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CONTINUING EDUCATION IN ONCOLOGY

The past and the future of cancer genetics

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Summary

Carcinogenesis represents a multistep process associated with accumulation of somatic mutations in the classes of genes that regulate cell proliferation, apoptosis as well as DNA repair. Oncogenes, positive regulators of cell proliferation are activated during carcinogenesis. On the contrary, tumor suppressor genes, negative regulators of cell proliferation have to be inactivated. Mutations in genes that function in the maintenance of genomic stability are manifested by increase in the mutation rate in cancer cells that drive tumor progression. In general, on the basis of malignant transformation lies the abrogation of the balance between cell proliferation and cell apoptosis. The genetic mechanisms included in the transformation of normally acting genes comprise a wide spectrum of events, such as gene mutation, gene and chromosome rearrangement and gene amplification. Besides the role of somatic gene alteration in the development of sporadic cancer, germline mutations are the basis of a substantial number of inherited cancer syndromes. The future decades will be marked with the expansion of data exploiting cancer genetics, epigenetics and genomics into clinical practice. Consequently, translational cancer research should provide the generating of new targeted therapies, since individual molecular profiling of a patient's tumor should increase efficacy of conventional anticancer therapies such as chemotherapy and radiotherapy.

Key words: cancer epigenetics, cancer genetics, gene mutation, genomic instability, protooncogenes, tumor suppressor genes

Introduction

It has long been recognized that cancer occurs as a consequence of several somatic mutations. The idea that cancer is a disease of the genome, first proposed by Theodor Bovery in 1914, has opened new insight in the genetic basis of cancer [1]. Bovery's work on the fertilization of sea-urchin eggs by two sperms instead of one, demonstrated that distribution of unequal numbers of chromosomes to the daughter cells produces their different characteristics depending on the random combination of chromosomes. Bovery had concluded that the individual chromosomes carry different information. He also suggested that tumors might arise as a consequence of abnormal segregation of chromosomes to daughter cells [2-4]. Most of the widely accepted concepts in cancer genetics were first mentioned in Bowery's work: cell cycle checkpoints, oncogenes and tumor suppres-

sor genes, tumor predisposition, and the relationship between genetic instability and cancer.

It is proposed that 4-7 independent mutational events must take place before the cell can be considered malignant [5]. In fact, cancer results from the accumulation of large numbers of somatic mutations (Figure 1). These mutations have two distinct consequences: they allow the inappropriate activation or expression of a gene, or they result in the functional inactivation of a gene or its protein product. Oncogenes are the genes that must be activated. Tumor suppressor genes, on the contrary, have to be inactivated by mutations. Simplifying their roles, it can be said that oncogenes are involved in signaling pathways which stimulate proliferation, while tumor suppressor genes code for proteins which normally act as checkpoints to cell proliferation or programmed cell death (apoptosis). The genetic mechanisms included in the transformation of normally acting genes comprise a wide spectrum of events, such as gene mutation, gene and chromosome rearrangement and gene amplification.

In an oversimplified way, in the genesis of a cancer cell five major pathways must be activated or inactivated:

- Development of independence in growth stimulatory signals
- Development of resistance to growth inhibitory signals
- Development of resistance to apoptosis
- Development of unlimited proliferative capacity
- Development of angiogenic potential

Besides the role of somatic gene alteration in the development of sporadic cancer, germline mutations are the basis of a substantial number of inherited cancer syndromes. Hereditary cancer is associated with alterations in tumor suppressor genes that are mainly inherited in a recessive manner.

Approximately 5 to 10% of cancer of all anatomic sites harbors a hereditary etiology. Although proportionally small, hereditary cancer possesses a major public health problem throughout the world, because of its health, social, psychological risk and high expense of antitumor treatment.

