Symposium: Diet, Natural Products and Cancer Prevention: Progress and Promise

Progress in Cancer Chemoprevention: Development of Diet-Derived Chemopreventive Agents[1]

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ABSTRACT   Because of their safety and the fact that they are not perceived as "medicine," food-derived products are highly interesting for development as chemopreventive agents that may find widespread, long-term use in populations at normal risk. Numerous diet-derived agents are included among the >40 promising agents and agent combinations that are being evaluated clinically as chemopreventive agents for major cancer targets including breast, prostate, colon and lung. Examples include green and black tea polyphenols, soy isoflavones, Bowman-Birk soy protease inhibitor, curcumin, phenethyl isothiocyanate, sulforaphane, lycopene, indole-3-carbinol, perillyl alcohol, vitamin D, vitamin E, selenium and calcium. Many food-derived agents are extracts, containing multiple compounds or classes of compounds. For developing such agents, the National Cancer Institute (NCI) has advocated codevelopment of a single or a few putative active compounds that are contained in the food-derived agent. The active compounds provide mechanistic and pharmacologic data that may be used to characterize the chemopreventive potential of the extract, and these compounds may find use as chemopreventives in higher risk subjects (patients with precancers or previous cancers). Other critical aspects to developing the food-derived products are careful analysis and definition of the extract to ensure reproducibility (e.g., growth conditions, chromatographic characteristics or composition), and basic science studies to confirm epidemiologic findings associating the food product with cancer prevention.   J. Nutr. 130: 467S–471S, 2000.

KEY WORDS: • *cancer chemoprevention* • *drug development* • *food-derived agents* • *breast* • *prostate* • *colon* • *lung*

In many major cancer targets, human cancer development requires 20–40 years or more (Kelloff et al. 1996i and 1997), and the scope of chemoprevention encompasses cohorts at all phases of this process—from healthy subjects at normal risk, to populations at intermediate risk resulting from environmental and lifestyle factors, genetic predisposition, and precancerous lesions, and then to previous cancer patients at high risk for second primaries. Because of their expected safety and because (unlike such agents as synthetic pharmaceuticals) they are not perceived as "medicine," food-derived products may find widespread, long-term use in the populations at normal risk; thus they are highly interesting for development as chemopreventive agents. As for other agents, characterization of efficacy and safety, biomarkers of efficacy and risk, and suitable cohorts for clinical intervention are critical to progress in chemoprevention with diet-derived agents.

Many food-derived agents are extracts containing multiple compounds or classes of compounds (e.g., tea, soy isoflavones or other soy fractions, curcuminoids). The National Cancer Institute (NCI) has advocated a science-based approach to their evaluation and development. Usually, a single or a few putative active compounds contained in the food-derived agent are isolated or synthesized and codeveloped with the food extract. For example, epigallocatechin gallate (EGCG)[3] is being codeveloped with green tea polyphenols. Once it has been determined that the cancer-related targets and effects of the putative active components and the extract are similar (e.g., dose-response curves are parallel), the more expensive

[3] Abbreviations used: EGCG, epigallocatechin gallate; GSH, glutathione; GST, glutathione *S*-transferase; HGPIN, high-grade prostatic intraepithelial neoplasia; IEN, intraepithelial neoplasia; NAC, *N*-acetyl-L-cysteine; NNK, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; PEITC, phenethyl isothiocyanate; PIN, prostatic intraepithelial neoplasia; PSA, prostate specific antigen; SERM, selective estrogen receptor modulators.

TABLE 1

Mechanisms for chemoprevention by diet-derived agents with possible molecular targets[1]

