metabolism.

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A COMPARTMENTED *IN SILICO* MODEL OF RAPESEED CENTRAL METABOLISM

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Systems level modeling of cellular metabolism has proven to be indispensable for the design of rational genetic modification strategies for the redistribution of the metabolic flux network towards desired end-products. The exploitation of whole-genome pathway databases in combination with the appropriate mathematical techniques and modern high-throughput measurement methods, gives new perspectives in terms of understanding and controlling the pleiotropic functionality of complex biological reaction networks. In this work a large-scale *in silico* model is constructed for the simulation of the central metabolism of Rapeseed (*Brassica napus*) embryos. Rapeseed is an organism of particular interest to the oil industry and the *in silico* reconstruction of its metabolism can help our understanding regarding the regulation of lipid biosynthesis. The model comprises 307 reactions and 249 metabolites extracted from the Aracyc and BRENDA databases. In order to validate this model, we performed constraint-based Flux Balance Analysis, through the application of linear optimization methods, and by incorporating relevant experimental data from literature. Further exploiting the derived model, an *in silico* gene deletion analysis and a systemic regulatory analysis were performed in order to evaluate and comprehend the plasticity of the real network and infer conclusions regarding its robustness as well as predict target for oil biomass overproduction.

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CHIP OVERRIDES MDM2-MEDIATED DEGRADATION OF P53 DURING REPLICATIVE SENESCENCE

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Senescence in human cells is accompanied by alterations in various biological processes including proteolysis and protein quality control. Senescence is known to be triggered by the activation of the tumor suppressor protein p53. The activity and stability of p53 are tightly regulated by various post-translational modifications, including ubiquitination and subsequent proteasomal degradation. Even though Mdm2 ubiquitin ligase is considered to play a pivotal role in stimulating the p53 turnover, a number of other E3 ligases of p53 have been identified, including the chaperone-associated ligase, CHIP. Moreover, CHIP deficiency has recently been shown to result in accelerated aging in mice, although the molecular basis of this phenotype is not completely understood. In this study, we aimed to analyze the role of CHIP ligase in regulating p53 levels and also the possible implication of this interaction in cellular senescence.

We demonstrate that CHIP ligase is gradually up-regulated in human fibroblasts undergoing replicative senescence, concomitant with a significant down-regulation in the levels of p53. We show that CHIP ligase partially translocates to the nucleus and acquires higher ubiquitination levels, thus possibly facilitating targeting of CHIP substrates to the proteasome for degradation in senescent fibroblasts. More importantly, the present findings suggest that CHIP overrides Mdm2-dependent degradation of p53 upon the onset of senescence. Taken together, our data imply that CHIP-dependent regulation of p53 stability is linked to specific p53-mediated responses such as the manifestation of cellular senescence.

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ENGINEERED BACTERIA FOR THE DISCOVERY AND ASSESSMENT OF HUMAN ENDOCRINE MODULATORS

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The nuclear hormone receptors comprise one of the largest classes of targets for drug discovery, as their function has been linked to many serious diseases, including several forms of cancer. Identifying novel compounds which modulate the function of these targets could lead to the development of effective therapeutics. In vivo sensors of ligand binding have emerged as tools that can greatly accelerate the lead identification process, allowing new drugs to be discovered more rapidly and cheaply. In this work, a novel sensor of nuclear hormone binding has been developed in *Escherichia coli* by constructing a fusion of the ligand-binding domain of the human estrogen receptor with a thymidylate synthase enzyme (TS). Expression of this fusion protein in TS-deficient bacterial cells resulted in growth phenotypes that were dependent on the presence of estrogen. Replacement of the estrogen receptor with the ligand-binding domain of the human thyroid hormone receptor led to specific thyroid hormone-enhanced growth. This biosensor was then challenged with a library of estrogen and thyroid hormone analogues, and it was observed that levels of cell growth correlate with ligand-binding affinity. Remarkably, this simple biosensor was able to discriminate between agonistic and antagonistic activities, as combinations of estrogen agonists had an additive impact on cell growth, whereas known estrogen antagonists were found to neutralize agonist effects. Furthermore, the ability of this system to assist the discovery of new estrogen-mimicking compounds was validated by screening a small compound library, which led to the identification of two structurally novel estrogen receptor modulators and the accurate prediction of their agonistic/antagonistic biocharacter in human cells. The ability of our sensor to detect ligand binding and recognize pharmacologically critical properties arises from allosteric communication between the artificially combined protein domains, where different ligand-induced conformational changes in the receptor are transmitted to the catalytic domain and translated to distinct levels of enzymic efficiency.

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DEVELOPMENT AND VALIDATION OF A QUANTITATIVE, PCR-BASED ASSAY FOR THE MEASUREMENT OF PLATINUM DNA DAMAGE FORMATION/REPAIR IN OVARIAN CARCINOMA. CLINICAL APPLICATION

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Background: DNA damage formation/repair plays an important role in the mechanism of action of genotoxic drugs and may therefore determine individual patient clinical outcome. In this report, the development and validation of a gene-specific method for measuring DNA damage formation/repair following exposure to platinum-based anticancer drugs used in the treatment of ovarian carcinoma is presented.

Materials and Methods: Cell cultures and peripheral blood mononuclear cells (PBMC) from healthy volunteers were treated with cisplatin or carboplatin. Gene-specific damage formation/repair was examined using a multiplex long quantitative PCR (Q-PCR) carried out in a 7kb fragment.

Results: Using both platinum drugs, the kinetics of intra- and interstrand cross-links formation/repair was examined using Southern blotting. Cisplatin-induced intrastrand cross-links levels reached a plateau within ~3h of treatment, while peak interstrand cross-links were observed at ~24h of exposure. Carboplatin-induced maximal levels of both intra- and interstrand cross-links were observed within 24h of drug incubation. The extent of Q-PCR amplification of the p53 fragment was conversely proportional to the treatment

concentrations of both platinum-based drugs examined, implying a dose-related inhibition by the DNA adducts formed. Parallel analysis of the same samples using both Southern blot and Q-PCR, showed that the adducts measured by Q-PCR correspond to the total platinum-induced lesions.

Conclusion: Using the current protocol of the Q-PCR method it is feasible to measure gene-specific damage formation/repair in PBMC from humans exposed to platinum-based drugs and to examine, at the level of individual patients, the relationship between the formation/repair of cytotoxic DNA damage and clinical outcome.

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THE IMPLICATION OF THE UBIQUITIN-PROTEASOME SYSTEM IN THE EARLY ONSET OF AGEING

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Several sophisticated DNA repair mechanisms exist in order to respond to constant threats on the genome. Nucleotide excision repair (NER) deals with helix-distorting lesions that generally obstruct transcription and normal replication. In humans, the consequences of a defective NER are illustrated by four autosomal disorders: xenoderma pigmentosum (XP), Cockayne syndrome (CS), trichothiodystrophy (TTD) and XPF-ERCC1 syndrome (XPF-ERCC1). Most XP patients have a 2000-fold elevated risk of skin cancer whereas CS, TTD and XPF-ERCC1 patients are progeroid syndromes. Mouse models that have been developed with defects in NER also demonstrate either accelerated ageing or increased cancer predisposition. Genome maintenance is determined by several other factors, including efficient proteolysis of damaged proteins. Protein degradation is predominantly catalyzed by the proteasome, whose function has been shown to decline with age. In order to further investigate the role(s) of the proteasome in the ageing process, we are currently analysing its function in mouse embryonic fibroblasts (MEFs) derived from NER deficient mice. Green Fluorescent Protein-fused reporters are used in order to estimate the *in vivo* function of the Ubiquitin proteasome system (UPS). Our results demonstrated a decline in the UPS function of MEFs derived from mutant mice demonstrating accelerated ageing whereas no such differences are observed in MEFs coming from mice with cancer predisposition. These preliminary data indicate an intriguing role of the UPS in the early onset of ageing and triggers further exploration in order to shed light on the traditional and possibly non-traditional roles of the proteasome in the physiological organismal ageing.

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TISSUE-SPECIFIC EXPRESSION OF A NEW HEAT SHOCK FACTOR IN ZEBRAFISH

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Background: Heat shock (transcription) factor 1 (HSF1) regulates the transcription of heat shock genes and various developmental events (1, 2). Until to date, three additional HSFs and several related proteins have been described and orthologs have been identified in various species; however not all of them were found to be directly involved with heat shock response (1, 2). HSF5 is a novel heat shock factor that was discovered and partially characterized by the Orban Lab (TLL, Singapore) recently (3). The expression of zebrafish hsf5 can be detected in the embryo and becomes testis-enhanced from 35 dpf (3). In adult, strong expression can be detected in the testis, with lower transcript levels in ovary, kidney and brain (3).