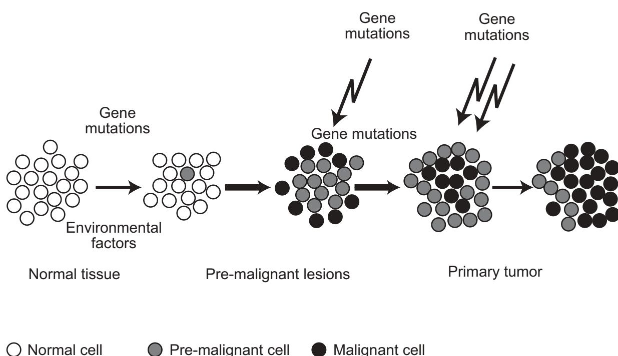

Figure 1. Multistep malignant transformation is a result of accumulated gene alterations.

Protooncogenes and cancer

Bovery noticed the existence of "growth-stimulatory chromosomes" and that unlimited growth of malignant tumor cells is attributable to a permanent increase in the number of these growth-promoting chromosomes. The concept of gain-of-function genetic alterations came from experiments that involved gene transfer into recipient cells: these cells could then be assayed for "transformation"- experimental approximation to a cancer phenotype. By the late 1960s, it had been shown that cells in culture could be transformed by several DNA viruses and retroviruses, and subsequently that a single gene from these viruses could carry out this information [6]. Genes related in sequence to those in transforming retroviruses were found in the DNA of normal cells: these genes had functions in the control of normal cell growth and differentiation, but their wrong activation could lead to cancer. The normal cellular genes were termed "protooncogenes", and their activated counterparts were termed "oncogenes" [7].

The discovery of methods to introduce foreign DNA into mammalian cells, some 30 years ago, provided the first powerful tools to examine gene function [8]. The introduction of exogenous DNA into mammalian cells resulted in integration of the exogenous DNA into the genome of the recipient cell. In that way it became possible to select cells that had a stable alteration in phenotype. The identification of the fragments of adenovirus type 5 DNA that harbor transforming activity was the first gain-of-function genetic screen in mammalian cells [9]. Ten years later, the introduction of sheared human tumor DNA into non-transformed NIH-3T3 cells led to the identification of the *ras* oncogene [10,11]. Similar experiments have since identified many more transforming oncogenes. A further line of evidence for the role of oncogenes in cancer came from superior techniques of chromosome analysis, starting with chromosome banding. In some tumors there were chromosomal translocations with breakpoints. Some of these breakpoints are located in or near to described protoonco genes, such as *c-myc* in Burkitt's lymphoma and *c-abl* in chronic myelogenous leukaemia [12]. The conclusion of these experiments was that these chromosomal events might result in increased expression of activity of the related genes.

In the past few years, the integration of genetic approaches into cancer research has been extraordinary. PCR-based technology allowed intensive screening of gene alterations due to its advantage concerning methodology simplification and sample quantity.

Additional studies on human tumors have pointed out the importance of a point mutation in a single gene, one of the members of the *ras* protooncogene family (*K-ras*, *H-ras* and *N-ras*). *Ras* gene represents a family of membrane signal transduction molecules, which interact, with large series of signal molecules with multiple functions including stimulation of proliferation. The active state of *ras* is produced by the binding of GTP, resulting with the conformational change of the molecule that allows interactions of *ras* with other downstream signaling molecules. The native protooncogene binds with GTP, hydrolyses it to GDP, which is then released and returns *ras* to its active state. Mutations in the *c-ras* decrease the ability of this molecule to act as a GTP-ase [13]. Since GTP is not released by mutated *ras* it now acts as permanently activated signal transduction molecule. The experiments have pointed out the mutational hotspots centered on codons 12, 13 and 61 [14]. The induction of *ras* mutations appears to be an early event in carcinogenesis. The presence of codon 12 mutations in the *ras* gene has been exploited recently as a sensitive indicator for the presence of pre-neoplastic cells in samples as diverse as feces for the detection of early colon cancer, in bronchial washings for lung cancer, and duodenal samples for pancreatic cancer [14]. Concerning *ras* family members, *K-ras* alterations are the most frequently found in pancreatic, colon and lung cancers, *H-ras* in breast and lung cancers, while *N-ras* alterations are almost exclusively associated with leukemia.

