Perspectives in Cancer Chemoprevention

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Cancer chemoprevention can be defined as prevention of cancer by the administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet. Based largely on the time period that chemopreventive agents exhibit activity in animal models of carcinogenesis, they can be classified as inhibitors of carcinogen formation, blocking agents, and suppressing agents. The majority of compounds that inhibit the formation of carcinogens prevent the formation of nitrosamines from secondary amines and nitrite in an acidic environment. Blocking agents are inhibitors of tumor initiation, while suppressing agents are inhibitors of tumor promotion/progression. Many well-characterized chemopreventive agents act at one or more steps in both tumor initiation and promotion/progression. The objective of this paper is to provide a general discussion of the mechanisms through which chemopreventive agents inhibit carcinogenesis. Examples of agents that act through these mechanisms are given; however, a complete listing of effective chemopreventive agents is not possible within the context of this paper. At the conclusion is a brief discussion of future prospects in cancer chemoprevention and obstacles to overcome. — *Environ Health Perspect* 105(Suppl 4):945–954 (1997)

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Introduction

Cancer chemoprevention can be defined as the prevention, inhibition, or reversal of carcinogenesis by administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet. Knowledge of chemoprevention science and its application in clinical studies has been growing rapidly over the past decade, as has been documented in reviews of the field prepared by us and by others (*1–8*). This paper serves as an update and brief commentary on the status and various aspects of chemoprevention.

Epidemiological studies indicate that approximately 80% of human cancer is caused by exposure to chemical carcinogens in tobacco smoke, in the diet, and in the workplace (*9,10*). Given these observations, at least three approaches to the prevention of cancer can be envisioned. First, reduce human exposure to environmental carcinogens through careful monitoring of the workplace and through educational approaches to encourage changes in lifestyle. Second, identify individuals at high risk for cancer development through predisposing genetic or biochemical factors, followed by appropriate clinical follow-up. Third, provide chemoprevention by dietary or synthetic means. For several reasons, chemoprevention has received growing consideration as a means of cancer control. In certain organ sites such as the lung, pancreas, stomach, ovary, and esophagus, the development of cancer leads to exceptionally low 5-year survival rates. Clearly, the considerable advances that have occurred in earlier detection and treatment of cancer have done little to improve the prognosis for patients diagnosed with cancer at certain organ sites. Primary cancer prevention requires removal of exposure to etiologic agents. Although this is an important approach to cancer prevention, it is not always effective, as evidenced by the marginal success of tobacco cessation programs. Moreover, numerous populations at high risk for certain types of cancer may already have received considerable exposure to etiologic agents, and many human cancers cannot be ascribed to specific agents. Thus, preventive strategies that do not require prior knowledge of specific etiological factors have great appeal. Additionally, the success obtained in chemoprevention of cancer in animal models provides a strong mandate for this approach to cancer prevention in humans.

Target Populations

The projected target populations for cancer chemoprevention consist of high-risk groups, such as the following: individuals with high exposure to carcinogens (e.g., tobacco smokers and populations that consume foodstuffs contaminated with fungal toxins and nitrosamines); those who are known to be genetically predisposed to the development of cancer (e.g., patients with familial colonic polyposis); individuals with premalignant lesions (e.g., oral leukoplakia, Barrett's esophagus, dysplastic nevi, etc.); individuals with occupational exposure to known carcinogens; and survivors of primary cancers with a high degree of recurrence or a marked tendency toward formation of second primary tumors. Some controversy remains as to whether or not chemopreventative strategies (other than certain dietary measures) will or should be used in the general population.

