

Chemoprevention of cancer

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In this short article, we review the conceptual basis for chemoprevention of cancer, the proven clinical efficacy of this concept, and current trends to develop new chemopreventive agents based on understanding of their mechanisms of action. Four classes of new agents, namely selective inhibitors of cyclooxygenase-2, selective estrogen receptor modulators, rexinoids (retinoids that bind selectively to the receptors known as RXRs) and ligands for the peroxisome proliferator-activated receptor- γ are discussed in detail. The importance of developing totally new classes of chemopreventive agents is stressed, with particular emphasis on the potential usefulness of new synthetic triterpenoids derived from naturally occurring molecules.

Introduction

The continuing magnitude of the cancer problem, and the failure of conventional chemotherapy of advanced invasive disease to effect major reductions in the mortality rates for the common forms of epithelial malignancy, such as carcinoma of the lung, colon, breast, prostate and pancreas, indicate that new approaches to the control of cancer are critically needed. Even though great advances have been made in basic scientific knowledge relating to cancer, as well as in the clinical treatment and cure of some malignancies (such as certain leukemias and lymphomas), the fact remains that the National Cancer Institute's stated goal of a 50% reduction in overall cancer mortality by the year 2000 has not been met (1). If anything, death rates from some of the common cancers continue to rise.

In this context, it is essential that we re-evaluate our basic assumptions about the nature of cancer, and begin to adopt a more intensive and imaginative approach to the prevention of this disease. Presently, both basic and clinical research on cancer are driven by the elusive goal of cure of advanced disease (the mythology of a magic bullet), an approach that is often unrealistic because of the genetic heterogeneity and extent of the tumor burden characteristic of late stage malignancy. Given the genotypic and phenotypic heterogeneity of advanced malignant lesions as they occur in individual patients, one wonders just exactly what are the specific molecular and cellular targets for the putative cure. A lesion that is anatomically defined in reality will contain many different types of cells, each with its own phenotype and genotype. As a consequence, thoughts that we will eventually treat invasive

cancer with some sort of single gene therapy seem pathetically naive. Which gene in which cell shall we fix?

Furthermore, the misperception of cancer as a disease whose most fundamental characteristic is excessive cell proliferation has led to an over-emphasis of testing and development of cytotoxic drugs that kill cancer cells. Unfortunately, most cytotoxic drugs used in cancer chemotherapy are also highly toxic to a wide spectrum of normal tissues, such as those found in the gastrointestinal tract, bone marrow, heart, lungs, kidney and brain; iatrogenic failure of these organs is a frequent cause of death from cancer.

As an alternative approach, we need to consider that cancer is ultimately the end stage of a chronic disease process characterized by abnormal cell and tissue differentiation. This process, which eventually leads to the final outcome of invasive and metastatic cancer, is carcinogenesis. We need to focus more effort on the control of carcinogenesis, rather than attempting to cure end-stage disease. Thus, our common sense tells us that it is easier to fix anything when the smallest numbers of its components are broken; unfortunately, this intuition has been ignored in our allocation of resources to deal with the cancer problem.

Chemoprevention, which is a pharmacological approach to intervention in order to arrest or reverse the process of carcinogenesis, attempts to address the issues outlined above. Although the carcinogenic process may be driven by mutation, there are clearly many epigenetic variables, particularly those relating to the action of autocrine, paracrine and endocrine regulatory molecules, which can also be important determinants during the 20 year (or more) latent period before invasion and metastasis occur. Pharmacologic modulation of these regulatory pathways, over and above the effective use of drugs and micronutrients that block mutagenic damage to DNA, thus offers great potential for prevention of cancer. In this short review, we will highlight some of the accomplishments that have already been made in the field of chemoprevention, as well as suggest some new opportunities for future research. This brief review is not intended to be comprehensive; for more detailed summaries, the reader is referred to recent surveys by Kelloff (2), Lippman *et al.* (3) or the timely report by the Chemoprevention Working Group to the American Association for Cancer Research (4).

Proven clinical efficacy of chemoprevention

The credibility of chemoprevention as a serious and practical approach to the control of cancer has been greatly enhanced by the publication, within the past 2 years, of the results of three major randomized clinical trials in the field of breast cancer. Three different agents, namely tamoxifen (5), raloxifene (6) and 4-hydroxyphenylretinamide (fenretinide) (7) have been shown to be effective agents for prevention of breast cancer in women of varying degrees of risk. The most definitive study, with tamoxifen, involved more than 13 000 women of relatively high risk, although they were free of detectable

Abbreviations: COX, cyclooxygenase; PPAR- γ , peroxisome proliferator-activated receptor- γ ; RAR, retinoic acid receptor; RXR, retinoid X receptor; SERM, selective estrogen receptor modulator.

invasive breast cancer at their entry into the study. Tamoxifen not only significantly reduced the subsequent occurrence of breast cancer in the treated group as a whole, but remarkably, it was effective in women with a history of lobular carcinoma *in situ* or atypical hyperplasia, indicating that this agent can prevent the further progression of established preneoplastic lesions in the breast.