Ras represents the oncogene most widely altered in human cancers with an incidence level ranging from 30% in lung cancer to 90% in pancreatic cancer. Because of this, specific therapies targeted to *ras* oncogene were recently developed. The most promising of this appears to be the development of drugs, which inhibit the association of *ras* with plasma membrane. This association is a result of the addition of farnesyl isoprenoid in a reaction catalyzed by the enzyme protein farnesyltransferase. Several inhibitors of this enzyme have been developed and their effects were investigated within clinical trials, Phase II and III, mostly in colorectal tumors [15]. Unfortunately, they appear to posses unacceptable side effects.

Normally, cell proliferation is regulated by external stimuli - growth factors. These factors include: epidermal growth factor (EGF), fibroblast growth factor (FGF), tumor growth factor-α (TGF-α) and platelet derived growth factor (PDGF). In order to exert their proliferative action, all these factors must bind to appropriate receptors and induce a cascade of responses most of which involve phosphorylation events. It was shown that cancer cells have mechanisms to enable constant activation of the receptors without external stimuli. The continued mitogenic stimulation is a consequence of the different genetic or epigenetic alterations, such as occurrence of mutations acting in a dominant negative manner. Also, there is a possibility of receptor gene amplification, resulting in overexpressed receptors, such as *c-erbB-2* in the majority of carcinomas. *c-erbB-2* is overexpressed in approximately 30% of breast carcinomas and is associated with worse clinical outcome. Once the mechanism by which the receptor expression is altered has been recognized, it has been exploited in cancer therapy. The strategies to block or downregulate receptors such as EGFRs and *c-erbB-2* have been developed. Trastuzumab, a monoclonal antibody to *c-erbB-2*, commercially named Herceptin, is now widely introduced into the treatment of breast cancer patients.

Furthermore, overexpression of *c-erbB-2* leads to increased secretion of vascular endothelial growth factors (VEGF), stimulating this way the angiogenesis necessary for progressive growth of the tumor [16]. The other way of inappropriate regulation of cell proliferation by growth factors is inappropriate expression of the growth factor itself.

The inappropriate activation or expression of transcription factors influence the synthesis of mutated proteins with altered functions affecting signal transduction pathways. One of these transcription factors is *c-myc*, regularly expressed in the S-phase of the cell cycle. In various human tumors, its appropriate expression may be lost, and *c-myc* can become wrongly expressed and/or overexpressed throughout the cell cycle, driving cells continuously towards proliferation. Since *c-myc* participates in many cellular functions including replication, growth, metabolism, differentiation and apoptosis, the major problem is the complexity of cellular events modified by *c-myc* [17,18]. The patterns of *c-myc* genetic alterations are different in hematological and solid malignancies. In hematological malignancies such as Burkitt's lymphoma, *c-myc* is altered by chromosomal translocation that fuses the *c-myc* gene on chromosome 8q24 with either the heavy chain, $\varkappa$ or λ locus of the immunoglobulin genes on chromosome14q23, 2p12 and 22q11 [19]. This translocation results in inadequate regulation of *c-myc.*

c-myc is overexpressed in numerous cases of breast cancer. It seems that this overexpression acts to facilitate the ability of *c-erbB-2* to cause cell pro-

liferation. Among the myc family members are also *N-myc*, overexpressed in neuroblastoma and *L-myc*, overexpressed in lung cancer [17].

The discovery of these genes and the determination of their roles in the normal processes of growth control, differentiation and development gave an important insight into the cell cycle function.

Tumor suppressor genes

It was known that cellular "protooncogenes", when mutationally deregulated or abnormally overexpressed, contribute to tumor formation. The present knowledge that many such genes encode proteins that govern the processes of cell proliferation, differentiation, and development and those mutations affecting their functions constitutively deregulate specific signaling pathways, provided us a clear insight into the functioning of cancer cells. The discovery of genetically dominant activated oncogenes supported the idea that a distinct class of antioncogenes might oppose their effect and block tumor development. If the 1970s and early 1980s were the era of oncogenes, the subsequent decade was marked by tumor suppressor genes [20,21]. The introduction of experiments involving somatic cell fusion and chromosome segregation had pointed to the existence of genes that could suppress tumorigenicity. In the last 15 years most tumor suppressor genes have been identified (Table 1).

