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Review

Animal Models of Carcinogenesis in the Digestive System

CHRISTOS TSIGRIS, MARIA KONSTANTAKAKI, CONSTANTINOS XIROMERITIS, NIKOLAOS NIKITEAS and ATHANASSIOS YANNOPOULOS

First Department of Surgery, University of Athens Medical School, Laikon Hospital, Athens, Greece

Abstract. *Digestive system malignancies are quite common, accounting for 25% of deaths from cancer in the European Union. Various etiological factors of carcinogenesis include hereditary mutations and susceptibility polymorphisms, inflammation due to infectious agents, environmental and dietary factors. Transgenic, knockout or mutant animal models are very useful in reproducing cancers of the digestive tract that occur in humans. They offer the opportunity to study the disease phenotype and the function of the underlying mechanisms of carcinogenesis. In addition, animal models are valuable tools in cancer treatment attempts with the combined use of gene targeting or chemotherapy. This review illustrates the importance of the numerous animal models that have been developed by various methods in order to study carcinogenesis in the digestive tract and test potential therapeutic treatments.*

Epidemiology of Digestive System Cancers

A variety of malignancies may develop throughout the digestive system, from the oral cavity to the anus. Topological and histological differences of carcinogenesis may indicate different etiological mechanisms, although some common alterations of certain pathways are possible. In 2004, cancers of the digestive system accounted for about 25% of all malignancy-related deaths in the European Union (1). The second most common type of cancer in the EU was colorectal carcinoma accounting for 13% of all cancer cases, as well as for 11.9% of deaths (203,700 deaths) (1). Stomach cancer was the fifth most common cancer (5.9% of cancer cases and 8.1% of deaths) (1). Oral cancer was the eighth most common cancer (3.4% of cases) causing 2.3% of deaths (1). Cancer of the esophagus was the eleventh most common cancer occurring in 1.5% of disease cases and causing 2.3% of deaths from cancer in the EU (1).

In a study of a representative adult population sample of 6,267 randomly selected subjects who were scheduled for examination from 1997 to 2001 (population-based cross-sectional study; response rate: 69%; age range 20 to 81 years), the prevalence of oral mucosal lesions was age-dependent (5.6% in 20 to 29-year-olds, 19.6% in 70 to 81-year-olds) with an overall prevalence of 11.8%, slightly higher in men than in women (2). Males also showed higher cumulative incidence risk of esophageal cancer at the age of 69 years compared with females in data from Cancer Incidence in Five Continents. Notably, males from Miyagi (Japan) and Bas-Rhine (France) showed remarkably high esophageal cancer risk, whereas Korean males from Los Angeles (USA) and Florence (Italy) showed low cancer risk (3).

Accordingly, males showed higher age-standardized rates of stomach cancer incidence than females in the Cancer Incidence in Five Continents registries (4). It should be noted though that a decreasing trend from the 1970s to the 1990s is observed in most registries (4).

On the contrary, a higher incidence in males than females is not observed in colorectal cancer. Although most colorectal cancers are similar histopathologically (adenocarcinomas), colon cancer is more common among women and rectal cancer is more common among men (5). The incidence of colorectal cancer begins to rise at age 40 and peaks between the ages of 60 and 75 (5). In age-adjusted incidence for this malignancy, the findings fall roughly into three categories: high risks in Canada and New Zealand; intermediate risks in most of Western Europe; lower risks in Finland, Eastern Europe, South America, Asia and Africa. Comparable data for carcinoma of the rectum reveal a similar ordering (5). There is a clear association in the level of risks between the two sites, although exceptions to the general rule are noted, particularly for males (5). Finally, in anal cancer, which is a rare disease, age-standardized incidence rates are higher for men than for women (6).

