Appendix V

The papers included in this section are classic review articles that document the evidence that 95-98% of all cancers are caused by somatic mutations in the genes that control cell growth. These papers are included here because they make a convincing argument that, instead of adding more and more mutagen to the environment, we should be doing the opposite in the name of improved public health. It is simply unfortunate that the general public does not know these facts nor the information presented in the previous appendices. Clearly, if the public understood the evidence it is unlikely that they would allow their neighborhoods to be sprayed with piperonyl butoxide and sumithrin.

Carcinogenesis vol.20 no.4 pp.519–527, 1999

REVIEW

Environmental factors as regulators and effectors of multistep carcinogenesis

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This review highlights current knowledge of environmental factors in carcinogenesis and their cellular targets. The hypothesis that environmental factors influence carcinogenesis is widely supported by both epidemiological and experimental studies. The fact that only a small fraction of cancers can be attributed to germline mutations in cancer-related genes further buttresses the importance of environmental factors in carcinogenesis. Furthermore, penetrance of germline mutations may be modified by either environmental or other genetic factors. Examples of environmental factors that have been associated with increased cancer risk in the human population include chemical and physical mutagens (e.g. cigarette smoke, heterocyclic amines, asbestos and UV irradiation), infection by certain viral or bacterial pathogens, and dietary non-genotoxic constituents (e.g. macro- and micronutrients). Among molecular targets of environmental influences on carcinogenesis are somatic mutation (genetic change) and aberrant DNA methylation (epigenetic change) at the genomic level and post-translational modifications at the protein level. At both levels, changes elicited affect either the stability or the activity of key regulatory proteins, including oncoproteins and tumor suppressor proteins. Together, via multiple genetic and epigenetic lesions, environmental factors modulate important changes in the pathway of cellular carcinogenesis.

Introduction

We will focus the discussion on three issues: (i) the evidence that events impinging on the organism from the outside foster, or protect against, carcinogenesis; (ii) mechanisms underlying environmental factors' abilities to exert their effects; and (iii) the contribution of endogenous factors to the impact of environmental factors.

Evidence that environmental factors influence carcinogenesis

A large body of compelling evidence either confirms or implicates various environmental factors in the development of a wide range of malignancies. Among the key factors are chemical and physical carcinogens, infectious agents and life-style. A long list of chemicals that occur in the environment has been implicated in tumor formation (reviewed in ref. 1). An increasing number of studies are documenting the ability of chemical mutagens to elicit changes at both the genomic and the protein level (discussed below).

Environmental factors known to play important roles in the etiology of human cancer include chemical carcinogens, such as those found in cigarette smoke, dietary contaminants, such as the mycotoxin aflatoxin B1, and physical carcinogens, such as UV irradiation, asbestos and radon. Other environmental factors include pathogenic bacteria and viruses, such as *Helicobacter pylori*, human papilloma virus (HPV), and human hepatitis B and C virus (HBV/HCV). Life-styles that ignore known risk factors, such as smoking, excess exposure to sunlight, fat consumption and stress are themselves integral environmental factors that contribute to cancer development. Conversely, life-style elements thought to reduce certain cancer risk include fiber ingestion, antioxidants and exercise.

Signature mutations

A signature mutation reflects the nature of adducts and DNA lesions formed by a specific mutagen, as confirmed for several chemical and physical mutagens (reviewed in refs 2–4). The comprehensive analysis of the *p53* tumor suppressor gene has enabled the establishment of the existence of signature mutations. Classic examples for signature mutations are UV-related C→T and CC→TT conversion (5), G→T changes caused by dietary aflatoxin B1 exposure (6,7), G→T and G→C mutations associated with tobacco derived carcinogens (8,9) and the A→T and T→A alterations associated with vinyl chloride exposure (10). The identification of so-called signature mutations has provided evidence that links specific environmental factors with the mutation spectrum associated with the etiology of tumor development.

Epidemiological findings

Several lines of epidemiological evidence support the role of environmental factors in malignant disease. Non-genetic factors in cancer development have been implicated by epidemiological studies that have identified the differences in incidence and tumor type among different ethnic and geographical populations. Such studies have provided the foundation for investigating the role of environmental factors in tumor development (11,12).

Among the better-characterized examples are differences in frequency of certain types of cancer between Japanese and Western populations (1,11–13). The rate of gastric cancer in Japan is as much as six times higher than in Western populations (13). Conversely, the incidence of breast cancer is three to four times lower and that of prostate cancer, seven times lower in Japan than in Western countries.