Since functionality of tumor suppressor genes requires the presence of a single functional gene, prototypic tumor suppressor genes are recessive, requiring "two-hit" inactivation of both alleles [22,23]. What are the implications of two mutations in tumorigenesis? Knudson proposed that the second event could be caused by intragenic mutation, whole gene deletion, chromosomal loss by nondisjunction or somatic recombination. The verification of this hypothesis came from introduction of DNA restriction fragment length polymorphism in the study of cancer.

The main features of tumor suppressor genes were first examined in retinoblastoma and Wilm's tumor. In these childhood malignancies in which hereditary features were manifested, the first tumor suppressor gene *Rb* was discovered. The retinoblastoma gene (*Rb*) was cloned and found to encode a nuclear protein, which control entry into the cell cycle [24,25]. *Rb* is a part of a gene family that includes two other members, p107 and p130, which collectively corepress genes that regulate programs governing cell cycle progression, apoptosis, and differentiation. The *Rb* family proteins exert their growth suppressive control during the G1 phase of the cell cycle [26]. *Rb* is normally not phosphorylated and associates with the transcription factor E2F. After mitogen stimulation, cyclin-dependent kinase phosphorylates *Rb* in the C-terminal region of the protein, which disrupts the binding region for E2F and causes its release. The transcription of genes required for cell cycle entry is allowed by disruption of the *Rb*/E2F complex. The loss of *Rb* function dissociates the cell cycle machinery from extracellular signals, dampening the ability of proliferating cells to exit the division cycle [27,28]. In retinoblastomas, osteosarcomas and small cell lung carcinomas, *Rb* protein is absent because of mutations that disable the *Rb* gene. In cervical carcinomas *Rb* protein is sequestered and marked for degradation by human papillomaviruses subtypes 16 and 18 [29,30].

Analysis of inactivating mutations in the *Rb* gene indicates that most are the result of C-T transitions at CpG dinucleotides (CpGs). Most of these mutations

Table 1. The most prominent tumor suppressor genes

Tumor suppressor	*Cancer type (hereditary, sporadic)*	*Function in normal cell*
Rb	Hereditary retinoblastoma, many other sporadic cancers	Cell cycle control Transcriptional corepression
p53	Li-Fraumeni syndrome, about 50% of all sporadic cancers	Transcription factor
p16	Hereditary melanoma, many other sporadic cancers	Cdk inhibitor (Rb activation)
ARF	Hereditary melanoma, many other sporadic cancers	Mdm2 antagonist (p53 activation)
BRCA1/2	Hereditary breast and ovarian cancer	DNA repair
PTEN	Cowden syndrome, sporadic glioblastoma, endometrial, thyroid and prostate cancer	Lipid phosphatase
APC	Familial adenomatous polyposis, sporadic colon cancer	Wnt/Wingless signaling
MSH2 and MLH1	Lynch syndrome (HNPCC), sporadic endometrial, ovarian, gastric, bladder cancer	DNA mismatch repair
ATM	Ataxia-telangiectasia (T-cell lymphoma)	DNA damage sensor
CHK2	Li-Fraumeni syndrome	Protein kinase (G1 checkpoint control)
VHL	Von Hippel-Lindau syndrome, sporadic renal cell carcinoma	E3 ligase recognition factor for HIFα

result in truncated proteins due to premature termination of protein synthesis either through the introduction of chain-termination sequences or altered splice sites resulting in changes in the processing of mRNA. Loss of *Rb* function is recessive and requires damage of both copies of the genes, which opens up the possibility that gene therapy may be used to reintroduce one or more copies of damaged genes. This approach has been successfully achieved in cell culture experiments [31].

In the past two decades, the far most investigated tumor suppressor gene was *p53*. *p53* gene is mutated in as many as half of all human tumors. About 15000 mutant *p53* alleles have been sequenced and have been found to carry inactivating mutants [32,33]. Mutations within this gene generate genetically aberrant cell clones with potential for malignancy. In normal cells *p53* is responsible for temporarily arresting cell growth at G1 and G2 checkpoints in response to certain types of molecular and biochemical damage until such damage can be repaired (Figure 2). Physiologic stress as well as other types of damage induces *p53*-dependent apoptosis, which eliminates the damaged cells [34].

