

The Promise of Natural Products for Blocking Early Events in Skin Carcinogenesis

Perspective on Stratton et al., p. 160, Kowalczyk et al., p. 170, and Katiyar et al., p. 179

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Abstract

This perspective on Stratton et al. (beginning on p. 160), Kowalczyk et al. (beginning on p. 170), and Katiyar et al. (beginning on p. 179) highlights the common theme of translational investigation of natural substances and their molecular effects and mechanisms in preventing skin squamous cell carcinoma, which has potentially severe clinical consequences. These studies comprise results of naturally occurring phytochemicals and green tea polyphenols in mouse models of UV-induced and chemically induced skin carcinogenesis and results of perillyl alcohol in a phase IIa clinical trial—all pointing to the great promise of this exciting approach for better understanding of and preventing skin cancer. *Cancer Prev Res*; 3(2); 132–5. ©2010 AACR.

*"Omnis cellula e cellula."*³

–Rudolf Virchow

As quoted in the epigraph, Virchow established in the 1850s the Biogenic Law, which asserts that cells arise from other cells, and so, by extension, it has been understood that tumor cells must arise from "normal" cells. Since that time, a sizable proportion of the world's biomedical research community has set about trying to understand the events surrounding the conversion of normal cells to cancer cells. Modern research in cancer prevention assumes that genetic events mediate this conversion, followed by the initiated cell or populations of initiated cells undergoing a series of promoting events leading to premalignancy and/or cancer.

Much of our current understanding of tumor initiation and promotion derives from studies within several mouse skin models, the most prominent of which are the two-stage chemical carcinogenesis protocol and the SKH-1 hairless mouse UVB tumorigenesis protocol (1–4). Both models have provided a framework for separating the biochemical events of initiation induced by 7,12-dimethyl-

benz(a)anthracene (DMBA; two-stage model) or UVB (SKH-1 model) from promoting events induced by repeated topical application of 12-O-tetradecanoylphorbol-13-acetate (TPA; two-stage model) or chronic UVB (SKH-1 model); they also have been useful for testing chemicals that can act specifically at these stages. These models are well suited for testing chemopreventive agents because of the accessibility of the target tissue before and immediately after initiation and throughout the tumor-promotion phase. Several potential skin cancer chemoprevention agents tested in these models have shown promise for suppressing early steps in tumor promotion.

Beyond its value for the study of carcinogenesis and chemopreventive agents and their mechanisms, nonmelanoma skin cancer is a major public health burden needing improved control. It is the most common cancer in the United States, with an estimated annual incidence of more than 1 million new cases of the two most common forms, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC; ref. 5). Skin SCC is the more clinically aggressive form (6) and has been increasing in incidence since the 1960s at annual rates of from 4% to as high as 10% in recent years. Advanced disease and treatment-related morbidity have a profound impact on the quality of life of nonmelanoma skin cancer patients. Chemoprevention is one approach showing promise for the much-needed improvement of control of advanced nonmelanoma skin cancer.

In this issue of the journal, Katiyar et al. (7), Kowalczyk et al. (8), and Stratton et al. (9) report on a group of promising natural products that are or have been in preventive development in the two-stage carcinogenesis and SKH-1/UVB mouse models. These two models more

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³ From cells come all cells.

closely mimic human SCC than BCC, which is more common. The molecular pathways leading to SCC and BCC differ markedly. SCC etiology can involve dysregulation of several different signaling pathways (discussed later), whereas nearly 100% of BCCs arise from defects in the Wnt/Sonic hedgehog (Shh) signaling pathway (10). It has been reported that 93% of BCCs display loss of heterozygosity at 9q21-q31, the location of the *Ptch* gene (11), the protein product of which is the cell surface receptor and negative regulator of Shh signaling. Until the relatively recent development of genetic mouse models for gain of function of Wnt/Shh pathway effectors (Shh, Smo, Gli1, and Gli2) or knockout of negative effectors (*Ptch* and *Sufu*), there were no animal models of BCC (10). For this reason, the SCC models used in the studies discussed here have been the most utilized for exploring, and have provided the greatest insight into the basic biology of, tumor initiation and promotion.

