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Molecular Mechanisms in Cancer Induction and Prevention

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Chemical and physical carcinogens, present in our environment and encountered in a variety of occupations, produce damage to DNA. X-rays produce direct ionizations and indirect hydroxyl radical attack. UV light in the short wavelength is specifically absorbed by unsaturated bonds in DNA, RNA, and proteins. There are a number of genetic sites that are specifically affected by environmental agents, and an increased sensitivity is found in certain genetic diseases. The development of a fully malignant tumor involves the activation or altered expression of oncogenes or the inactivation of tumor-suppressor genes that control normal cellular development. Mutations in the *p53* tumor-suppressor gene are common in diverse types of cancer and could perhaps provide clues to the etiology of some cancers and to the effect of various environmental and occupational carcinogens in cancer development. The fact that environmental factors are involved to a great extent in cancer suggest that cancer may be preventable. Experimental as well as epidemiological data indicate that a variety of *nutritional factors can act as anticarcinogens and inhibit the process of cancer development and reduce cancer* risk. The interaction of cells with a number of environmental and occupational genotoxic substances such as X-rays, UV light, and a variety of chemicals including ozone results in an enhanced generation of free oxygen radicals and in modified pro-oxidant states. A number of nutritional factors such as vitamins A, C, E, β-carotene, and micronutrients such as selenium act as antioxidants and anticarcinogens. Certain hormones such as thyroid hormones enhance oxidative processes and act as a co-transforming factor in carcinogenesis. A number of bioactive lipids act as cancer preventive agents. Sphingolipids act on signal transduction pathways and inhibit protein kinase C and multistep carcinogenesis. Sphingolipids are found in dairy products and milk. Omega-3 fatty acids suppress X-ray induced transformation as well as promotion. They also inhibit transformation by the *ras* oncogene. The ω-3 fatty acids act in part by reducing prostaglandin synthesis. In addition, the ω-3 fatty acids alter the composition of membrane fatty acids that are released from one or more phospholipids, causing remodeling of cellular phospholipids and reduced arachidonate-containing species. Such remodeling interferes with transformation.

Introduction

Over the years, epidemiological data have shown that most cancers are due to environmental factors including lifestyle practices and specific occupations (*1,2*). Experimental data confirmed the role of environmental agents in multistep carcinogenesis (*3*), though genetic predisposing factors remain important determinants (*4*). The development of a malignant phenotype involves complex interactions among endogenous and external factors. The complex multistep process can occupy over one-half of the life span of the organism in both human and experimental animal models. These and other properties of the carcinogenic process predict that multicellular genes and multiple mechanisms are involved in the conversion of normal cells to fully malignant tumor cells (*4*). Known carcinogens include ionizing radiation, ultraviolet light, chemicals, and viruses.

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The interaction of radiation with the cell takes place within a fraction of a second. The interaction of cells with chemical carcinogens is more prolonged (*3*). Neither radiation nor chemicals, in contrast to viruses, can introduce new genetic information into target cells. They must, therefore, modify the function of normal cellular genes.

The initiation of the neoplastic process involves DNA alteration, which may be heritable in progeny cells. These alterations include mutations, oncogene activation, rearrangements, amplifications, and methylation. The initiation of the transformation process is irreversible and is modified by agents that may act as promoters. Tumor promoters have been shown to act at different stages and induce a clonal expansion of initiated cells (*3*). Because promotion, and later progression, take place in multiple stages, it is in principle possible to intervene at various stages and modify the course and frequency of the neoplastic process. Initiation and promotion give rise to an abnormal expression of cellular genes. The events that take place appear to be highly dependent on permissive factors. Permissive factors include both genetic susceptibility as well as physiological permissiveness, including a

number of growth factors and hormones that regulate cellular differentiation and are involved in the malignant process. A wide number of specific oncogenes have been recognized as associated with specific growth modulators of the cell (*4*).

The interactions of growth factors and hormones with cellular receptors trigger a cascade of intracellular biochemical signals resulting in the activation and repression of various subsets of genes. Aberrations in growth-factor signaling pathways are closely linked to the induction of cancer. Malignant cells arise as a result of a stepwise progression of genetic events that include unregulated expression of growth factors or components of their signaling pathways (*4*).

Radiation As a Cancer-Causing Agent

Ionizing Radiation

Radiation is a fact of life. It occurs in nature and pervades the environment. It can also be produced artificially and as such has been used for several decades. Radiation is called "ionizing" when it possesses sufficient energy to remove electrons from their orbits in atoms or molecules constituting the irradiated material. This leads to the breaking of chemical bonds and results in permanent changes. In most cases ionization occurs through electrically charged particles of nuclear components such as protons or α particles. These are directly ionizing radiations. They may originate from external or internal forces. They can also be generated inside the irradiated matter after exposure to indirectly ionizing radiation including electromagnetic quanta (or photons) such as X-rays and γ-rays, or electrically neutral particles such as neutrons (*3*). Ionizations result in short-lived (10^{-10} sec) ion pairs that go on to produce free radicals with a somewhat longer life (10^{-5} sec).