Mechanism	Possible molecular targets	Representative agents
Antimutagenesis		
Inhibit carcinogen uptake	Bile acids (bind)	Calcium
Inhibit formation/activation of carcinogen	Cytochromes P450 (inhibit)	PEITC, tea, indole-3-carbinol, soy isoflavones
	PG synthase hydroperoxidase, 5-lipoxygenase (inhibit)	Curcumin
	Bile acids (inhibit)	Ursodiol
Deactivate/detoxify carcinogen	GSH/GST (enhance)	NAC, garlic/onion disulfides
Prevent carcinogen-DNA binding	Cytochromes P450 (inhibit)	Tea
Increase level or fidelity of DNA repair	Poly(ADP-ribosyl)transferase (enhance)	NAC, protease inhibitors (Bowman-Birk)
Antiproliferation/antiprogression		
Modulate hormone/growth factor activity	Estrogen receptor (antagonize)	Soy isoflavones
	Steroid 5α-reductase (inhibit)	Tea
	IGF-I (inhibit)	Soy isoflavones
Inhibit oncogene activity	Farnesyl protein transferase (inhibit)	Perillyl alcohol, limonene, DHEA
Inhibit polyamine metabolism	ODC induction (inhibit)	Retinoids, curcumin, tea
Induce terminal differentiation	TGFβ (induce)	Retinoids, vitamin D, soy isoflavones
Restore immune response	Cyclooxygenases (inhibit)	Tea, curcumin
	T, NK lymphocytes (enhance)	Selenium, tea
	Langherans cells (enhance)	Vitamin E
Increase intercellular communication	Connexin 43 (enhance)	Carotenoids (lycopene), retinoids
Induce apoptosis	TGFβ (induce)	Retinoids, soy isoflavones, vitamin D
	RAS farnesylation (inhibit)	Perillyl alcohol, limonene, DHEA
	Telomerase (inhibit)	Retinoic acid
	Arachidonic acid (enhance)	Curcumin, tea
	Caspase (activate)	Retinoids
Inhibit angiogenesis	FGF receptor (inhibit tyrosine kinase)	Soy isoflavones
	Thrombomodulin (inhibit)	Retinoids
Correct DNA methylation imbalances	CpG island methylation (enhance)	Folic acid
Inhibit basement membrane degradation	Type IV collagenase (inhibit)	Protease inhibitors
Inhibit DNA synthesis	Glucose 6-phosphate dehydrogenase (inhibit)	DHEA

[1] Abbreviations: PEITC, phenethyl isothiocyanate; PG, prostaglandin; GSH, glutathione; GST, glutathione *S*-transferase; NAC, *N*-acetyl-L-cysteine; IGF, insulin-like growth factor; DHEA, dehydroepiandrosterone; ODC, ornithine decarboxylase; TGFβ, transforming growth factor β; NK, natural killer; RAS, *ras* oncogene protein product; FGF, fibroblast growth factor.

and possibly more toxic purified agent may be dropped from development in favor of the more nearly natural product. Alternatively, the purified product may be more potent and, even if more toxic, suitable for use in higher risk populations, such as patients with premalignant disease or previously treated cancers.

A second important concept in the development of food-derived chemopreventive agents is careful characterization of the active substance(s) and the technology to ensure reproducible preparations. For example, definition of growth conditions (e.g., hours of sunlight or soil nutrients) may be important, as may be the precise extraction conditions and spectrophotometric characteristics of the preparation to ensure the similarity of different preparations of the agent.

Identifying promising chemopreventive agents in the diet

Many genetic lesions and other cellular constituents have been implicated in the initiation and progression of precancers. Possible mechanisms for chemoprevention involve interfering with the expression and/or activity of these molecules; examples of the mechanisms, their possible molecular targets and dietary agents that act at these targets are listed in **Table 1** (Kelloff et al. 1994, 1995a, 1996a and 1997).

Systematic evaluation of classes of dietary agents acting at

such molecular targets is one strategy for identifying and characterizing new potential chemopreventive agents (Kelloff et al. 1996b). Many promising agents have multiple chemoprevention-associated molecular activities, some of which are interrelated, for example, the effects of the soy isoflavones on various components of the growth factor–induced signal transduction pathways. In addition, a single activity, even if it is the agent's predominant pharmacologic activity, may not be the most important or the only one required for chemoprevention. Therefore, besides testing agents against molecular targets, another approach is to evaluate efficacy at the cellular and tissue levels, in particular, evidence that agents prevent hyperproliferation or inhibit mutagenesis. These observations imply that molecular targeting should not be the only approach used to identify potential chemopreventive drugs (Kelloff et al. 1996a).

Experimental and epidemiologic carcinogenesis studies, showing that >90% of cancers are associated with mutagens and mitogens (Kelloff et al. 1996a and 1997), suggest a complementary empirical approach, i.e., searching for agents that inhibit or reverse cellular processes derived from mutagenesis and mitogenesis as follows: *1)* decreased programmed cell death (from senescence, or in response to damage or environmental conditions such as overpopulation or hormone with-

drawal); 2) decreased maturation or differentiation; and 3) increased proliferation.