Classes of Chemopreventive Agents

Absolute classification of chemopreventive agents is difficult because the precise mechanisms of action are not known for many compounds. In addition, many chemopreventive agents act through more than one mechanism, making it difficult, if not impossible, to establish the most effective mode of action. The classification scheme developed by Wattenberg (*11*) is based essentially on the time period during which agents appear to exhibit activity in animal models of carcinogenesis. On this basis,

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Abbreviations used: AAF, 2-acetylaminofluorene; ADPRT, poly(ADP-ribosyl)transferase; BPDE, benzo[*a*]pyrene diol epoxide; BHA, butylated hydroxyanisole; DFMO, α-difluoromethylornothine; DMBA, 7,12-diemthylbenz[*a*]anthrancene; FDA, U.S. Food and Drug Administration; GST, glutathione *S*-transferase; I3C, indole-3-carbinol; IGF-I, insulinlike growth factor I; MNU, methylnitrosourea; NAC, *N*-acetylcysteine; NCI, National Cancer Institute; NMBA, *N*-nitrosomethylbenzylamine; NK, natural killer; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NSAID, nonsteroidal anti-inflammatory drug; ODC, ornithine decarboxylase; PKC, protein kinase C; TGF-β, transforming growth factor β; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; UDC, urine diphosphate.

Table 1. Inhibitors of carcinogen formation.

Chemical class	Inhibitor
Reductive acids	Ascorbic acid
Phenols	Caffeic acid, ferulic acid, gallic acid
Sulfhydryl compounds	*N*-Acetylcysteine
Amino acids	Proline, thioproline

Table 2. Categories of blocking agents.[a]

Mechanism	Examples
Inhibition of cytochrome P450	Dithiocarbamates, ellagic acid, diallyl sulfide, isothiocyanates
Induction of cytochrome P450	Indole-3-carbinol, β-naphthoflavone
Induction of phase II enzymes	
Glutathione *S*-transferase	Allyl sulfides, dithiolethiones, isothiocyanates
UDP-glucuronyltransferase	Polyphenols
Glutathione peroxidase	Selenium
Scavenge electrophiles	Ellagic acid, *N*-acetylcysteine
Scavenge free radicals	Sodium thiosulfate, polyphenols, vitamin E
Increase overall levels of DNA repair	Vanillin
Increase poly(ADP-ribosyl)transferase	*N*-Acetylcysteine
Suppress error-prone DNA repair	Protease inhibitors

[a]After Morse and Stoner (*1*) and Kelloff et al. (*70*).

chemopreventive agents are classified as inhibitors of carcinogen formation, blocking agents, and suppressing agents. Blocking agents are inhibitors of tumor initiation, while suppressing agents are inhibitors of tumor promotion/progression. Examples of the three major classes of chemopreventive agents are given below.

Inhibitors of Carcinogen Formation

Chemopreventive agents that inhibit the formation of carcinogens act predominantly to prevent the formation of nitrosamines from secondary amines and nitrite in an acidic environment. A list of these agents is given in Table 1. When present in appreciable amounts, ascorbic acid decreases nitrosamine production from secondary amines and nitrite in the stomach (*12*), thus leading to a diminished lung tumor response in mice (*13*). Other compounds that inhibit nitrosamine formation include phenols such as ferulic, gallic, and caffeic acids (*14*), as well as several sulfhydryl compounds (*15*). Proline and thioproline scavenge nitrite by reacting with it to form nonmutagenic nitrosamines (*16*). Compounds of this class may have utility when incorporated into the diet of populations with suspected high rates of endogenous formation of nitrosamines.

Blocking Agents

There are several means of chemical intervention at the initiation stage of carcinogenesis. It is well known that most environmental procarcinogens must first be metabolically activated to electrophilic forms that damage DNA while to some extent avoiding pathways of metabolic detoxification. The electrophilic species reacts with DNA, forming adducts that result in base mispairing and mutation. On this basis, most blocking agents can be assigned to one or more of five major categories (Table 2): inhibitors of cytochrome P450 enzymes; inducers of cytochrome P450 enzymes; inducers of phase II enzymes such as glutathione *S*-transferase (GST), urine diphosphate (UDP)-glucuronyltransferase, and glutathione peroxidase; scavengers of electrophiles and free radicals; and inducers of DNA repair.