Radiogenic Cancer

The carcinogenic potential of X-rays in humans was realized within the first decade after their discovery by Roentgen in 1895 (*5*). In 1896, Thomas Edison tried to use the X-ray tube to develop a fluorescent illuminating lamp. He abandoned these efforts, however, explaining later,

> ... I started to make a number of these lamps, but I soon found that the x-ray had affected poisonously my assistant, Mr. Dally so that his hair came out and his flesh commenced to ulcerate. I then concluded it would not do and that it would not be a very popular kind of light so I dropped it ...

By 1902 the degenerative changes in Dally's skin progressed to a carcinoma requiring the amputation of his left hand above the wrist. The disease progressed to both arms and led to Dally's death from metastases in 1904.

The carcinogenic potential of X-rays was confirmed in later years through epidemiological data, the largest single source of information being from Hiroshima and Nagasaki (*6*). Enhanced rates of cancer in workers in uranium mines (*7*), as well as the enhanced rates of cancer in individuals exposed to asbestos and radon, have been recorded (*7*). The data provided good evidence to suggest that various forms of cancer represent the most significant late effects when human populations are exposed to substantial doses of radiation (*6*).

Data in cell culture showing transformation *in vitro* by radiation made it possible to establish the direct action of radiation at the cellular level in its ability to convert a normal cell to a malignant cell (*3,8–10*). Further advance came in the ability to show that DNA from radiation-transformed cells can induce transformation in normal cells upon transfection (*11*). The work indicated that DNA is a target of radiation carcinogenesis induced at the cellular level *in vitro*. The experiments also indicated that malignant radiogenic transformation *in vitro* involved the activation of unique, dominant oncogenes, induced by *in vivo* exposure to radiation (*11*). These differ from the *ras* genes activated in some tumors (*12*).

UV Irradiation

Of the nonionizing radiations, UV radiation from sunlight is the major cause of skin cancers, including malignant melanoma, and accounts for the high rates of these cancers in southern climates (*2*). Studies suggest that intermittent exposure (recreational and childhood exposures associated with sunburn) are important in melanoma, whereas cumulative (occupational) exposures appear more closely related to nonmelanoma skin cancer (*2*).

Occupation

Occupational exposure accounts for about 5% of cancer deaths, but the percentage varies with region and tumor (*2*). Asbestos is probably the main occupational carcinogen in many countries due to its induction of lung cancer and mesotheliomas (*7*). Bladder cancer is also influenced by occupational exposures and appears to be higher in areas with chemical and petrochemical industries (*2*).

Pollution

Pollutants in urban atmospheres have long been suspected in the etiology of lung cancer. Polycyclic hydrocarbons and ozone, which is the most ubiquitous pollutant in urban atmospheres, may be of special concern (*13*). Indoor pollution by radon gas and tobacco smoke, in conjunction with external pollution by hydrocarbons and ozone, is a serious threat to human health (*7*). Several of these agents, such as hydrocarbons and ozone, produce free radicals when they interact with a variety of macromolecules in the cell (*13*). These processes have been associated with in part with their neoplastic potential (*15,16*).

Molecular Mechanisms of DNA Damage from Various Radiations

The damage produced in DNA by ionizing and nonionizing radiations results from complex alterations to the

reactive sites in DNA. X-ray induces ionizations and indirect hydroxyl radical attack, all of which damage many sites in the purine and pyrimidine rings, destroy deoxyribose residues, break one or both strands of DNA, and produce DNA–protein crosslinks (*16*). Oxygen, which is a ubiquitous, stable, free radical, enters into the radiation-induced free-radical reactions extensively and tends to exacerbate the biological effects.

Ultraviolet light in the short wavelength range (260–280 nm), in contrast, is specifically absorbed by the unsaturated chemical bonds in DNA, RNA, and proteins. Its biological effects are the consequence of chemical changes produced by specific excitations (*16*).

DNA Double-Strand Break, Repair, and Deletion Mutagenesis

A more important lesion produced in mammalian cells by ionizing radiation appears to be the DNA double-strand break. Double-strand breaks are rejoined very rapidly, but it is unlikely that many are repaired accurately. In mammalian cells, the favored mechanism for double-strand repair is not homologous recombination because sister chromosomes are rarely physically close enough for homologous events to be possible (*16*).

Mutagenic Effects of DNA Damage

Mutations are one of the end products of a cascade of metabolic processes initiated by radiation or chemically induced damage to DNA. Ionizing radiation generates a high frequency of large deletions compared to point mutations (*16*), whereas UV light produces point mutations almost exclusively (*16*). These extremes illustrate that the discrimination of widely different agents is possible on the basis of the mutation spectra they generate.