Notably, changes in environment may be associated with major shifts in cancer prevalence. Thus the incidence of gastric cancer decreased markedly among Japanese people who migrated to Western countries (11). The risk of breast

Abbreviations: CREB, cyclic AMP response element binding protein; EGFR, epidermal growth factor receptor; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HPV, human papilloma virus; IGFR, insulin-like growth factor receptor; MAPK, mitogen activated protein kinase; NO, nitric oxide; PKA, protein kinase A; PKC, protein kinase C; ROS, reactive oxygen species; TFIIH, transcription factor IIH; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Summary

Whether drug-based or target-based screens are used, it is possible to exploit the detailed information gathered for several model organisms that are genetically tractable. Such approaches are well suited to identifying drugs that have a selective killing capacity for the tumor context. They allow us to escape from strategies that are based on inhibiting the activities of oncogene products, or attempting to restore the lack of activity resulting from the inactivation of a tumor suppressor gene product. Because such genetic approaches allow an alignment of particular molecular defects with "specific" drugs, there is a high probability that the serious side effects associated with many currently used chemotherapeutics will be less problematic. Although the utility of genetics and model organisms is potentially quite broad, three inadequacies will continue to limit clinical applications. The first stems from the current difficulties in understanding the complexities of the mammalian cell signaling circuitry, the second stems from our still limited methods of assessing molecular alterations in tumors, and the third stems from relatively ineffective ways of conditional gene inactivation in mammalian cells. Finally, as more therapies are developed for particular molecular defects, there will be increased need as well as incentive to improve methods for detecting these alterations.

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8 September 1997; accepted 3 October 1997

Environment and Cancer: Who Are Susceptible?

Frederica P. Perera

Acting in concert with individual susceptibility, environmental factors such as smoking, diet, and pollutants play a role in most human cancer. However, new molecular evidence indicates that specific groups—characterized by predisposing genetic traits or ethnicity, the very young, and women—may have heightened risk from certain exposures. This is illustrated by molecular epidemiologic studies of environmental carcinogens such as polycyclic aromatic hydrocarbons and aromatic amines. Individual genetic screening for rare high-risk traits or for more common, low-penetrant susceptibility genes is problematic and not routinely recommended. However, knowledge of the full spectrum of both genetic and acquired susceptibility in the population will be instrumental in developing health and regulatory policies that increase protection of the more susceptible groups from risks of environmental carcinogens. This will necessitate revision of current risk assessment methodologies to explicitly account for individual variation in susceptibility to environmental carcinogens.

Most cancer results from the interaction of genetics and the environment (*1*–*3*). That is, genetic factors by themselves are thought to explain only about 5% of all cancer (*3*). The remainder can be attributed to external, "environmental" factors that act in conjunction with both genetic and acquired susceptibility. This is an optimistic message for cancer prevention in that exposure to environmental carcinogens—tobacco smoke, dietary constituents, pollutants (in the workplace, air, water, and food supply), drugs, radiation, and infectious agents—is theoretically preventable. But it challenges scientists to document environment-susceptibility interactions and policy-makers to rapidly

Human Cancer Syndromes: Clues to the Origin and Nature of Cancer

Eric R. Fearon

More than 20 different hereditary cancer syndromes have now been defined and attributed to specific germline mutations in various inherited cancer genes. Collectively, the syndromes affect about 1 percent of cancer patients. An individual who carries a mutant allele of an inherited cancer gene has a variable risk of cancer that is influenced by the particular mutation, other cellular genes, and dietary, lifestyle, and environmental factors. Though hereditary cancer syndromes are rare, their study has provided powerful insights into more common forms of cancer. Somatic mutations in sporadic cancers frequently alter the inherited cancer genes, and the functions of cell signaling pathways have been illuminated by study of the affected genes. Further investigation of inherited mutations that affect susceptibility to cancer will aid efforts to effectively prevent, detect, and treat the disease.

Cancer is a genetic disease, arising from an accumulation of mutations that promote clonal selection of cells with increasingly aggressive behavior. The vast majority of mutations in cancer are somatic and are found only in an individual's cancer cells. However, about 1% of all cancers arise in individuals with an unmistakable hereditary cancer syndrome. These individuals carry a particular germline mutation in every cell of their body. Although rare, the inherited cancer syndromes are of vast biological importance. Studies of the specific mutations responsible for these syndromes and the cellular signaling pathways disrupted by the mutant proteins have begun to provide unprecedented insights into the molecular origins and pathogenesis of inherited and sporadic forms of cancer. I discuss (i) the strategies that have led to successful isolation of inherited cancer genes; (ii) the cellular signaling pathways that are disrupted by the mutant genes; (iii) the roles of allelic variation and modifier genes in cancer development; and (iv) some of the future challenges and opportunities for the field of cancer genetics.