Inhibitors of Cytochrome P450 Enzymes. One of the first cytochrome P450 inhibitors shown to exhibit chemopreventive activity was disulfiram, which inhibits the activation of dimethylhydrazine (*17*) and colon cancer induced by this compound. The isothiocyanates are strong P450 inhibitors and among the most potent chemopreventive agents known (*18–28*). For example, dietary phenethyl isothiocyanate, at a concentration of 3 mmol/kg diet, can inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumors in F344 rats by approximately 50% (*20*). This concentration completely inhibits *N*-nitrosomethylbenzylamine (NMBA)-induced esophageal tumors in F344 rats (*24*). 6-Phenylhexyl isothiocyanate inhibits NNK-induced lung tumorigenicity by > 80% in strain A mice when administered at a dose of 50-fold lower than NNK (*23*). Unfortunately, 6-phenylhexyl isothiocyanate appears to promote azoxymethane-induced colon tumors and NMBA-induced esophageal tumors in F344 rats (*28*). These results illustrate the importance of utilizing more than one animal model system in evaluating the efficacy of chemopreventive agents. Diallyl sulfide, a naturally occurring constituent of *Allium* vegetables, inhibits carcinogen activation and tumorigenesis in several animal models (*29–32*). Ellagic acid inhibits benzo[*a*]pyrene metabolism *in vitro* (*33*), NMBA metabolism *in vivo* and *in vitro* (*34,35*), and inhibits NMBA-induced esophageal tumors (*36,37*).

Inducers of Cytochrome P450 Enzymes. Another mechanism of action of blocking agents is through induction of cytochrome P450. Inducers of cytochrome P450 act either by increasing the metabolic activation of carcinogens in nontarget tissues or by enhancing oxidative detoxification at any tissue site. Indole-3-carbinol (I3C) is a potent inducer of P450 enzymes and has chemopreventive activity in several animal models (*38–44*). Compounds that induce P450 enzymes, however, may promote cancer at other organ sites through enhanced carcinogen activation at these sites; this may, at least in part, account for the known cocarcinogenic or promotional activity of I3C (*45–47*).

Inducers Phase II Enzymes. Inducers of phase II detoxifying enzymes are preferred to cytochrome P450 inducers as chemopreventive agents because they are less likely to produce cancers themselves. Sulforaphane, an isothiocyanate found in broccoli (*48*), is a potent inducer of GST and inhibits chemically induced mammary cancer in rats (*49*). Another potent inducer of GST is the dithiolethione, oltipraz, which inhibits carcinogen-induced tumorigenesis in a number of animal models (*50–56*). Butylated hydroxyanisole (BHA) stimulates UDP-glucuronyltransferase activity, and this appears to be the mechanism by which BHA inhibits benzo[*a*]pyrene tumorigenesis in the mouse forestomach (*57,58*).

Scavengers of Electrophiles and Free Radicals. Scavenging or trapping agents are compounds that physically react with the activated (electrophilic) forms of carcinogens and oxygen free radicals. Ellagic acid reacts directly with the diolepoxide of benzo[*a*]pyrene (BPDE) to form both *cis*- and *trans*- adducts (*59*); such activity may account for its inhibition of BPDE-induced mutagenicity and carcinogenicity (*60,61*). The sulfhydryl moiety of *N*-acetylcysteine (NAC) can accept electrophilic species, which may account for its antimutagenic and anticarcinogenic effects (*62–64*).

