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 progression. Many well-characterized chemopreventive agents act at one or more steps in both tumor initiation and 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 colon cancer 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 chemopreventive 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 Wartenberg (11) is based essentially on the time period during which agents appear to exhibit activity in animal models of carcinogenesis. On this basis,
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)-glucuronosyltransferase, 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 dimethyldihydrazine (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-Phenylethyl isothiocyanate inhibits NMBA-induced lung tumorigenicity by >80% in strain A mice when administered at a dose of 50-fold lower than NNK (23). Unfortunately, 6-phenylethyl 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.

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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, dithiolethenes, isothiocyanates |
| UDP-glucuronosyltransferase                    | Polyphenols                                   |
| Glutathione peroxidase                         | Selenium                                      |
| Scavenger electrophiles                        | Ellagic acid, N-acetylcysteine                |
| Scavenger 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                           |

aAfter Morse and Stoner (7) and Kelloff et al. (70).

Inducers Phase II Enzymes. Inducers of phase II detoxifying enzymes are preferred to cytochrome P450 inducers 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 diihiolethione, oltipraz, which inhibits carcinogen-induced tumorigenesis in a number of animal models (50-56). Butylated hydroxyanisole (BHA) stimulates UDP-glucuronosyltransferase activity, and this appears to be the mechanism by which BHA inhibits benzo[α]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 diolepoxy of benzo[α]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, peroxyl 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-ribose)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.

### Suppressing Agents

Table 3. Categories of suppressing agents.

| Mechanism | Examples |
|-----------|----------|
| Inhibit polyamine metabolism | DFMO, polyphenols, substituted putrescines |
| Induce terminal cell differentiation | Calcium, retinoids, vitamin D3 |
| Modulate signal transduction | Glycyrrhetinic acid, NSAIDs, polyphenols, retinoids |
| Modulate hormonal/growth factor activity | NSAIDs, retinoids, tamoxifen |
| Induce 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 |

*After Kelloff et al. (70).*

**Inhibitors 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, phosphatidylcholine 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 D3 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 D3 induces differentiation in a variety of human and animal tissues (106–109). The effects of calcium and vitamin D3 may be mediated by the same signal transduction pathway, involving the vitamin D3 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/antiproliferation 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-1 (115). Tamoxifen lowers blood concentrations of IGF-1 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 methyl nitrosourea (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-aminoacaproic 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-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-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 normal breast epithelial cells.
MECHANISMS OF CANCER CHEMOPREVENTION

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 B₁₂ 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 lipoxigenase converts arachidonic acid to leukotrienes and hydroxyicosatetraenoic 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 lipoxigenase, such as vitamin E, inhibit tumor promotion in mouse skin. Likewise, lipoxigenase 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 chemopreventive 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.