Alterations of *p53* gene are mostly induced by point mutations. Analysis of *p53* mutations revealed mutational hotspots localized in evolutionary conserved regions, indicating that these regions were central to *p53* function - about 90% of all *p53* mutations are detected in the core DNA-binding domain (exon 5-8). The mutations can either eliminate critical contact with DNA or destabilize key protein structures required for DNA binding. Loss of DNA binding is critical for its biological activity [35,36].

Intact *p53* can be deactivated at the protein level. The key regulator of *p53* activity is the complex

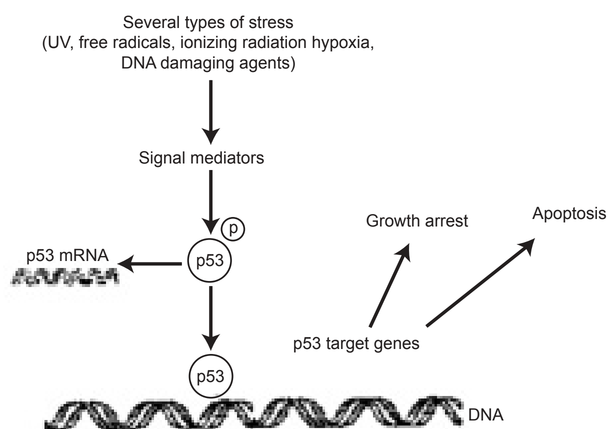

Figure 2. Activation of p53 induced by stressful stimuli results in apoptosis or growth arrest.

interplay between *p53* and *mdm2*, which inactivates *p53* and targets it for degradation. Any disturbance of this delicate balance may also abrogate *p53* function [37]. Similarly to *Rb* gene, human papillomaviruses (HPV) oncogenic subtypes such as 16, 18, and 31 can inhibit *p53* function via their protein product E6 and E7. Thus, by inactivating these two cell cycle regulators, HPV virus creates clones of epithelial cells with enhanced proliferation rate which are resistant to apoptosis. This type of *p53* deactivation is very frequent in some epithelial cancer types such as cervical and head and neck cancer [30].

The evidence indicates that *p53* plays a critical role in implementing apoptosis in response to treatment with DNA-damaging chemotherapeutics, as well as ionizing radiation. Whether *p53* is a significant independent predictor for response to treatment remains unclear and is the subject of current ongoing clinical trials.

The high incidence of *p53* mutations among some malignant tumor types and the resulting resistance to apoptosis, which would normally be triggered by genome instability or by chemotherapeutic agents makes restoration of *p53* an attractive target for gene therapy. Concerning *p53* gene therapy, different approaches are used. The first approach is based on viral vector delivery of wild type *p53*. This approach is limited by the fact that *p53* acts as tetramer, the presence of mutated p53 can frequently act in a dominant manner and inhibit actions of the wild type protein [38]. The second approach is quite different. It is based on exploiting the difference between normal cells expressing wild type *p53* and *p53* mutant tumor cells. A modified adenovirus construct ONYX-015 finishes its replicative cycle with consequent cell lysis only in *p53*-deficient cells. Normal cells with wild type *p53* are spared from lysis. Clinical success has been achieved after direct administration of ONYX-015 into the tumor mass, in combination with conventional chemotherapy, in patients with head and neck tumors [39]. The most promising work in the field suggested that small-molecule drugs can be designed to activate *p53* by preventing the binding of the negative *p53* regulator *mdm2*. The first small molecule, inhibitor of mdm2, has been synthesized this year by the Roche Research Center and named Nutilin [40].

Inherited *p53* mutations in one gene allele have been identified in cancer-prone families (the Li-Fraumeni syndrome). Like in sporadic tumors, the majority (about 75%) of inactivating mutations are located in exons 5-8. While many cancer predisposition syndromes are characterized predominantly by site-specific cancers such as breast cancer, colorectal cancer or melanoma, Li-Fraumeni syndrome is associated with a variety of different tumor types occurring over a wide

age range, including childhood. Although very rare, the occurrence of germline *p53* mutations is associated with very high incidence of certain cancer types such as breast carcinoma, soft tissue sarcoma, osteosarcoma, brain tumors, adrenocortical carcinoma, Wilms' tumor and phyllodes tumor [41].

