Identification of Candidate Cancer Chemopreventive Agents and Their Evaluation in Animal Models and Human Clinical Trials: A Review

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Abstract
A search of the literature using National Library of Medicine databases and individual cancer journal articles yielded over 500 compounds with published chemopreventive activity in animals. From these, an initial 16 agents or agent combinations have been evaluated in the following animal tumor models: mouse skin papillomas/carcinomas induced by 7,12-dimethylbenz(a)anthracene/12-O-tetradecanoylphorbol-13-acetate; rat breast adenocarcinoma induced by N-methyl-N-nitrosourea or 7,12-dimethylbenz(a)anthracene; hamster lung carcinoma induced by N-methyl-N-nitrosourea or diethyl nitrosamine; mouse bladder papillary carcinoma induced by N-butyl-N-(4-hydroxybutyl)nitrosamine; and rat and mouse colon cancer induced by azoxymethane/methylazoxymethanol acetate. Some of the most interesting positive results observed include 4-hydroxyphenyl retinamide plus tamoxifen in breast cancer, piroxicam in colon cancer, dimethylfluoroorinthine in breast and bladder cancer, oltipraz in lung cancer, dehydroepiandrosterone in colon cancer, and molybdate in bladder cancer. Eighteen human intervention trials in progress are described that involve the following agents: β-carotene (eight trials), Retinol/retinoic acid (seven trials), vitamins C and E (three trials), 4-hydroxyphenyl retinamide (one trial), piroxicam (one trial), and calcium (one trial). By organ site these studies involve cancer of the lung (six studies), skin (five studies), colon (four studies), breast (one study), and uterine cervix (two studies).

Introduction
Chemoprevention refers to the administration of chemical agents to prevent the initiational (mutational) and promotional events that occur during the process of neoplastic development. This “prescription” approach to cancer prevention supplements the conventional “proscription” approach of eliminating or avoiding carcinogens in the environment, when they can be identified, and of screening for the early detection of precancerous and cancerous lesions. The Chemoprevention Branch of the Prevention Program, Division of Cancer Prevention and Control, National Cancer Institute, has developed a program to do the following: (a) identify and characterize agents with proven efficacy in preventing carcinogenes in animals or with a high probability of preventing human cancer based on epidemiological studies, (b) conduct efficacy and toxicity testing of agent compounds in animal model systems, and (c) conduct human intervention trials of potential chemopreventive agents. Current progress along these lines is presented below.

Classification of Chemopreventives According to Mechanism of Action

Chemopreventives That Block or Suppress the Effect of Mutation
Agents that suppress prostaglandin synthesis, in addition to being antipromotional, may also be antimutational because of blocking the generation of mutagenic alkoxy free radicals during prostaglandin hydroperoxide synthesis.

Chemopreventives Whose Mechanism Is Undetermined
Agents which act through uncertain mechanisms are grouped here. Molybdate is an example.
Literature Search for Candidate Chemopreventive Compounds

SRI International and CCS Associates both of Menlo Park, CA, have assisted the Chemoprevention Branch by identifying potential cancer chemopreventive agents through a comprehensive search of the literature that is being continuously updated. So far over 500 compounds have been compiled into a master list, based on published reports of apparent cancer inhibitory activity in humans or animals. The best 50 of these agents were selected and prioritized using the criteria of high efficacy and activity in humans or animals. The best 50 of these compounds, β-carotene and 4-HPR, plus an additional six not screened in animals because they are considered safe and have some indication of efficacy from the epidemiology or experimental literature, were accepted for immediate intervention trials in human, as detailed later.

Animal Models Used by the Chemoprevention Program

The Chemoprevention Branch has developed the following battery of in vivo model systems for screening the efficacy of candidate chemopreventive compounds.