Correspondence to: Professor Christos Tsigris, First Department of Surgery , University of Athens Medical School, Laikon General Hospital, Mikras Asias 75, Athens GR-11527, Greece, Tel: +30 210 7713158, Fax: +30 210 7713158, e-mail: ctsigkri@med.uoa.gr

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Animal Models of Carcinogenesis

Upon completion of the human genome project, traditional reverse genetics approaches (linkage and positional cloning) may still be employed to characterize a few of the genetic susceptibility agents. Nevertheless, the contemporary challenges include identifying and cataloguing the functional variations within genes and their impact in normal physiology and pathology of humans. In this context, animal models are useful in reproducing diseases that occur in humans, under controlled conditions. This may be achieved by studying disease phenotypes produced either by genetic alteration (mutant, knockout animals, transgenic) or by environmental (*e.g.* chemical, viral, radiation) induction of carcinogenesis.

Various mutant animals have been characterized which lack an important factor of cell cycle control. For instance, somatic mutations resulting in spontaneous tumor development have been made in the mouse version of distal colon carcinoma model after treatment with the DNA mutagen azoxymethane of heterozygous Cdx2+/– mice (7). In contrast to the surrounding intestinal epithelium, the neoplastic cells do not express Cdx2 from the remaining allele. That study provided experimental evidence that Cdx2 is a tumor suppressor gene involved in distal colon cancer, and that a Cdx2 mutation is the primary event in carcinogenesis (7).

Gene targeting is also being exploited by scientists to obtain information about the function of individual genes and to create models of human disease. Knockout animals are genetically engineered animals that have had one or more of their genes made inoperable through gene targeting. For example, studies of forkhead M1 box transcription factor knockout mice suggest that this factor is very important for the proliferation and growth of colorectal cancer (8).

Transgenic animals are created by inserting foreign genes into their genome in order to study gene function and to identify genetic elements that determine in which tissue and at what stage of development a gene is normally activated. Oncomice, transgenic mice with an activated oncogene, aim to significantly increase the incidence of a cancerous phenotype. For example, transgenic mice were constructed harboring a portion of the human CEACAM family gene locus in a bacterial artificial chromosome. They treated the transgenic mice and their wild-type littermates with the carcinogen azoxymethane in order to induce colon tumor formation. The transgenic mice showed more than a twofold increase in mean tumor load relative to their wild-type littermates. The expression of CEACAM genes was found in 'normal' crypts adjacent to the tumors, thus closely mimicking the situation in human colon tumorigenesis. These results show that expression of the human CEACAM family genes predisposes mice to acquire and retain essential mutations necessary for sporadic colon tumor development (9). These transgenic mice serve both as models for human colon tumorigenesis and as sources of oncogenic proteins.

Sometimes additional copies of a gene transferred in an animal model using a viral vector may reveal the possible carcinogenic effect of its gene product when it is in abundance. For example, because cyclin D1 plays an important role in the multistep process of gastrointestinal tumorigenesis, its targeted overexpression by normal enterocytes injected into nude mice confers a transformed phenotype. When nude mice were injected with non-tumorigenic intestinal epithelial cells, previously transfected by a vector encoding cyclin D1, intestinal tumors were generated after 6-8 weeks (10). Such models may be useful for proving that certain genes can act as oncogenes *in vitro* and *in vivo*, as well as for understanding their role and interrelationships in carcinogenesis.

A common environmental mode for inducing carcinogenesis is the use of chemical carcinogens. Genotoxic carcinogens may require endogenous metabolism to generate the mutagenic compound species or may directly damage DNA by causing mutations or chromosomal alterations, which result in tumor development. On the other hand, non-genotoxic carcinogens (also called epigenetic carcinogens or tumor promoters) do not interact with DNA and trigger cell signal transduction pathways to increase rates of DNA synthesis, cell proliferation, genomic instability and resistance to apoptosis (11).

Animal Model Studies of Carcinogenesis in the Digestive System

Etiologies of carcinogenesis

Cancer may be caused by various factors and is a multistage process as shown in Figure 1. Various genes, including oncogenes, tumor suppressor genes and modifying genes, contribute to cell cycle control and cell proliferation mechanisms which underlie many common malignancies. Such mutant genes are sometimes inherited obeying known Mendelian patterns and result in hereditary cancer disorders.