REFERENCES

1. Morse MA, Stoner GD. Cancer chemoprevention: principles and prospects. Carcinogenesis 14:1737–1746 (1993).
2. Kelloff GJ, Boone CW, Malone WF, Steele V. Recent results in preclinical and clinical drug development of chemopreventive agents at the National Cancer Institute. In: Cancer Chemoprevention (Wattenberg L, Lipkin M, Boone CW, Kelloff GJ, eds). Boca Raton, FL:CRC Press, 1992:41–56.
3. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Sigman CC. Chemopreventive drug development: perspectives and progress. Cancer Epidemiol Biomarkers Prev 3:85–98 (1994).
4. Kelloff GJ, Boone CW, Steele VE, Crowell JA, Lubet R, Sigman CC. Progress in cancer chemoprevention: perspectives on agent selection and short-term clinical intervention trials. Cancer Res 54:2015–2024s (1994).
5. Greenwald P, Kelloff G, Burch-Whitman C, Kramer BS. Chemoprevention. CA Cancer J Clin 45:31–49 (1995).
6. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Doody LA. Surrogate endpoint biomarkers for phase II cancer chemoprevention trials. J Cell Biochem Suppl 19:1–9 (1994).
7. Boone CW, Kelloff GJ. Development of surrogate endpoint biomarkers for clinical trials of cancer chemopreventive agents: relationships to fundamental properties of preinvasive (intraepithelial) neoplasia. J Cell Biochem Suppl 19:10–22 (1994).
8. De Flora S, Izzo A, Bennicelli J. Mechanisms of antimutagenesis and anticarcinogenesis: role in primary prevention. Basic Life Sci 61:1–16 (1993).
9. Harris CC. Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res 5(Suppl):5023s–5044s (1991).
10. ACS. Cancer Facts and Figures, 1995. Atlanta, GA:American Cancer Society, 1995; 1–3.
11. Wattenberg LW. Chemoprevention of cancer. Cancer Res 45:1–8 (1985).
12. Mirvish, SS. Ascorbic acid inhibition of N-nitroso compound formation in chemical, food and biological systems. In: Inhibition of Tumor Induction and Development (Zedeck MS, Lipkin M, eds). New York:Plenum, 1981:101–126.
13. Hartman PE, Shankel DM. Antimituagens and anticarcinogens: a survey of putative interceptor molecules. Environ Mol Mutagen 15:145–182 (1990).
14. Kuenzig W, Chau J, Norkus E, Holowaschenko H, Newmark H, Mergens W, Conney AH. Caffeic acid and ferulic acid as blockers of nitrosamine formation. Carcinogenesis 5:309–314 (1984).
15. Shenoy NR, Choughuley ASU. Inhibitory effect of diet related sulphydryl compounds on the formation of carcinogenic nitrosamines. Cancer Lett 65:227–232 (1992).
16. Wakanabashi K, Nagao M, Sugimura T. Heterocyclic amines, lipopolitic ascorbic acid, and thiprolino: ubiquitous carcinogens and practical anticarcinogenic substances. In: Cancer Chemoprevention (Wattenberg L, Lipkin M, Boone CW, Kelloff GJ, eds). Boca Raton, FL:CRC Press, 1992:313–325.
17. Fiala ES, Bobotas G, Kulakis C, Wattenberg LW, Weisburger JH. The effects of disulfiram and related compounds on the in vivo metabolism of the colon carciogen 1,2-dimethylhydrazine. Biochem Pharmacol 26:1765–1768 (1977).
18. Wattenberg LW. Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. J Natl Cancer Inst 58:395–398 (1977).
19. Wattenberg LW. Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine or benzo[a]pyrene on pulmonary and forestomach neoplasia in A/J mice. Carcinogenesis 8:1971–1973 (1987).
20. Morse MA, Wang C-X, Stoner GD, Mandal S, Conran PB, Amin SG, Hecht SS, Chung F-L. Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone DNA adduct formation and tumorigenicity in the lung of F344 rats by dietary phenethyl isothiocyanate. Cancer Res 49:549–553 (1989).
21. Morse MA, Eklind KI, Amin SG, Hecht SS, Chung F-L. Effects of aromatic isothiocyanates on tumorigenicity, O₆-methylguanine formation, and metabolism of the tobacco-specific nitrosamine 4-(methylisothio- cyanamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. Cancer Res 49:2894–2897 (1989).
22. Morse MA, Eklind KI, Hecht SS, Jordan KG, Choi C-I, Desai DH, Amin SG, Chung F-L. Structure–activity relationships for inhibition of 4-(methylisothiocyanamino)-1-(3-pyridyl)-1-butanone (NKK) lung tumorigenesis by phenethyl isothiocyanates in A/J mice. Cancer Res 51:1846–1850 (1991).
23. Stoner GD, Morrissey DT, Heur Y-H, Daniel EM, Galati AJ, Wagner SW. Inhibitory effects of phenethyl isothiocyanate on N-nitrosobenzylamine carcinogenesis in the rat esophagus. Cancer Res 51:2063–2068 (1991).
24. Morse MA, Eklind KI, Amin SG, Chung F-L. Effects of frequency of isothiocyanate administration on inhibition of 4-(methylisothiocyanamino)-1-(3-pyridyl)-1-butanone-induced pulmonary adenoma formation in A/J mice. Cancer Lett 62:77–81 (1992).
25. Morse MA, Stoner GD. Dose-related inhibition by dietary phenethyl isothiocyanate of esophageal tumorigenesis and DNA methylation induced by N-nitosomethylbenzylamine in rats. Cancer Lett 72:103–110 (1993).
26. Wilkinson JT, Morse MA, Kresty LA, Stoner GD. Effect of alkyl chain length on inhibition of N-nitrosomethylbenzylamine-induced esophageal tumorigenesis and DNA methylation by isothiocyanates. Carcinogenesis 16:1011–1015 (1995).
27. Stoner GD, Siglin JC, Morse MA, Desai DH, Amin SG, Kresty LA, Toburen AL, Heffner EM, Francis DJ. Enhancement of esophageal carcinogenesis in male F344 rats by dietary phenethyl isothiocyanate. Carcinogenesis 16:2473–2476 (1995).
28. Wargovich MJ. Diallyl sulfide, a flavor component of garlic (Allium sativum), inhibits dimethylhydrazine-induced colon cancer. Carcinogenesis 8:487–489 (1987).
29. Spannins VL, Barany G, Wattenberg LW. Effects of organosulfur compounds from garlic and onions on benzo[a]pyrene-induced neoplasia and glutathione S-transferase activity in the mouse. Carcinogenesis 9:131–134 (1988).
30. Wargovich MJ, Woods C, Eng VWS, Stephens LC, Gray K. Chemoprevention of N-nitrosomethylbenzylamine-induced esophageal cancer in rats by the naturally-occurring thioether, diallyl sulfide. Cancer Res 48:6872–6875 (1988).
31. Tadi PP, Teel RW, Lau BHS. Organosulfur compounds of garlic modulate mutagenesis, metabolism, and DNA binding of aflatoxin B₁. Nutr Cancer 15:87–95 (1991).
32. Dixit R, Teel RW, Daniel FB, Stoner GD. Inhibition of benzo[a]pyrene and benzo[a]pyrene-trans-7,8-diol metabolism and DNA binding in mouse lung explants by ellagic acid. Cancer Res 45:2951–2956 (1985).
34. Mandal S, Shivapurkar NM, Galari AJ, Stoner GD. Inhibition of N-nitrosobenzylmethylene metabolism and DNA binding in cultured rat esophagus by ellagic acid. Carcinogenesis 9:1313–1316 (1988).