Oxygen free radicals are produced by the metabolism of several carcinogens and by inflammatory cells (*65*). Numerous

chemopreventive agents exhibit antioxidant activity through their ability to scavenge oxygen radicals, including, for example, singlet oxygen, peroxy radicals, superoxide anion, and hydroxyl radicals. For example, NAC and other chemopreventive thiols are known to react with hydroxyl radicals (*66*). The reaction of β-carotene with singlet oxygen and its participation in other free radical-trapping reactions is well documented (*67,68*). Phenolic antioxidants are known to scavenge peroxy radicals; in particular, vitamin E is known to scavenge peroxy radicals, singlet oxygen, and superoxide radicals (*69*). Other phenols such as ellagic acid, curcumin, caffeic acid phenyl ester, and the tea polyphenols are particularly active oxygen radical scavengers, due likely to the presence of hydroxyl groups on adjacent carbons in these compounds. Nonphenolic antioxidants also scavenge oxygen free radicals. For example, glutathione reacts with alkyl–peroxy radicals (*69*). A disadvantage of scavenging agents is that they must be present at sufficient concentrations in target tissues at all times during which carcinogens or free radicals are present.

Inducers of DNA Repair. There are three possible chemopreventive mechanisms that involve DNA repair (*70,71*). The first is an increase in the overall level of DNA repair. An example of a naturally occurring chemical that increases the level of DNA repair is vanillin, which inhibits mammalian cell mutagenicity (*72*). The mechanisms through which vanillin promotes DNA repair have not been determined. Second, the enzyme poly(ADP-ribosyl)transferase (ADPRT) is involved in modulation of DNA damage (*73,74*), and the level of this enzyme is reduced by chemical carcinogens (*75*). *N*-Acetylcysteine prevents the decrease in ADPRT caused by the carcinogen 2-acetylaminofluorene (AAF) (*75*). The third mechanism is suppression of error-prone DNA repair. Protease inhibitors depress error-prone repair in bacteria (*76*), and it has been suggested that they could prevent carcinogenesis by inhibiting an error-prone repair system activated by proteases that, in turn, are induced by tumor promoters (*77*).

Many would argue that the use of blocking agents is not a feasible approach to chemoprevention in humans, since all members of high risk groups have presumably received some exposure to initiating agents. The work of Vogelstein et al. (*78*) and Fearon and Vogelstein (*79*) on colorectal cancer, however, indicates that human cancer is not adequately represented by the traditional initiation/promotion model, but more likely involves an accumulation of mutational events in key genes such as the oncogenes and tumor suppressor genes. If that is so, then administration of blocking agents should prove of some value, since many individuals at high risk (e.g., smokers and the occupationally exposed) are continually exposed to genotoxic carcinogens. Moreover, it could also be important to inhibit further mutational events in individuals who have a reduced exposure to carcinogens but remain at higher risk for cancer development (e.g., former tobacco smokers). Individuals who are genetically predisposed to cancer must avoid further mutational events that could trigger the carcinogenesis process; such individuals are excellent candidates for prophylactic treatment with blocking agents. Also, the administration of inhibitors of promotion/progression will be helpful in combating the effects of exposure to a wide range of carcinogens, no matter what model human carcinogenesis follows. Co-administration of blocking and suppressing agents is a promising strategy for optimizing efficacy.

Table 3. Categories of suppressing agents.[a]

Mechanism	Examples
Inhibit polyamine metabolism	DFMO, polyphenols, substituted putrescines
Induce terminal cell differentiation	Calcium, retinoids, vitamin D_3
Modulate signal transduction	Glycyrrhetinic acid, NSAIDs, polyphenols, retinoids
Modulate hormonal/growth factor activity	NSAIDs, retinoids, tamoxifen
Inhibit oncogene activity	Genistein, NSAIDs, monoterpenes
Promote intracellular communication	Carotenoids, polyphenols, retinoids
Restore immune response	NSAIDs, selenium, vitamin E
Induce apoptosis	Butyric acid, genistein, selenium, sulindac sulfone, retinoids
Correct DNA methylation imbalances	Folic acid, choline, methionine
Inhibit basement membrane degradation	Protease inhibitors
Inhibit arachidonic acid metabolism	Glycyrrhetinic acid, *N*-acetylcysteine, NSAIDs, polyphenols

[a]After Kelloff et al. (*70*).