Materials and Methods: In this collaborative project, we are studying the distribution and abundance of HSF5 protein in zebrafish embryos and tissues using whole mount immuno-histochemistry and sections of different developmental stages.

Results: Our results confirm the expression data by detecting a large amount of HSF5 protein in adult testis and lower levels in the ovary.

Conclusion: These experiments will yield valuable information about the HSF5 protein localization in zebrafish gonads at different developmental stages and they might also provide indications for the potential role of the protein in the gonad.

1 Pirkkala L, Nykanen P and Sistinen L: Roles of the heat shock factorsin regulation of the heat shock response and beyond. FASEB J *15*: 1118-1131, 2001.

2 Akerfelt M, Trouillet D, Mezger V and Sistonen L: Heat shock factors at a crossroad between stress and development. Ann N Y Acad Sci *1113*: 15-27, 2007.

3 Hossain MS: Molecular analysis of gonad differentiation and function in zebrafish, PhD. Thesis, National University of Singapore, January, 2010.

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ROLE OF 19S PROTEASOME SUBUNITS IN DNA DAMAGE AND STRESS-INDUCED PREMATURE SENESCENCE

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The DNA damage response (DDR) has evolved in order to optimise cell survival following DNA damage. It includes the orchestrated recruitment of DNA-repair proteins to sites of damage and checkpoint events that delay or arrest cell-cycle progression, until the damage is removed. On the other hand, the proteasome is responsible for the degradation of various regulatory proteins involved in signalling cascades, including the DDR. The proteasome consists of the 20S catalytic "core" and the 19S regulatory cap, which is further comprised of the lid and the base. In this study we addressed the role of the 19S proteasome in the DDR. Applying oxidative stress results initially in acute DDR demonstrated by γH2AX foci formation and, if not repaired, eventually led to Stress Induced Premature Senescence (SIPS). Unlike during acute damage, there was a major increase in the protein levels of the 19S subunit components under SIPS. Confocal microscopy demonstrated that under both stress conditions there was a clear translocation of the 19S subunits from the cytoplasm to the nucleus, whereas core proteasome subunits' localisation remained unchanged. In particular, Rpn7, Rpn11 and Rpn12, lid subunit components, showed a distinct co-localisation with γH2AX foci. Interestingly, when cells were released from stress, there was a coincidental removal of both Rpn11 and Rpn7 from DDR foci. The data presented indicate a possible involvement of the various 19S subunits in DNA repair.

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OLIVE OIL-BASED W/O NANO-EMULSIONS: A NOVEL BIOMIMETIC SYSTEM FOR STUDYING OXIDATIVE ENZYMATIC REACTIONS

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Virgin olive oil (VOO) can be considered as a fine emulsion of a small quantity of water dispersed in a continuous medium of triglycerides, stabilized by various endogenous ionic and nonionic amphiphilic components (1). Water-in-oil (w/o) nano-emulsions, composed of olive oil as the continuous oil phase, have been successfully used as model biomimetic media to carry out enzymatic reactions that may naturally occur in olive oil since detectable amounts of lipoxygenase, poly-phenoloxidase and peroxidase activities have been detected in olive oil samples (2, 3). Oxidative enzymatic reactions using horseradish peroxidase (HRP) were carried out in w/o nano-emulsions composed of olive oil/lecithin/1-propanol/water. The substrates used (gallic acid, octyl gallate and ABTS) span a range of hydrophobicities and possible locations in the nano-emulsion system. HRP reactivity in these systems with reference to the substrate hydrophobicity is examined. The nature of the enzyme microenvironments was examined using Dynamic Light Scattering (DLS) and Differential Scanning Calorimetry (DSC). While the location of the various enzymatic substrates in the microemulsion phase was assessed by solubility measurements and by determining pressure-area isotherms of mixed monolayers of the substrates with dipalmitoyl-phosphatidylcholine (DPPC), which is a major constituent of lecithin.

1 Sotiroudis TG, Sotiroudis GT, Varkas N and Xenakis A: The role of endogenous amphiphiles on the stability of virgin olive oil-in water emulsions. J Am Oil Chem Soc *82*: 415-420, 2005.
2 Georgalaki MD, Sotiroudis TG and Xenakis A: The presence of oxidizing enzyme activities in virgin olive oil. J Amer Oil Chem Soc *75*: 155-159, 1998.
3 Tzika ED, Sotiroudis TG, Papadimitriou V and Xenakis A: Partial purification and characterization of peroxidase from olives (Olea europaea cv. Koroneiki). Eur Food Res Technol *228*: 487-495, 2009.

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ASSOCIATION BETWEEN POLYMORPHISM IN THE CARD8 GENE AND MYOCARDIAL INFARCTION IN MEN

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Objective: Ischemic heart disease is one of the leading causes of death in the Western world. One of the major factors for myocardial infarction is inflammation and crucial for this process is the assembly of the inflammasome. Several inflammasomes are known, but the NALP3 inflammasome is

the one best characterized. The NALP3 inflammasome consists of the protein products of the *NLRP3*, *ASC* and *CARD8* genes, which together activates proinflammatory cytokines like IL-1b, IL-18 and IL-33. Previous studies have found associations between polymorphisms in the *NLRP3* and *CARD8* genes and inflammatory diseases. In the present study the association between these polymorphisms and myocardial infarction was Investigated.

Materials and Methods: 550 cases with myocardial infarction and 1013 controls were genotyped for the Q705K and C10X polymorphisms in the *NLRP3* and the *CARD8* genes respectively, using the TaqMan® SNP genotyping assay followed by allelic discrimination.

Results: A significant association was found between the A-allele in the CARD8 gene and myocardial infarct in men (p=0.016). Two A-alleles compared to one or no A-allele were more prevalent in the disease group compared to controls, but of borderline significance (p=0.051). No significant association was found between the A-allele in the *NLRP3* gene.

Conclusion: Genetic alterations in the *CARD8* gene seem to be a risk factor for the development of myocardial infarction in men. The correct assembly of the inflammasome may therefore be of significance to the development of myocardial infarction. The knowledge of a person's genotype may therefore in the future be informative in evaluating the risk for myocardial infarction.

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A LARGE-SCALE EUROPEAN STUDY REVEALS THE ROLE OF GENETIC AND ENVIRONMENTAL FACTORS ON HEALTHY AGING AND LONGEVITY

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Under the framework of the European Union-Integrated Project "GEnetics of Healthy Aging" (GEHA) which aims to identify genes involved in healthy aging and longevity, we have collected data (health status & biological sample) from 400 subjects (90+ sibpairs and younger unrelated controls) revealing that human longevity is clustered in families enriched in long-lived parents and ancestors. Epidemiological data from different European regions show a lower prevalence of cancer, CVD, insulin-resistance and diabetes, and a delay of about 1-2 decades of the onset of other pathologies, such as dementia and hip fractures. Most of them do not show insulin-resistance and have anthropometric (BMI) and metabolic (cholesterol, LDL-C, HDL-C, triglycerides, *etc*.) features that are optimal for their age. Smoking is extremely rare, and even when it occurs among them, it is correlated almost exclusively to bad health conditions and non-auto sufficiency.

Furthermore, the results indicate that offspring of long-lived sibling pairs have a lower mortality risk already at middle age, whereas their spouses, with whom they have shared in part a common environment, do not show this survival benefit. Linkage analysis has also been performed on a dataset of 2564 sibling pairs using a panel of 6090 highly polymorphic SNPs evenly distributed over the entire genome revealing regions potentially enriched in longevity genes. Finally the analysis of demographic data demonstrated regions in Greece with an extreme rate of longevity and validated data highlighted Ikaria as a "blue zone", one of the four regions worldwide with exceptional longevity.

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EFFECTS OF PERFLUORINATED COMPOUNDS ON HEPATIC FATTY ACID OXIDATION IN AVIAN EMBRYOS USING A TRITIUM RELEASE ASSAY

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Objective: Due to high persistence and bioaccumulation perfluorinated compounds (PFCs) are found globally in various types of wildlife samples and also human samples[1]. According to our laboratory studies, perfluorooctane sulfonate (PFOS) has caused early mortality in chicken embryos at doses close to concentrations found in eggs of the Baltic guillemot[2]. We have designed a method in which hepatic embryonic tissue from chicken (*Gallus domesticus*) is used to investigate the effects of PFCs on the β-oxidation of fatty acids.

