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Review

Current Mechanistic Approaches to the Chemoprevention of Cancer

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The prevention of cancer is one of the most important public health and medical practices of the 21st century. We have made much progress in this new emerging field, but so much remains to be accomplished before widespread use and practice become common place. Cancer chemoprevention encompasses the concepts of inhibition, reversal, and retardation of the cancer process. This process, called carcinogenesis, requires 20-40 years to reach the endpoint called invasive cancer. It typically follows multiple, diverse and complex pathways in a stochastic process of clonal evolution. These pathways appear amenable to inhibition, reversal or retardation at various points. We must therefore identify key pathways in the evolution of the cancer cell that can be exploited to prevent this carcinogenesis process. Basic research is identifying many genetic lesions and epigenetic processes associated with the progression of precancer to invasive disease. Many of these early precancerous lesions favor cell division over quiescence and protect cells against apoptosis when signals are present. Many oncogenes are active during early development and are reactivated in adulthood by aberrant gene promoting errors. Normal regulatory genes are mutated, making them insensitive to normal regulatory signals. Tumor suppressor genes are deleted or mutated rendering them inactive. Thus there is a wide range of defects in cellular machinery which can lead to evolution of the cancer phenotype. Mistakes may not have to appear in a certain order for cells to progress along the cancer pathway. To conquer this diverse disease, we must attack multiple key pathways at once for a predetermined period of time. Thus, agent combination prevention strategies are essential to decrease cancer morbidity. Furthermore, each cancer type may require custom combination of prevention strategies to be successful.

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Mechanisms of cancer chemoprevention can be classified into two major types: antimutagic (Table 1) and antiproliferative (Table 2). Antimutagens can act to inhibit carcinogen uptake. For example, calcium can block bile acid uptake into colonic epithelial cells (Wargovich *et al.*, 1983; Newmark *et al.*, 1984). Also there are a large number of chemicals which can modulate the activity of the cytochrome P450 enzymes (Wolf *et al.*, 1996). These agents can block the activation of procarcinogens by a wide variety of mechanisms (Hecht *et al.*, 2002). Antimutagens can also block carcinogen activation by cyclooxygenases and lipoxygenases. Some agents can enhance conjugation or binding of activated carcinogens to glutathione or other molecules to inactivate them and facilitate their removal. Other agents can block the binding of activated carcinogens to DNA thus reducing the formation of DNA adducts (Garcia *et al.*, 2000; Egner *et al*., 2001). The removal/repair of DNA adducts is the process which typically leads to the bulk of the DNA defects.

Antioxidants such as the polyphenols found in green and black teas have the potential to absorb free electrons and thus quench free radicals (Steele *et al.*, 2000). Free radicals can damage DNA by forming adducts such as 8-deoxyguanosine.

DNA synthesis itself is a very complex process requiring a multitude of factors, cofactors, enzymes, co-enzymes, protein synthesis, energy, repressors and co-repressors, repair enzymes, ligases, polyamines and nucleotides, winding and unwinding machinery, nucleases, error checking enzymes, and many more molecules. This process is required to correct defects and reproduce the DNA strand correctly. If this process does not proceed correctly then either the cell will quiesce, sensece, or replicate uncontrollably.

The progression of clonal evolution in epithelial cancers is well documented and described (Boone *et al.*, 1992; Altucci and Gronemeyer, 2001). There are quite a large number of diverse mechanisms which could effectively block or retard

Table 1. Antimutagenesis mechanisms, possible targets, and promising agents

Mechanisms	Targets	Agents
Inhibit activation of carcinogens	Cytochrome P450	Tea, indole-3-carbinol
	Cyclooxygenases	NSAIDs, celecoxib
Detoxify activated carcinogens	Glutathione-*S*-transferase	Oltipraz, *N*-acetylcysteine
Inhibit carcinogen uptake	Bile acids	Calcium, usodiol
Increase fidelity of DNA repair	Poly(ADP-ribosyl)transferase	NAC, protease inhibitors
Inhibit free radical DNA damage	Free radicals	Tea, soy isoflavones