Suppressing Agents

Classification of suppressing agents is more difficult because the critical events and their exact sequence in the processes of tumor promotion and progression are not well understood. However, as described by Morse and Stoner (*1*), De Flora and Ramel (*80*), and Kelloff et al. (*70,71*), many current suppressing agents can be classified as compounds that inhibit polyamine metabolism; induce terminal cell differentiation; modulate signal transduction; modulate hormonal/growth factor activity; inhibit oncogene activity; promote intercellular communication; restore immune response; induce apoptosis; correct DNA methylation imbalances; inhibit basement membrane degradation; and inhibit arachidonic acid metabolism (Table 3).

Inhibiters of Polyamine Metabolism. The polyamine content of cells is correlated to their proliferative, and often their neoplastic, capabilities (*81*). A key enzyme in the polyamine biosynthetic pathway, ornithine decarboxylase (ODC), catalyzes the conversion of ornithine to putrescine (*82*). The levels of ODC and polyamines are frequently elevated in tumor tissues relative to their normal counterparts. In addition, phorbol ester tumor promoters such as 12-tetradecanoylphorbol-13-acetate (TPA) cause increased ODC activity and accumulation of polyamines in affected tissues (*83*). Inhibitors of polyamine metabolism include the suicide inhibitor of ODC, α-difluoromethylornithine (DFMO) (*84*). DFMO inhibits tumorigenesis induced by a number of different carcinogens (*85–92*). Other chemopreventive agents such as the tea polyphenols, ellagic acid, and curcumin, inhibit ODC activity; presumably, this is one mechanism through which these compounds inhibit TPA-induced tumor promotion in mouse skin. Due to the rapid turnover of ODC (*81*), constant levels of a given ODC inhibitor must be maintained at the target organ to achieve the desired antiproliferative activity.

Inducers of Terminal Cell Differentiation. Terminal differentiation is one of the steps in the normal, regulated cell proliferation in epithelial tissues. Cancer cells often have lost the ability to differentiate (*93*). Abundant evidence indicates that restoring the ability of abnormally proliferating cells to differentiate suppresses carcinogenesis. Vitamin A and the retinoids are the most extensively

studied differentiation agents. It has been known for many years that vitamin A deficiency causes squamous metaplasia and keratinization; both are signs of uncontrolled proliferation (*94*). Studies in hamster trachea (*95–97*) show that treatment of squamous keratinizing epithelium with vitamin A restores normal mucociliary differentiation. Retinoids appear to control differentiation via intracellular binding proteins and nuclear receptors (*98–100*).

Calcium and vitamin D_3 are differentiating agents that also inhibit carcinogenesis in animal models. Calcium induces differentiation in a number of epithelial tissues, including mouse skin (*101*), rat esophagus (*102*), human colon (*103*), and human mammary gland (*104,105*). Vitamin D_3 induces differentiation in a variety of human and animal tissues (*106–109*). The effects of calcium and vitamin D_3 may be mediated by the same signal transduction pathway, involving the vitamin D_3 nuclear receptor with calcium as the messenger (*93*).

Modulators of Signal Transduction. The components of signal transduction pathways provide multiple sites for chemopreventive activity by restoring normal cellular growth control. In fact, many of the antipromotion/antiprogression activities important to chemoprevention impact one or more components of signal transduction pathways. For example, one of the steps in signal transduction involves activation of protein kinase C (PKC) by diacyl glycerol. Several chemopreventive agents, such as the flavonoids and glycyrrhetinic acid, have inhibited PKC activity leading to suppression of carcinogenesis (*70,71*).

Further, invocation of the signal transduction pathways provides a mechanistic rationale for the multiple chemopreventive effects of some agents. For example, agents such as the retinoids and PKC inhibitors, which affect activities at the cell membrane, cytoplasmic, and nuclear membrane levels, can also affect other connected events such as growth factor expression and polyamine metabolism (*70,71*).