Using both approaches, several thousand agents have been reported to have chemopreventive activity (Bagheri et al. 1989, Kelloff et al. 1994 and 1995). Since 1987 in the NCI chemoprevention testing program, >1000 agents and agent combinations have been selected and evaluated in preclinical studies of chemopreventive activity, ranging from in vitro mechanistic and cell-based transformation assays (Steele et al. 1996) to carcinogen-induced (Steele et al. 1994) and transgenic animal models. More than 40 promising agents and agent combinations are being evaluated clinically as chemopreventive drugs for major cancer targets. A few agents have been in vanguard, large-scale intervention trials, for example, the diet-derived agents vitamin E (Heinonen et al. 1998) and selenium (Clark et al. 1998) in prostate, and calcium (Baron et al. 1999) in colon.

An advantage of diet-derived products in cancer prevention is that they also have apparent benefit in other chronic diseases (e.g., protection from heart disease); green and black tea polyphenols (Kelloff et al. 1996c), lycopene (Gann et al. 1999, Giovannucci et al. 1995a), soy isoflavones (Kelloff et al. 1996d), curcumin (Kelloff et al. 1996e), phenethyl isothiocyanate (PEITC) (Kelloff et al. 1996f), indole-3-carbinol (Kelloff et al. 1996g), and perillyl alcohol (Kelloff et al. 1996h) have shown preventive potential against several chronic diseases.

The potential of single chemopreventives is limited by potency and, more importantly, toxicity at efficacious doses. Simultaneous or sequential administration of multiple agents can increase efficacy and reduce toxicity. For example, differences in the chemopreventive mechanisms among the agents can provide additive or synergistic efficacy; thus, adequate efficacy may be observed at lower and presumably less toxic doses of the individual agents. An example of a diet-derived combination under evaluation is that of vitamin E + selenium in prostate. In addition, mechanistic data may suggest the potential synergy of two agents; an example is the enhancement of electrophile-trapping activity (hence, carcinogen-detoxifying activity) that might be achieved by combination of an agent such as N-acetyl-L-cysteine (NAC), which provides substrate for glutathione (GSH) synthesis, with agents such as oltipraz or garlic/onion disulfides, which enhance GSH S-transferases (GST) (Kelloff et al. 1994c and 1995a).

Chemopreventive agent development strategies using intermediate biomarkers of carcinogenesis

The multidisciplinary approach of the NCI to chemopreventive agent development and collaboration with the Food and Drug Administration to provide consensus guidance for applying this approach have been described previously (Kelloff et al. 1995b, 1996i and 1997). It is an applied drug development science effort that begins with the identification of candidate agents for development and the characterization of these agents in in vitro and animal chemopreventive efficacy screens. Promising agents, including diet-derived substances, are then evaluated further in animal models to design regimens for clinical testing and use. Agents judged to have potential as human chemopreventives are subjected to preclinical toxicity and pharmacokinetic studies, and then Phase I clinical safety and pharmacokinetic trials. The most successful agents progress to clinical chemoprevention trials.

The impracticality of cancer incidence reduction as an endpoint is a major challenge in designing chemoprevention efficacy trials. Increased understanding of the molecular and phenotypic progression in carcinogenesis has provided a means

of overcoming this obstacle, i.e., with intermediate biomarkers that can be validated as surrogate endpoints for cancer. Primary intermediate biomarkers and targets of chemoprevention are intraepithelial neoplasia (IEN), which are almost always cancer precursors. In the NCI chemopreventive drug development program, Phase II and small Phase III clinical chemoprevention trials are conducted in patients with current or previous IEN. A major goal of the studies is characterization and standardization of quantitative measurements of chemopreventive agent–induced morphometric and cytometric changes in these lesions. Results showing reversion, slowed progression or inhibition of recurrence of the target lesions can be obtained within 3–24 mo.

Further, an important component of clinical (and preclinical) studies in chemoprevention is identification of earlier intermediate biomarkers in IEN that reflect carcinogenesis/chemopreventive mechanisms, i.e., proliferation (e.g., proliferating cell nuclear antigen, MIB-1), differentiation signals (e.g., actins, vimentin or blood group antigens), and genetic/regulatory changes (e.g., apoptosis, DNA methylation, oncogene or tumor suppressor expression) (Kelloff et al. 1994a). The early intermediate biomarkers can be very distant developmentally from the cancer; therefore, standardized methods for their sampling and measurement, and their validation against IEN are critical. Also, it is anticipated that the reliability of early biomarkers as endpoints for clinical trials may be improved by using them in batteries that model carcinogenesis.

Considerations in development of diet-derived chemopreventive agents at major cancer targets—breast, prostate, colon, lung

The following discussion reviews promising diet-derived agents and specific considerations in the development of chemoprevention strategies in breast, prostate, colon and lung.

