

Mechanism-Based Cancer Prevention Approaches: Targets, Examples, and the Use of Transgenic Mice

Stephen D. Hursting, Thomas J. Slaga, Susan M. Fischer, John DiGiovanni, James M. Phang

Humans are exposed to a wide variety of carcinogenic insults, including endogenous and man-made chemicals, radiation, physical agents, and viruses. The ultimate goal of carcinogenesis research is to elucidate the processes involved in the induction of human cancer so that interventions may be developed to prevent the disease, either in the general population or in susceptible subpopulations. Progress to date in the carcinogenesis field, particularly regarding the mechanisms of chemically induced cancer, has revealed several points along the carcinogenesis pathway that may be amenable to mechanism-based prevention strategies. The purpose of this review is to examine the basic mechanisms and stages of chemical carcinogenesis, with an emphasis on ways in which preventive interventions can modify those processes. Possible ways of interfering with tumor initiation events include the following: i) modifying carcinogen activation by inhibiting enzymes responsible for that activation or by direct scavenging of DNA-reactive electrophiles and free radicals; ii) enhancing carcinogen detoxification processes by altering the activity of the detoxifying enzymes; and iii) modulating certain DNA repair processes. Possible ways of blocking the processes involved in the promotion and progression stages of carcinogenesis include the following: i) scavenging of reactive oxygen species; ii) altering the expression of genes involved in cell signaling, particularly those regulating cell proliferation, apoptosis, and differentiation; and iii) decreasing inflammation. In addition, the utility for mechanism-based cancer prevention research of new animal models that are based on the overexpression or inactivation of specific cancer-related genes is examined. [J Natl Cancer Inst 1999;91:215-25]

The major stages of carcinogenesis were deduced over the past 50 years, primarily from animal model studies (particularly in the mouse skin); these stages are termed initiation, promotion, and progression (1,2) and are shown in Fig. 1. Tumor initiation begins when DNA in a cell or population of cells is damaged by exposure to exogenous or endogenous carcinogens. If this damage is not repaired, it can lead to genetic mutations. The responsiveness of the mutated cells to their microenvironment can be altered and may give them a growth advantage relative to normal cells. In the classic two-stage carcinogenesis system in the mouse skin, a low dose of 7,12-dimethylbenz[*a*]anthracene (DMBA) causes permanent DNA damage—the initiating event—but does not give rise to tumors over the lifespan of the mouse unless a tumor promoter, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), is repeatedly applied (3). The tumor-promotion stage is characterized by selective clonal expansion

of the initiated cells, a result of the altered expression of genes whose products are associated with hyperproliferation, tissue remodeling, and inflammation (2). During tumor progression, preneoplastic cells develop into tumors through a process of clonal expansion that is facilitated by progressive genomic instability and altered gene expression (4).

The classic view of experimental carcinogenesis, in which tumor initiation is followed by tumor promotion and progression in a sequential fashion, remains conceptually important to experimental carcinogenesis research. However, while the processes involved in each stage of experimental carcinogenesis also appear to be involved in human carcinogenesis (5), the temporal nature of initiation, promotion, and progression events in the natural world is complex. For instance, we know from the work of Fearon and Vogelstein (6) and of Sugimura (7) that multiple mutational events are involved in the formation of a tumor. Humans are generally exposed to mixtures of agents that can simultaneously act at different stages of the carcinogenesis process, and it has become clear that promotional events—which frequently increase cellular proliferation or decrease apoptosis—can influence subsequent initiation events. It is also increasingly apparent that an individual's genetic background can dramatically affect his or her susceptibility to a carcinogen (8-10).

Thus, rather than occurring in three discrete stages in a predictable order, human carcinogenesis is best characterized as an accumulation of alterations in genes regulating cellular homeostasis, such as oncogenes, tumor suppressor genes, apoptosis-regulating genes, and DNA repair genes (11). Most animal models used in carcinogenesis research were developed before the identification of the major cancer-related genes, the recognition of the importance of host susceptibility to a carcinogenic insult, or the realization that mitogenesis and apoptosis together regulate cell number. Nonetheless, these animal models have contributed significantly to our current understanding of carcino-

Affiliations of authors: S. D. Hursting, Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, and Department of Carcinogenesis, The University of Texas M. D. Anderson Cancer Center, Science Park-Research Division, Smithville; S. M. Fischer, J. DiGiovanni, Department of Carcinogenesis, The University of Texas M. D. Anderson Cancer Center, Science Park-Research Division, Smithville; T. J. Slaga, Center for Cancer Causation and Prevention, The AMC Cancer Research Center, Denver, CO; J. M. Phang, Laboratory of Nutritional and Molecular Regulation, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD.

Correspondence to: Stephen D. Hursting, Ph.D., M.P.H., Department of Epidemiology, Box 189, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030 (e-mail: shursting@request.mdacc.tmc.edu).

See "Note" following "References."