The prospect of identifying a particular mutagen as a causative agent for a particular individual mutation is difficult because most mutations are observed at low frequency in a natural environment and under conditions of exposure to a mixture of chemicals or radiation. The requirement for generating a mutation spectrum is the ability to derive a large range of mutations within a well-characterized gene.

Mutations can be classified, in general, as a single-gene mutation or a chromosomal or multiple-gene mutation. Point mutations and small deletions are considered to be genetic effects involving a small number of bases (no more than about 20 in a single region), whereas large deletions and rearrangements are considered genetic effects spanning many hundreds of thousands of base pairs (*16*). Radiation produces a significant frequency of chromosomal or multiple-gene mutations. Point mutations and deletions are formed by rapid rejoining of DNA breaks and a round of DNA replication to fix damage leading to a point mutation. The proportion of deletions increases with the density of ionizations. Point mutations are less than 50 base pairs and are probably predominantly GC to AT base substitution mutations (*16*).

Genetic Predisposition to Cancer

Genetic determinants that predispose individuals to cancer are more difficult to identify by clinical and epidemiological means. There are a number of genetic diseases that predispose individuals to cancer. Many cancers show small familial risks on the order of 2- to 3-fold, but in some cases there is a remarkable aggregation of cancer that seems consistent with autosomal dominant inheritance; for example, families in which the susceptibility may affect the variety of cancer types such as the Li-Fraumeni syndrome of sarcomas, breast cancer, and other tumors (*2*), which has been associated with the mutation in the tumor-suppressor gene *p53* (*17*).

Human diseases that are known to have major or minor alterations in their radiation response frequently exhibit altered radiation sensitivities with increased carcinogenesis (*16*). These diseases include xeroderma pigmentosum (XP) and ataxia telangiectasia (AT). The increase in sensitivity is large and is an integral feature of the disease (Table 1).

Genetic and Environmental Factors in Breast Cancer

Of particular importance for X-ray damage is the disease ataxia telangiectasia. There is considerable evidence that heterozygotes are predisposed to breast cancer; they may constitute 9–18% of all persons with breast cancer in the United States (*18*). Of the various anatomical sites, breast tissue is considered the most sensitive to the effect of ionizing radiation (*6*). To date, exposure to environmental factors such as radiation (*6*) in conjunction with some lifestyle factors such as smoking and alcohol consumption (*1*) could clearly modify the incidence of the disease. Base modification, which arises from free-radical induced hydroxylation and cleavage reaction of purine rings, has been suggested to play a role in the initiation of breast cancer (*19*). Other types of genetic effects that could be induced by environmental agents are mutations in specific genes.

To date, at least nine genetic alterations have been characterized in breast cancer, including amplification of *myc* and *neu/erbB2*, an oncogene homologous to the epidermal growth factor receptor, and mutations in *p53* (*17*). Alterations in *p53* in human breast tissue have been shown to occur at the level of gene structure, mRNA, or protein by mechanisms involving point mutation. The time at which these mutations occur in the neoplastic process is not established (*17*).

Table 1. Human diseases showing hypersensitivity to radiation or carcinogens (*16*).

Disease	Agent
Xeroderma pigmentosum	UV, chemical carcinogens
Ataxia telangiectasia	X-rays, γ-rays
Cockayne syndrome	UV light
Retinoblastoma	X-rays
Huntington's chorea	X-rays

Although genetic susceptibility and environmental factors appear to be important in the etiology of cancer (*20*), diet can modulate the rate of the disease (*1,21,22*). The role of micronutrients and vitamins in cancer prevention, which act by means of interfering with free oxygen radical mechanisms, has been studied in human populations as well as in the laboratory. Serum levels of vitamins E, C, β-carotene, and selenium appear to be inversely proportionate to cancer risk in different sites (*22,23*).

In Vitro Induction of Environmental Carcinogenesis and Its Modulation

Transformation of rodent and human cells *in vitro* provides a powerful tool for defining stages in neoplastic development (i.e., initiation and promotion). Transformation affords the opportunity to identify co-carcinogenic interactions among various agents and to determine cellular and molecular factors that initiate and alter the course and frequency of malignant transformation (*3,24,25*) (Fig. 1).