The second trial, involving more than 7000 post-menopausal women with known osteoporosis (but with no specific risk factors for breast cancer other than age), showed that raloxifene dramatically decreased risk of development of breast cancer. The third trial, with fenretinide, was conducted in a cohort with particularly high risk, namely women who had undergone surgery for a previous breast cancer and who thus were at exceptional risk for development of a second (primary) breast malignancy. Although fenretinide treatment had no statistically significant effect in the cohort as a whole on the incidence of a second breast malignancy, the subgroup of pre-menopausal women treated with fenretinide had a significantly lower incidence of new breast cancers than the corresponding control group. Since pre-menopausal breast cancer is often characterized by particularly aggressive invasive and metastatic behavior and is therefore particularly difficult to manage, these results with fenretinide are of special interest.

The importance of the above three trials for the future of the entire field of chemoprevention cannot be overstated. They are landmark studies for several reasons. First of all they clearly demonstrate that the most common form of cancer in women can be controlled by a preventive approach. Secondly, they demonstrate that a rational approach to prevention, based on the use of agents whose mechanism of action is understood, can be used to develop drugs for chemoprevention. Thus, both tamoxifen and raloxifene are estrogen receptor antagonists in the breast, an organ in which estrogen is a known promoting agent for carcinogenesis. Tamoxifen and raloxifene bind to both estrogen receptors (ER- α and ER- β). Furthermore, tamoxifen and raloxifene had been conclusively shown to prevent breast cancer in experimental animal models before they were used clinically for this purpose (8–10). Finally, in terms of their clinical utility, they are good examples of the selective estrogen receptor modulator (SERM) concept, in that they can act selectively in different tissues and organs either as estrogen antagonists (to suppress the undesirable cancer-promoting effects of estrogen in the breast) or as estrogen agonists (to enhance the desirable growth-promoting effects of estrogen in bone). With respect to its overall SERM profile, raloxifene appears to be the superior agent, since it is not uterotrophic and does not cause endometrial carcinoma, as does tamoxifen. However, the relative efficacy of tamoxifen and raloxifene in preventing breast cancer in a single clinical trial, in which they are directly compared, remains to be determined; this is presently underway in the 'STAR' trial sponsored by the National Cancer Institute.

The third agent, fenretinide, is a synthetic retinoid which was shown many years ago to be a differentiating agent for tracheobronchial and mammary epithelium, as well as to be an effective drug for preventing experimental breast cancer (11). There is abundant literature on the successful use of retinoids to prevent cancer at many epithelial target sites in experimental animals, as well as in humans (12,13). Interestingly, fenretinide and tamoxifen act synergistically in suppressing mammary carcinogenesis in rats (14), although the

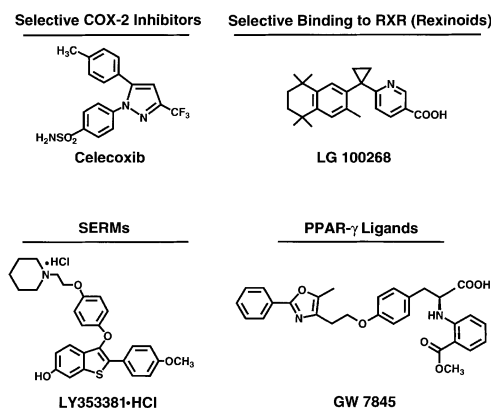


Fig. 1. Representative members of four classes of new chemopreventive agents, whose mechanism of action is known.

efficacy of this combination for prevention of breast cancer in women remains to be determined.

The major benefits that have been demonstrated clinically for tamoxifen, raloxifene and fenretinide are in marked contrast to the unfortunate results that have been obtained with β -carotene in several prevention trials (15–17). The failure of the β -carotene trials represents a good example of the results of entering putative chemopreventive agents into clinical trials before there are adequate mechanistic and animal data to support their use in men and women. In the case of β -carotene, there were essentially no data from animal studies that this agent would prevent any important form of cancer in an experimental animal. The impetus to use β -carotene as a chemopreventive agent came from epidemiologic studies, and, even in these studies, there was no direct evidence that β -carotene, rather than a large number of other substances occurring in fruits and vegetables, might be responsible for any benefit (18). Thus, it is essential to realize that epidemiological data alone do not provide a sufficient basis for the selection of a new agent for a major clinical chemoprevention trial. The future of this field will depend on the development of new agents whose mechanism of action is well understood, and we will now turn to a discussion of this problem.