Breast. Control of estrogen exposure is a key factor in breast cancer chemoprevention strategies. Many risk factors for breast carcinogenesis are associated with prolonged cyclical or high levels of estrogen exposure (e.g., early menarche, late menopause or nulliparity). It is expected that estrogen exposure would couple with genetic predisposition (e.g., BRCA or Li-Fraumeni mutations) and other factors in determining an individual's risk (Kelloff et al. 1996a). One strategy for reducing estrogen effects is by treatment with selective estrogen receptor modulators (SERM). For example, the promise of the antiestrogen tamoxifen is widely known on the basis of its success in reducing the risk of breast cancer in women at high risk (Fisher et al. 1998). Soy isoflavones, which are phytoestrogens, also appear to have chemopreventive potential as SERM. Of equal interest is the potential protection SERM may offer against other chronic diseases. Depending on the estrogen receptor response elements they affect, protection against cardiovascular disease, bone loss and brain function are also seen.

Epidemiologic studies have shown that consumption of cruciferous vegetables such as broccoli, cauliflower, cabbage, and brussels sprouts is associated with decreased cancer risk in humans (Kelloff et al. 1996g). Indole-3-carbinol, an autolysis product of glucobrassicin, is one component of cruciferous vegetables that may reduce breast cancer incidence through modulation of cytochrome P450-dependent estradiol metabolism by enhancing 2-hydroxyestrone (estrogen receptor antagonist) at the expense of 16α-hydroxylation (estrogen receptor agonist) (Kelloff et al. 1996g). Also, induction of phase II enzymes by indole-3-carbinol may increase estrogen conjugation and excretion, and indole-3-carbinol metabolites, such as

indolo[3,2-b]carbazole, may exhibit direct antiestrogenic activity by down-regulation of the estrogen receptor (Kelloff et al. 1996g).

Prostate. Several dietary products have promise as chemopreventive agents in prostate including SERM (soy isoflavones), retinoids, vitamin E, organoselenium (and the combination of selenium and vitamin E), lycopene, perillyl alcohol and vitamin D. One particularly interesting finding from epidemiologic studies suggests that increased serum levels of lycopene, the most abundant serum carotenoid, is associated with a decreased relative risk of prostate cancer (Gann et al. 1999, Giovannucci et al. 1995a, Kelloff et al. 1999). Lycopene is now in preclinical toxicity and pharmacodynamics studies to determine its distribution to prostate, and it will soon be in Phase I clinical studies.

Prostate specific antigen (PSA) and prostatic intraepithelial neoplasia (PIN) are considered to be primary intermediate biomarkers for evaluating prostate cancer risk and, potentially, chemopreventive efficacy; however, there are issues in their use. Serum PSA is well-established as a biomarker of prostate cancer, but it is not specific to neoplasia, and the data do not suggest that the level is related directly to the degree of neoplastic progression. Many studies indicate that other measurements of PSA, especially density and velocity of PSA rise, may correlate better with progression than serum level alone. The validation of PSA as an intermediate biomarker awaits further data, some of which may be obtained in the large NCI Prostate, Lung, Colorectal, and Ovary Cancer Screening Trial in which PSA is being monitored over several years in >30,000 men. Even without further refinement, PSA may prove useful in identifying clinical cohorts at risk as subjects for chemoprevention studies.

There is abundant evidence that PIN is a precursor of prostatic adenocarcinoma, suggesting subjects with PIN as cohorts for chemoprevention studies. One such cohort includes individuals with high-grade PIN, but without demonstrable prostatic carcinoma. These subjects are treated with a chemopreventive agent for approximately 2 y and then evaluated by transrectal ultrasound–directed biopsy every 3–6 mo to determine the modulation of PIN, changes in proliferation indices and nuclear abnormalities. Because PIN is nearly always also observed in conjunction with prostatic adenocarcinomas, patients with newly diagnosed early-stage prostate cancers form a cohort for biomarker studies using PIN. Such patients are usually not scheduled for prostatectomy until 3–8 wk after diagnosis. Chemopreventive intervention can be made in the period between diagnosis and prostatectomy. The removed prostate gland is analyzed for PIN modulation and other potential biomarkers.