Most attempts to develop chemopreventive strategies for epithelial cancer have focused on targeting the signaling pathways known to be deregulated in transformed cells in the two-stage and SKH-1 models. Such pathways are critical for the control of cell proliferation, survival, and differentiation. The activator protein-1 (AP-1) signaling pathway is clearly implicated in early promotion. AP-1 is a transcription factor complex comprising heterodimers of jun (*c-jun*, *junB*, and *junD*) and fos (*c-fos*, *fosB*, *fra1*, and *fra2*) protein family members. AP-1 is a positive regulator of cell proliferation and transformation, and TPA stimulates its activity in the skin (12). The promotion step likely involves AP-1 because the oncogenic forms of *c-jun* and *p21^{ras}* cooperate in converting normal keratinocytes to SCCs (13) and, as shown more recently, expression of a dominant-negative form of *c-jun* in skin inhibits tumor formation in the SKH-1 model (14). Agents that suppress tumor formation, such as the vitamin A metabolite *all-trans* retinoic acid in the two-stage model and the isothiocyanate sulforaphane in the UVB model, can block AP-1 signaling in these model systems (15, 16). Another naturally occurring chemical, perillyl alcohol, also suppressed tumor formation in the UVB model, and this effect correlated with AP-1 suppression (17). In this issue of the journal, Stratton et al. report their extension of these studies into the clinic (9). They conducted a randomized, double-blind phase IIa study of two doses of perillyl alcohol versus placebo (applied topically in cream twice daily) for 3 months in patients with sun-damaged skin. The primary end point, reversal of actinic damage, was determined by histopathologic scoring and karyometric analysis of skin sections. The histopathologic score was reduced in patients receiving the lower dose (0.3% w/w; borderline statistical significance) but not the higher dose (0.76% w/w) of perillyl alcohol. The higher dose, however, produced a statistically significant reduction in abnormal nuclei. These interesting findings provide incentive to conduct larger, well-controlled studies and to develop topical pro-drug formulations that would improve dermal penetration and bioavailability.

The numerous other chemopreventive targets that have been identified and/or tested in the UVB and two-stage models include *Ha-ras* (18), the transcription factor signal transducer and activator of transcription 3 (19–22), the B-Raf/Mek/Erk kinase cascade (23), estrogen receptor (24), cytochrome P450 enzymes (25), Polo-like kinase 1 (26), the phosphoinositide-3 kinase inhibitor phosphatase and tensin homologue (27), the anti-apoptotic Bcl-2 family member Bcl-X(L) (28), the NF- κ B transcription factor complex (29), superoxide dismutase (30), and several others. The list of agents that have been implicated in blocking signaling through these pathways is equally long and includes many natural compounds found in the diet. These molecular targets and related agents all pertain to SCC; however, *PTCH* heterozygous knockout mice (*Ptch* +/-), which develop BCCs, recently also became available for testing chemopreventive agents. Both tazarotene (targeting retinoic acid receptors β/γ) and celecoxib (targeting cyclooxygenase-2) have shown promising results for suppressing BCC development (10, 31).