Recent data have expanded the concept that inflammation is a critical component of tumor development and progression. It is now becoming clear that the tumor microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. Many types of cancer arise from sites of infection, chronic irritation and inflammation. In addition, tumor cells have co-opted some of the signalling molecules of the innate immune system, such as selectins, chemokines and their receptors for invasion, migration and metastasis (12).

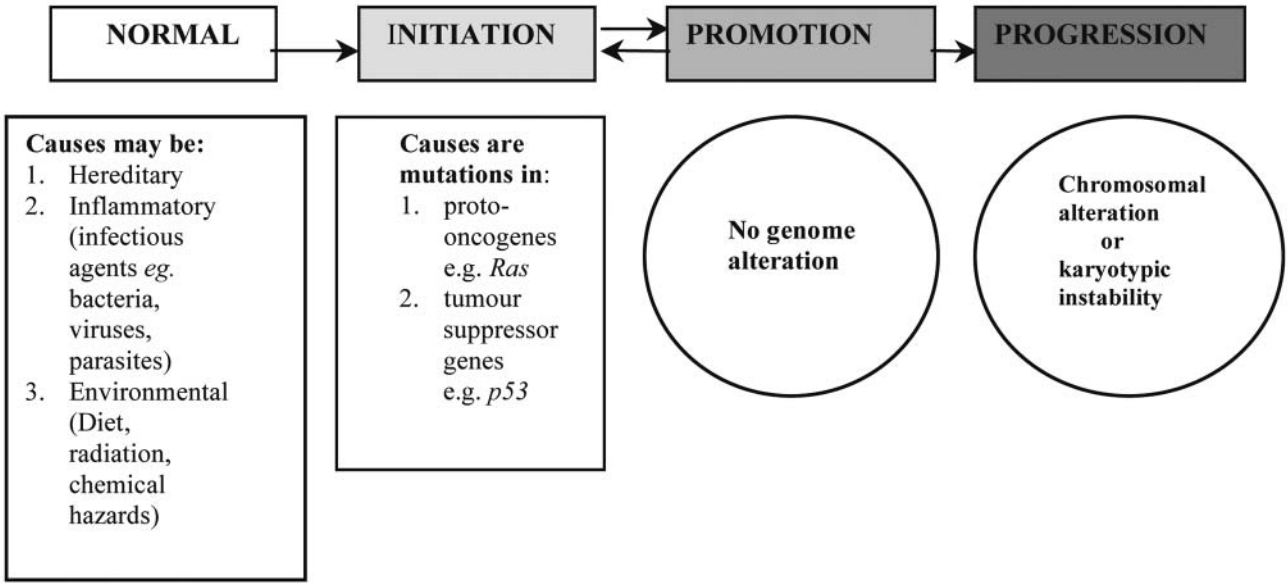

Figure 1. *Major stages of carcinogenesis in the digestive system with their characteristics. The initiation stage is irreversible, the promotion stage is operationally reversible, while the progression stage is also irreversible.*

Epidemiological data on geographical and temporal variations in cancer incidence, as well as studies of migrant populations and their descendants who acquire the pattern of cancer risk of their new country, indicate that environmental exposure and the convergence towards local cancer rates seen among immigrants excludes a genetic explanation for these differences. By the 1960s, cancer epidemiologists had therefore concluded that most cancers are in principle preventable and many could be avoided by a suitable choice of lifestyle and environment (13).

Hereditary cancer

Highly penetrant hereditary conditions such as polyposis coli, Li-Fraumeni syndrome and familial retinoblastoma cause at most a few per cent of the majority of cancers (13). From an epidemiological perspective, the genetic basis of the roughly twofold increase in incidence of the same type of cancer in first-degree relatives of patients with the most common types of cancer is a more important question (14).