The appearance of colorectal cancer is associated with two classes of genes, which have to be inactivated. Germ-line mutations in these genes were identified in families with cancer clustering. In hereditary non-polyposis colorectal cancer (HNPCC), mutations in the genes coding for enzymes of mismatch repair are present [42]. Mutations inactivating the adenomatous polyposis coli (APC) gene are responsible for the familial adenomatous polyposis (FAP), a disease in which hundreds of adenomatous polyps arise in the colon and rectum of affected individuals [43]. If FAP is untreated, colorectal cancer develops invariably, relatively early in the lifetime. Although germline mutations in APC account for the early appearance of colorectal tumors in FAP patients, somatic mutations in APC gene also occur as early events in >80% of sporadic, nonhereditary colorectal cancers.

Another two tumor suppressor genes named BReast CAncer Gene 1 and 2 (BRCA1 and BRCA2) are identified in individuals with familial clustering of breast and/or ovarian cancer. The discovery of the association between breast and ovarian cancer and BRCA1 and BRCA 2 genes made it possible to screen women for this genetic predisposition to develop either one or both of these diseases. BRCA1 and BRCA2 genes encode proteins that normally function to mediate integrity after DNA damage. The majority of the mutations in BRCA genes are frameshift mutations caused by small insertions and deletions. When mutations in these genes occur, they disrupt their normal functions in regulating cell turnover and DNA integrity, thus increasing the risk of cancer. Escalating risk associated with BRCA1 and BRCA2 is the consequence of inheritance of one mutated allele, usually in autosomal-dominant manner. The offspring of mutation carriers have a 50% chance of inheriting a mutant allele from either parent [44].

BRCA1 gene is located on chromosome 17q21. It is composed of 24 exons. Exon 1 is noncoding and exon 11 is unusually large. BRCA1 gene encodes a 1863 amino acid protein with a "zinc-finger" motif, suggesting that it may function as a transcription factor. Mutations are located throughout BRCA1 gene with little evidence for clustering or "hot spots". Most of the revealed mutations are capable of disrupting BRCA1's tumor suppressor function, and have been reported scattered along the gene. It is estimated that only about 20% of BRCA1 mutations are recurrent [45].

About half of all hereditary breast cancers are attributed to mutations in BRCA1 gene with 50% of all cases diagnosed by the age of 41 years. A woman with such a mutation has a 56% to 87% lifetime risk of developing breast cancer, although some new studies suggested lower risk - up to 50%. The majority of hereditary ovarian cancers are associated with inherited mutations in BRCA1 with a lifetime risk up to 44% [46].

A second breast cancer gene (BRCA2) is localized on chromosome 13. This is a large gene with 27 coding exons. As is the case with BRCA1 mutations, only a few have been observed to be recurrent. Besides female breast and ovarian cancer, BRCA2 mutations are strongly associated with the occurrence of male breast cancer. BRCA2 mutations are also associated with an increased risk of ovarian cancer, but the incidence of ovarian cancer is significantly less than that observed in women with BRCA1 mutations. Cancers of the prostate, pancreas and colon have been reported in BRCA2-associated cancer-prone families [47].

Genomic instability

The spontaneous mutations rate in normal cells is relatively low i.e. mutations in a normal cell are relatively rare events. The mutation rate in normal cells can be insufficient to account for the number of mutations observed in human cancers. It has been proposed that the occurrence of numerous mutations in cancer cells is the consequence of genome instability. Sporadic tumors need to acquire some form of inherent genome instability: a mutator phenotype. This phenotype is the result of mutations in genes that function for the maintenance of genomic stability such as DNA repair and chromosomal segregation genes. Mutations of these genes have no direct selective advantage or disadvantage; they rather affect the mutation rates of the other genes.