Materials and Methods: The embryos were exposed in ovo to PFCs. On day 10 embryo livers were incubated *in vitro* with tritiated fatty acids. Fatty acid oxidation was then calculated from the tritium released into water, using a scintillation counter.

Results: Our studies suggest a small but significant increase of the β-oxidation of fatty acids in chicken embryonic liver tissue *in vitro* after in ovo exposure to PFOS. The β-oxidation was significantly induced after embryo exposure to 1 mg/kg PFOS (p=0.003) and 10 mg/kg PFOS (p=0.04), being 39% and 34% higher, respectively compared to control.

Conclusion: The results show that in ovo exposure in combination with an *in vitro* method using a tritium release assay to detect effects on the β-oxidation of fatty acids in avian embryo hepatic tissue could be a useful method in elucidating possible mechanisms behind avian developmental toxicity.

1 Heckster FM, Laane RWPM and de Voogt P: Environmental and toxicity effects of perfluoroalkylated substances. Rev Environ Contam Toxicol *179*: 99-121, 2003.
2 Engwall M, Andersson Å and Järnberg U: Toxicity of perfluorosulfonate (PFOS) in chick embryos-LOAEL is close to concentrations in piscivorous avian top predators in the Baltic Sea. Organohalogen Compounds *68*: 414-417, 2006.

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STRUCTURAL STUDIES OF HCINAP: AN ATYPICAL MAMMALIAN NUCLEAR ADENYLATE KINASE WITH ATPASE ACTIVITY

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Human Coilin Interacting Nuclear ATPase Protein (hCINAP) is a nuclear factor that directly interacts with coilin, a marker protein of Cajal bodies, nuclear organelles involved in the maturation of UsnRNPs and snoRNPs. hCINAP has been designated as an adenylate kinase (AK6), but exhibits unusually broad substrate specificity, atypical structural features and additional ATPase activity. Its enzymatic mechanism and biological function have remained poorly characterised. Structural analysis, rigid- and induced-fit docking calculations and enzyme kinetic analysis coupled with site-directed mutagenesis have revealed that besides a structure typical for an AK, hCINAP also displays features characteristic of ATPase/GTPase proteins (Walker motifs A and B) and provide a detailed examination of its dual enzyme activity.

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ABERRANT TYROSINE TRANSPORT IN FIBROBLASTS FROM PATIENTS WITH BIPOLAR TYPE-1 DISORDER

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Aberrant tyrosine transport across cell membranes has been found in patients with schizophrenia in several studies (1) and recently also in patients with autism (2). The amino acids tyrosine and tryptophan are precursors of the neurotransmitters dopamine and serotonin and their availability in the brain may influence neurotransmission. Disturbed neurotransmitter systems, such as the dopaminergic, noradrenergic and serotoninergic systems are indicated in patients with various neuropsychiatric disorders, including bipolar disorder (Figure 1). The aim of the present study was therefore to explore whether an abnormality in the transport of tyrosine and/or tryptophan is also present in patients with bipolar disorder.

Patients and Methods: Fibroblast cell lines from patients with bipolar type-I disorder (n=10) and healthy controls (n=10) were included in this study. All patients fulfilled the DSM-IV diagnostic criteria. The transport of amino acids across the cell membranes was measured by the cluster tray method. The kinetic parameters, maximal transport velocity (V_{max}) and affinity constant (K_m) were determined by the Lineweaver-Burke plot equation.

Results: A significantly lower V_{max} of tyrosine transport (p=0.027) was found in patients with bipolar type-I disorder in comparison to healthy controls, but the K_m of tyrosine transport did not differ significantly between the two groups. There were no significant differences for tryptophan transport in either V_{max} or K_m between patients with bipolar type-1 disorder and healthy controls (Figure 1).

Conclusion: The fibroblasts from patients with bipolar type-I disorder show a decreased V_{max} of tyrosine transport, This findings implies that the transport systems have lower capacity for amino acid uptake, which could be due to lower expression of transporting proteins and/or a point mutation in one of the genes coding for the transporters involved. It could also be due to a disturbed membrane phospholipid composition (MPC), indicated in both bipolar disorder and schizophrenia, which could be altering the structure of transporter proteins that in turn might change the functionality of the transporters. The decreased tyrosine transport (low V_{max}) may indicate less access of dopamine in the brain, resulting in disturbed dopaminergic and/or noradrenergic neurotransmission that secondarily could lead to disturbances in other central neurotransmitter systems, such as the serotoninergic system. A low V_{max} and/or K_m have repeatedly been found in patients with schizophrenia. As bipolar disorder and schizophrenia share many epidemiologic features it is speculated whether the two disorders are related. The present results are in accordance with previous findings in

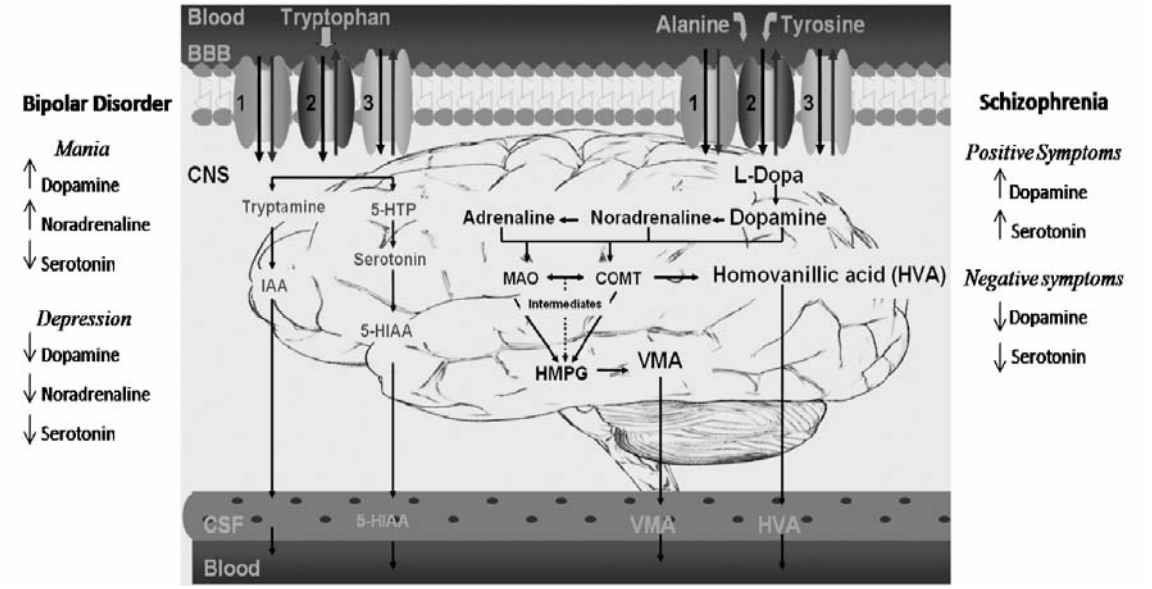

Figure 1. *Schematic drawing showing the transport of amino acids across the blood brain barrier (BBB) through different amino acid transporters (1, 2, 3) to form neurotransmitters and their metabolites in the brain. The text on either side of the figure, mentions the variations in neurotransmitter levels in the central nervous system of bipolar disorder and schizophrenia,respectively. 5-HTP: 5-Hydroxytryptophan, IAA: Indoleacetic acid, 5-HIAA: 5-Hydroxyindoleacetic acid, MAO: Monoamine oxidase, COMT: Catechol-o-methyl transferase, HMPG: 4-Hydroxy 3-methoxy phenyl glycol, VMA: 3-Methoxy-4-hydroxymandelic acid, CSF: Cerebrospinal fluid.*

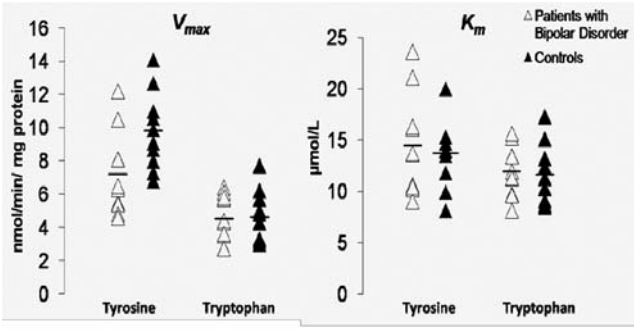

Figure 2. *Individual values and mean values (horizontal bars) for V_{max} and Km of tyrosine and tryptophan transport in fibroblasts from patients with Bipolar type-1 disorder (n=10) and controls (n=10).*

schizophrenic patients indicating the existence of a decreased tyrosine transport in both disorders. The two disorders present with different clinical symptoms, Therefore a shared amino acid transport aberration may be implicated in bipolar disorder and schizophrenia.