Molecular genetic studies of hereditary cancer predisposition syndromes have led to the discovery of genes whose mutations are responsible for colon carcinogenesis. A paradigm of a hereditary colon cancer syndrome with dominant inheritance is familial adenomatous polyposis (FAP).

Mouse models for hereditary polyposis colon cancer. FAP is a hereditary disease that causes colon polyps. The adenomatous polyposis (*APC*) gene, located on human chromosome 5q, is commonly deleted in FAP families. If *APC* is mutated, its protein product does not phosphorylate *N*-terminal serine/threonine residues of β-catenin, stabilised β-catenin accumulates in the cytoplasm and activates transcription factors, which then induce their target genes (15). Moreover, experiments on Apc^{Min}, $Apc^{\Delta 716}$ and Apc^{1638N} mutant mice strongly suggest that APC protein is essential for the proliferative zone cells to migrate along the crypt-villus axis. All *Apc* mutant mice develop adenomatous polyps, apart from the additional mutations in the *Apc* mice (15).

Homeobox genes play essential roles in specifying the fates of different cell types during embryogenesis. In the mouse, the caudal homologue Cdx2 has been implicated in directing early processes in intestinal morphogenesis and in the maintenance of the differentiated phenotype. A recent study showed that Cdx2 null mutation was embryonically lethal, whereas Cdx2+/– mice developed multiple intestinal polyps in the proximal colon in addition to developmental defects (16). In support of the above, the expression pattern of some homeobox genes was investigated in various human colon cell lines (polyposis coli Pc/AA adenoma cells, Caco-2, HT-29 and LS174T adenocarcinoma cell lines), which represent various stages of colon cancer progression and differentiation. Most of these cell lines demonstrated a pattern of deregulated expression of the HOX and CDX-1 homeobox genes which resembled that of colorectal cancer. This suggests a possible role of these genes in colorectal cancer development (17).

Mouse models for hereditary non-polyposis colon cancer. Various colon cancer models have been constructed with mutations in different genes. For example, a knockout

mutation in the TGF-β1 gene introduced into *Rag2* mutant mice causes adenocarcinomas with strong local invasion (18). Moreover, with the use of the promoter for the villin gene, whose expression is specific to the intestinal epithelium, transgenic mice have been constructed that express the activated mutant of *K-ras* V12G. Most of these transgenic mice develop single or multiple lesions, ranging from microadenomas to invasive adenocarcinomas. Targeted expression of oncogenic K-ras in the intestinal epithelium causes spontaneous tumorigenesis in mice. Similar to the *cis*-compound $Apc^{\Delta 716}Smad4$ mutant mice, none of the adenocarcinomas in this model metastasize to distant loci, although the tumors are highly invasive locally (19). In agreement with the involvement of the ras protein in intestinal oncogenesis, an immunohistochemical analysis which has been employed to study the ras p21 oncoprotein in a total of 88 gastric carcinomas suggests an association of the expression of ras p21 with metaplastic and neoplastic gastric mucosa (20).

Inflammation

Although it is now clear that proliferation of cells alone does not cause cancer, sustained cell proliferation in an environment rich in inflammatory cells, growth factors, activated stroma and DNA-damage-promoting agents certainly potentiates and/or promotes neoplastic risk. During tissue injury associated with wounding, cell proliferation is enhanced while the tissue regenerates. Proliferation and inflammation subside after the assaulting agent is removed or the repair completed. In contrast, proliferating cells that sustain DNA damage and/or mutagenic assault continue to proliferate in microenvironments rich in inflammatory cells and growth/survival factors that support their growth. In a sense, tumors act as wounds that fail to heal (21).