35. Barch DH, Fox CC. Dietary ellagic acid reduces the esophageal microsomal metabolism of methylnitrosourea. Cancer Lett 44:39–44 (1989).

36. Mandal S, Stoner GD. Inhibition of N-nitrosobenzylmethylene-induced esophageal tumorigenesis in rats by ellagic acid. Carcinogenesis 11:55–61 (1990).

37. Daniel EM, Stoner GD. The effects of ellagic acid and 13-cis-retinoic acid on N-nitrosobenzylmethylene-induced esophageal tumorigenesis in rats. Cancer Lett 56:117–124 (1991).

38. Wattenberg LW, Loub WD. Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally-occurring indoles. Cancer Res 38:1410–1413 (1978).

39. Nixon JE, Hendricks JD, Pawlowski NE, Pereira CB, Sinnhuber RO, Bailey GS. Inhibition of aflatoxin B1 carcinogenesis in rainbow trout by flavone and indole compounds. Carcinogenesis 5:615–619 (1984).

40. Goege DE, Shelton DW, Hendricks JD, Bailey GS. Mechanisms of anti-carcinogenesis by indole-3-carbinol: effect on the distribution and metabolism of aflatoxin B1 in rainbow trout. Carcinogenesis 7:2025–2031 (1986).

41. Dashwood RH, Arbogast DN, Fond AT, Hendricks JD, Bailey GS. Mechanisms of anti-carcinogenesis by indole-3-carbinol: detailed in vivo DNA binding dose–response studies after dietary administration with aflatoxin B1. Carcinogenesis 9:427–432 (1988).

42. Dashwood RH, Arbogast DN, Fond AT, Pereira C, Hendricks JD, Bailey GS. Quantitative inter-relationships between aflatoxin B1, carcinogen dose, indole-3-carbinol anti-carcinogen dose, target organ DNA addition and final tumor response. Carcinogenesis 10:175–181 (1989).

43. Tanaka T, Mori Y, Morishita Y, Hara A, Ohno T, Kojima T, Mori H. Inhibitory effect of sinigrin and indole-3-carbinol on diethylstilbestrol-induced hepatocarcinogenesis in male ACI/rats. Carcinogenesis 11:1403–1406 (1990).

44. Morse MA, LaGreca SD, Amin SG, Chung F-L. Effects of indole-3-carbinol on lung tumorigenesis and DNA methyltransferase induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and on the metabolism and disposition of NNK in AJR mice. Cancer Res 50:2613–2617 (1990).