Chemoprevention based on mechanism

The appropriate use of a chemopreventive agent ultimately depends on the understanding of its mechanism of action at all levels, namely at the molecular, cellular, tissue and organ levels, as well as in the animal as a whole. Without this knowledge we can only make intuitive decisions in selecting preventive agents and hope that a useful clinical result will be forthcoming. The trend in the field of chemoprevention has therefore been to develop new agents based on their mechanism of action, and we will mention four such categories of new agents, which hold great promise for eventual clinical use (Figure 1).

Agents in the first category are selective inhibitors of inducible cyclooxygenase (COX-2), which is a key enzyme responsible for the synthesis of inflammatory prostaglandins from arachidonic acid. Although the concept that inflammation and carcinogenesis are related processes is by no means new (19), the immediate relevance of this notion to the world of modern molecular genetics was demonstrated by the finding that overexpression of the gene for COX-2 is an early and important event in colon carcinogenesis (20) and that knockout

of the gene for COX-2 could suppress colon carcinogenesis in mice that were genetically predisposed to develop this condition (21). This in turn has led to the development of new pharmacologic agents, such as celecoxib (Figure 1), that are selective for inhibition of the enzymatic activity of COX-2, while not affecting the constitutive form of the enzyme (COX-1). Celecoxib has been shown to prevent colon carcinogenesis caused by azoxymethane in a standard rat model (22), and is now in clinical trial in cohorts of patients at high risk, such as those with familial adenomatous polyposis or hereditary non-polyposis colorectal cancer syndromes. Hopefully, agents that are selective for COX-2 inhibition will be much better tolerated during prolonged chronic administration than the non-selective non-steroidal anti-inflammatory drugs (including aspirin) that have been used in the past. The recent demonstration of the overexpression of COX-2 in many other forms of epithelial cancer (23–26) now also offers promise that selective COX-2 inhibitors might be used to suppress carcinogenesis in organs other than the colon.

A second category of important new agents are the SERMs. Although tamoxifen and raloxifene are both in clinical use, they represent agents that were synthesized >20 years ago, before the cloning of the estrogen receptors, and before structural biology has allowed us to make accurate molecular models of the binding of estrogen and estrogen analogs to these receptors. The demonstration of the occurrence of ER- β , as contrasted with ER- α , in the prostate, colon and ovary also suggest that it may be useful to develop estrogen analogs that will be selective for binding to this isoform (27). The estrogen receptor in the prostate is a particularly interesting target for chemoprevention, and indeed it has already been shown that tamoxifen is a highly effective agent for suppression of experimental prostatic carcinogenesis in the rat (28). Many new SERMs have been synthesized, but they have yet to be used effectively for chemoprevention. One agent that is highly promising is LY353381.HCl (Figure 1), which is significantly more potent than raloxifene in many assays, including prevention of breast cancer induced in the rat with nitrosomethylurea (NMU) (29).

The retinoids that are selective for binding to the three retinoid X receptors (RXRs), whereas not binding to the three retinoic acid receptors (RARs), are now called 'rexinoids' (30), and represent a third important new category for chemoprevention. The RXRs are of particular importance in the nuclear receptor superfamily because of their ability to heterodimerize with many other members of this family, including the RARs, the vitamin D receptor, the thyroid receptor, as well as relatively newly discovered 'orphan' receptors, such as peroxisome proliferator-activated receptor- γ (PPAR- γ), liver X receptor (LXR) and farnesoid X-activated receptor (FXR) (31–33). RXRs thus play a central, integrative role in the cellular physiology of the nuclear receptor superfamily by virtue of their ability to modulate the activity of many other receptors. It is therefore not surprising that rexinoids have been found to be potent chemopreventive agents in experimental animals. The first of the rexinoids to be used for this purpose was LGD1069 (targretin) for prevention of mammary carcinogenesis in the rat (34). However, targretin is not totally specific for binding to RXR, and at high concentrations it will bind to the RARs. Therefore new rexinoids, which have essentially no affinity for RARs, have been synthesized; LG100268 (Figure 1) is a prototype of this new class; it is highly effective in the rat mammary carcinogenesis prevention model.