Mouse models of infection for colon cancer associated with inflammatory bowel disease. Recently, a transgenic mouse strain was constructed that mimicks early inflammatory changes in *Helicobacter pylori* infection and develops hyperplastic gastric tumors. Colon cancer associated with inflammatory bowel disease is different from regular colon cancer. Malignant cancer develops long and sustained inflammation in the intestines. As models for this type of colon cancer, several mutant mouse strains have been constructed. For example, IL-10 deficient mice produce aberrant cytokines (especially γ-interferon) and invasive adenocarcinoma develops in the colon in 80% of mice by 6 months of age (15). Interestingly, null mutant mice for one of the G proteins, Gαi2, also develop similar colitis and adenocarcinoma of the colon. However, introduction of dominant negative *N*-cadherin causes inflammatory bowel disease and adenomas, but not carcinomas (15).

Molecular mechanism for gastric cancer associated with infection. Gastric cancer is one of the most deadly types of cancer in most countries (22). Although there is an established epidemiological correlation between *H. pylori* infection and gastric carcinogenesis (23), the molecular mechanism that leads to gastric cancer remains to be elucidated, although various animal models have been developed to clarify gastric carcinogenesis. It is rather more reasonable to assume that sustained infection with *H. pylori* causes a precancerous condition that can lead to carcinogenesis after additional molecular insults. Based on such an indirect hypothesis, the role of prostaglandin E_2 in earlier stages of gastric carcinogenesis has been particularly studied. As is well known *Helicobacter* infection induces COX-2 and Mpges-1 in the gastric mucosa (24, 25). COX-2 is also induced in gastric cancer tissues (26). Notably, the COX-2 gene mutation is also implicated dramatically in decreasing hereditary intestinal polyposis in Apc polyposis mice (27-29).

Environmental compounds causing oncogenesis

Synthetic or natural chemical compounds in the environment may cause genetic changes that result in many types of human cancer. Chemical carcinogenesis involves the interaction of xenobiotics with the body. Chemical carcinogens obey essentially the same pharmacokinetic principles of absorption, distribution and elimination as therapeutic drugs. Some examples of the implication of the environment in the development of cancer are discussed.

Examples of environmental factors involved in carcinogenesis. The most important discovery in the history of cancer epidemiology is the carcinogenic effect of tobacco. For many years the carcinogenic effects of tobacco were thought to be restricted largely to the lung, pancreas, bladder and kidney, and (synergistically with alcohol) the mouth, the larynx, the pharynx (except nasopharynx) and esophagus. More recent evidence indicates that several other types of cancer, the most important of which is gastric carcinoma, are also increased by smoking (11).

Another example is oral squamous cell carcinoma (OSCC), a cancer strongly affected by environmental factors, such as tobacco smoking and heavy alcohol consumption. In OSCC, significant gene-environment interactions are involved between tobacco, alcohol and gene polymorphisms (30-53). Even rarer oral cancers, such as those of the major and minor salivary glands, with a largely unknown origin, probably involve several environmental factors, such as certain types of jobs, ionizing and ultraviolet radiation, as well as diet (55-59). A high cholesterol intake has been related to an increased risk of salivary gland cancer (57, 58).

Of course, dietary epidemiology is notoriously complex owing to the variety of foods and their many constituents

and to intercorrelations and temporal changes in their patterns of use. Nevertheless, accumulating epidemiological and biochemical evidence suggests that a high intake of total fat, mainly from saturated animal sources, increases the susceptibility to cancer at different sites, particularly the breast and colon (60, 61). It should be remarked that cholesterol is associated with a saturated fat consumption which is expected to induce cell hyperproliferation, a fact coinciding with the protumorigenic activity of the non-essential oleic acid (62).

Animal Models of Sequential Stages of Carcinogenesis

The development of neoplasias in mammalian organisms is now understood to occur in a series of at least three definable stages which have been termed initiation, promotion and progression (63). In general, the initiation stage has been implied on the basis that preneoplastic and neoplastic lesions develop following administration of an initiating agent or complete carcinogen. Altered expression or mutational events in specific genes can be identified and quantified in single putatively initiated cells or their very early progeny (64). It is reasonable to argue that initiation of neoplasia in the gastrointestinal tract of both rodents and humans does arise from single, probably genetically altered, cells (63). The efficiency of the initiation may differ in different portions of the gastrointestinal tract, depending on the effectiveness of cell proliferation in establishing or fixing the initiation stage.