45. Morse MA, Wang C-X, Amin SG, Hecht SS, Chung F-L. Effects of dietary sinigrin or indole-3-carbinol on O5-methylguanine-DNA-transmethlase activity and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA methylation and tumorigenicity in F344 rats. Carcinogenesis 9:1891–1895 (1988).

46. Pence BC, Budding F, Yang SP. Multiple dietary factors in the enhancement of dimethylhydrazine carcinogenesis: main effect of indole-3-carbinol. J Natl Cancer Inst 77:269–276 (1986).

47. Bailey GS, Hendricks JD, Shelton DW, Nixon JE, Pawlowski NE. Enhancement of carcinogenesis by the natural anticarcinogen indole-3-carbinol. J Natl Cancer Inst 78:931–934 (1987).

48. Zhang Y, Talalay P, Cho C-G, Posner GH. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci USA 92:2399–2402 (1995).

49. Zhang Y, Kessler TW, Cho C-G, Posner GH, Talalay P. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci USA 91:3147–3150 (1994).

50. Wattenberg LW, Buening E. Inhibitory effects of 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) on carcino- genesis induced by benzo[a]pyrene, diethylnitrosamine, and uracil mustard. Carcinogenesis 7:1379–1381 (1986).

51. Kessler TW, Egner PA, Trush MA, Buening E, Groupman JD. Modification of aflatoxin B1 binding to DNA in vivo in rats fed phenolic antioxidants, ethoxyquin and a dithiolthione. Carcinogenesis 6:759–763 (1985).

52. Kessler TW, Egner PA, Dola PM, Groupman JD, Roebuck BD. Mechanism of protection against aflatoxin tumorigenicity in rats fed 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) and related 1,2-dibutyl-3-thiones and 1,2-dithiol-3-thiones. Cancer Res 47:4271–4277 (1987).

53. Liu, Y-L, Roebuck, BD, Yager, JD, Groopman, JD, Kessler, TW. Protection by 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) against the hepatotoxicity of aflatoxin B1 in the rat. Toxicol Appl Pharmacol 53:442–451 (1988).

54. Davidson NE, Egner PA, Kessler TW. Transcriptional control of glutathione S-transferase gene expression by the chemoprotective agent 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) in rat liver. Cancer Res 50:2251–2255 (1990).

55. Roebuck BD, Liu Y-L, Rogers AE, Groopman JD, Kessler TW. Protection against aflatoxin B1-induced hepatocarcinogenesis in F344 rats by 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz): predictive role for molecular dosimetry. Cancer Res 51:5501–5506 (1991).

56. Rao CV, Tokomo K, Kelloff G, Reddy BS. Inhibition by dietary oltipraz of experimental intestinal carcinogenesis induced by azoxymethane in male F344 rats. Carcinogenesis 12:1051–1055 (1991).

57. Wattenberg LW, Jerina DM. Lam LKT, Yagi H. Neoplastic effects of oral administration of (+/-)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene and their inhibition by butylated hydroxyanisole. J Natl Cancer Inst 62:1103–1106 (1979).

58. Sato K, Kitahara A, Yin Z, Waragi F, Nishimura K, Hatayama I, Ibina T, Yamazaki T, Tsuda H, Ito N. Induction by butylated hydroxyanisole of specific molecular forms of glutathione S-transferase and UDP-gluconuronyltransferase and inhibition of development of gamma-glutamyl transpeptidase-positive foci in rat liver. Carcinogenesis 5:473–477 (1984).

59. Sayer JM, Yagi H, Wood AW, Conney AH, Jerina DM. Extremely facile reaction between the ultimate carcinogen benzo[a]pyrene-7,8-diol-9,10-epoxide and ellagic acid. J Am Chem Soc 104:5562–5564 (1982).

60. Wood AW, Huang M-T, Chang RL, Newmark NL, Lehre R, Yagi H, Sayer JM, Jerina DM, Conney AH. Inhibition of the mutagenicity of bay-region diol-epoxides of polycyclic aromatic hydrocarbons by naturally occurring plant phenols: exceptional activity of ellagic acid. Proc Natl Acad Sci USA 79:5513–5517 (1982).

61. Chang RL, Huang M-T, Wood AW, Wong C-Q, Newmark HL, Yagi H, Sayer JM, Jerina DM, Conney AH. Effect of ellagic acid and hydroxylated flavonoids on the tumorigenicity of benzo[a]pyrene and (+/-)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene and their inhibition by butylated hydroxyanisole. J Natl Cancer Inst 62:1103–1106 (1979).