Modulators of Hormonal/Growth Factor Activity. Chemopreventive agents may inhibit neoplastic cell proliferation by directly regulating the induction and activity of specific hormones and growth factors that initiate steps in signal transduction. This regulation may occur at membrane level receptors (for growth factors and peptide hormones) or through cytoplasmic and nuclear receptors (for the steroid family of receptors). For example, transforming growth factor-β (TGF-β) has antiproliferative activity in both normal and neoplastic cells *in vitro* and *in vivo* (*110–113*). Neoplastic cells such as A549 human lung carcinoma cells produce TGF-β, but in a latent form that cannot bind to its membrane receptor; these cells are responsive to the antiproliferative effects of activated TGF-β (*110*). Antiestrogens such as tamoxifen bind to nuclear estrogen receptors, preventing the binding and activity of estrogens (*114*). There is also evidence of crossregulation among membrane and nuclear receptors. For example, insulinlike growth factor I (IGF-I) stimulates cell replication in various tumors (*115,116*). Human breast cancer cells have membrane receptors for and excrete IGF-I (*115*). Tamoxifen lowers blood concentrations of IGF-I in breast cancer patients, which may in part be responsible for its antitumor activity (*115*).

Inhibitors of Oncogene Activity. Most studies on the ability of chemopreventives to inhibit oncogene activity have concerned the *ras* gene. To be activated, the *ras* gene protein must first be farnesylated. *ras* Oncogenes are involved in mammary gland carcinogenesis induced by methylnitrosourea (MNU) and, to a lesser extent, by 7,12-dimethylbenz[*a*]anthracene (DMBA). Gould and colleagues (*117,118*) showed that D-limonene, found in citrus oils, inhibits the progression of mammary tumors induced in rats by either MNU or DMBA. They also showed that D-limonene inhibits farnesylation of small G proteins; these data suggest that D-limonene prevents oncogene activation by inhibiting posttranslational farnesylation of the *ras* p21 protein (*119*).

Investigations *in vitro* indicate inhibition of oncogene expression as a mechanism for inhibitory activity of protease inhibitors and retinoids. For example, the protease inhibitors 6-aminocaproic acid, leupeptin, and antipain inhibit transformation of NIH-3T3 cells transfected with activated H-*ras* oncogenes (*120*). Retinoic acid also inhibits H-*ras*-induced transformation in NIH-3T3 cells (*120*).

Promoters of Intercellular Communication. Communication between cells is mediated through gap junctions. Gap junctions are pores or channels in the cell membrane, which join channels of adjacent cells; when open, these channels allow passage of molecules up to approximately 1000 d in size (*121,122*). Lowenstein (*123*) and Mehta et al. (*124,125*) have proposed that gap junctions allow growth regulatory signals to move between cells. Numerous studies have shown that inhibition of gap-junctional communication between cells occurs during carcinogenesis. Several carotenoids such as β-carotene and canthaxanthine, and retinoids such as [E]-4(2,5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-l-propenyl)-benzoic acid and vitamin A, have been shown to enhance gap junctional communication in chemically treated C3H10T1/2 cells *in vitro* (*126*). This enhancement of communication correlated with inhibition of transformation of these cells and was mediated by upregulation of connexin proteins involved in gap-junction formation (*126*).

Restorers of Immune Response. Chemopreventive agents influence the immune response through a number of mechanisms. For example, retinoic acid increases cell mediated and natural killer (NK) cell cytotoxicity; retinoids also cause leukemic promyelocytes to differentiate to mature granulocytes comparable to mature neutrophils (*127*). These effects might be partially responsible for the activity of retinoids against established tumors (*128*). Both thymocytes and NK cells from selenium-deficient mice have a decreased ability to destroy tumor cells *in vitro* (*129*). Supplementation with 0.5 or 2 ppm selenium enhances the ability of rat NK cells to kill tumor cells.