1 Flyckt L, Venizelos N *et al*: Aberrant tyrosine transport across the cell membrane in patients with schizophrenia. Arch Gen Psychiatry *58(10)*: 953-958, 2001.

2 Fernell E, Karagiannakis A, Edman G, Bjerkenstedt L, Wiesel FA and Venizelos N: Aberrant amino acid transport in fibroblasts from children with autism. Neurosci Lett *418*: 82-86, 2007.

3 Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, Sullivan PF and Hultman CM: Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet *373*: 234-239, 2009.

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INTERLEUKIN-1BETA INHIBITS TYROSINE TRANSPORT IN FIBROBLASTS FROM PATIENTS WITH SCHIZOPHRENIA AND HEALTHY CONTROLS

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The amino acid tyrosine is the precursor of the neurotransmitters dopamine and norepinephrine and its availability in the brain may thus influence dopaminergic and noradrenergic neurotransmission. Tyrosine is actively transported through plasma membranes, like the blood brain barrier (BBB), mainly by the amino acid transport systems L (LAT1) and A (ATA2). Decreased transport of tyrosine has been a repeated finding in patients with schizophrenia, in particular when using a fibroblast cell model, in which a lower maximal transport velocity (V_{max}) and/or affinity constant (K_m) has been

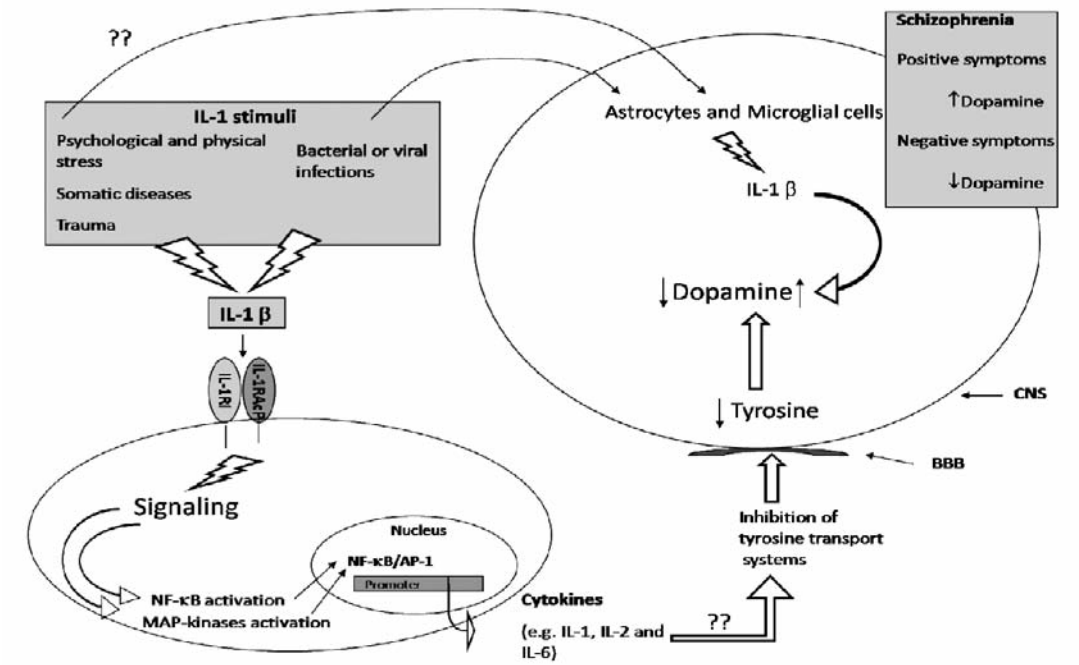

Figure 1. *A simplified proposed illustration of the interleukin (IL)-1-induced signal transduction pathways and the potential relationship between IL-1β, tyrosine transport systems, dopamine synthesis in the central nervous system (CNS) and schizophrenia.*

demonstrated (1). However, the reason for this transport aberration has still not been elucidated. Altered levels of the pro-inflammatory cytokine interleukin-1beta (IL-1β) have been found in patients with schizophrenia and IL-1β has shown to have inhibitory effects on amino acid transport systems (2, 3). Hence, the aims of this study were to investigate the effect of IL-1β on tyrosine uptake in fibroblasts from schizophrenic patients and healthy controls and to study any difference in tyrosine uptake between patients and controls (Figure 1).

Materials and Methods: Fibroblast cells were incubated with different concentrations (0.01 ng/ml to 10 ng/ml) of IL-1β for different time periods (1 hour to 24 hours) to optimize the effect of IL-1β on tyrosine uptake. The optimized conditions were used to study any difference in tyrosine uptake between patients and controls. Fibroblast cell lines from schizophrenic patients (n=10) and healthy controls (n=10) were treated with 5 ng/ml of IL-1β for 6 hours. Fibroblasts untreated with IL-1β were used as controls in all studies. Uptake of 14C(U)-L-tyrosine was measured using the cluster tray method.

Results: IL-1β significantly inhibited the tyrosine uptake in fibroblasts of patients with schizophrenia by 43% ($p<0.001$) and controls by 42% ($p<0.001$). The inhibition of tyrosine uptake by IL-1β was abolished in the presence of the IL-1β receptor antagonist. No difference in the uptake levels between fibroblasts of patients with schizophrenia and controls was evident (Figure 2).

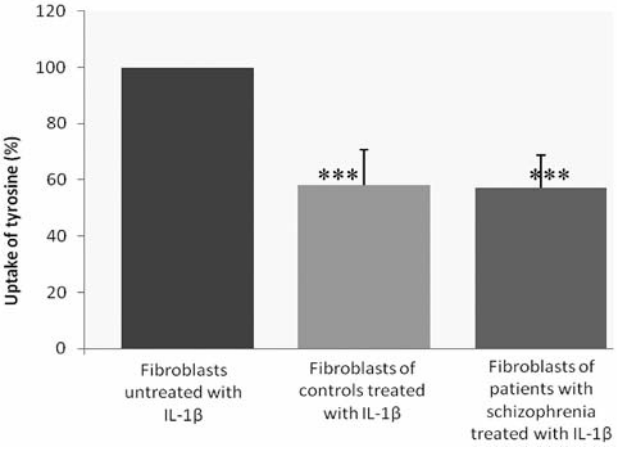

Figure 2. *Effect of interleukin-1beta (IL-1β) on tyrosine uptake in fibroblasts of patients with schizophrenia and controls. The results are shown in percentage as mean with standard deviation. ***p<0.001 untreated with IL-1β condition vs. treated with IL-1β condition.*

Discussion and Conclusion: The main finding in this study was that the pro-inflammatory cytokine IL-1β has a strong inhibitory effect on tyrosine transport systems. This study was carried out without discriminating between the tyrosine transport systems L and A, but since previous findings have shown that IL-1β inhibits both of these

systems, we speculate that the IL-1β-mediated inhibition of tyrosine uptake affected both of them. However, only 10% of tyrosine is transported by system A (ATA2) and since we observed an IL-1β-mediated inhibition effect on tyrosine uptake of approximately 40%, the inhibition should mainly be due to an IL-1β-mediated effect on system L, but this needs to be further explored. A decreased tyrosine uptake, mediated by the presence of IL-1β, indicates that increased levels of IL-1β in patients with schizophrenia can be a factor of importance for the availability of tyrosine in the brain. Since the brain is the only organ for which amino acid transport is limited, a limited transport of tyrosine caused by IL-β could thus lead to disturbances in dopamine and norepinephrine metabolism. Further studies are needed to elucidate the significance of these findings.

1 Flyckt L, Venizelos N *et al*: Aberrant tyrosine transport across the cell membrane in patients with schizophrenia. Arch Gen Psychiatry *58(10)*: 953-958, 2001.

2 Theodoropoulou S *et al*: Cytokine serum levels, autologous mixed lymphocyte reaction and surface marker analysis in never medicated and chronically medicated schizophrenic patients. Schizophr Res *47(1)*: 13-25, 2001.

3 Thongsong B, Subramanian RK *et al*: Inhibition of amino acid transport system a by interleukin-1beta in trophoblasts. J Soc Gynecol Investig *12(7)*: 495-503, 2005.