Table 1. Examples of dietary factors or chemopreventive agents that target specific stages of carcinogenesis

Prevention strategy	Examples
Tumor initiation	
Inhibit carcinogen activation	Epigallocatechin gallate (EGCG), selenium, phenyl-isothiocyanate (PEITC), indole-3-carbinol, coumarins, ellagic acid, resveratrol, genistein
Scavenge electrophiles	Ellagic acid, EGCG
Enhance carcinogen detoxification	Oltipraz, diallyl sulfide, PEITC, EGCG, N-acetylcysteine, resveratrol
Enhance DNA repair	Calorie restriction, EGCG, selenium
Tumor promotion/progression	
Scavenge reactive oxygen species	Antioxidants (carotenoids, α -tocopherol, ascorbic acid, EGCG), selenium, calorie restriction
Alter gene expression	Retinoids (all- <i>trans</i> retinoic acid, fenretinide), calorie restriction, monoterpenes (i.e., <i>d</i> -limonene), dehydroepiandrosterone (DHEA), fluasterone, genistein
Decrease inflammation	Nonsteroidal anti-inflammatory drugs, calorie restriction, DHEA, fluasterone, antihistamines
Suppress proliferation	Calorie restriction, difluoromethylornithine, selenium, tamoxifen, DHEA, fluasterone, genistein, retinoids
Induce differentiation	Retinoids, calcium, sodium butyrate
Encourage apoptosis	DHEA, fluasterone, fenretinide, sodium butyrate

metabolism of xenobiotic compounds, as well as by ultraviolet radiation and gamma radiation—can also cause extensive DNA damage. For instance, proto-oncogenes and tumor suppressor genes are normal cellular genes that can be mutated to cause uncontrolled cell growth or other characteristics that increase the probability of neoplastic transformation (11–13).

Metabolic activation of procarcinogens (i.e., carcinogens requiring enzymatic conversion to DNA-reactive intermediates) is generally catalyzed by cytochrome P450 enzymes through oxidation. More than 100 distinct mammalian P450 enzymes have been identified (14). In addition, there are other enzyme systems involved in carcinogen activation such as peroxidases (including the cyclooxygenases, which will be discussed in more detail below) and certain transferases such as N-acetyltransferase and sulfotransferase (15,16). Each of these enzymes provides a potential target for modulating carcinogen activation.

One common feature of the metabolic activation of all procarcinogens is that their ultimate DNA-reactive carcinogenic species are electrophilic (17). In addition, many direct-acting carcinogens damage DNA through electrophilic intermediates (18). Thus, the electrophilicity of the ultimate carcinogenic species serves as a shared intervention target for most chemical carcinogens. The electrophilic metabolites may themselves be ROS and interact as such with DNA (19). Oxygen-free radicals may also be involved in a step required for activation of a procarcinogen, and thus the reactions involved in metabolic activation of carcinogens may release ROS that can in turn attack DNA (19). Thus, directly scavenging DNA-reactive intermediates with antioxidants or other agents that can scavenge electrophiles constitutes a plausible strategy for modulating this early stage of carcinogenesis.

Carcinogen Detoxification

In addition to the carcinogen-activating enzymes, a series of enzymes (the so-called phase II enzymes) detoxify activated carcinogens, thus preventing their binding to DNA. The induction of the glutathione S-transferases (GSTs) is an important response for the detoxification of xenobiotics (20). This class of enzymes couples a number of diverse substrates to glutathione to excrete them from the body. GSTs are segregated into three classes based on their sequence homology and specificity for substrates (21). Other detoxification enzymes include uridine diphosphate-glucuronosyl transferase, quinone reductase, and the epoxide hydrolases (22,23). The efficiency with which these and other enzymes detoxify carcinogens is a critical factor in determining the carcinogenicity of a particular xenobiotic.

DNA Repair Processes

The generation of DNA-reactive intermediates by most chemical carcinogens leads to the production of DNA adducts or other types of damage. As reviewed by Mitchell et al. (24), normal mammalian cells can efficiently remove DNA damage induced by carcinogens. Cells use different strategies to repair DNA damage, depending on the structure of the damage and its location in the genome. For example, small lesions (such as alkylated DNA bases) can be repaired by a mechanism termed base excision repair (25). This process involves removal of the damaged base followed by a “small cut-and-patch” repair involving removal of a few nucleotides. When methylation occurs at either the O^6 or O^4 positions of guanine or thymine, the modified bases can be repaired by the direct transfer of the methyl group to a methyl transferase (26). Bulky carcinogen-induced DNA adducts and ultraviolet light photodimers can be repaired through a “large cut-and-patch” mechanism involving a region of approximately 27–29 nucleotides that includes the damaged bases; this is termed nucleotide excision repair (27). The integrity of the genetic information is threatened not only by various environmental exposures but also by errors produced during normal DNA replication, for example, non-Watson-Crick base-pairing and slippage during DNA replication. To correct the errors resulting from such mis-replication, cells have also developed a mismatch repair mechanism (28).