Molecular Mechanisms in Radiation Carcinogenesis *in Vitro*

Changes in the DNA of somatic cells after exposure to radiation or chemicals are the genetic events that transform a normally regulated cell into one that grows without responding to controls. These changes have been assigned to a group of genes called proto-oncogenes (*4*). When proto-oncogenes are activated by mutation, they are altered into oncogenes whose products cause an inappropriate pattern of cell growth. One group of such oncogenes constitutes the *ras* gene family, which is activated in radiation-induced tumors (*12*). Activation occurs by point mutations mostly in codons 12, 13, and 61 (*12*). By contrast to tumors induced *in vivo* in which a percentage of tumors show a *ras* gene activation, rodent cells exposed *in vitro* to ionizing radiation do not show the activation of any of the *ras* genes (*11*) (Fig. 2). Exposure of C3H 10T1/2 cells or hamster embryo cells *in vitro* resulted in the activation of distinct, dominant transforming genes (*11*). A study of more than 11 oncogenes suggested that transfor-

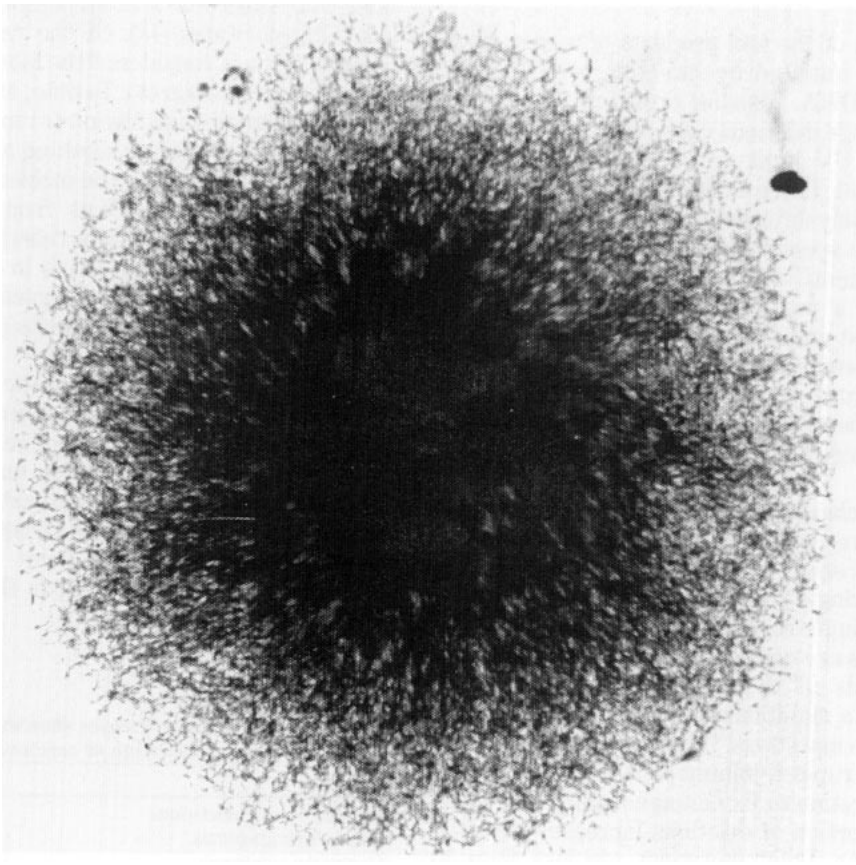

FIGURE 1. Colony of radiation-transformed hamster embryo cells.