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RELEASED SUBSTANCES FROM LACTOBACILLI INFLUENCE IMMUNE RESPONSES IN HUMAN EPITHELIAL CELLS

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Objective: Lactobacilli are a major constituent of the human microbiota where they influence the normal immune development and maintain homeostasis. Numerous lactobacilli have been isolated and used as health promoting, or probiotic, bacteria. While most are used for gastrointestinal ailments, some lactobacilli strains are also available for urogenital use. The urogenital probiotic *Lactobacillus rhamnosus* GR 1 is commercially available together with *L. reuteri* RC 14. These two strains are used in concert to normalize the vaginal microbiota and protect against urogenital diseases, including urinary tract infections (1). Although these two strains have a scientifically established clinical effect, the mechanism of action, as for most probiotic microbes, remains largely unknown. Some lactobacilli are able to modify and help shape the immune system. Although generally considered safe for human consumption, these microbes can have a profound influence on how immune responses are mounted. We have studied the role of released products from the two urogenital probiotic strains, *L. rhamnosus* GR 1 and *L. reuteri* RC 14, and their ability to modulate immune responses against the uropathogenic *Escherichia coli* GR12 *in vitro*. The nuclear factor-kappa B (NF-κB) transcription factor is a key regulator of cytokine and chemokine expression from bladder cells during uropathogenicity, and is important for the subsequent eradication of pathogens from the urinary tract. In this study, we have analyzed NF-κB activation within bladder epithelial cells after stimulation with heat killed (HK) uropathogenic *E. coli* and the secreted products from probiotic *L. rhamnosus* GR 1 and *L. reuteri* RC 14.

Materials and Methods: T24 bladder cells were stimulated with released products from *L. rhamnosus* GR 1 or *L. reuteri* RC 14 isolated in phosphate buffered saline. Cells were challenged with lactobacilli products in the absence or presence of HK uropathogenic *E. coli* GR12. To assess immunomodulatory action of these products, cells were transiently transfected with a vector carrying NF-κB enhancer regions with a reporter gene to be used for the quantification of NF-κB activation.

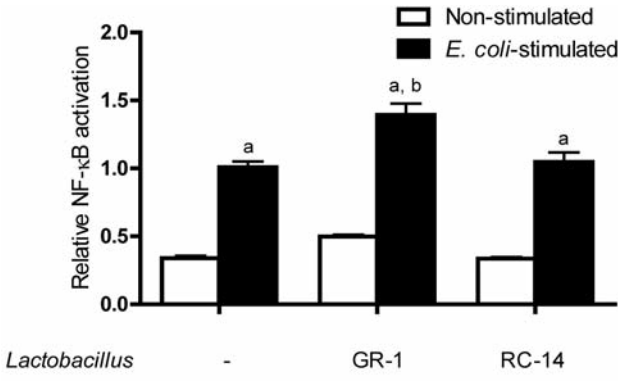

Figure 1. *T24 cells were challenged for 24 hours with released products from L. rhamnosus GR 1 or L. reuteri RC 14. Closed bars indicate cells stimulated with HK E. coli GR12. Bars labeled "a" are significantly different from cells not stimulated with HK E. coli, and bar labeled "b" is significantly different from cells stimulated with HK E. coli alone (P-values<0.05). Error bars represent the standard error of the means.*

Results: Stimulation of T24 cells with HK uropathogenic *E. coli* significantly increased NF-κB activation, mimicking an *in vivo* situation of bladder infection. However, isolated released products from *L. rhamnosus* GR 1 or *L. reuteri* RC 14 altered NF-κB activation in the bladder cells. Although these isolated

released substances from lactobacilli were unable to activate NF-κB alone, the products from *L. rhamnosus* GR 1 potentiated the *E. coli* induced NF-κB activation two-fold. *L. reuteri* RC 14 material did not result in any increased activation of NF-κB during co-stimulation with HK *E. coli* compared to cells stimulated with *E. coli* alone (Figure 1).

Conclusion: Immunomodulation is regarded as an important attribute for probiotic effect. The increased immune response evoked by some lactobacilli can represent an improved ability of the cells to defend themselves against microbial pathogens. Although most pathogens are associated with disease manifestations such as inflammation, many harmful microbes including uropathogenic *E. coli*, effectively subvert host immunity. The immunomodulatory effect observed by *L. rhamnosus* GR 1 released products on epithelial cells *in vitro* may represent a potential mechanism of how these bacteria support urogenital health. The strong response against incoming pathogens seen after stimulation with released products from *L. rhamnosus* GR 1 can be a successful strategy to prevent infection and may be responsible for the probiotic action of this strain.

1 Reid G and Burton J: Use of Lactobacillus to prevent infection by pathogenic bacteria. Microbes Infect *4*: 319-324, 2002.

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ENVIRONMENTAL INFLUENCES ON INFLAMMATORY RESPONSES

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Objective: Evaluation of the effects of pharmaceuticals on induced inflammatory responses is necessary for the evaluation of potential human risk. Since NF-κB and MAPK are the main pathways known to be critical regulators of inflammatory responses, intracellular signalling and downstream targets as well as modulatory factors were examined *in vitro* using Jurkat T-cells and T24 bladder epithelial cells.

Materials and Methods: Activation of the inflammatory regulators NF-κB and AP-1 was examined in T24 cells exposed to heat killed *E. coli* GR12 using Luciferase reporter assays. Furthermore, involvement of intracellular proteins (PKC and Bcl10) affecting the transduction of signals in the pathways under investigation was determined using western blotting. In addition, cytokine and chemokine levels were measured by ELISA and multiplex assays. The results were verified by using inhibitors targeting important mediators of inflammation, namely NF-κB, PKC and JNK.

Results: Pharmaceuticals are regularly released into the environment; in particular non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics, including ibuprophen, diclofenac, ketoprofen, erythromycin and sulphamethoxazole. These substances are detected at relatively low concentrations and are considered to not affect cellular activities. However, several pharmaceuticals, including naproxen and atenolol, were reported to be stable for up to 1 year in the environment, which increases the risk for accumulation. In order to determine the effects of the following environmental pollutants, pharmaceuticals were tested individually and in combination according to the measured concentrations in water samples collected in river Viskan, Borås, Sweden. Among the analyzed pharmaceuticals, NSAIDs were shown to significantly reduce NF-κB activity in T24 cells, by using environmentally relevant concentrations1. Most pharmaceuticals are found as biologically active compounds in sewage treatment plants (STP), which suggest that an increased use and improper disposal has led to an accumulation of these compounds in the aquatic environment. Furthermore, insufficient elimination of these compounds in STPs may also be a contributing factor to the elevated levels. Alteration of immune responses by these compounds may therefore lead to progressive infections since interleukin expression is mainly controlled by a cooperative activity of NF-κB and AP-1. Analysis of NF-κB- and AP-1 signalling pathways and the regulatory mechanisms of cytokine release were therefore examined in Jurkat T-cells. Short-term exposure to PMA resulted in a rapid NF-κB activation, while extended treatment suppressed NF-κB and activated AP-1. Suppression of NF-κB activity may be due to PKC-dependent Bcl10 degradation2, which was shown to decrease in response to PMA and was equivalent to NF-κB activity. However, regulation of cytokine expression revealed that NF-κB was essential for IL-6 but not CXCL8 expression following specific inhibition of NF-κB, without affecting AP-1 activity. T24 cells respond to a wide variety of bacterial products following recognition by TLRs. Both NF-κB activation and IL-6 expression was induced in a dose-dependent manner in T24 cells exposed to heat killed *E. coli* GR12 (Figure 1).

Conclusion: Alterations in pro-inflammatory cytokine and chemokine levels such as TNF, IL-6 and CXCL8 are associated with several human diseases including cystic fibrosis, pulmonary fibrosis and AIDS. The complexity of regulatory systems of inflammation suggest that there is a need to further evaluate the signalling pathways involved in order to provide a better understanding of cellular responses to foreign substances, but also to offer an insight into possible development of alternative treatments for human diseases with elevated cytokine/chemokine levels. The presence of pharmaceuticals, even at low concentrations in the environment, induce inflammatory responses as demonstrated in the human cell studies and should therefore be further studied in order to understand the effects on both humans and water-living organisms.