62. De Flora S, Bennicelli C, Zannachi P, Camoirano A, Morelli A, De Flora A. In vitro effects of N-acetylcysteine on the mutagenicity of direct-acting compounds and procarcinogens. Carcinogenesis 5:505–510 (1984).

63. De Flora S, Bennicelli C, Camoirano A, Serra D, Romano M, Rossi GA, Morelli A, De Flora A. In vivo effects of N-acetylcysteine on glutathione metabolism and on the biotransformation of carcinogenic and/or mutagenic compounds. Carcinogenesis 5:1735–1745 (1985).

64. De Flora S, Astengo M, Serra D, Bennicelli C. Inhibition of urethan-induced lung tumors in mice by dietary N-acetylcysteine. Cancer Lett. 32:235–241 (1986).

65. Frenkel K. Carcinogenesis-mediated oxidant formation and oxidative DNA damage. Pharmacol Ther 53:126–166 (1992).

66. Pryor WA. Why is the hydroxyl radical the only radical that commonly adds to DNA? Hypothesis: It has a rare combination of high electrophilicity, high thermochemical reactivity, and a mode of production that can occur near DNA. Free Radical Biol Med 4:253–263 (1988).

67. Foote CS, Chang YC, Denny RW. Chemistry of singlet oxygen. X: Carotenoid quenching parallels biological protection. J Am Chem Soc 92:5216–5218 (1970).

68. Packer JE, Mahood JS, Mora-Arellano VO, Slater TF, Willson RL, Wolffenden BS. Free radicals and singlet oxygen scavengers: reaction of a peroxy radical with beta-carotene, diphenyl furan.
952 Environmental Health Perspectives

71. Tabor CW, Tabor BK, Bey P, Danzig NJ. Inhibition of mouse skin tumor promotion and of promoter-stimulated epidermal polyamine biosynthesis by α-difluoromethylornithine. Cancer Res 43:2545–2549 (1983).

72. Takigawa M, Verma AJ, Simmsiman RC, Boutwell RK. Inhibition of muscle tumor growth and of promoter-stimulated epidermal polyamine biosynthesis by α-difluoromethylornithine. Cancer Res 43:3732–3738 (1983).

73. Homma Y, Kakizoe T, Samma S, Oyasu R. Inhibition of N-butylnitrosamine-induced rat urinary bladder carcinogenesis by α-difluoromethylornithine. Cancer Res 47:6176–6179 (1987).

74. Reddy BS, Nayini J, Tokumo K, Rigotty J, Zang E, Kellogg G. Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal antiinflammatory drug with D1,α-difluoromethylornithine, an ornithine decarboxylase inhibitor; dose-dependent reduction in 4-nitroquinoline 1-oxide-induced tongue neoplasms in rats. Cancer Res 53:772–776 (1993).

75. Whitfield JF. Calcium: driver of cell cycles, trigger of differentiation, and killer of cells. In: Cellular and Molecular Targets for Chemoprevention (Steele VE, Stoner GD, Boone CW, Kellogg GJ, eds). Boca Raton, FL: CRC Press, 1992:257–311.

76. De Luca LM. Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. Fed Am Soc Exp Biol J 5:2924–2933 (1991).

77. Newton DL, Henderson WR, Sporn MB. Structure–activity relationships of retinoids in hamster tracheal organ culture. Cancer Res 40:3413–3425 (1980).

78. Huang FL, Roop DR, De Luca LM. Vitamin A deficiency and keratin biosynthesis in cultured hamster trachea. In Vitro Cell Dev Biol 22:223 (1986).

79. McDowell EM, Ben T, Newkirk C, Chang S, De Luca L. Differentiation of tracheal mucociliary epithelium in primary cell culture recapitulates normal fetal development and regeneration following injury in hamsters. Am J Pathol 129:511–522 (1987).

80. Basbor MM, Toft DO, Chylit F. In vitro binding of retinol to rat tissue components. Proc Natl Acad Sci USA 70:3483–3487 (1973).

81. Sani BP, Hill DL. A retinoic acid-binding protein from chick embryo skin. Cancer Res 56:409–413 (1996).

82. Sani BP, Singh RK, Meek P, Isolation, partial purification and characterization of nuclear retinoic acid receptors from chick skin. Arch Biochem Biophys 283:107–113 (1990).