Vitamin E also produces stimulatory effects on the immune system. Pharmacological doses of vitamin E fed with normal animal diets increases humoral antibody production, especially IgG (*130*). Vitamin E also stimulates cell mediated immunity (*131*) and prevents the carcinogen-induced decrease in the density of macrophage-equivalent cells (Langerhans cells) in the oral cavity of DMBA-treated hamsters (*132*).

Inducers of Apoptosis. Apoptosis (programmed cell death) is a well-regulated function of the normal cell cycle (*133,134*). Tumor suppressors, such as wild-type *p53* (*135,136*), and growth factors, such as TGF-β (*137*), have been implicated as inducers of apoptosis. Apoptosis is inhibited by tumor promoters such as TPA (*136,137*) and other chemicals that stimulate cell proliferation, such as hormones (*134,138,139*). These results suggest that induction of apoptosis may inhibit tumor formation. Although there have not been a large number of reports as yet, certain chemopreventive agents have been demonstrated to induce cellular apoptosis. For example, tamoxifen induces programmed cell death in human

mammary cancer MCF-7 cells (*134*). Apoptosis in colonic tissues is induced by sulindac sulfone, a metabolite of the nonsteroidal anti-inflammatory drug (NSAID) sulindac (*140*). This may be a major mechanism by which sulindac inhibits development of polyps in the human colon (*141,142*).

Correctors of DNA Methylation Imbalances. A number of studies have shown that methyl-deficient diets increase cell turnover and promote the development of carcinogen-induced liver tumors in rats and mice (*143–146*). In contrast, methyl-rich (fortified with choline and methionine) diets prevent or reduce these effects in the liver (*147–149*). Changes in the expression levels of protooncogenes and decreased expression of growth factors and growth factor receptors occur in animals on methyl-deficient diets (*143,150,151*). The increased protooncogene expression correlates with hypomethylation of the protooncogenes (*143,150*). Collectively, these data suggest that hypomethylation of DNA results in changes in the expression of genes involved in cellular growth control (*143,148*). Certain compounds that serve as methyl group donors inhibit tumorigenesis. Methionine, which is involved with choline, folic acid, and vitamin B_{12} in regulating intracellular methyl metabolism, inhibits chemically induced mammary cancer in rats; choline inhibits chemically induced liver tumors in rats (*143,145*).

Inhibitors of Basement Membrane Degradation. Cancer cells produce various enzymes that digest the basement membrane and allow the cells to invade through normal tissues. These enzymes include the proteases collagenase, hyaluronidase, cathepsin B, elastase, and plasminogen activators (*120,152*). Protease inhibitors inhibit the activities of type IV collagenase and thrombin, which are among the proteases that participate in the destruction of the basement membrane during tumor invasion (*120*). Thus protease inhibitors may exert their protective effects in part by inhibiting the degradation of the basement membrane.

Inhibitors of Arachidonic Acid Metabolism. Among the multiple events that occur during experimentally induced tumor promotion is an increased metabolism of arachidonic acid, which contributes to an overall inflammatory response (*81*). The cyclooxygenase pathway converts arachidonic acid to prostaglandins, prostacyclins, and thromboxanes, while lipoxygenase converts arachidonic acid to leukotrienes and hydroxyeicosatetraenoic acids (*153*). Activated oxygen species and alkylperoxy species are formed throughout this process. Relative to these events, the cyclooxygenase inhibitors such as NSAIDs (e.g., aspirin, indomethacin, ibuprofen, piroxicam) and certain antioxidants (e.g., flavonoids) are effective inhibitors of carcinogenesis (*153–157*). Compounds that inhibit lipoxygenase, such as vitamin E, inhibit tumor promotion in mouse skin. Likewise, lipoxygenase inhibitors that are stable one-electron donors—which competitively inhibit the production of unstable free radicals and electrophiles by prostaglandin H synthase (e.g., curcumin, the tea polyphenols, the flavonoids)—also inhibit tumor promotion in mouse skin (*158–160*). Since the products of arachidonic acid metabolism could contribute to both the initiation and promotion/progression stages of carcinogenesis, inhibitors of arachidonic acid metabolism may act as either blocking agents or suppressing agents.