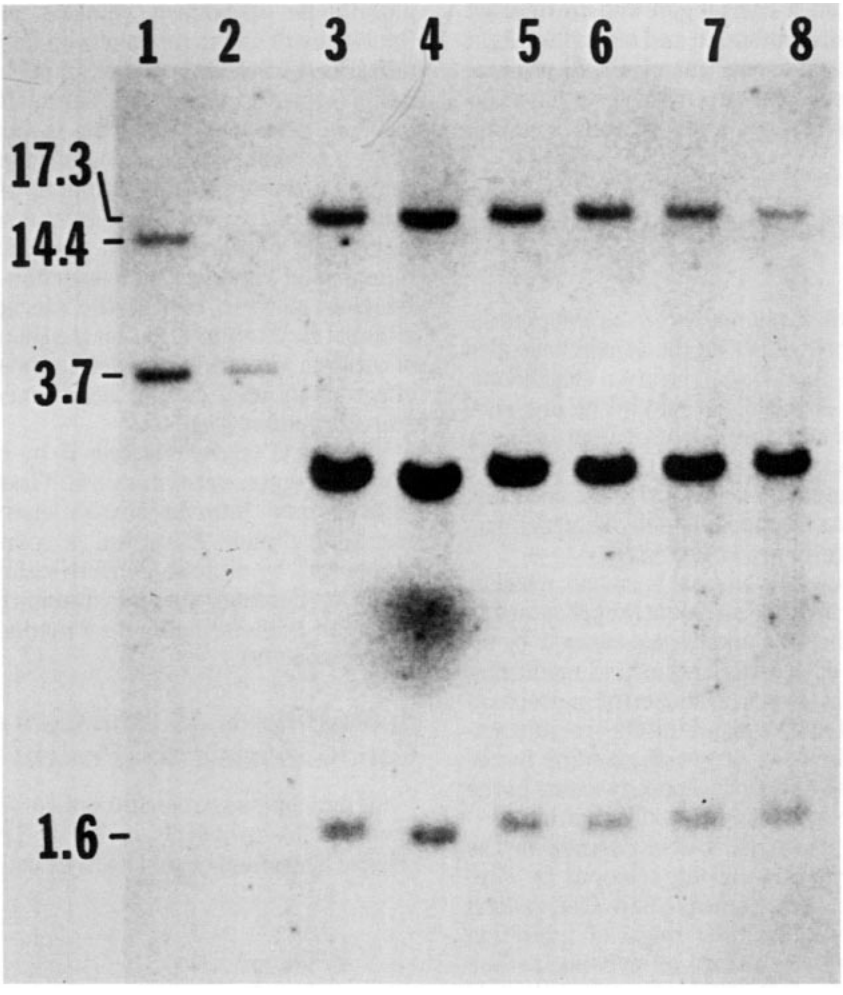

FIGURE 2. Analysis of K-*ras* sequences important in DNA of independent mouse transformants from *in vitro* transformed hamster embryo and C3H 10T1/2 mouse cells. (Lane 1) DNA of normal hamster embryo cells; (lane 2) DNA of X-ray-transformed hamster embryo cells; (lane 3) 3T3 transformants obtained with DNA of X-ray-transformed hamster embryo DNA; (lane 4) DNA from 3T3 cells; (lanes 5 and 6) 3T3 transformants obtained with DNA from X-ray-transformed C3H 10T1/2, lanes 1 and 2, respectively; (lane 7) DNA from X-ray-transformed C3H 10T1/2; (lane 8) DNA from normal C3H 10T1/2 cells. No activated *ras* genes were found in the following X-ray transformation *in vitro*. From Borek et al. (*11*).

mation involved activation of unique transforming genes that differ in the hamster and the mouse and excluded a wide variety of genes associated with the focus formation in rat 2 cells, 10T1/2 cells, or 3T3 cells, such as the *ras neu*, and *trk* oncogenes (*11*). Transformation of human cells by radiation also indicated that the *ras* genes were not involved in the process of transformation (*26*).

Thyroid Hormone as a Cotransforming Factor

Hormones have long been known to exert an important influence on neoplastic transformation and cancer induction (*21*). Thyroid hormone has been shown, in experimental systems, to play a critical role in cellular transformation by radiation, chemical carcinogens, tumor viruses (*3*).

An additional mechanism for thyroid hormone regulation of transformation resides in the ability of the hormone to modify the pro-oxidant state of the cell (*3,24*). This function is underscored by the fact superoxide dismutase suppresses the effects of triiodothyridine in potentiating radiogenic transformation as well as inhibiting the promoting action of phorbol esters that generate free oxygen species.

Oxygen Radicals in Carcinogenesis

There is a growing volume of data that provides evidence that oxygen radicals play an important role in the

neoplastic process (*27–30*). Ozone, the largest pollutant in the atmosphere and the chief component in oxygen smog, has been shown to act as a carcinogen and to interact synergistically with ionizing radiation and ultraviolet light (*14,15*). X-rays, UV light, and some chemicals all produce oxygen radicals and free-radical intermediates upon interaction with tissues and enhanced levels of lipid peroxidation.

Arachidonate and Prostaglandins in Transformation

The role of oxygen radicals in multistep carcinogenesis is mediated in part by their effect on the arachidonic acid cascade. Phospholipase A_2 (PLA_2) cleaves arachidonic acid from phospholipids. Arachidonic acid in the presence of oxygen radicals goes on to produce a range of prostaglandiens via the cyclo-oxygenase route and leukotrienes via the lipoxygenase route (*31*). In addition, arachidonic acid gives rise to malondialdehyde, which has been shown to be involved in promotion (*32*).

Our recent findings show that thyroid hormone, which is a cotransforming factor involved in oxidative processes in the cells, plays a role in the arachidonic acid cascade by increasing the production of prostaglandins and modifying arachidonic acid synthesis (Borek, manuscript in preparation). Vitamin E, by contrast, which inhibits transformation (*30*), also reduces the level of prostaglandins in the cell, thus antagonizing the effect of triiodothyronine in the oxidative processes (Borek, manuscript in preparation).

The inhibition of the arachidonic acid cascade in the intervention in transformation can be achieved by suppressing the level of oxygen radicals and free-radical intermediates in the cells. Another route of inhibition would be by attenuating the effect of prostaglandins (eicosanoids) by introducing fatty acids that are analogs or arachidonate, which would reduce the production or action of the normal eicosanoids (*33*).