Table I. *A summary of the work performed with the different HIV antigens in transgenic plants. An x corresponds to completed result, an empty box shows ongoing work, and * represents completed, but unpublished, trials.*

	Arabidopsis					Carrot				
Antigen	Gene	mRNA	Protein	Feeding	Ref.	Gene	mRNA	Protein	Feeding	Ref.
p24	x	x	x	x	1					
p24_SEKDEL	x	x	x	*	2	x	x	x	*	2
p17	x									
p17_SEKDEL	x	x								
tat/nef	x	x								
nef	x									

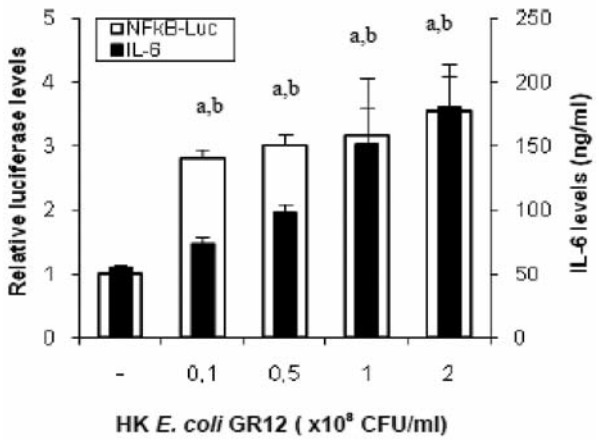

Figure 1. *Induction of inflammatory responses. NFκB activation and IL-6 expression were determined in T24 bladder epithelial cells following heat killed E. coli GR12 treatment for 24h. Statistical significant differences from the control were determined using two-tailed Student's t-test (NF-κB activation (a), IL-6 expression (b), p<0.05; n=4).*

1 Khalaf H *et al*: *In vitro* analysis of inflammatory responses following environmental exposure to pharmaceuticals and inland waters. Sci Total Environ *407*: 1452-1460, 2009.

2 Scharschmidt E, Wegener E, Heissmeyer V, Rao A and Krappmann D: Degradation of Bcl10 induced by T-cell activation negatively regulates NF-kappa B signaling. Mol Cell Biol *24*: 3860-3873, 2004.

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PLANT-BASED PRODUCTION OF HIV ANTIGENS: TOWARDS A CANDIDATE FOR AN EDIBLE VACCINE

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For several decades, enormous efforts have been devoted to the development of traditional prophylactic vaccines against HIV with very little success so far. Principally, novel angles now have to be tried with the final aim to develop a complete vaccination regimen against this sexually transmitted pathogen. One of these new angles involves efforts to muster the large amount of immune tissue present in the human intestine, the so-called gut-associated lymphoid tissue (GALT). This mucosally located immune tissue is drained through the same lymphatic nodes as the genital mucosal immune tissue and, thereby, immunization *via* GALT should give rise to immunity also in the genital mucosa, in addition to more systemic immune responses. Immunization *via* GALT can principally be accomplished by oral administration of a vaccine formulation with the pre-condition that the vaccine antigen survives the acidic conditions in the stomach. One way to deliver the vaccine orally and to circumvent acidic denaturation of the antigen would be to encapsule the antigen within a plant cell. By genetic transformation of plants and consumption of the resulting antigen-carrying transgenic plants, proof-of-principle of the development of immune responses have been shown for a number of different infectious agents, including hepatitis B and measles, both in laboratory animals and in humans.

In our research, we have identified four different HIV proteins that we believe can be used as antigens, either on their own or in combination, in an edible HIV prototype vaccine. These four proteins are the HIV capsid proteins p17 and p24 and the regulatory proteins tat and nef. We have cloned all four of these proteins and their chimeric combinations into the pGreen transformation vector normally used for *Agrobacterium tumefaciens*-mediated transformation of plants. In this vector, the expression of the HIV antigen nucleotide sequences are under control of the constitutive over-expression promoter 35S from the cauliflower mosaic virus (CaMV). In the case of p24, different expression constructs, with or without the endoplasmatic reticulum Ser-Glu-Lys-Asp-Glu-

Leu (SEKDEL) retention signal added C-terminally to the p24 protein, have been introduced either into the well-known edible model plant *Arabidopsis thaliana* (1, 2) or into carrot (2). In the case of Arabidopsis expressing the p24 antigen, laboratory mice were fed these plants and responded to this feeding by eliciting p24-specific IgG antibodies in serum (1). A large number of transgenic lines were obtained, both for carrot and Arabidopsis (1, 2), containing different numbers of inserts of the transgenes. Also, these transgenic plants were shown to be genetically stable over a number of generations with respect to the p24 insert (1).

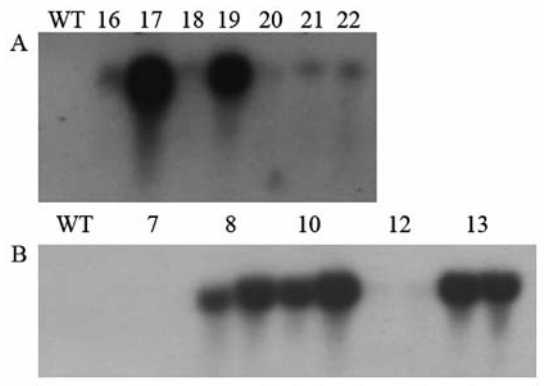

Figure 1. *Northern blots of mRNA isolated from transgenic Arabidopsis lines, WT is the negative control (mRNA from non-transformed Arabidopsis). A) Transgenic lines, numbers 16-22, of p17_SEKDEL. All lines are positive for p17_SEKDEL mRNA. However, lines 17 and 19 are showing greater signal than the others. B) Transgenic lines 7, 8, 10, 12, and 13 carrying the genetic tat/nef construct. Lines 8, 10, and 13 are producing tat/nef mRNA (two lanes of mRNA/plant line).*

For p17, p17_SEKDEL (see above), nef, and the tat/nef chimaer, we have introduced over-expression constructs into Arabidopsis in the same manner as described previously. We have also been able to show by PCR that incorporation of transgenes have occurred in all cases. Furthermore, expression of the transgenes in the form of mRNA transcripts occurs in a number of transgenic Arabidopsis lines for both p17_SEKDEL (see the Northern blot in Figure 1A) and tat/nef (Figure 1B). A summary of the progress of these studies for the different genetic constructs and plant species is shown in Table I.

At present, we are improving the expression yield of HIV antigens in plants by gene gun-mediated transformation of the plastid genome.

We thank Rody Egah and Denise Paltian for technical assistance with the p17, nef, and tat/nef constructs.

1 Lindh I, Kalbina I, Thulin S, Scherbak N, Sävenstrand H, Bråve A, Hinkula J, Strid Å and Andersson S: Feeding of mice with *Arabidopsis thaliana* expressing the HIV-1 subtype C p24 antigen gives rise to systemic immune responses. APMIS *116*: 985-994, 2008.

2 Lindh I, Wallin A, Kalbina I, Sävenstrand H, Engström P, Andersson S and Strid Å: Production of the p24 capsid protein from HIV-1 subtype C in *Arabidopsis thaliana* and *Daucus carota* using an endoplasmic reticulum-directing SEKDEL-sequence in protein expression constructs. Prot Expr Purif *66*: 46-51, 2009.

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HEPATOCYTE GROWTH FACTOR IN PATIENTS WITH CORONARY ARTERY DISEASE: A DIAGNOSTIC MARKER FOR EVALUATION OF THE DISEASE STATE?

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Background: Hepatocyte growth factor (HGF) is an angiogenic and cardio protective factor with important roles in tissue and vascular repair. High levels of HGF are associated with CAD and periodontitis. However, the biological activity of HGF may be decreased, possibly due to protease activity of periodontal bacteria (*e.g. Porphyromonas (P.) gingivalis*) and/or the host response. The aims of this study were to analyze serum concentration, and evaluate the biological activity of HGF before and after intervention in patients with CAD, and to study correlations with periodontal status and the presence of *P. gingivalis* in the tooth pocket.

Patients and Methods: Serum samples from 46 patients with CAD, collected before and in the course of 12 months after angiography, percutaneous coronary or coronary artery bypass graft intervention, were analyzed. The periodontal state of the patients was examined and classified into 3 groups and the presence of *P. gingivalis* in the tooth pocket was detected by PCR. Serum levels of HGF were measured by ELISA and biological activity was analyzed by ligand-binding capability using Biacore. Serum from 53 healthy age-matched blood donors were used as control.

Results: Patient serum levels of HGF were at all time points higher than control levels ($p<0.001$) and did not differ between different sampling times. The biological activity of HGF before intervention was significantly lower ($p<0.038$) compared to controls, and after 24 hours it decreased further. The activity of HGF increased reaching a peak 1 month ($p<0.003$ *vs.* 24 hours) after intervention, but decreased almost to the same level as

baseline after 12 month (P=0.015 *vs*. 24 hours). Patients positive for *P. gingivalis* showed slightly reduced HGF activity, which also agreed with patients with severe periodontitis.