Kelloff et al. (*70,71*) have discussed other mechanisms by which suppressing agents might inhibit molecular and cellular events associated with the promotion/progression stages of carcinogenesis; e.g., restoration of tumor suppressor function, inhibition of angiogenesis, and activation of antimetastasis genes. Although these are logical targets for chemoprevention, at present there is little evidence to suggest that known chemopreventive agents act through these mechanisms.

Future Prospects and Obstacles to Overcome

The large body of information on carcinogenesis and chemopreventive mechanisms that has been summarized in this report has been developed, for the most part, in the past 15 to 20 years. This information provides a strong base for future mechanistic studies in chemoprevention as well as for the design and development of clinical investigations. Indeed, a number of phase I, II, and III clinical trials of chemopreventive agents are underway and some success has already been achieved. For example, Hong et al. (*161*) showed that isotretinoin inhibited the development of second primary tumors in patients treated for primary cancers of the head and neck. Garewal et al. (*162*) showed regression of oral leukoplakia in individuals treated with β-carotene. Several studies have demonstrated the ability of the nonsteroidal anti-inflammatory agent, sulindac, to cause regression of colonic polyps (*141,142*). However, the results of some clinical trials have not been as promising, and future success in clinical trials is needed to further establish chemoprevention as a plausible approach to the prevention of human cancer. In this respect, the progressive increase in research activity on the basic mechanisms of action of chemopreventive agents during the past few years is gratifying and is likely to result in an even stronger database from which to design clinical trials in the future.

In a previous report (*1*), we discussed in considerable detail some of the obstacles to be overcome in the field of cancer chemoprevention. Among these is the relative lack of participation of the pharmaceutical industry. A major concern of the pharmaceutical industry is the length of time and the cost to conduct phase III clinical trials of efficacy of chemopreventive agents. To some degree, this problem could be overcome by U.S. Food and Drug Administration (FDA) approval of the use of chemopreventives in populations at high risk to cancer based on the successful modulation of surrogate end point biomarkers in phase II trials (*3,4,6,7*). To this end, the National Cancer Institute (NCI) and the FDA have produced consensus guidance on the development of chemopreventive agents that emphasizes the evaluation and validation of such surrogate end points (*163*).

Another obstacle is that of subject compliance and recruitment. Subject compliance with the chronic dosing regimens of chemopreventative clinical trials could be a considerable problem. Also, early withdrawal of subjects from multiyear protocols conducted at a single site can be a frequent occurrence in a highly mobile society. Finally, recruitment of a sufficient number of subjects for large-scale clinical trials can be difficult if the subjects are not highly motivated.

Another obstacle to chemoprevention is funding for basic research and for clinical trials. As we described previously, the NCI Chemoprevention Branch has a comprehensive, science-based chemopreventive agent drug development program ranging from drug discovery through phase III clinical trials (*2–7,163*). Similarly, other components of the NCI Cancer Prevention Research Program fund large chemoprevention clinical trials. However, such efforts represent only a fraction of those required to make rapid progress in chemoprevention. In 1990, in the United States alone, total costs associated with neoplastic diseases have been estimated at > $100 billion (*164*). The

costs of treating cancer increase annually at a rate greater than inflation. A reduction in cancer incidence of only 10% would result in substantial savings. The impressive advances made with chemopreventive agents in experimental models and the encouraging results of some of the clinical trials clearly warrant increased research in chemoprevention. Those engaged in research in chemoprevention must become more involved in funding decisions that affect the field.

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