Interestingly, the new rexinoids do not have the classic toxicologic profile of typical retinoids; thus they do not elicit the same sort of mucocutaneous toxicity, and they are very weak teratogens.

The orphan nuclear receptor, PPAR- γ , represents another new target for development of ligands for chemoprevention. PPAR- γ is now an important target for development of drugs that are used to treat type 2 diabetes, such as the thiazolidinediones (TZDs), because ligands for PPAR- γ can sensitize cells to the adipogenic action of insulin (35,36). Because of the ability of PPAR- γ to bind both fatty acids and prostaglandins, it has also become a target for investigation in colon carcinogenesis. Recent studies have led to conflicting results, with two studies (37,38) showing that the TZD, troglitazone, enhanced colon carcinogenesis in *Min* mice (in these mice there has been loss of a single copy of the tumor suppressor *APC* gene), whereas a third study (39) indicated that in cell culture troglitazone suppressed the growth of human colon cancer cells as well as the expression of several genetic markers associated with colon cancer. Furthermore, other studies have indicated that troglitazone can induce differentiation in human liposarcoma cells (40).

Because the ability of ligands for PPAR- γ to suppress experimental mammary carcinogenesis had not been investigated, we chose to study the activity of a member of a new class of PPAR- γ ligands (tyrosine analogs) in the standard rat system that uses NMU as carcinogen. Accordingly, we have shown recently (41) that the new agent, GW7845 (Figure 1), which is significantly more active as a PPAR- γ ligand than troglitazone, is highly effective in preventing breast cancer in the rat. Ligands for PPAR- γ have been shown to have marked synergism with rexinoids in treatment of experimental diabetes (30). It would now seem reasonable to suggest that similar synergisms between these two classes of agents in chemoprevention of cancer will also be found. Indeed, it will be surprising if useful chemopreventive synergisms do not exist among all four classes of agents shown in Figure 1.

Thus, combination chemoprevention, whereby one achieves significant synergism of two or more drugs to obtain a desired preventive effect, while minimizing the toxic side effects of the individual components of the combined regimen, represents an important challenge in this entire field. There are numerous examples of such synergy in experimental chemoprevention of cancer (2,4,13), and it is likely that the future practical development of chemopreventive regimens will rely on the use of this principle.

Development of new classes of chemopreventive agents

The need for new agents with novel mechanisms of action to prevent cancer is perhaps the most urgent need in the entire field of chemoprevention. Although proof of principle of chemoprevention has been clearly demonstrated, in both animal and clinical studies, none of the existing chemopreventive agents is ideal, either because of lack of efficacy and potency, or because of toxic side effects that preclude widespread, long-term use. Discovery of new agents is therefore of vital importance. Overall strategies for new drug discovery in chemoprevention have been reviewed at length elsewhere (2,4), and we will not discuss the broader aspects of this problem here. Rather, as a single example of approaches that can be taken in this general area, we will briefly summarize some recent attempts in our own laboratory to develop a

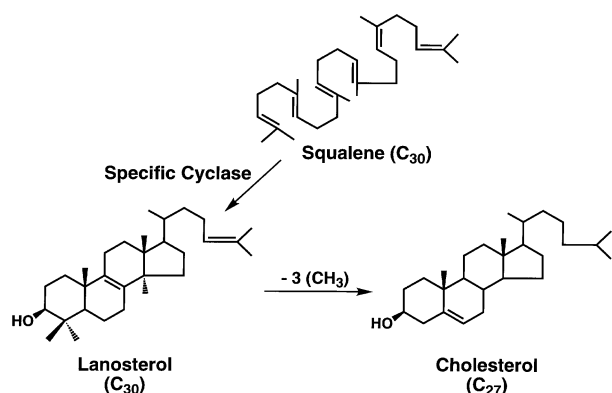


Fig. 2. Simplified diagram of biosynthesis of cholesterol from squalene. Not shown are the epoxidation of squalene to 2,3-oxidosqualene and the 19 enzymatic steps for the conversion of lanosterol to cholesterol.

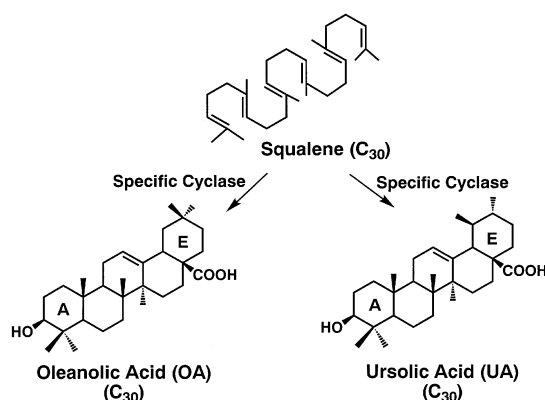


Fig. 4. Oleanolic and ursolic acids, which have been used as chemopreventive agents, are both derived from squalene. Note that the two structures differ only in the location of the two methyl groups in the E-ring.