Table 2. Antiproliferative mechanisms, possible targets and promising agents

Mechanisms	Targets	Agents
Induce apoptosis	Caspase activity	Retinoids
Inhibit angiogenesis	VEGF expression	Retinoids, tamoxifen, NSAIDs
Inhibit polyamine synthesis	Ornithine decarboxylase	Difluoromethylornithine
Inhibit oncogene activity	Farnesyl transferase	Perillyl alcohol, FTIs
Induce terminal differentiation	TGF beta	Retinoids, vitamin D
Inhibit DNA synthesis	Topoisomerase II	Oltipraz, genistein
Balance DNA methylation	Methyl transferase enzyme	Folic acid, budesonide
Modulate growth hormone activity	Estrogen receptor	Tamoxifen, soy isoflavones
	Androgen receptor	Flutamide
	Aromatase	Vorozole, arimidex
	5-alpha Reductase	Finasteride, eprosteride

the progression phase of the carcinogenesis process. One simple process is to induce terminal differentiation, thereby removing the cell from replicating pool and producing more progeny. Retinoids are key examples of agents which induce terminal differentiation in epithelial cells (Sun and Lotan, 2002). Other agents include sodium butyrate and vitamin D.

Cell proliferation can be induced by the inflammatory process. A major pathway in this process to produce cytokines is the arachidonic acid metabolism pathway . Arachidonic acid metabolism diverges down two main pathways, the cyclooxygenase (COX) and the lipoxygenase (LOX) pathways. The COX pathway leads to prostaglandin and thromboxane production and the LOX pathway leads to the leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs). These classes of inflammatory molecules exert profound biological effects which enhance the development and progression of human cancers. A large number of synthetic drugs and natural products have been discovered that block many of these key pathways. Much experimental evidence in animals has shown that inhibition of the key enzymes which drive these pathways can, in fact, prevent, slow or reverse the cancer process. There are a number of potential COX activities which could be target mechanisms for chemoprevention strategies. Through its peroxidase activity, COX can activate many procarcinogens into ultimate carcinogens. As an example, COX can oxidize the dihydodiol of benzo[a]pyrene to its epoxide form , which is believed to be the ultimate carcinogen capable of binding to DNA and producing mutations. The same COX enzymes can also activate aromatic amines to their active mutagenic form. COXs can also indirectly produce free radicals which activate c-*fos*, c-*myc*, and c-*jun* and thus stimulate cell proliferation which fosters the progression of the cancer process (Chen *et al.*, 1994; Ristimaki *et al.*, 1994). Inhibiting COX-2 activity in particular has also been linked to a number of anticancer mechanisms. For example COX-2 inhibition can cause reductions in angiogenic activity (Prescott and White, 1996) and can induce apoptosis in colon cancer cells and other tissues (Dubois *et al.*, 1994). Reducing prostaglandin production may also stimulate the immune system (Hempel *et al.*, 1994). The data are convincing in a number of organ sites including colon, breast, lung, bladder and skin.

Likewise the inhibition of LOX activity may have profound anticancer effects. Since LOX-produced 12(S)-HETE is a critical intracellular signaling molecule for PKC and cytokines, as stated above, gene products needed for neoplastic cell growth may be absent or greatly reduced. LOX inhibition of 12 (S)-HETE levels may reduce the production of cathepsin B, a cysteine protease involved in tumor metastasis and invasion (Smith *et al.*, 1996) and reduce cell adhesion by reducing the surface expression of integrin receptors (Williams and DuBois, 1996).

One key oncogene activation pathway that has been exploited recently in the prevention and therapy areas (Scharovsky *et al.*, 2000). The *ras* oncogene protein requires farnesylation to activate it. Drugs which can inhibit the farnesylation enzyme from acting blocks the activity of this protein in stimulating aberrant cell proliferation. A number of

natural and synthetic agents block this farnesylation activation process. Limonene and perillyl alcohol from citrus fruits can block the farnesylation pathway by decreasing the availability of farnesol. Synthetic proteins can also bind to the farnesylation activation site and block activation.

The modulation hormone activity is a major target for cancer chemoprevention. Selective estrogen receptor modulators (SERMs) have both agonist and antagonist properties. Agonist properties of SERMs are useful against cardiovascular disease and osteoporosis, while endometrial and breast cancer treatment requires the antagonist properties. Currently the NCI is sponsoring human clinical trials using SERMs such as tamoxifen, raloxifene, and arzoxifene. One of tamoxifen's serious side effects is an increased rate of endometrial cancer. The number of cases are few, but they are serious. Newer SERMs, such as raloxifen and arzoxifen, have minimized this problem. Other SERMs under development include: Faslodex [ICI 182,780] (Robertson, 2001), GW5638 (Dardes *et al.*, 2002), and lasofoxifene (Cohen *et al.*, 2001). Steroid aromatase inhibitors block estrogen biosynthesis and thus modulate the estrogen levels in the body (Kelloff *et al.*, 1998).