The intermediate promotion stage does not involve alterations in the structure of the genome, but rather in its expression. In a number of experimental situations, the promotion stage may be bypassed as a result of extensive genetic damage due to very high and/or sustained doses of DNA-damaging carcinogens. The distinctive feature of this stage is its operational reversibility which makes it the most important stage for cancer prevention efforts (63). Oral leukoplakia, a known preneoplastic lesion, may regress spontaneously, especially on cessation of smoking or chewing tobacco products, or as a result of active administration of vitamin E (65).

The progression stage also involves structural alterations in the genome, but of a much more complex nature than the relatively simple mutational events which are characteristically seen in the progression stage (66). The characteristic genetic alterations of the progression stage involve chromosomal alterations that range from being subtle to extremely complex (Figure 2) (67). Furthermore, the critical characteristic of this stage is the evolution of karyotypic instability, which results in numerous genetic abnormalities including gene amplification, chromosomal translocation with the formation of fusion genes, chromosomal deletion and/or duplication, and a

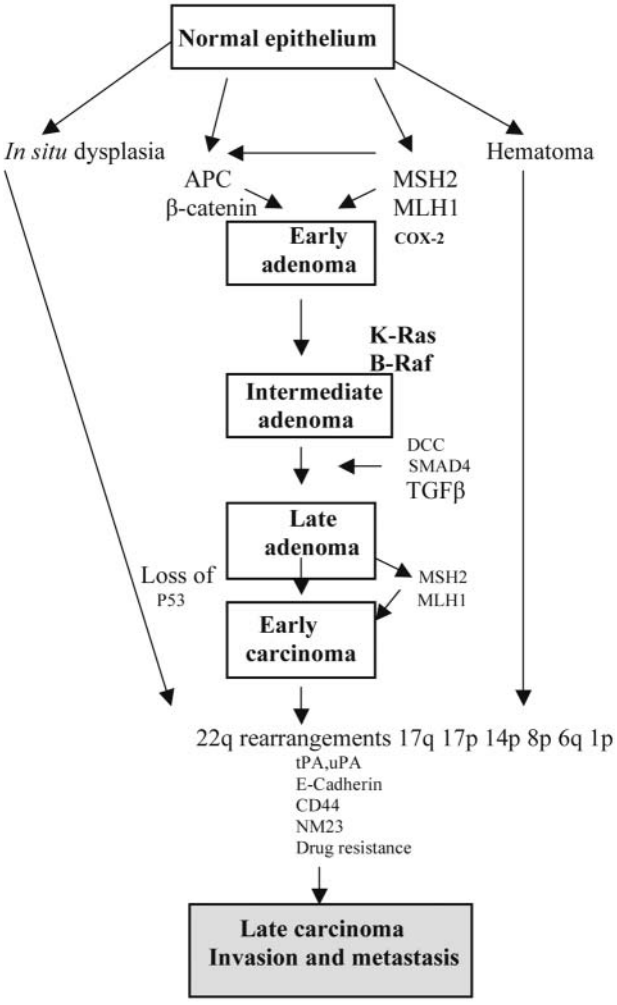

Figure 2. *The adenoma to carcinoma sequence in the development of colorectal cancer. As an example of the complex multistage process of carcinogenesis, most colorectal carcinomas are thought to develop from adenomas. Adapted with alterations (67).*

dramatically enhanced capacity for gene transfection. The evolution of karyotypic instability, at least in the early phases of progression, is not random but rather assumes specific pathways apparently unique to the cell type from which a particular histogenetic neoplasm arises. The initiation event in the development of human colon cancer has been correlated with alteration of the APC gene structure, with subsequent karyotypic and further mutational events occurring in a sequence that parallels the morphological development of preneoplastic to neoplastic lesions in the progression stage.