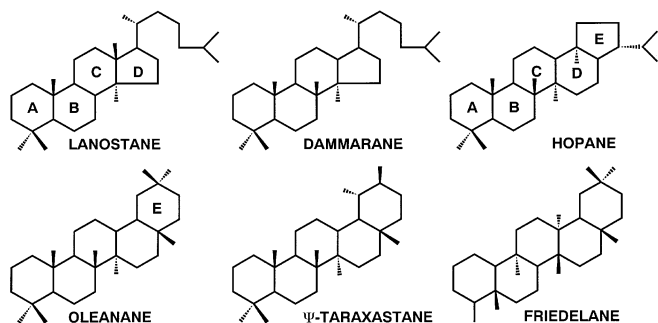


Fig. 3. Six patterns for the cyclization of squalene are shown here; numerous other variations exist in nature.

new class of chemopreventive agents. These new agents are triterpenoids that, together with their close chemical relatives, the steroids, are members of a larger family of related structures that we would like to call cyclosqualenoids.

In the bacterial, plant and animal worlds, squalene, which is an open-chain 30-carbon isoprenoid molecule, is cyclized (either directly, or via its metabolite, 2,3-oxidosqualene) to form tetracyclic or pentacyclic derivatives. There are at least 20 different folding patterns resulting from this enzymatic cyclization, and each pattern of folding is believed to be catalyzed by a unique enzyme (or set of enzymes), known as cyclases. The most familiar of these cyclization schemes is that which converts squalene to the proximate tetracyclic 30-carbon molecule, lanosterol (Figure 2), which can then be further oxidatively and catabolically metabolized to the precursor of all the common steroids, namely cholesterol. However, this well known cyclization of 2,3-oxidosqualene to lanosterol represents only a small fraction of the cyclosqualenoid diversity that exists in nature. Thus, in bacteria and plants there exist thousands of tetracyclic or pentacyclic derivatives of squalene that are not derived from lanosterol (42,43). This huge set of cyclosqualenoid molecules has been derived from squalene by the initial action of a diverse set of cyclases (distinct from the oxidosqualene-lanosterol cyclase), each of which contributes to the total diversity of the folding patterns that are obtained. We have chosen to show only six patterns for the folding of squalene in Figure 3, but this small example illustrates a few important points. We have included hopanoids to emphasize the evolutionary age of the folding of squalene, since molecules with a hopane folding pattern are found in primitive bacteria (43). We have included the

dammarane, taraxastane, oleanane and friedelane patterns to emphasize the use of various cyclosqualenoid molecules derived from plants as medicinal or antimicrobial agents, as documented at length (42). For our own research in this area, we have focused on derivatives of either oleanolic acid and ursolic acid (Figure 4), since both these agents have been shown to have definite anti-inflammatory and anti-carcinogenic activities (44,45), although their potency and efficacy are relatively weak. Our underlying hypothesis has been that the inflammatory process and the carcinogenic process are mechanistically related (19), and that it should be possible to design agents for chemoprevention of cancer, based on their ability to block the *de novo* synthesis of two key anti-inflammatory enzymes, namely inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2).

We have therefore undertaken a program to synthesize analogs of ursolic and oleanolic acid, and to assay these new derivatives for their ability to suppress the expression of the genes for iNOS and COX-2. Many of these new analogs are significantly more potent than either ursolic or oleanolic acid (46), and one of them, 2-cyano-3,12-dioxolean-1,9-dien-28-oic acid has shown exceptional activity; it is 1000 times more potent than oleanolic acid in primary mouse macrophages in suppressing the production of nitric oxide induced by interferon- γ (47). This new agent also shows interesting activity in differentiating a variety of human leukemia cells, as well as blocking the growth of many human cancer cells. Its use as a chemopreventive agent *in vivo* is presently being evaluated.

There is no question that the entire family of cyclosqualenoids represent a fruitful area for future synthesis and development of new chemopreventive agents. The parent structures that are found in plants have been in the biosphere for millions of years, and it is highly likely that evolution has led to some molecular fit of these agents as ligands for receptors that are found in animals. The development of this area on a mechanistic basis remains an intriguing challenge for the future.

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