Skin (Mouse)

This has become the classic model for the experimental study of mechanisms of carcinogenesis. Excellent reviews are available (2). The two-stage protocol involves the sequential application of DMBA to the dorsal skin, followed 1 week later by twice weekly applications to the same area of the tumor promoter TPA. Papillomas begin to appear after 6–7 weeks, and the number of mice with papillomas, as well as the number of papillomas per mouse, continue to increase with time as long as TPA treatment continues. Squamous cell carcinomas also appear. The SENCAR mice used were specifically inbred for sensitivity to skin tumor induction.

Breast (Rat)

Two well-known models are in use (3, 4) involving either the induction of mammary adenocarcinomas in virgin rats by a single intragastric dose of DMBA or by an i.v. dose of MNU. The use of both models permits testing a candidate chemopreventive agent against initiation by a direct acting carcinogen (MNU) or one requiring activation (DMBA). The latency for mammary tumor formation is about 10 weeks and tumors develop in 60–80% of animals by 33 weeks after dosing with carcinogen.

Lung (Hamster)

Two hamster models are in use. One consists of the induction of invasive squamous cell carcinomas in the trachea by the application of MNU directly to the tracheal mucosa through the use of specially designed catheters (5). Treatment once a week for 15 weeks produces tumors in about 70% of animals within 6 months. The other hamster model consists of multiple s.c. injections for 20 weeks to produce tracheal carcinomas in 90–100%, and lung tumors in 60–70%, of animals. Serial sacrifice studies have shown that the lung tumors originate from the pulmonary Clara cells and endocrine cells while the tracheal tumors are derived from respiratory epithelium-basal cells (6, 7). Since human small cell cancer has features of pulmonary endocrine cells, this model is especially appropriate.

Bladder (Mouse)

In this model, OH-BBN given by intragastric instillation to (C57BL/6 × DBS/2)F1 mice produces invasive transitional cell carcinomas of the bladder that morphologically resemble their human counterparts (8, 9). When OH-BBN is given once a week for 10 weeks, 60% or more of mice develop bladder tumors within 6 months, depending on the dose.

Colon (Rat and Mouse)

1,2-Dimethylhydrazine will produce adenocarcinomas of the colon in both rats and mice when given i.p. It is activated in vivo first to AOM and then to the ultimate carcinogen MAM. In the rat model, a single s.c. dose of AOM given to 7-week-old Fisher 344 male rats produces adenomas and adenocarcinomas of the colon within 40 weeks (10, 11). Low dose and high dose AOM groups are used. In the mouse model, female CF1 mice are given MAM acetate i.p. four times in 11 days (low dose) and eight times in 22 days (high dose). Colon tumors are produced within 38 weeks after dosing (12).

Compounds Currently Being Tested for Chemopreventive Activity in Animal Models

The compounds listed below are currently being tested for chemopreventive activity by the Chemoprevention Branch, NCI. After the name of each compound, the animal tumor models in which it is being tested is indicated by the following abbreviations: S, mouse skin; M, rat mammary; L, hamster lung; C, mouse colon; B, mouse bladder.

β-Carotene (L,B) and 4-HPR (S,B)

See section below on compounds being tested in clinical trials.

Selenium Compounds: Selenite, Selenate, and Selenomethionine (S,M,L,C,B)

There is substantial evidence from both epidemiological studies (13) and many animal experiments (14) that both inorganic selenium, such as selenite, and organic selenium, such as selenomethionine, are chemopreventive. In February of 1985, the Chemoprevention Branch sponsored a workshop called "Strategies Needed to Develop Selenium Compounds as Cancer Preventive Agents." Many of the manuscripts presented at this workshop have been published (15). A committee formed from members of this workshop was able to reach a consensus that selenite and L-selenomethionine should be the forms of selenium selected for further development. Accordingly, the Chemoprevention Branch has implemented preclinical toxicological testing of selenite and L-selenomethionine.