Interestingly, diabetes type I has been shown to affect some mechanisms of oral carcinogenesis in a rat model (68-75). Based on this animal model, diabetes was found to induce the activation of the Ras/Raf/MAPK signal transduction pathway, mainly *via* activation of erbB2 and

erbB3 cell surface receptors, leading to increased cell proliferation (68, 70-72, 75).

Stages of carcinogenesis. The progressive accumulation of genetic changes in both oncogenes and tumor suppressor genes parallels the clinical and histopathological progression from normal colonic epithelium through benign adenomas to colon cancer. A similar progression is postulated in the transition from normal squamous epithelium to metaplastic mucosa (Barrett's esophagus) and subsequently through dysplasia to adenocarcinoma of the esophagus. A common link between colorectal cancer and Barrett's esophagus or esophageal carcinoma might be explained by either genetic predisposition or common environmental risk factors. The multistep nature of oncogenesis is most directly illustrated by molecular experimental genetic studies which demonstrate that the progression from adenoma to colon carcinoma results from the accumulation of molecular genetic alterations involving 3 main factors: activation of oncogenes, inactivation of tumor suppressor genes and abnormalities in genes involved in DNA mismatch repair. Changes in oncogenes encoding four distinct groups of proteins (peptide growth factors, protein kinases, signal transducting proteins and nuclear transcriptional regulatory proteins) can contribute to colon carcinogenesis. In addition, various carcinogens may act at different stages of this model, affecting somatic mutations and resulting in additional genetic alterations. Other promoters, including hormones, may enhance the likelihood of these events through the stimulation of the rate of cell turnover. Diseased detoxification processes may also play a role in carcinogenesis (76).

Stages of oral carcinogenesis. An exhaustive example of all the stages of oral carcinogenesis is provided by the use of the hamster as a model system. Induction of carcinogenesis was effected by 9,10-dimethyl-1,2-benzanthracene (DMBA) on left buccal pouches of animals for 10 to 14 weeks (77). The histological status of the resulted lesions was defined after examination of the complete section under light microscopy and the tissue profiles were classified in the following categories: normal, hyperkeratosis, hyperplasia (acanthosis), dysplasia, early invasion, well-differentiated carcinoma, moderately differentiated carcinoma. In every sample all possible different lesions were evaluated. All sections were used for immunohistochemical detection of tyrosine kinase receptors (EGFR, erbB2, erbB3, FGFR-2, FGFR-3), cytoplasmic proteins (H-ras, N-ras), apoptosis-related proteins (Bax, Bcl-2), cell proliferation markers (Ki-67), nuclear transcriptional factors (p53, c-myc, c-fos, c-jun, ets-1) and cell cycle proteins (p16).

Tyrosine kinase receptors, which are responsible for subsequent signal transduction, were expressed in substantially high levels in the initial stages of oral oncogenesis (78-80). It has also been observed that both N-ras and H-ras expression decreases in the early stages of oncogenesis (81). The analysis of the Bcl-2/Bax ratio in the various histological categories indicated increased apoptosis during initial stages and decreased apoptosis in later stages of oncogenesis. In addition, Ki-67 was sharply increased in initial stages of oral oncogenesis, consequently decreased in well- and moderately differentiated OSCC, but remained well above the levels in normal mucosa (77). In light of the synergism of tumour suppressor p53 and oncogene c-myc in both induction of apoptosis and tumor formation, the findings suggest a similar pattern of both p53 and c-myc expression in the initial stages of oral oncogenesis, indicating a possible strong correlation between them (82). While c-fos was found significantly increased in the last stage of moderately differentiated OSCC (83), c-jun was found progressively increased from the precancerous stage of hyperplasia during oral oncogenesis (78). In addition, ets-1 expression appears to be a frequent event in oral carcinogenesis; it rises in early stages of tumor formation where it may play a central role in invasiveness by inducing matrix metalloproteinases, while it remained relatively stable in following OSCC stages (81). Inactivation of tumour suppressor protein p16, a negative regulator of cell proliferation, occurs at the early stage of oral mucosal dysplasia in the multistep process of oral cancer progression, before the acquisition of an invasive phenotype. Therefore, loss of p16 function in precancerous oral lesions may be considered as a prognostic marker for progression of malignancy (84). Conclusively, the findings of the hamster experimental model indicated increased apoptosis and cell proliferation in early stages of oral oncogenesis, as well as a different signaling pathway in the pathogenesis of oral squamous cell carcinoma without the involvement of ras proteins.