83. Hennings H, Michael D, Cheng C, Steinert P, Holbrook K, Yusa SH. Calcium regulation of growth and differentiation of mouse epidermal cells in culture. Cell 19:245–254 (1980).

84. Babcock MS, Marino MS, Gunning WT III, Stoner GD. Clonal growth and serial propagation of rat esophageal epithelial cells. In Vitro 19:403–415 (1983).

85. Scalmat A, Lipkin M, Newmark H. Relationships of calcium and vitamin D to colon cancer. In: Cancer Chemoprevention (Wattenberg L, Lipkin M, Boone CW, Kellogg GJ, eds). Boca Raton, FL: CRC Press, 1992:239–262.

86. McGrath CM, Sani BP. Isolation, partial purification and characterization of nuclear retinoic acid receptors from chick skin. Arch Biochem Biophys 283:107–113 (1990).

87. Soule HD, McGrath CM. A simplified method for passage and long-term growth of human mammary epithelial cells. In Vitro 22:8–12 (1986).

88. Abe F, Miyaura C, Sakagami H, Takeda M, Konno K, Yamazaki T, Yoshiaki S, Suda T. Differentiation of mouse myeloid leukemia cells induced by 1α,25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 78:4990–4994 (1981).

89. Miyaura C, Abe E, Kuribayashi T, Tanaka H, Konno K, Nishii Y, Suda T. 1α,25-Dihydroxyvitamin D3 induces differentiation of human myeloid leukemia cells. Biochem Biophys Res Commun 102:937–943 (1981).
108. Osterm VK, De Luca HF. The vitamin D-induced differentiation of HL-60 cells: structural requirements. Steroids 49:73–102 (1987).

109. Goldman R. Synergism and antagonism in the effects of 1α,25-dihydroxyvitamin D₃, retinoic acid, dexamethasone, and a tumor-promoting phorbol ester on the functional capacity P388D1 cells: phagocytosis and transglutaminase activity. Cancer Res 45:3118–3124 (1985).

110. Sporn MB. Transforming growth factor β. Adv Cancer Res 51:107–145 (1988).

111. Silberstein GB, Daniel CW. Reversible inhibition of mammary gland growth by transforming growth factor-β. Science 237:291–293 (1987).

112. Sporn MB, Roberts AB, Glick AB, Luckettt PH, Pollard M. Interactions of retinoids and transforming growth factor β in the chemoprevention of cancer. In: Control of Growth Factors and Prevention of Cancer (Sporn MB, ed). New York: Springer-Verlag, 1992;37–46.

113. Russell WE, Coffrey RJ Jr, Ouellette AJ, Moses HL. Type β transforming growth factor reversibility inhibits the early proliferative response to partial hepatectomy in the rat. Proc Natl Acad Sci USA 85:5126–5130 (1988).

114. Jordan VC. The strategic use of antiestrogens to control the development and growth of breast cancer. Cancer 70:977–982 (1992).

115. Pollak MN, Huyhn HT, Lefebvre SP. Tamoxifen reduces serum insulin-like growth factor I (IGF-I). Breast Cancer Res Treat 22:91–100 (1992).

116. Macauley VM. Insulin-like growth factors and cancer. Br J Cancer 65:311–320 (1992).

117. Elson CE, Malzman TH, Boston JL, Tanner MA, Gould MN. Anti-carcinogenic activity of 3-dimethoxacetone during the initiation and promotion/progression stages of DMBA-induced rat mammary carcinogenesis. Carcinogenesis 9:331–332 (1988).

118. Russin WA, Hoey JD, Elson CE, Tanner MA, Gould MN. Inhibition of rat mammary carcinogenesis by monoterpenoids. Carcinogenesis 10:2161–2164 (1989).

119. Crowell PL, Chang RR, Ren Z, Elson CE, Gould MN. Selective inhibition of isoprenylation of 21–26 kDa proteins by the anticarcinogenic 3-dimethoxacetone and its metabolites. J Biol Chem 266:17679–17685 (1991).

120. Troll W, Kennedy AR. Protease inhibitors as cancer chemopreventive agents. Cancer Res 49:499–502 (1988).

121. Klauing J, Ruch RJ. Role of inhibition of intercellular communication in carcinogenesis. Lab Invest 62:135–146 (1990).