Sodium Molybdate (S,M,L,C,B)

One epidemiological study has associated low levels of molybdenum exposure via food and/or drinking water with an increased rate of esophageal cancer (16). Two rat carcinogenesis studies indicate that sodium molybdate in the drinking water inhibits the appearance of esophageal and forestomach tumors and mammary tumors (16).
DHEA (S,C,B)

In a 10-year prospective study of 5000 healthy women, those with subnormal excretion of androsterone and etiocholanolone, two metabolites of DHEA, exhibited a significantly increased risk of breast cancer (17). Additionally, women with breast cancer were shown to have lower plasma levels of DHEA than normal women (17). There is also experimental data showing chemopreventive activity of DHEA in animal models for cancer of the skin (17), breast (18), colon (19), and lung (20). Furthermore, administration of DHEA to mice will prevent the binding of carcinogens to DNA in their skin (21). Thus there are indications that DHEA has chemopreventive activity against both the initiation and promotion phases of carcinogenesis. These effects appear to be the result of the strong and specific inhibition of glucose-6-phosphate dehydrogenase by DHEA, with associated lowering of the NADPH cellular pool. NADPH is a required cofactor for the activation of procarcinogens by cytochrome P-450 mixed-function oxidases and for the synthesis of deoxysphingosine-1,2-diols required for cell replication. DHEA can be metabolized into testosterone and estrogen, which leads to uterine enlargement in female rats and to seminal vesicle enlargement in male mice. It also produces hepatomegaly.

However, two synthetic analogues of DHEA, 16α-fluoroestradiol (27), rat bladder (28), and rat mammary gland (29). These effects appear to be the result of the strong and specific inhibition of glucose-6-phosphate dehydrogenase by DHEA, with associated lowering of the NADPH cellular pool. NADPH is a required cofactor for the activation of procarcinogens by cytochrome P-450 mixed-function oxidases and for the synthesis of deoxysphingosine-1,2-diols required for cell replication. DHEA can be metabolized into testosterone and estrogen, which leads to uterine enlargement in female rats and to seminal vesicle enlargement in male mice. It also produces hepatomegaly.

Flucinolone Acetonide (M,L,C,B)

Flucinolone acetonide was shown to be the most potent of a series of synthetic glucocorticoids for inhibiting tumor promotion in the mouse skin model (23).

DFMO (M,L,C,B)

DFMO is an enzyme-activated irreversible inhibitor of ornithine decarboxylase, and early enzyme in the synthetic pathway of polyamines such as spermidine and spermine, which have mitogenic and tissue growth-promoting effects (24). DFMO has been shown to be chemopreventive in mouse skin (25, 26), mouse colon (27), rat bladder (28), and rat mammary gland (29).

Piroxicam (C)

Piroxicam is the only oxicam approved by the FDA for the treatment of rheumatoid arthritis and osteoarthritis. It has antiinflammatory, antiproliferative, analgesic, and antipyretic properties, presumably based on the inhibition of prostaglandin synthesis. The inhibition by Piroxicam of rat colon tumors induced with MAM acetate has been reported in a preliminary study (30).

Sulfasalazine (C)

Sulfasalazine, which is composed of sulfapyridine conjugated to 5-aminosalicylate, is the most widely prescribed drug worldwide for the treatment for chronic ulcerative colitis. The mechanism for its beneficial effect in this disease has been suggested to be through the inhibition of prostaglandin synthesis in the colonic mucosa (31, 32). Thus, it is possible that sulfasalazine may suppress colon carcinogenesis in the same way as does indomethacin, another prostaglandin synthesis inhibitor (33).

Oltipraz (S,M,L,C,B)

Oltipraz is an antioxidant which is structurally related to diithiobiones found in cruciferous vegetables (34). It increases reduced glutathione levels in tissues and induces glutathione-S-transferase, which catalyzes the inactivation by glutathione of eutrophic carcinogenic compounds that are formed by the action of cytochrome P-450 mixed-function oxidases.