Clues to Heritable Forms of Cancer

Family history has long been recognized as an important component of cancer risk, yet the identification of specific genes that affect cancer risk is a formidable task. Of critical importance in the discovery process has been the establishment of clear criteria for recognizing families and individuals who are not only likely to be affected by an inherited cancer syndrome, but who are also suitable for genetic studies. For instance, genetic studies have a greater likelihood of success in families in which multiple affected and unaffected individuals in two or more generations are available for analysis, than in families in which only a few individuals can be studied. A complicating factor in genetic studies is that cancer is not a single disease, even when it arises in the same organ site. Rather, it is a collection of many diseases, some of which are very common and others extremely rare. Thus, families in which multiple members develop a rare form of cancer, such as retinoblastoma or osteosarcoma, are much more likely to be segregating a mutation in an inherited cancer gene than are families affected by more common cancers, such as adenocarcinomas of the lung, breast, prostate, and colon. Nonetheless, an inherited cancer syndrome should be considered when numerous family members develop cancer at an especially young age or affected individuals develop multiple primary cancers, even if they are common cancers. Families in which those with cancer also manifest other rare conditions, particularly congenital abnormalities, should also arouse suspicion of a cancer syndrome.

However, in many families segregating a mutant copy (also known as a mutant "allele") of a major inherited cancer gene, none of these striking features will be evident, perhaps because of small family size, uncertain family history, or the absence of cancer in family members who carry the mutant allele (termed "incomplete penetrance"). Confounding matters further, in some families with an inherited cancer syndrome, sporadic cancers of the same type may arise in individuals who do not carry the mutant allele (termed "phenocopies"). Incomplete penetrance and phenocopies can make it difficult to distinguish true mendelian forms of cancer from chance familial aggregations. The number of mendelian forms of cancer is not known, but more than 20 distinct inherited syndromes have been defined (Table 1).

The term "inherited cancer genes" will be used here to describe those genes for which certain mutant alleles have been demonstrated to cause highly penetrant cancer syndromes when transmitted through the germline. As discussed below, the likelihood that an individual who carries a mutant allele of an inherited cancer gene will ultimately develop cancer is variable and dependent on the particular mutant allele; various other cellular genes that can influence the likelihood, age of onset, and severity of cancer (called modifier genes); and poorly understood dietary, lifestyle, and environmental factors. Hence, because variant alleles of modifier and other genes have a meaningful role in cancer development, the inherited cancer genes constitute only a subset of a larger class of genes that affect the cancer risk of an individual. This larger, more inclusive class of genes might be termed cancer susceptibility genes. Certain variant alleles of cancer susceptibility genes would be, by definition, associated with increased cancer risk. Either singly or collectively, these variant alleles may have an important role in sporadic cancers and familial aggregations of cancer that do not present as highly penetrant syndromes.

Mapping Inherited Cancer Genes

Linkage analysis remains the mainstay of efforts to map inherited cancer genes. This approach usually requires study of large, multigenerational families to establish that genetic markers from a particular chromosomal region cosegregate in unambiguous fashion with the development of cancer. Although linkage analyses have proven quite successful, they are sometimes limited by problems of variable penetrance and phenocopies, as noted above. Another obstacle is genetic heterogeneity, which refers to the fact that germline mutations in several different inherited cancer genes at unique chromosomal locations can give rise to essentially indistinguishable clinical syn-

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citations (*7*), and magnetization plateaus are to be expected as a result. The first of these appears at a value of 1/8 of the saturation magnetization. The required magnetic field of 27 T is quite large but within range of the NMR facility of the Grenoble High Magnetic Field Laboratory. The unique combination of high fields and low temperatures at this facility enabled Kodama *et al.* (*5*) to observe the magnetic superlattice as a dense series of lines in the Cu NMR spectrum at 35 mK. Analysis of the magnetization in the large supercell implied by the high order of the commensurability (1/8) required a numerical solution of the Shastry-Sutherland model.

The spectra could be well fit by the magnetization pattern shown in the figure. One in eight of the dimers is strongly polarized parallel to the external field. But the magnetization pattern is much richer than a simple polarization of 1/8 of the dimers. This more complex pattern can be attributed to the high magnetic polarizability of the singlet ground state of the dimer lattice. As a result, the dilute superlattice of spin triplet dimers is accompanied by a background magnetic polarization. The transition into the superlattice state as the field is increased appears to be first order, which favors an interpretation of it as a crystallization of a dilute bosonic fluid.