Dietary Antioxidants and Dietary Factors in Cancer Chemoprevention

The interaction of cells with a number of environmental carcinogens and mutagens including X-rays, UV light, and a variety of chemicals including ozone results in enhanced generation of free oxygen species and free-radical intermediates (*3*). The result is a loss in the optimal cellular balance between oxidative challenge, a source of DNA damage, and the inherent mechanisms that protect the cell from excess oxidative stress. A large number of antioxidants prevail in the cells, which protects the cells under normal metabolic conditions (*24,29*).

Experiments *in vitro* indicated that selenium, a component of glutathione peroxidase, and vitamin E, a powerful antioxidant in the component of the cell membrane, act as potent anticarcinogens (*30*). When C3H 10T1/2 and hamster cells are pretreated with vitamin E, selenium, or the combined agents and exposed to X-rays, benzo[*a*]pyrene, tryptophan pyrolysates, ozone, or ultraviolet light, suppression of transformation is observed. Cellular pretreatment with selenium results in increased levels of cellular glutathione peroxidase, catalase, and nonprotein thiols (glutathione) and in an enhanced destruction of peroxides (*30*) (Fig. 3). Selenium and vitamin E act synergistically to inhibit transformation (*30*). Vitamin E protects by inhibiting lipid peroxidation and the formation of malondialdehyde, a compound with oncogenic potential (*29*). Selenium acts as a true protector. Addition of selenium at various times before exposure to X-rays results in a suppressive action that diminishes with time (Fig. 4). The metabolic functions of vitamin E and selenium are interrelated, and selenium plays a role in the storage and transport of vitamin E. Vitamin E action is also closely related to that of vitamin C, which appears to increase the antioxidant effect of vitamin E and acts synergistically to inhibit transformation (*29*).

Vitamin C spares vitamin E by reducing the vitamin radical to regenerate vitamin E. Vitamin E scavenges lipid radicals and interferes with chain propagation. The resulting vitamin E radical is reduced by ascorbate to regenerate by vitamin E. Both vitamin E and vitamin C act as anticarcinogens, and vitamins E and C act in concert to inhibit transformation in a manner that appears to be synergistic (*29*).

Sphingolipids As Inhibitors of Multistep Carcinogenesis and Protein Kinase C

Sphingolipids are constituents of foods, especially dairy products. Their hydrolysis during digestion is reported to release long chain bases that may act as a protective factor

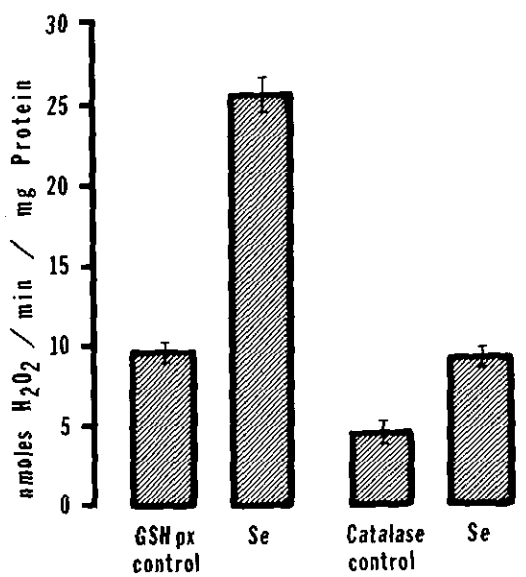

FIGURE 3. The effect of selenium in enhancing levels of glutathione peroxidase and catalase after pretreatment with selenium and X-irradiation. Selenium enhances the inherent levels of glutathione peroxidase and catalase and increases the breakdown of hydrogen peroxide in the cells. From Borek et al. (*30*).