Animal Models for Cancer Treatment

Animal models may be very useful in experimental trials of new therapeutical approaches of cancer treatment. Chemotherapy with anticancer agents such as cytokines that promote immune response, or gene therapy with introduction of viral vectors containing an anticancer gene, such as a tumor suppressor, are promising strategies that are evaluated for the first time *in vivo* in animals.

Possible use of cytokine IL-2 as potential therapeutic agent. Local therapy with interleukin-2 (IL-2) may be very effective in the treatment of different forms of cancer because this cytokine induces antitumor immunity and also increases the number of regulatory T-cells, which suppress antitumor immune responses. In a study of regional

application of IL-2 in rats with colon cancer, all studied parameters, such as weight, neoplastic/non-neoplastic tissue index of the spleen, mitotic index and vascular density of splenic and hepatic lesions, showed statistically significant differences in treated and untreated animals (85). Another study showed that IL-2-induced antitumor immunity is enhanced by regulatory T-cell depletion and is due to expansion of the tumor-infiltrating cytotoxic T-cell population (86).

Possible combination of oncolytic and immune therapy. The use of viral vectors combining oncolytic therapy and immunotherapy are promising agents in treatment of colorectal carcinoma. An interesting example is the use of a multimutated oncolytic herpes simplex virus that attempts to combine these two anticancer strategies (87). *In vivo* efficacy of the viral vector against murine colorectal carcinoma was tested in subcutaneous tumors in immune-competent mice, demonstrating potent antitumor activity (87).

Possible new mechanisms of therapy. Introduction of homozygous mutation for the inducible nitric oxide synthase gene reduced the polyp number in Apc^{Min} mice by less than half in the small intestine and to about 10% of the control in the colon, suggesting a possibility for its use in colonic polyposis prevention with inducible nitric oxide synthase-selective inhibitors (88).

Homeobox genes as potential therapeutic targets. Homeobox CDX2 gene expression has been coupled with the cell proliferation rate in various types of gastric cancer by immunostaining, indicating that CDX2 may be used as prognostic marker (89). Furthermore, in gastric cancer and colon cancer cell lines cultured *in vitro*, sodium butyrate acts to oppose the malignant behaviour, stimulating cell differentiation and apoptosis (90, 91). Intestine-specific transcription factors Cdx1 and Cdx2 can be induced by butyrate. Thus, Cdx1 and Cdx2 homeobox genes are direct targets of regulation by nutrients or butyrate in intestinal cells (92). Many methylation-prone genes were found to be associated with tissue-specific genes, including homeobox genes such as Cdx2 (54). As a family, the homeobox genes are frequently down-regulated in association with aberrant methylation in human cancer cells (93, 94). Epigenetic changes are a potential "hotspot" for therapeutic research with inhibitors of DNA methylation. Homeobox genes could possibly become new therapeutic targets in developing anticancer drugs. This could be accomplished through treatment of animal models of gastroinstestinal malignancies with demethylating agents, such as an aza-nucleotide analogue, which would retrigger the expression of genes such as CDX2 (94, 54).

Conclusion

Many animal models have been constructed that provide useful information on the initiation, progression and expansion of digestive system cancers, as well as regards their more effective treatment. These experimental models seem to be extremely valuable tools in an effort to effectively treat malignancies with poor prognosis in the 21st century. However, since most deaths of digestive tract cancer patients are due to the development of metastases, one of the remaining outstanding goals is to create animal models of cancer metastasis to the liver, lung and lymph nodes.

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