The inhibition of new blood vessel growth, called angiogenesis, can prevent the growth or progression of a benign tumor. New blood vessel growth is necessary for the nourishment of cells within neoplasms multiple cell layers deep. Thus, disrupting tumor-associated blood vessel formation by targeting endothelial cells, should inhibit tumor growth with little of no systemic toxicity or cancer cell-associated drug resistance. Voest (1996) suggested that antiangiogenic activity of chemopreventive agents may prove to be a novel approach to preventing cancer progression. Several studies have suggested that the vascular endothelial growth factor (VEGF) receptor may be a new target for blocking angiogenesis. There was a strong correlation between the microvessel density in cervical intraepithelial neoplasia and VEGF expression (Dobbs *et al.*, 1997). A recent study by Sharma *et al*., (2001) has shown that several classes of agents, including nonsteroidal antiinflammatory drugs (NSAIDs), retinoids and SERMS have antiangiogenic activity.

A novel way slow the progression of cancer is to select chemopreventive agents based on their anti-topoisomerase II activity. Topoisomerases I and II are enzymes involved in DNA synthesis. Topoisomerase I is the cancer therapy target of camptothecin and its analogues. Such agents are essentially cell poisons and could not be used in a prevention setting. However catalytic topoisomerase II inhibitors are potential chemopreventives due to their antiproliferative and cell differentiating activities governed by nontoxic mechanisms. Recent studies have shown the topoisomerase II inhibitors were effective chemopreventive agents in whole animal studies with a positive predictive value of 91% (Cho *et al.*, 2000). Examples of topoisomerase II inhibitors include thiones, such are oltipraz, phenols, such as genistein and ellagic acid, and other agents, such as indole-3-carbinol and sulforaphane.

In normal tissue about 5% of the DNA is methylated as 5-methylcytosine, while in tumor tissue of various types the methylation of DNA is decreased by 30% or more. The DNA hypomethylation of oncogenes or hypermethylation of tumor suppressor genes are alternations found in dysplastic tissues that might be reversed by chemopreventive agents. In a mouse lung cancer model investigators found that IGF-II and *myc* genes were hypomethylated (Tao *et al.*, 2002). Budesonide, a glucocorticoid, given in the diet significantly reduced the cancer incidence in mice treated with vinyl carbamate. At the same time budesonide reduced the expression of IGF-2 and *c-myc* genes and increased methylation of their promoters. Alteration in methylation patterns could be used as surrogate endpoint biomarkers in clinical trial without having to follow cancer progression to a invasive tumor endpoint.

A wide variety of mechanisms can be activated or inactivated to induce apoptosis or programmed cell death. Caspase activity can be reduced by exposure to retinoids. Arachadonic acid metabolism can be reduced by administration of NSAIDs and lipoxygenase inhibitors. Ras farnesylation can be inhibited by perillyl alcohol, limonene, and synthetic farnesylation inhibitors (FTIs). Vitamin D and retinoids can induce apoptosis by inducing TGF-beta.

Cancer progression can also be slowed by inhibition polyamine synthesis which has the effect of decreasing cell proliferation. There are two ways of doing this. The first is by inhibiting a key enzyme, ornithine decarboxylase, by the irreversible binding of difluoromethylornithine. This inactivates the enzyme and results in lower production of putrescine and spermine. The second way is to inhibit the production of the enzyme. Several agents have been found to do this, including retinoids and NSAIDs.

As our understanding of the key mechanisms of cancer grows, we can better devise strategies to block two or more key pathways in the cancer process and thus limit or reverse the clonal evolution of many types of cancer. The use of combination chemopreventive strategies should also allow the use of even lower doses of natural or synthetic agents and thus lower concomitant adverse effects, if any. Newer genomic and proteomic tools are being used to accelerate this process of drug discovery and development. Basic genomic research is being translated into clinical trials with a new age of molecular interventions, such as antisense therapy. The future is bright for preventing cancer at its earliest stages and reversing it at later stages before it becomes invasive.

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