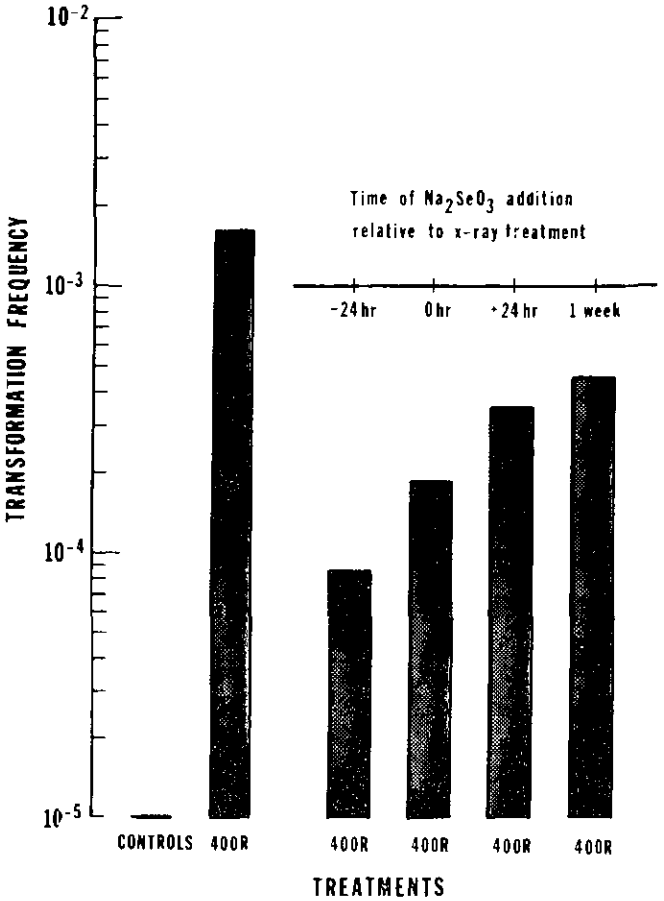

FIGURE 4. The effects of selenium as a true protector. When selenium is added to the cells before radiation, there is maximal effect of protection. When added at later stages, the protective effect is reduced and transformation levels are increased in C3H 10T1/2 cells (*30*).

for the small and large intestine (*25*). We tested the ability of long chain bases (sphingosine and sphinganine) to inhibit multistage carcinogenesis in mouse (C3H 10T1/2) cells exposed to γ-rays and the phorbol ester phorbol myristate acetate (PMA) and to suppress protein kinase C, the major cellular receptor for phorbol ester tumor promoters (*34*). Protein kinase C (PKC) plays a central role in signal transduction and growth control (*34*). We found that the long chain bases inhibit transformation by radiation as well as promotion by the tumor promoter PMA. They also inhibited PKC activity in radiation-treated and PMA-treated cells.

The suppression of radiation-induced transformation by long chain bases in groups that were not treated with PMA, but only with radiation, may reflect an inhibition of PKC activation by endogenous factors such as diacylglycerol, unsaturated fatty acids, hormones, and various naturally occurring compounds (*25*). These may act as endogenous tumor promoters in cells exposed to initiating environmental carcinogens. Transformation of cells by radiation is associated with the activation of cellular oncogenes and one mechanism by which oncogenes transform cells has been postulated to be by way of constituent activation of PKC (*25*).

ω-3 Fatty Acids as Cancer Preventive Agents

Diets high in fat have been shown to increase the incidence of certain types of experimental cancers; in particular diets rich in essential fatty acids of the ω-6 family enhance carcinogenesis. Thus, not only the amount of dietary fat, but also its composition, is a factor in carcinogenesis (*35*). The cancer-enhancing effect of fats can be inhibited by substances that attenuate the synthesis of prostaglandins (*36*). This also points to an involvement of ω-6 fatty acids in carcinogenesis. Individuals such as Eskimos living in a non-Westernized environment have a low incidence of cancer (*37,38*), an observation that led to the investigations of the components of their diet that might be responsible. It was found that their diet is rich in fat of marine mammals that contain low amounts of ω-6 and high amounts of ω-3 fatty acids, in particular eicosa-pentaenoic acid (EPA) and docosahexaenoic acid (DHA). In a number of experimental animals, ω-3-rich diets reduced the incidence and multiplicity of chemically induced tumors and ultraviolet-light-induced skin tumors (*39*). These observations suggest that arachidonate metabolites are involved in the progression of cancer and that arachidonate analogs such as EPA block the action of arachidonate.

In experiments designed to test this hypothesis, we found that EPA and DHA inhibited radiation-induced transformation and the enhancement of transformation by the phorbol ester PMA (Tables 2 and 3), and suppressed the transformation of cells by the H-*ras* oncogene [(*33*)

Table 2. Effect of polyunsaturated fatty acids on radiogenic transformation in C3H 10T1/2 cells. (*3*).

Treatment	Frequency of transformation, $\times 10^{-4}$
Control	0
AA	0
EPA	0
DHA	0
4 Gy	8.6
4 Gy + AA	8.7
4 Gy + EPA	1.2
4 Gy + DHA	0

Abbreviations: AA, arachidonate (ω-6); EPA, eicosanpentaenoate (ω-3); DHA, decosahexaenoate (ω-3).

Table 3. Inhibitory effects of EPA on radiogenic transformation and on promotion by ester PMA (*33*).

Treatment	Frequency of transformation, $\times 10^{-4}$
Control	0
PMA + EPA	0
4 Gy	5.1
4 Gy + PMA (0.15 M)	9.2
4 Gy + EPA (100 μM)	1.4
4 Gy + EPA + PMA	1.4

Abbreviations: EPA, eicosapentaenoate ω-3; PMA, phorbol myristoyl acetate.

Table 4. Effect of polyunsaturated fatty acids on transfection with pT24 in C3H 10T1/2 cells (33).