However, PIN demonstrates the difficulty of tissue sampling for biomarkers. In men aged ≥50 y, high-grade PIN (HGPIN) incidence is 50%. However, of all sextant prostate biopsies taken in these men, only 5% HGPIN incidence is detected (i.e., 10% of the expected cases). In the general population, <1% HGPIN incidence is detected by sextant prostate biopsies. These low detection rates are probably due to inability to visualize the prostate adequately and demonstrate the need for standardized measurement methods (e.g., number and location of biopsies). The situation is improved when the whole gland is available after prostatectomy. Even in this case, the number and location of samples from invasive cancer, HGPIN and adjacent normal-appearing tissue, as well as the thickness/number of histologic sections processed and scored are important parameters that will affect variability, accuracy and reproducibility.

Colon. The developmental path for most colorectal cancer is well-documented. Histopathologically, it starts with hyperproliferation in colon mucosa, formation of adenomas (IEN) with varying degrees of malignant potential, and finally adenocarcinoma (see, e.g., Hamilton 1992). In contrast to prostate, this well-documented histopathology and the accessibility of tissue at all stages of colon carcinogenesis facilitate the evaluation of chemopreventive activity in colon (Kelloff et al. 1996i).

Because their risk of new adenomas is high (37–60%, 1–4 y after polypectomy), patients with previous adenomatous polyps are a feasible cohort for clinical chemoprevention studies (Winawer et al. 1990). In the National Polyp Study, a recurrence rate of 29–35% was seen in patients after removal of all synchronous adenomas. Anti-inflammatories, including diet-derived agents such as curcumin and tea polyphenols as well as nonsteroidal anti-inflammatory drugs (aspirin, sulindac, ibuprofen, piroxicam) and the selective cyclooxygenase-2 inhibitors, show potent chemopreventive activity in animal colorectal carcinogenesis models (primarily against azoxymethane- or 1,2-dimethylhydrazine-induced cancers in rats and mice) (Kawamori et al. 1998, Steele et al. 1994), and epidemiologic data show reduced colorectal cancer incidences among subjects who use aspirin regularly (Gann et al. 1993, Giovannucci et al. 1995b, Thun et al. 1991). In fact, one complicating factor in estimating sample size required for adenoma prevention trials for any agent is the widespread use of low dose aspirin as a cardioprotective in the target population. To detect their effects in short-term trials among aspirin users, the dietary agents might have to be particularly potent or large numbers of subjects may be required.

Lung. Tobacco use is by far the greatest risk factor for lung cancer, and chronic smokers are a primary target for chemopreventive strategies in lung (Kelloff et al. 1994b). Potential diet-derived lung chemopreventives, NAC, dithiolthiones and PEITC, have pronounced antimutagenic activity against tobacco carcinogens. PEITC is an analog and potent inhibitor of the metabolic activation of 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) (Kelloff et al. 1996g). Mutated p53 is observed in >50% of lung cancers (Hollstein et al. 1991); the tobacco carcinogens B(a)P and nitrosamines such as NNK, which mutate p53, are inhibited by oltipraz (Puisieux et al. 1991). On the basis of animal studies, other antioxidant/anti-inflammatory agents, such as lipoxygenase inhibitors, also have potential as lung cancer chemopreventives.

The concept of local delivery of chemopreventive agents is also useful for dietary chemopreventive agents. For example, local application in target tissues such as colon and upper aerodigestive tract is feasible for natural products. In fact, a study of topical tea polyphenols in prevention of actinic keratosis has recently begun.

Future progress in chemoprevention with diet-derived substances

Our understanding of carcinogenesis is increasing rapidly on the basis of new findings in cancer-related functional genomics and proteomics. Basic and translational research using the findings and the new technologies will contribute to the characterization of molecular and genomic cancer biomarkers that can be used to evaluate cancer risk in prospective cohorts as well as surrogate endpoints in clinical studies. Leads to new animal models of carcinogenesis that mimic human disease (including transgenic and gene knockout mice) can be used to validate surrogate endpoints. New treatment regimens to improve the therapeutic ratio of chemopreventives will also

be important. As suggested by the discussion above on topical delivery, the development of systems allowing local delivery to cancer targets is one possibility. Others include agent combinations and pharmacodynamically guided dosing regimens. Use of foods and dietary supplements present a safe chemopreventive strategy. In addition to epidemiologic studies, basic science research to detect mechanisms and evaluate the chemopreventive potential of food components is necessary. Talalay's research on phase II enzyme induction by molecular components of broccoli sprouts is the prototype of what is required to demonstrate chemopreventive potential of foods (Fahey et al. 1997). The results of epidemiologic (Gann et al. 1999, Giovannucci et al. 1995a) and molecular studies of lycopene are another example.

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