Ellagic Acid (M,M,C,B)

Ellagic acid is related to the coumarins, a subclass of lactones found in a wide variety of fruits and vegetables in the human diet. It specifically binds to and inactivates carcinogenic bay-region diol epoxides of benz(a)pyrene produced by the action of P-450 monoxygenase and thereby prevents them from forming mutational adducts to DNA. Because benz(a)pyrene is an important carcinogen that is ubiquitous in the environment, ellagic acid has potential for use in high risk groups chronically exposed to increased concentrations of benz(a)pyrene in the air, water, or food. Ellagic acid has been shown to inhibit carcinogen-induced neoplasia in mouse skin, rat mammary gland, and mouse forestomach (35, 36).

Benzylisothiocyanate (S,L,C)

Benzylisothiocyanate is one of the major isothiocyanate compounds found in cruciferous plants. It reportedly inhibits DMBA-induced mammary tumor formation in rats and benz(a)pyrene-induced tumor formation in the forestomach of the mouse (37), but was ineffective in preventing dimethylhydrazine-induced colon tumors in mice (38).

IC (S,L,C,B)

IC is one of three major indole components of cruciferous plants. It reportedly inhibits DMBA-initiated mammary tumors in rats and forestomach tumors in mice (39). The mechanism of action of IC is unclear. It increases the activity of rat liver aryl hydrocarbon hydroxylase and may therefore speed detoxification of procarcinogens by augmenting their solubilization and elimination. However, it should be kept in mind that increased aryl hydrocarbon hydroxylase (AHH) activity may also speed activation reactions that convert procarcinogens to ultimate carcinogens.

Results of Screening Candidate Chemopreventive Compounds in Animal Models

Table 1 depicts the results on some compounds currently being tested in the animal models described above. Preliminary data on some of the positive experiments are presented in Figs. 1, 2, and in Table 2. In Fig. 1, the effect of 4-HPR and tamoxifen on percentage incidence of rats with a second cancer following excision of the first mammary cancer is shown. The carcinogen was MNU administered i.v. at 50 days of age. Note that the combination of 4-HPR and tamoxifen was much more efficacious than either compound alone. Fig. 2 shows the effect of DFMO on the number of palpable tumors per rat in female Sprague-Dawley rats given DMBA intragastrically. Note the significant suppression of tumor formation when the DFMO is given during carcinogen administration (Schedule A), after
Table 1  Chemical compounds under test in animal models

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Skin</th>
<th>Breast</th>
<th>Lung</th>
<th>Colon</th>
<th>Bladder</th>
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<tr>
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<td>P</td>
<td>N*</td>
<td>N</td>
<td>N</td>
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<td>4-HPR</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Selenium compounds</td>
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<td>N</td>
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<td>N</td>
</tr>
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<td>X</td>
<td>N</td>
<td>P</td>
<td>P</td>
</tr>
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<td>P</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
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<td>X</td>
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<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Difluoromethylornithine</td>
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<td>P</td>
<td>P</td>
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<tr>
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<td>P</td>
<td>P</td>
<td>X</td>
<td>P</td>
<td>P</td>
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<td>Ellagic acid</td>
<td>P</td>
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<td>X</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Benzylisothiocyanate</td>
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<td>N</td>
<td>N</td>
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<tr>
<td>Indole-3-carbinol</td>
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<td>X</td>
<td>N</td>
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<tr>
<td>4-HPR &amp; tamoxifen</td>
<td>P</td>
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<td></td>
<td></td>
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<tr>
<td>4-HPR &amp; selenium</td>
<td>N</td>
<td>P</td>
<td>P</td>
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</tr>
</tbody>
</table>

* N, completed experiment with negative results; P, completed experiment with positive results; X, experiment in progress.

carcinogen administration (Schedule B), or both during and after carcinogen administration (Schedule C). In Table 2 is shown the effect of Piroxicam on azoxymethane-induced colon tumors in male F344 rats starting 1 week after carcinogen treatment. Note the dose-dependent suppression of tumor formation, expressed both as the number of animals with tumors, and the number of tumors per animal.