Turning our attention back to the quantum ground state, which appears when the kinetic terms dominate, new results on another set of copper salts, $KCuCl_3$ and $TlCuCl_3$, have recently been obtained. Initially, their crystal structure seemed to imply that the dimers formed the rungs of ladders with only weak interladder interactions. However, a detailed mapping of the energy dispersion of the triplet excitations showed a fully three-dimensional network of exchange interactions (*8*).

These salts do not show magnetization plateaus but instead show a continuous rise starting at a threshold magnetization value and ending at the saturation magnetization. The Bose-Einstein condensed state in the intermediate range is characterized by a coherent superposition of the singlet and $S_z = +1$ triplet component on each dimer (*9*). This generates a staggered magnetization transverse to the external field.

The phase in the complex superposition determines the orientation of staggered moments in the *xy*-plane. Elastic neutron-scattering measurements observe a staggered magnetization with long-range ordering with a finite ordering temperature (*10*). Recently, Ruegg *et al.* (*11*) examined the dynamics of the condensate by inelastic neutron scattering and observed a mode with linear dispersion above the threshold magnetization. As shown by Matsumoto *et al.* (*12*), this mode can be nicely interpreted as the well-known collective oscillation (or Goldstone mode) of the Bose-Einstein condensate.

As these recent experiments illustrate, quantum magnetism in a magnetic field offers exemplary systems for exploring the competition between the classical and quantum ground states for interacting bosons—a subject of current research also for the dilute atomic bosonic clouds.

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PERSPECTIVES: CANCER BIOLOGY

A Matter of Dosage

Riccardo Fodde and Ron Smits

Knudson's classic two-hit model of tumorigenesis stipulates that mutation of both alleles of a tumor suppressor gene is needed to trigger tumor formation (*1*). This recessive nature of tumor suppressor genes has been challenged by a growing number of reports (see the table) including recent papers in *Science* and *Nature Genetics* (*2–4*). These studies show that mutation or loss of a single allele may be sufficient to exert a cellular phenotype that leads to tumorigenesis without inactivation of the second allele. This gene-dosage effect is called haploinsufficiency and has been demonstrated by at least two different experimental approaches. Individuals or mice carrying a heterozygous mutation that inactivates only one allele of a tumor suppressor gene exhibit an increased incidence of tumors, a subset of which develop without loss or mutation of the second normal allele (*5*, *6*).

Enhanced online at www.sciencemag.org/cgi/content/full/298/5594/761

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Alternatively, haploinsufficiency can modify cancer risk in humans or mice that either already carry a heterozygous mutation in a separate tumor suppressor gene (known to comply with Knudson's two-hit model) (*7*) or that have been challenged by exposure to radiation or viruses (*8*). Tumors influenced by haploinsufficiency usually have a later age of onset when compared with those caused by inactivation of the second allele (loss of heterozygosity).

Although it is well documented that gene-dosage effects cause developmental defects in model organisms and in certain inherited human diseases, their importance in tumor biology has been overlooked. Morphogen gradients modulate cell proliferation, differentiation, and apoptosis in developing organisms. Exposure to different doses of these diffusible factors is rate-limiting for the determination of cell fate. Likewise, in the presence of a heterozygous loss-of-function mutation in a tumor suppressor gene, fluctuations in gene dosage below tissue-specific thresholds may interfere with the control of fundamental cellular processes (see the table). This results in either the direct triggering of tumorigenesis or modification of the cellular environment so that additional mutations or epigenetic changes in other genes can successfully promote tumor growth (see the figure).

Some tumor suppressor genes are "gatekeepers," that is, they carry out a crucial cellular function that when abrogated leads directly to tumorigenesis. However, there also exists a subset of tumor suppressor genes that are "caretaker" genes involved in DNA repair or chromosomal segregation. Haploinsufficiency at these caretaker genes may result in defective DNA repair and increased genetic instability leading to somatic mutations in other tumor suppressor genes and oncogenes. The recent *Science* and *Nature Genetics* papers (*2–4*) support the notion of DNA repair haploinsufficiency.

Bloom syndrome is a rare recessive disorder characterized by a predisposition to a broad spectrum of tumors. It is caused by loss-of-function mutations in the *BLM* gene, which encodes the DNA repair enzyme recQ helicase. Gruber *et al.* (*2*) genotyped two large series of colorectal cancer patients of Ashkenazi Jewish ancestry and showed that carriers of the BLM^{Ash} founder mutation had a significantly increased risk of developing large-bowel tumors. However, they did not analyze whether the second normal allele was mutated in the colorectal cancers. Thus, they could not discriminate whether the increased colorectal cancer risk was