122. Bertram JS, Hossain MZ, Zhang L-X. Use of cell culture systems for mechanistic studies of chemopreventive agents. In: Cellular and Molecular Targets for Chemoprevention (Steele VE, Stoner GD, Boone CW, Kelloff GJ, eds). Boca Raton, FL:CRC Press, 1992;43–62.

123. Lowenstein WR. Junctional intercellular communication and the control of growth. Biochem Biophys Acta 560:1–65 (1979).

124. Mehta PP, Bertram JS, Lowenstein WR. Growth inhibition of transformed cells correlates with their junctional communication with normal cells. Cell 44:187–196 (1986).

125. Mehta PP, Bertram JS, Lowenstein WR. The actions of retinoids on cellular growth correlate with their actions on gap junctional communication. J Cell Biol 108:1053–1065 (1989).

126. Zhang L-X, Cooney RV, Bertram JS. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in CHO/H101/12 cells: relationship to their cancer chemopreventive action. Carcinogenesis 12:2109–2114 (1991).

127. Breitbart TR, Selonick SE, Collins SJ. Induction of differentiation in human promyelocytic leukemia cell line (HL-60) by retinoic acid. Proc Natl Acad Sci USA 77:2936–2940 (1980).

128. Hill DL, Grubb CJ. Retinoids and cancer prevention. Annu Rev Nutr 12:161–182 (1992).

129. Kiremidjian-Schumacher L, Stotosky G. Selenium and immune responses. Environ Res 42:277–303 (1987).

130. Tengerdy RP. Effect of vitamin E on immune function. In: Vitamin E—A Comprehensive Treatise (Machlin LJ, ed). New York: Marcel Dekker, 1980;429–444.

131. Corwin RM, Gordon RK. Vitamin E and immune regulation. Ann NY Acad Sci 393:437–451 (1982).

132. Schwartz J, Odukoya O, Stout F, Shklar G. Alpha-tocopherol alters the distribution of Langerhans cells in DMBA-treated hamster cheek pouch epithelium. J Dent Res 64:117–121 (1985).

133. Sen S, D’Incalci M. Apoptosis. Biochemical events and relevance to cancer chemotherapy. FEBS Lett 307:122–127 (1992).

134. Bursch W, Oberhammer F, Schulte-Herrmann R. Cell death by apoptosis and its putative role against disease. Trends Pharmacol Sci 13:245–251 (1992).

135. Yonish-Rouach E, Resnitsky D, Lotem J, Sachs L, Kimchi A, Oren M. Wild-type p53 induces apoptosis of myeloid leukemia cells that is inhibited by interleukin-6. Nature 352:345–347 (1991).

136. Oren M. The involvement of oncopgenes and tumour suppressor genes in the control of apoptosis. Cancer Metastasis Rev 11:141–148 (1992).

137. Oberhammer F, Bursch W, Parzefall W, Breit P, Erber E, stadler M, Schulte-Herrmann R. Effect of transforming growth factor β on cell death of cultured rat hepatocytes. Cancer Res 51:2478–2485 (1991).

138. Kyriamou N, English HF, Isaacs JT. Programming cell death during regression of PC-82 human prostate cancer following androgen ablation. Cancer Res 50:3478–3753 (1990).

139. Bursch W, Liehr JG, Sirbasku DA, Putz B, Taper H, Schulte-Herrmann R. Control of cell death (apoptosis) by diethylstilbestrol in an estrogen-dependent kidney tumor. Carcinogenesis 12:855–860 (1991).

140. Piazza GA, Rahm ALK, Krutzsch M, Sperl G, Paranka NS, Gross PH, Brendel K, Burt RW, Alberts DS, Pamuku R et al. Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. Cancer Res 55:3110–3116 (1995).

141. Waddell WR, Gasen GF, Cerise EJ, Loughry RW. Sulindac for polyposis of the colon. Am J Surg 157:175–179 (1990).

142. Labayle D, Fischer D, Vielh P, Drouhin F, Pariente A, Bories C, Duhamel O, Trousset M, Attali P. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology 101:635–639 (1991).

143. Wainfan E, Poirier LA. Methyl groups in carcinogenesis: effects of DNA methylation and gene expression. Cancer Res 52:2071s–2077s (1992).

144. Mikol YB, Hoover KL, Creasia D, Poirier LA. Hepatocarcinogenesis in rats fed methyl-deficient, amino acid-defined diets. Carcinogenesis 4:1619–1629 (1983).