Kowalczyk et al. report their analysis of the synergistic effects of combinations of naturally occurring phytochemicals in suppressing inflammatory hyperplasia in mice undergoing twice-weekly applications of DMBA (two-stage model) for 4 weeks (8). The study combinations were dietary elagic acid or calcium D-glucarate plus topical resveratrol, or grape seed extract and dietary grape seed extract plus topical resveratrol. Each combination was given 2 weeks before and during the 4 weeks of DMBA treatment. All combinations showed synergistic effects in reducing DMBA-induced hyperplasia. The effect was linked to several other parameters of tumor initiation and promotion, including the A-to-T mutation in codon 61 of the *Ha-ras* gene, which is the most common initiating event in the two-stage model. All combinations were strikingly effective in inhibiting *Ha-ras* mutations, whereas single agents had only a modest effect. These findings suggest that combinations may be more effective than single agents in blocking initiation-related events. All combinations were also better than any single agent in suppressing expression of the AP-1 component *c-fos*, along with several AP-1 targets. The collective data from these analyses suggest that phytochemicals act synergistically in combinations in suppressing early tumor-promoting events. This study used a short-term protocol that mimicked a complete carcinogenesis protocol (i.e., repeated DMBA application), making the separation of initiation from promotion events problematic. Furthermore, a tumor end point was not evaluated in this study; thus, further work is needed to determine whether the tested or similar combinations would be effective in suppressing tumor development.

Another set of promising cellular targets for chemoprevention of skin cancer are DNA repair systems, in particular nucleotide excision repair (NER), which primarily repairs UV-induced DNA damage (32). It is well established that UV radiation can cause DNA damage through formation of cyclobutane pyrimidine dimers, and that this damage is associated with immunosuppression

(33). Furthermore, there is a connection between immunosuppression and an increased risk of human skin cancer (34, 35). A previous study showed that oral green tea polyphenols (GTP) can suppress both UV-induced and chemically induced skin tumors in SKH-1 mice (36), and Katiyar and colleagues previously reported that the tumor-suppressive effect of GTP correlated with reductions in angiogenesis and expression of matrix metalloproteinase-2 (MMP-2) and MMP-9 and increases in apoptosis and numbers of CD8⁺ T cells in tumors (37).

In this issue of the journal, Katiyar et al. report new work that extends their earlier studies, showing that the mechanism of suppression by GTPs of UVB-induced skin cancer is due, at least in part, to inhibition of UV-induced immunosuppression (7). Using xeroderma pigmentosum complementation group A (XPA)-deficient (XPA^{-/-}) mice, which lack NER capacity, they showed that the NER system is required for GTP prevention of UVB-induced immunosuppression. They saw similar results in NER-deficient fibroblasts from XPA patients, in which GTPs did not enhance repair of UVB-induced DNA damage, whereas GTPs enhanced repair in normal donor fibroblasts. They also showed that GTPs could enhance expression of NER genes. In other words, oral GTPs can block UVB-induced tumor formation through enhancing NER in mice. Of importance, the authors calculated that the GTP dose providing a protective effect against UVB in mice would be equivalent to the human consumption of five to six cups of green tea. These studies reveal a novel mechanism by which green tea may block UVB-mediated skin cancer. This mechanism joins several others that have been linked to the actions of green tea (38). Indirect evi-

dence from a clinical trial in XPA patients (39), however, suggests that NER enhancement may be a critical mechanism of beneficial GTP effects. A topical liposomal preparation of the bacterial DNA repair enzyme T4 endonuclease reduced the number of skin premalignancies (actinic keratoses) to an average of 8.2 per XPA patient compared with 25.9 for control patients ($P = 0.004$). GTPs represent a simpler, natural product-based means of enhancing NER.

Since Virchow's articulation of the Biogenic Law in the 19th century, we have come a long way in our understanding of the molecular events surrounding the initiation, promotion, and progression stages of tumor development, or how tumor cells evolve. Many promising chemopreventive agents can be found in dietary components known for centuries to have health benefits. Investigating the chemopreventive mechanisms of these agents is at an early and exciting stage and promises to greatly benefit people at the highest risk of cancer or its recurrence in the skin or other sites. Advances in the understanding of skin SCC and BCC signaling alterations, including their differences and potential similarities, could lead to more effective targeted preventive approaches (e.g., combined agents) for controlling both disease processes and their potential for severe disease- and treatment-related morbidity (5, 6).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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