Treatment	Foci/ng DNA
Control ± 100 μM EPA	0
T24 (2 ng/pL)	0.85
T24 + 25 μM EPA	0.75
T24 + 50 μM EPA	0.19
T24 + 100 μM EPA	0.06
T24 + 100 μM EPA for first 2 weeks	0.66

Abbreviations: AA, arachidonate; EPA, eicosapentaenoate; DHA, docosahexaenoate.

Table 4]. The dramatic inhibition of transformation, which was time related, was associated with decreased prostaglandin production in cells grown in the presence of either ω-3 fatty acid (*33*).

The inhibitory effect of ω-3 fatty acid on transformation was associated in an alteration of the composition of molecular species of the cellular phospholipids (*33*). These included phosphotidylcholine, phosphotidylethanolymine, phosphotidylserine, and phosphatidylinositol. Phosphotidylcholine (PC) gives rise to arachidonic acid via the action of PLA_2 and hence to eicosanoids. Diacylglycerol (DAG) is an activator of protein kinase C, which plays a role in promotion (*34*). Phosphotidylserine also activates protein kinase C. Phosphotidylinositol plays a major role in signal transduction (*40*), giving rise to DAG and subsequently to arachidonic acid and eicosanoids, as well as activating (via DAG) protein kinase C, resulting in cellular responses (*34*).

Control cells grown on medium with fetal calf serum without added polyunsaturated fatty acids showed a low content of ω-3 containing molecular species, as did cells grown on arachidonic acid. Cells grown in medium containing arachidonic acid showed a dramatic increase in ω-6-containing molecular species. Cells grown in medium containing EPA showed a dramatic shift to ω-3-containing molecular species (*33*).

Mechanisms of ω-3 Fatty Acids as Chemopreventive Agents

The ω-3 fatty acids EPA and DHA may act by modifying the conversion of arachidonate to prostaglandins. They may act as competitive inhibitors of cyclo-oxygenase or lipoxygenase. They may act as competitive substrates of these enzymes to yield products whose properties are different from those derived from arachidonate. Another mechanism that may be possible is the attenuation of the activation of phospholipases and protein kinases that play a role in transformation. The ω-3 fatty acids may act in cancer prevention by modifying the affinity of membrane receptors compared to membranes containing predominantly ω-6 fatty acids.

Conclusion

Epidemiological studies provide evidence that environmental factors (external agents such as chemicals, radiation, and viruses) play a major role in the causation of the majority of human cancers. The proliferation of normal cells is thought to be regulated by growth-promoting proto-oncogenes counterbalanced by growth-constraining tumor-suppressor genes. Genetic lesions that inactivate suppressor genes liberate the cell from constraints imposed by these genes, resulting in unconstrained growth of the cancer cell. DNA damage can be induced by a variety of environmental agents and can occur at high rates in conditions where genetic disease prevails.

The multistep process of carcinogenesis is mediated via complex signal pathways that can be modified by growth factors as well as hormones. This process could be initiated by a number of environmental agents that produce oxygen radicals, play a role in regulating cellular metabolism and converting arachidonic acid to eicosanoids and other products, and play a role in carcinogenesis.

The ideal method of cancer control is prevention or intervention. Both can be achieved by dietary factors which appear, in part, to act by inhibiting oxygen free radicals and free-radical intermediates as well as modifying signal transduction pathways in the cell. A number of vitamins that act as antioxidants can act as anticarcinogens, and these include vitamin E, selenium, vitamin C, and β-carotene. Other agents such as sphingolipids found in milk can act as cancer-preventive agents by modifying cellular pathways, for example, by inhibiting protein kinase C, which is the receptor for the phorbol ester tumor promoters. Other agents such as ω-3 fatty acids can also modify transformation by interfering with the production of prostaglandin synthesis as well as modifying molecular species in cellular phospholipids.

Normal growth or absence of growth occurs when cells produce a pattern within certain latitudes of phospholipids, autocrine growth factors, and responses to external growth factors. Transformation causes remodeling of this pattern so that the cells depend less on external growth factors, including inhibitors of growth, and they rely more on autocrine factors. The ω-3 fatty acids such as EPA and DHA cause remodeling of cellular phospholipids, so that they are substantially reduced in arachidonate-containing species (*33*), and they inhibit transformation. These fatty acids act as inhibitors at concentrations that are in the range of plasma concentrations that can be obtained readily. For example, human subjects given a single dose of 20 mL of EPA oil have plasma concentrations of 20 μM after 4 hr. Although environmental factors and carcinogens encountered in a variety of occupations may affect cancer rates, the message is optimistic because there are certain dietary factors that could modify cellular responses to these environmental agents at a cellular and molecular level and prevent the onset and the progression of neoplastic development.

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