Conclusion: High circulating levels of HGF in patients with CAD are a marker of anatherothrombotic event. The results presented here show consistent elevated HGF concentrations up to 12 months after intervention. Despite increased serum HGF concentration the biological activity is decreased, but improves temporarily 1 month after opening of the closure. An underlying infection, as with the periodontal bacterium *P. gingivalis*, could result in an inflammatory process causing elevated HGF levels. *P. gingivalis* positive serum showed slightly lower HGF activity suggesting that infection affects the degradation of HGF biological activity.

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$BRAF^{V600E}$ AN HSP90 CLIENT PROTEIN, AS A SENSITIZER TO TRAIL INDUCED APOPTOSIS

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For a long time it has been appreciated that the presence of KRAS mutations highly correlates with colorectal cancer (CRC) progression and decreased patient survival. The more recently identified BRAF mutations in CRC, do not co-exist with those in KRAS and display a more potent transforming activity (Oikonomou *et al*. 2009b; Oikonomou *et al*. 2009a). In contrast to KRAS mutation, genetic alterations in the BRAF kinase have the ability to cause genomic rearrangements in colon cells that can potentially sensitize them to apoptosis, a major advantage in cancer therapeutics, since deregulation of apoptosis can lead to growth advantage in cancer cells. Prominent among cell surface molecules capable of initiating and tightly controlling apoptosis only in cancer cells is the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) (MacFarlane 2003; Walczak *et al*. 1999). The resistance of some colon cancer cells to TRAIL-induced apoptosis impedes its successful clinical application. TRAIL sensitivity can be modulated by the MAPK pathway and facilitated through combination with other therapeutics. The MAPKs play a central role in the transduction of signals for growth and differentiation and also act as important modulators of various apoptosis-inducing signals, while the protective effect of ERK1/2 on DR-induced apoptosis has also been described (Tran *et al*. 2001; Seger and Krebs 1995). Deregulation of this pathway by RAS and more recently BRAF oncoproteins induce constitutive ERK activation, thereby promoting cell growth and survival. Manipulation of the MAPK signalling pathway could be a powerful means of treatment for tumours with BRAF mutation especially those resistant to TRAIL. Since the constitutive activation of $BRAF^{V600E}$ recapitulates the effect on growth and apoptosis by implicating the MAPK pathway which can modulate TRAIL sensitivity, we aim to elucidate the apoptotic program activated by this oncoprotein. Human colon late adenoma cells (Caco-2) overexpressing $BRAF^{V600E}$ were committed to apoptosis after treatment with TRAIL at monolayer and 3D culture conditions. Engagement of apoptosis is dependent on rapid activation of DISC complex and characterised by overexpression and rapid accumulation of pro-apoptotic molecules. Despite the fact that during colorectal carcinogenesis, a marked increase in sensitivity to the apoptotic effects of TRAIL has been reported there are some human colon adenocarcinoma cell lines like HT29 cells (type II) that remain partially resistant (Hague *et al*. 2005). Even though HT29 cells bear a $BRAF^{V600E}$ mutation, they are characterised by low pBRAF activity regardless reported impact of this mutation on the MAPK pathway (Ikenoue *et al*. 2003; Oikonomou *et al*. 2009b). The synergistic effect of TRAIL and a geldanamycin derivative, 17-allylamino-17-demethoxygeldanamycin (17-AAG), commits HT29 cells to apoptosis demonstrating how targeting $BRAF^{V600E}$, with Hsp90 inhibitor 17-AAG, leads to amplification of apoptotic events. Although CRAF and ARAF are degraded in cells that are exposed to 17-AAG, $BRAF^{WT}$ is not found in an Hsp90 complex whereas, activated mutant BRAF acquires a dependence on Hsp90 for its stability. Degradation of $BRAF^{V600E}$ leads to MAPK inhibition, cell-cycle arrest and apoptosis with concomitant antitumour activity *in vivo* (Grbovic *et al*. 2006). Apoptosis in our case was mediated through DR5 since DR4 was subjected to cytoplasmic proteasomal processing by the Hsp90-ubiquitin-proteasome pathway, and MAPK dependent transcriptional down-regulation. Blocking DR5 with a selective antibody demonstrated its prevalent role in TRAIL-induced apoptosis. Incorporating BRAFmutshRNA in HT29 cells, the dependence of TRAIL-induced apoptosis upon the constitutive activation of this mutant and how much $BRAF^{V600E}$ is dependent for folding and stability on the Hsp90 was emphasised. $BRAF^{V600E}$ sensitized TRAIL-induced apoptosis by facilitating DISC complex formation and amplification of pro-apoptosis molecules, while the combination treatment of TRAIL with 17-AAG in a $BRAF^{V600E}$ mutated background sensitised human colon cancer cells to apoptosis *via* DR5 and could therefore prove beneficial towards new anti-cancer strategies.

1 Oikonomou E, Kosmidou V, Katseli A, Kothonidis K, Mourtzoukou D, Kontogeorgos G *et al*: TRAIL receptor up-regulation and the implication of KRAS/BRAF mutations in human colon cancer tumors. Int J Cancer *125*: 2127-2135, 2009.

2 Oikonomou E, Makrodouli E, Evagelidou M, Joyce T, Probert L and Pintzas A: BRAF(V600E) efficient transformation and induction of microsatellite instability *versus* KRAS(G12V) induction of senescence markers in human colon cancer cells. Neoplasia *11*: 1116-1131, 2009.
3 MacFarlane M: Toxicol Lett *139*: 89-97, 2003.
4 Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M *et al*: Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. Nat Med *5*: 157-163, 1999.
5 Tran SE, Holmstrom TH, Ahonen M, Kahari VM and Eriksson JE: MAPK/ERK overrides the apoptotic signaling from Fas, TNF, and TRAIL receptors. J Biol Chem *276*: 16484-16490, 2001.
6 Seger R and Krebs EG: The MAPK signaling cascade. FASEB J 9: 726-735, 1995.
7 Ikenoue T, Hikiba Y, Kanai F, Tanaka Y, Imamura J, Imamura T *et al*: Functional analysis of mutations within the kinase activation segment of B-raf in human colorectal tumors. Cancer Res *63*: 8132-8137, 2003.
8 Grbovic OM, Basso AD, Sawai A, Ye Q, Friedlander P, Solit D and Rosen N: V600E B-Raf requires the Hsp90 chaperone for stability and is degraded in response to Hsp90 inhibitors. Proc Natl Acad Sci USA *103(1)*: 57-62, 2006.

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CORRECTING THE CHLORIDE TRANSPORT ABNORMALITY IN THE AIRWAY EPITHELIUM OF CYSTIC FIBROSIS PATIENTS

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Objective: Cystic fibrosis (CF) is a hereditary, congenital, life-threatening disease, due to a mutation in a chloride channel in epithelial cells, the CF transmembrane conductance regulator (CFTR). Most of the patients have a mutation type where CFTR is synthetized, but on its way from the Golgi system to the cell membrane, is ubiquinated and destroyed by the proteasomes. Although the disease affects several organs, the main clinical problem is abnormally viscous mucus in the airways, resulting in frequent airway infections that ultimately destroy the lungs. The goal of our research is to find a pharmacological treatment for CF by either avoiding the destruction of the mutated CFTR, or by activating an alternative (Ca^{2+}-activated) chloride channel.

Materials and Methods: As a first step, potential compounds were tested on bronchial epithelial cell lines. The cells were loaded with a fluorescent indicator, MQAE, the fluorescence of which was inversely related to the intracellular chloride concentration. Chloride efflux from the cells washence observed as an increase in fluorescence. Wild-type CFTR is activated by cAMP, and in non-CF cells, chloride efflux could be induced by substances that increase the intracellular cAMP level. In CF cells, these substances did not cause chloride efflux.

Results: In the course of the project, we have identified several compounds that promote chloride efflux from CF cells. One of these is S-nitrosoglutathione (GSNO), a compound that normally occurs in the airway surface liquid, but occurs at abnormally low levels in CF airways. GSNO increases the expression of the mutated CFTR in the cell membrane, and stimulates chloride efflux *via* CFTR (1). Unfortunately, this compound is not stable. Recently, we found that N-acetylcysteine, often used as a mucolytic, had a similar effect (2). Other compounds that were found to increase chloride efflux from CF airway epithelial cells were azithromycin, genistein, and colchicine. Some compounds that had been suggested as possible drugs to treat CF were shown not to increase chloride efflux from CF airway epithelial cells significantly: curcumin, duramycin, and lubiprostone.