Compounds Being Tested in Clinical Trials

**Vitamin A, β-Carotene, and 4-HPR**

Many epidemiological studies have appeared to show an association between low dietary intake of vitamin A and the development of cancer, specifically, lung (40), oropharyngeal and laryngeal (41, 42), esophageal (43), gastric (44), colorectal (45), and prostate cancers (46). These studies are based on the dietary intake of vitamin A with foods that are also high in β-carotene, a precursor of vitamin A. An inverse association with lung cancer has been found for β-carotene, but not vitamin A (47).

Increased serum β-carotene levels (but not vitamin A) have been reported to be associated with decreased rates of lung cancer (48, 49) and gastric cancer (48). Two reports have appeared suggesting a possible association between dietary vitamin A intake and prostatic adenocarcinoma (50, 51), which, if confirmed, would limit the potential chemopreventive application of retinoids in older men. At this time, vitamin A derivatives and β-carotene are viewed by many as having the greatest potential for immediate application as chemopreventive agents in humans. For this reason, the Chemoprevention Branch is associated with a number of clinical trials of vitamin A, β-carotene, or both (see below). Derivatives of retinoic acid such as 13-cis-retinoic acid (isotretinoin) and 13-cis-ethylretinamide, though proved effective in animal model systems have demonstrated a level of toxicity that precludes their use except in subjects at higher risk of cancer. The Chemopreventive Branch is seeking to test newer retinoid derivatives which retain their chemopreventive activity but are less toxic. One such derivative, 4-HPR appears to fulfill these criteria (52) and is being tested in Phase I and Phase II clinical trials.

**Vitamin C**

In animals, vitamin C blocks nitrosation by reducing nitrate to the compound NO in the digestive tract, thereby preventing the reaction of nitrate with amines and amides to form carcinogenic nitrosamines and nitrosamides (53) and prevents the formation of tumors in bladders implanted with 3-hydroxyanthranilic acid (54). In humans, foods containing vitamin C showed a protective effect against development of cancers of esophagus (55), stomach (56) and cervix (57). Colonic polyps regressed with vitamin C therapy in one small study (58), were reduced in size in another (59), and appeared with decreased frequency after polypectomy in a third (60). Other studies supporting the effect of vitamin C in reducing cancer risk have been recently reviewed (61).

**Vitamin E**

Being fat soluble, the antioxidant vitamin E remains in the lipid compartment of cell membranes and quenches fat soluble free radicals that might otherwise produce promotional and mutational events. A recent review finds the evidence suggestive but not conclusive that vitamin E exerts a protective effect
against carcinogen-induced tumors (62). In humans, in both prospective and case control studies, no relationship was found between vitamin E blood levels and risk for cancer (63, 64). However, low vitamin E levels have correlated with increased risk of cancer of breast (65), lung (66), and intestine (67), but not ovarian cancer (68).

Calcium

In animals, increased dietary calcium suppresses the hyperproliferation of colonic epithelium induced by bile salts and fatty acids (69–71), and exerts an antipromotional effect which decreases the yield of carcinogen-induced tumors of the colon (72, 73). In humans, diet supplementation with calcium reduces colonic cell hyperplasia and increases the number of maturing crypt cells in subjects with familial colon cancer patients. These findings appear to justify intervention trials which measure the effect of dietary calcium supplementation on colon risk in patients with familial polyposis.

Vitamin B12 and Folate

Administration of oral folic acid was associated with improvement of cervical dysplasia in users of oral contraceptives (74). The potential of folic acid to inhibit cancer development was analyzed during chemical carcinogenesis of the hamster buccal pouch (75).

Chemoprevention of Clinical Trials

The predictive value to humans of animal model results is uncertain and can only be resolved by clinical trials. Epidemiology studies also lack specificity in that the studied populations differ in several factors and it may not be apparent which factor is etiologically most important. The only approach to determine the efficacy and safety of these agents in humans, is through the conduct of clinical intervention trials (76, 77).