145. Ghoshal AK, Farber E. The inhibition of liver cancer by dietary deficiency of choline and methionine without added carcinogens. Carcinogenesis 5:1367–1370 (1984).

146. Yokoyama S, Sells MA, Reddy TV, Lombardi B. Hepatocarcinogenic and promoting action of a choline-derivative diet in the rat. Cancer Res 45:2834–2842 (1985).

147. Brada Z, Altman NH, Hill M, Bulba S. The effect of methionine on the progression of hepatocellular carcinoma induced by ethionine. Res Commun Chem Pathol Pharmacol 38:157–160 (1982).

148. Hoffman RM. Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis—a review and synthesis. Biochem Biophys Acta 738:49–87 (1984).

149. Wainfan E, Dizik M. Suppression by methionine and choline of onco-fetal patterns of liver mRNA methyltransferase activities in carcinogen treated rats. Carcinogenesis 8:615–617 (1987).

150. Dizik M, Christman JK, Wainfan E. Alterations in expression and methylation of specific genes in livers of rats fed a cancer-promoting, methyl-deficient diet. Carcinogenesis 12:1307–1312 (1991).

151. Wainfan E, Dizik M, Shiekhejedad G, Christman JK. Early changes in nucleic acid methylation and gene expression in livers of rats fed cancer-promoting, methyl-deficient diets. Fed Am Soc Exp Biol J 4:A1043 (1990).

152. Hocman G. Chemoprevention of cancer: protease inhibitors. Int J Biochem 24:1365–1375 (1992).
153. Moncada S, Flower RJ, Vane JR. Prostaglandins, prostacyclin, and thromboxane A₂. In: The Pharmacological Basis of Therapeutics. 6th ed (Gilman AG, Goodman LS, Gilman A, eds). New York:McMillan, 1980;668–681.

154. Reddy BS, Maruyama H, Kelloff G. Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. Cancer Res 47:5340–5346 (1987).

155. Kudo T, Narisawa T, Abo S. Antitumor activity of indomethacin on methylazoxymethanol-induced large bowel tumors in rats. Gann 71:260–264 (1980).

156. Narisawa T, Sato M, Tani M, Kudo T, Takahashi T, Goto A. Inhibition of development of methylnitrosourea-induced rat colon tumors by indomethacin. Cancer Res 41:1954–1957 (1981).

157. Boone CW, Steele VE, Kelloff GJ. Screening for chemopreventive (anticarcinogenic) compounds in rodents. Mutat Res 267:251–255 (1992).

158. Nakadate T, Yamamoto S, Aizu E, Kato R. Inhibition by lipooxygenase inhibitors of 7-bromomethylbenz[a]anthracene-caused epidermal ornithine decarboxylase induction and skin tumor promotion in mice. Carcinogenesis 10:2053–2057 (1989).

159. Huang M-T, Lusz T, Ferraro T, Abidi TF, Laskin JD, Conney AH. Inhibitory effects of curcumin on in vitro lipooxygenase and cyclooxygenase activities in mouse epidermis. Cancer Res 51:813–819 (1991).

160. Huang M-T, Smart RC, Wong C-Q, Conney AH. Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-O-tetradecanoylphorbol-13-acetate. Cancer Res 48:5941–5946 (1988).

161. Hong WK, Lippman SM, Itri LM, Karp DD, Lee JS, Byers RM, Schantz SP, Kramer AM, Lotan R, Peters LJ et al. Prevention of second primary tumors with isotretinoin in squamous cell carcinoma of the head and neck. N Engl J Med 323:795–801 (1990).

162. Garewal HS, Meyskens FL Jr, Reeves D, Kirsch TA, Elletson H, Strosberg A, King D, Steinbrom K. Response of oral leukoplakia to beta-carotene. J Clin Oncol 8:1715–1720 (1990).

163. Kelloff GJ, Johnson JJ, Crowell JA, Boone CW, DeGeorge JJ, Steele VE, Mehta MU, Temeck JW, Schmidt WJ, Burke G et al. Approaches to the development and marketing approval of drugs that prevent cancer. Cancer Epidemiol Biomark Prev 4:1–10 (1995).

164. Brown ML. The national economic burden of cancer: an update. J Natl Cancer Inst 23:1811–1814 (1990).