NUTRITIONAL MODULATION AND CHEMOPREVENTION OF TUMOR INITIATION PROCESSES: EXAMPLES

Inhibiting Carcinogen Activation

Fruits, vegetables, herbs, and other foodstuffs (as well as inedible plants) contain numerous chemical constituents known to affect the metabolic activation of chemical carcinogens. Examples of food sources containing agents that decrease carcinogen activation are the cruciferous vegetables, such as cauliflower, broccoli, and cabbage. The crucifers are sources of isothiocyanates, which are known to interfere with the metabolism of nitrosamines. Studies by Chung et al. (29), Hecht (30), Stoner and Mukhtar (31), and Morse et al. (32,33) have shown conclusively that the metabolism and carcinogenicity of the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone are decreased by the administration of phenylisothiocyanate. Extensive structure-activity studies by these

Table 1. Overall mortality ratios among current smokers and former smokers, relative to never smokers, according to sex, duration of abstinence, and cigarette intake*

	Duration of abstinence, y						≥16
	0 (current smokers)	<1	1-2	3-5	6-10	11-15	
All smokers							
Males							
1-20 cigarettes/day	2.22	2.49	2.38	2.03	1.63	1.38	1.00
≥21 cigarettes/day	2.43	2.77	2.64	2.25	2.04	1.77	1.27
Females							
1-19 cigarettes/day	1.60	1.58	1.96	1.41	1.14	1.10	1.01
≥20 cigarettes/day	2.10	3.39	2.58	2.03	1.60	1.38	1.15
Smokers with no current illness†							
Males							
1-20 cigarettes/day	2.34	2.06	2.05	1.89	1.48	1.29	1.01
≥21 cigarettes/day	2.73	1.85	2.15	1.90	1.77	1.65	1.19
Females							
1-19 cigarettes/day	1.82	1.76	1.26	1.42	1.01	1.09	1.00
≥20 cigarettes/day	2.46	3.33	2.15	1.44	1.46	1.18	0.99

*Data from American Cancer Society's Cancer Prevention Study II, appearing in: USDHHS. Reducing The Health Consequences of Smoking: 25 Years of Progress: a Report of the Surgeon General. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Centers for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 1989.

†Former smokers with heart disease, cancer, stroke, or other serious illness at the time of enrollment in the study were excluded.

Table 2. Smoking and lung cancer: Causative agents*

Carcinogens	Modifying agents
Strong evidence†	
NNK‡	Co-carcinogens (catechols)
PAH§ (benzo[a]pyrene, benzo[b,j, and k]fluoranthenes, 5-methylchrysene, dibenz[a,h]anthracene, and Indeno[1,2,3-cd]pyrene)	Tumor promoters (phenols and others)
	Toxic aldehydes (acrolein)
	Diet
Weak evidence	
Oxidative damage and free radicals	
²¹⁰ Po, Cr, Cd, and Ni	
Aldehydes	

*Modified from: Hoffmann D, Hecht SS. Advances in tobacco carcinogenesis. In: Cooper CS, Grover PL, editors. Handbook of Experimental Pharmacology, Heidelberg: Springer-Verlag, 1990:63-102.

†Criteria: animal carcinogenicity, presence in smoke, and biochemical studies in animal and human lungs.

‡Nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

§Polynuclear aromatic hydrocarbons.

their role as major factors in the carcinogenic process has been established (30,31). These same DNA adducts are found in the lungs of smokers (30,31), and the role of specific adducts in causing permanent mutations has been elucidated (32). The

bulky adducts resulting from the metabolic activation of BaP and NNK cause G to T mutations, while the methyl adducts formed from NNK produce G to A mutations. These mutations have been detected in K-ras oncogenes and p53 tumor suppressor genes isolated from lung tumors in smokers, and a dose-response relationship has been noted between G to T mutations in p53 and cigarette smoke exposure (33,34). As shown in Fig. 1, dependence on nicotine is a prerequisite to the multistage process of lung carcinogenesis, in which these mutations play a critical role (35). Moreover, in addition to their role in lung cancer, the nitrosamines, are also considered major causative factors for cancers of the esophagus and pancreas. Both PAH and nitrosamines have been causally related to oral cancer, and aromatic amines have been associated with bladder cancer in smokers (26,36).

Virtually all known carcinogens in tobacco products require metabolic activation for binding to DNA. There are competing detoxification reactions. The balance between metabolic activation and detoxification in an individual will, in part, determine that person's risk for cancer upon carcinogen exposure. This balance is in large measure determined by individual levels and activities of carcinogen metabolizing enzymes, such as cytochromes P450, glutathione S-transferases, N-acetyltransferases, and uridine diphosphoglucuronosyl transferases (37).

Assessment of specific carcinogen metabolites in urine of

Fig. 1. Scheme linking nicotine addiction to lung cancer through the major pulmonary carcinogens of tobacco smoke—polynuclear aromatic hydrocarbons (PAH) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