Initial trials are intended to provide information on both toxicological and pharmacological aspects of the agent in humans. The dose level for a chemoprevention clinical trial is chosen to select high risk lung cancer populations include dysplastic sputum cytology (one study), smoking history (three studies), and asbestos exposure (three studies). The agents that are included are folie acid/vitamin B12, retinol, β-carotene, 13-cis-retinoic acid, and etretinate. Five studies are concerned with prevention of skin cancer, the precancerous condition actinic keratosis, and a specific population group which is at high risk for skin cancer, Albinos in equatorial Africa. The spectrum of agents being evaluated in these studies includes β-carotene, retinol, and 13-cis-retinoic acid, and selenium.

There are four studies on prevention of colon cancer. Selection factors are either familial polyposis or adenomatous polyps. The agents being studied include β-carotene, vitamins C and E, calcium and fiber, and piroxicam. One study concerned with breast cancer prevention is designed to evaluate the effectiveness of 4-HPR in preventing new primary lesions of the contralateral breast in women who have had surgery for breast cancer. The incidence of contralateral breast cancer is about 0.8% per year and a group of approximately 5000 subjects are being recruited to test the effectiveness of the agent in reducing the rate of a second primary in the opposite breast. One study on cervical cancer involves topical application of retinoids and another the systemic administration of folic acid.
Participants are selected on the basis of dysplasia seen on Pap smear.

Considerations Regarding Selection of Chemopreventive Compounds in Relation to Their Mechanism of Action and to Current Models of Carcinogenesis

During the course of selecting candidate chemopreventive compounds for further development, we had to consider questions concerning mechanisms of carcinogenesis because we wanted to obtain a balanced selection that would act at many different points in the neoplastic process. Should we, for instance, choose an equal number of "antiinitiators" and "antipromoters," or should we follow the conclusion reached by a committee of the National Research Council in 1982, that "... only the young would benefit if we removed the initiators from our environment," appears to be a direct result of animal experiments involving skin painting with a carcinogen, using initially the rabbit ear (78, 79) and later mouse skin (80, 81).

The Single-continuum Model

There is another well-known but less considered model of carcinogenesis, which may be called the “Single-continuum” Model, that is based on the landmark observations of human cancer biology at the clinical and histological level, and of the mouse mammary tumor (82), made by Lester Foulds. It may not be adequately appreciated that the term “progression” was first used by Foulds to describe the entire neoplastic process as a single continuum from its earliest inception. Only later was the term “progression” adapted for use with more restrictive meaning as the name of a “step” in the Multistep Model.

The Clonal Evolution Model

The clonal evolution of human tumors at the chromosomal level described by Nowell (83) consists of an initial mutation and clonal overgrowth followed, possibly slowly at first, by repeated mutations and selective clonal overgrowths leading to a multiclonal population with expanding phenotypic diversity that is increasingly able to escape growth controls and finally invade the surrounding normal tissue, i.e., to behave in a malignant manner.

With regard to the question of the relative emphasis to be placed on the selection of “antiinitiators” versus “antipromoters,” the conclusion of the National Research Council referred to above, that “... only the young would benefit if we removed the initiators from our environment,” appears to be a direct
inference from animal experiments involving the one-time initial application of a mutagenic carcinogen, and is difficult to apply to many, if not most, types of human experience where there is continuous or repeated exposure to initiators, i.e., mutagens, of variable type and dose over a lifetime.

In the literature describing the Multistep Model, we found that the boundary between the “promotion” step of premalignant tumors and the “progression” step of malignant ones, as it applied to human carcinogenesis, was obscure. Indeed, an entire symposium has recently been dedicated to this question (84). To us this distinction was especially important, because the development of “antipromoters” would be considered chemoprevention, but the development of “antiprogrossors” would be chemotherapy and out of our purview, according to the Multistem Model.

On the other hand, in the “Single-continuum” Model of Foulds, which is based on clinical and histological studies of human as well as experimental animal tumors, no such problem of “boundaries” arises because there are no boundaries. We eventually found that Fould’s “Single-continuum” Model and Nowell’s “Clonal Evolution” Model were more applicable than the “Multistem” Model as an aid in selecting candidate chemopreventive compounds.

References


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