Studies on HNPCC revealed defect in genes that control genetic stability at the level of short repeat sequences. The tumor cells with the greatest numbers of changes in the length of repetitive sequences were subsequently shown to possess mutations in mismatch repair (MMR) genes [48]. HNPCC is caused by *MSH2* and *MLH1* mutations within the mismatch repair system. Failure of the MMR system to correct errors made by DNA polymerase during coping of repetitive sequences, gives rise to the length changes – microsatellite instability. Even more than 100 000 repetitive sequences per genome are altered in HNPCC [49].

Microsatellite instability has been also found in sporadic colon cancer. Most of the additions and deletions occur in sequences between repetitive ele-

ments. Repetitive sequences have been found within the coding regions of several genomic stability and growth regulatory genes, including *hMSH3*, *hMSH6*, *TGF-β*, *APC*, *IGF-RII* and *bax*. Repetitive sequences may represent the region of mutation clustering that is able to emphasize mutagenesis [50].

It is likely that genomic instability provides faster progression throughout the many stages of tumorigenesis. However, it seems that genetic stability is not the initiating event in the growth of sporadic tumors. Recently obtained experimental data suggest that early tumors have normal mutation rate. Genetic instability may occur later in the genetic evolution of cancer cells, but the exact mechanism under which genetic instability appears is still unclear.

Cancer epigenetics

In the era of intensive cancer genetics research, cancer epigenetics was neglected. The term "epigenetics" comprises cellular alterations other than those in DNA structure, which are heritable during cell division.

Three main epigenetic events have been reported: DNA methylation, genomic imprinting and histone modification. The most important of them for tumorigenesis is aberrant methylation. Cytosine DNA methylation is a covalent modification of DNA, in which a methyl group is transferred from S-adenosylmethionine to the C-5 position of cytosine by a family of cytosine-methyltransferases. In cancer, hypo and hypermethylation was observed. The first reported epigenetic abnormality has been the loss of DNA methylation at CpG dinucleotide. Hypomethylation, as a mechanism of deactivation, affects mainly oncogenes such as *H-ras* [51].

It has been confirmed that gene promoter hypermethylation is implicated in silencing of tumor suppressor genes. So far, hypermethylation is considered as the most frequent gene alteration in human cancers. Key tumor suppressor proteins including *p53, p16*, *MLH1,* the von *Hippel-Lindau (VHL)* tumor suppressor, *IGF-2* and *E cadherin* were eliminated both in the cell lines and in primary cancers by epigenetic pathway that correlates with CpG hypermethylation of their gene promoter. For instance, promoter hypermethylation and loss of expression of *MLH1* is commonly observed in sporadic colon cancer [52].

Other epigenetic mechanisms relevant to cancer, in the first place genomic imprinting by which one of inherited allele of a certain gene is silenced, is now intensively investigated. The impact of chromatin modifications on cancer development has been recently recognized. All together, intensive investigations of epigenetic events in cancer in past two decades pointed out that cancer arises as the consequences of genetic, as well as epigenetic alterations.

Future directions

So far, for the majority of human cancers, a characteristic single cancer gene, such as APC in colorectal cancer, can not be defined. Introduction of novel microarray-based technologies such as cDNA-based, oligonucleotide-based and high-throughput proteomics approaches to detect changes in gene expression, or BAC (bacterial artificial chromosome) microarrays for high-resolution detection of tumor gene alterations, into cancer research should facilitate the characterization of the complex network of the signaling pathway implicated in cancer development. However, the translation of experimental data into clinical practice is still the major obstacle. Future translational research has to identify genes differently expressed in tumor *versus* normal tissue, then to characterize the gene and its protein product and to define the biological role of the examined gene in tumorigenesis. After that, an antibody or other molecule must be created to block the activity of the examined gene protein product. Finally, it is necessary to evaluate the success of such an approach in the clinical setting. Molecularly targeted cancer therapies are much more effective, with fewer side effects, than the other currently used anticancer therapies. Translational research, on the other hand, must provide individual molecular profiling of a patient's tumor that should increased efficacy of conventional anticancer therapies such as chemotherapy and radiotherapy. An example is the status of p53 gene concerning DNA damaging chemotherapeutics. Finally, exploiting data on cancer genetics, gene expression, epigenetics and genomics to clinical practice, will aid the identification of individuals at risk and the design of targeted cancer-preventive therapies.

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