Conclusion: Studies performed by our group and others have now suggested a large number of substances that potentially could correct the chloride transport abnormality in CF. However, to be useful in practice, drugs should be stable, and not have deleterious side effects. Transgenic mice with the same mutation in CFTR as human CF-patients are available, but are not very suitable to test the effect of potential drugs on airway symptoms, since in rodent airways, chloride efflux is primarily *via* Ca^{2+}-activated chloride channels. Hence, clinical studies need to be carried out directly on patients.

1 Servetnyk Z, Krjukova J, Gaston B, Zaman K, Hjelte L, Roomans GM and Dragomir A: Activation of ΔF508 CFTR in CF airway epithelial cell lines and CF nasal epithelial cells by S-nitrosoglutathione. Respir Res *7*: 124, 2006.
2 Varelogianni G, Oliynyk I, Roomans GM and Johannesson M: The effect of N-acetylcysteine on chloride efflux from airway epithelial cells. Cell Biol Int *34*: 245-52, 2010.

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CELLULAR DNA DAMAGE-RESPONSE FOLLOWING EXPOSURE TO THERAPEUTIC DRUGS. THE CASE OF MULTIPLE MYELOMA

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Background: The development of cancer is driven by the accumulation of scores of alterations affecting the structure and function of the genome. Equally important in this process are genetic alterations and epigenetic changes. Whereas the former disrupt normal patterns of gene expression, sometimes leading to the expression of abnormal, constitutively active proteins, the latter deregulate the mechanisms such as transcriptional control leading to the inappropriate silencing or activation of cancer-associated genes. In this report, the effect of differential epigenetic alterations on the clinical outcome in multiple myeloma (MM) patients is presented.

Patients and Methods: Peripheral blood mononuclear cells (PBMC) were obtained from twelve human healthy volunteers (7M/5F; median age 41 years, range 28 to 52 years) and 32 MM patients (14M/18F; median age 59 years, range 23 to 71 years) prior to therapeutic treatment with high-dose melphalan (HDM; 200 mg/m^2) supported by autologous stem cell support (ASCT) as part of their first line therapy. Twenty-three patients achieved an objective response post-ASCT while 9 patients did not respond according to IMWG criteria. Chromatin condensation (using micrococcal nuclease digestion), transcription activity (steady-state levels using RNA slot-blots and rates of transcription using run-off assay), as well as *in vitro* melphalan-induced DNA damage formation/repair (monoadducts and interstrand cross-links using Southern blot) were measured in three transcriptionally active genes (the housekeeping β-actin gene, the tumor suppressor p53 and the protooncogene N-ras) as well as in the non-transcribed β-globin gene.

Results: In all MM patients examined, a close association was observed between the locus-specific repair efficiency, the transcriptional activity and the chromatin condensation.

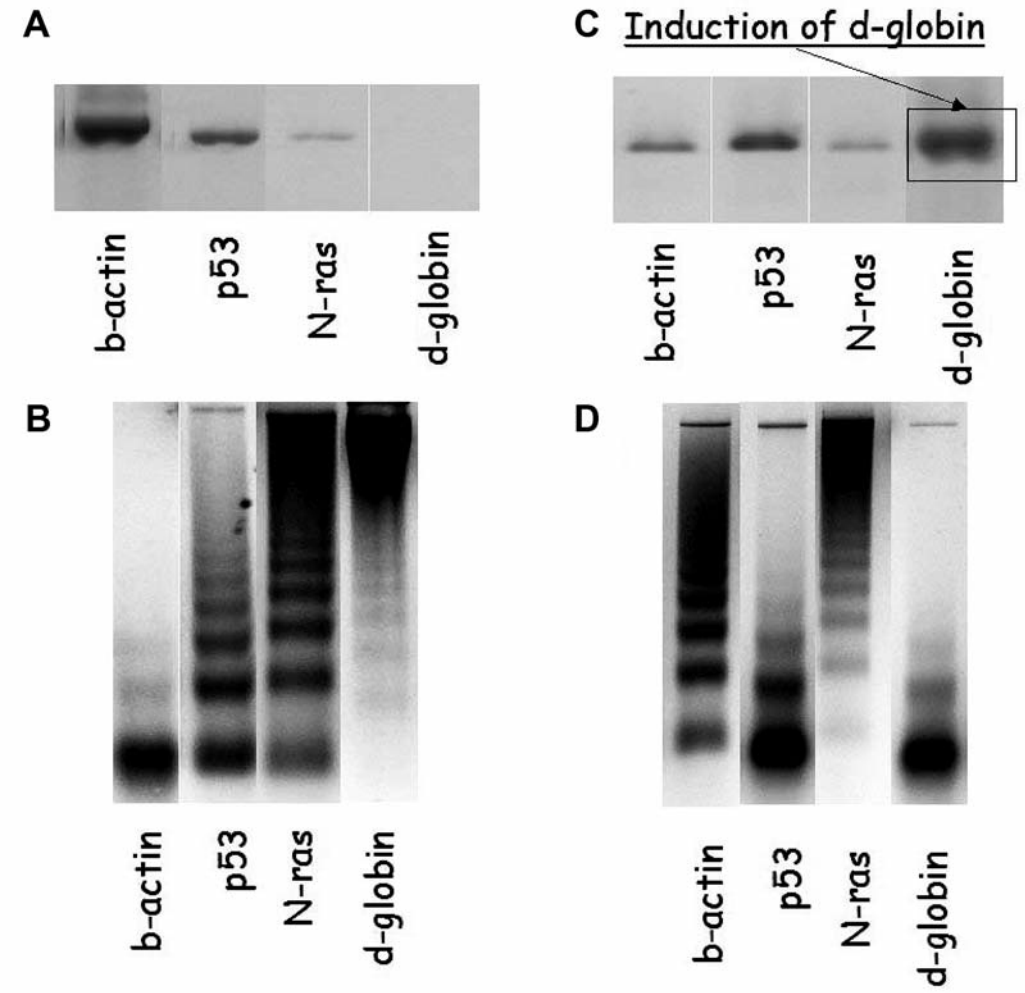

Figure 1. *Steady-state RNA levels (A, C) in the four different genes of untreated PBMC from responders and healthy volunteers (A) as well as from non-responders (C) to melphalan therapy. Autoradiograms showing micrococcal nuclease sensitivity (B, D) of the four different genes of untreated PBMC from responders and healthy volunteers (B) as well as from non-responders (D) to melphalan therapy.*

Strikingly, the order of variation of DNA repair efficiency, transcriptional activity and local chromatin structure between different genes was b-actin>p53>N-ras>β-globin in all healthy volunteers and in 95% of responders to chemotherapy (Figure 1A, B), while a perturbation of this order was found in 80% of non-responders (Figure 1C, D). Interestingly, in the β-globin gene, an induction of the transcription activity, a higher "looseness" of the local chromatin structure and an increase in the repair efficiency was observed specifically in MM patients who did not respond to melphalan therapy (Figure 1C, D). No difference between responders and non-responders was found in regions on both sides of the genes as well as at the overall genome repair, suggesting that in non-responders the repair system active in the removal of melphalan-induced adducts was not grossly affected. In agreement with our previous reports (Dimopoulos *et al*, J Clin Oncol *23*: 4381-4389, 2005; Dimopoulos *et al*, Haematologica *92*: 1505-1512, 2007) in the p53, N-ras and β-globin gene, responders to HDM showed higher melphalan-induced damage and lower rates of repair compared to non-responders (Table I). No difference in the repair activity between responders and non-responders was observed in the β-actin gene. Also, in all genes examined, DNA damage formation and repair induced by *in vitro* melphalan treatment of PBMC taken from MM patients prior to therapy, correlated with the respective data obtained *in vivo*, *i.e.* after therapeutic administration of HDM.

Conclusion: Our study suggests that the DNA repair efficiency, the transcriptional activity and the local chromatin structure in different gene loci are tightly connected, and can be used as molecular markers to select those patients with MM who are more likely to benefit from HDM therapy.

Table I. *Correlation of in vitro damage/repair in the p53 gene of PBMC taken from 32 MM patients prior to therapy, with "clinical response" after HDM, using the "cut-off" value (736 adducts/10^6 nucleotides x h)*

DNA damage (adducts/10^6 nucleotides × h)	Clinical response		
	Responders (# patients)	Non-responders (# patients)	Total # patients
>736	18	0	18
<736	5	9	14
Total	23	9	32

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(Figures refer to abstract numbers)