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Targeting Ornithine Decarboxylase for the Prevention of Nonmelanoma Skin Cancer in Humans

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Abstract

Bailey et al. report in this issue of the journal (beginning on page *XXX*) one of the first successful trials of basal cell carcinoma (BCC) prevention. Oral -difluoromethyl-dl-ornithine (DFMO) reduced new BCCs in patients with a prior history of non-melanoma skin cancer. DFMO is an inhibitor of ornithine decarboxylase, a key enzyme in the polyamine biosynthetic pathway. This perspective on Bailey et al. discusses our knowledge of the contribution of polyamines to BCC pathogenesis, how this knowledge advanced the development of a new method to prevent BCCs, and prospects for future studies of DFMO in BCC prevention.

The nonmelanoma skin cancers (NMSCs) basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common malignancies in whites worldwide. It is estimated that 1.0–1.3 million new NMSCs are diagnosed in the United States each year, only slightly fewer than the number of all other cancers combined. Although rarely lethal, these lesions are locally destructive and are a tremendous economic burden to the health care system. In 2004, the estimated cost of treating NMSCs in the United States was about \$1.5 billion; adding actinic keratoses, premalignant lesions that can progress to SCCs, would increase the estimated cost to over \$2.3 billion (1). The vast majority of NMSCs are caused by excessive exposure to ultraviolet (UV) radiation. In contrast to the stabilized or declined incidences of most other cancers, the rate of NMSCs continues to rise (2,3). The reasons for this rise relate, at least in part, to increased time people have for outdoor recreational activities, more frequent use of artificial light sources by the lay public for cosmetic purposes, and the increasing proportion of aging individuals in the general population. NMSCs are becoming more frequent in younger people as well. A population-based retrospective study found that the incidence of BCCs and SCCs in patients younger than forty years of age increased significantly between 1976 and 2003 (4).

Experts usually group BCCs and SCCs together because both occur on exposed areas of the skin, have a low mortality rate, and are frequently caused by chronic exposure to UV radiation. Despite these similarities, however, they also have distinguishing clinical and histological characteristics and biochemical and molecular differences. Consider mutations in *p53*, for example, which occur in most cutaneous SCCs. Inactivation of *p53*-dependent tumor suppressor functions related to apoptosis induction and other critical processes are considered to be important in the pathogenesis of SCC (5). Although approximately 50% of BCCs have *p53* mutations, 90% of BCCs are associated with unrepaired UV-induced mutations in patched

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or smoothened genes (5), which belong to the sonic hedgehog (Shh) pathway. Mutations in the Shh pathway are thought to be the *sine qua non* of BCCs. Therefore, non-surgical treatment and prevention approaches for BCCs may differ from those for SCCs.

BCC and SCC prevention has focused mainly on limiting the amount of UV radiation that reaches the skin. Reducing UV exposure has been accomplished chiefly through public-awareness campaigns that warn people about adverse effects of sun exposure and have influenced the public to protect themselves from sun exposure during peak hours of UV intensity (i.e., 10:00 a.m.–4:00 p.m.) and to use protective hats and clothing, avoid artificial UV radiation sources (e.g., tanning beds), and regularly apply sunscreens.

It is important to note that the efficacy of sunscreens is determined by their ability to protect against UV-induced sunburns, and the SPF number on sunscreen labels represents its “sunburn protection factor.” The value of sunscreens for protecting humans against NMSCs, melanoma, photo-aging, and UV-induced immune-suppression remains largely undetermined. Although sunscreens may play an important role in preventing skin cancer, it should be noted that they also have a number of limitations. There is inconsistent compliance in the use of most sunscreens because they are greasy and messy and stain clothing. Although large amounts of sunscreen are generally required to achieve the full SPF value displayed on the label, most people apply only about 25% of this amount (6). Furthermore, UVB radiation is required for the cutaneous photoconversion of 7-dehydrocholesterol to previtamin D, and sunscreens may limit this conversion (7). Potential effects of sunscreens on vitamin D metabolism may not concern most people, but they may be an issue for the elderly and other people with vitamin D deficiency. Last, sunscreens have no effect on prior UV-mediated skin tissue damage.

The few reported studies of sunscreen effects suggest that sunscreens reduce the incidence of SCCs and actinic keratoses but are much less effective in preventing BCCs (8–11). Regular application of sunscreen for seven months significantly reduced the ratio of new actinic keratoses to total actinic keratoses (versus vehicle) in individuals with prior actinic damage (8). Another controlled trial found that people who applied sunscreen regularly for five years had a statistically significant 35% lower incidence of cutaneous SCCs but not a statistically significantly lower incidence of BCCs (compared with placebo) (9,10). A more recent controlled trial lasting 24 months in 120 heart, lung, and kidney transplant recipients showed a significant reduction in actinic keratoses and SCCs in people who used a highly protective sunscreen regularly compared with individuals who did not. BCCs also declined in the sunscreen group, but not statistically significantly (12).

Researchers have shown great interest in identifying new ways other than sunscreen to prevent UV-induced NMSCs. Oral retinoids (13,14) and topical DNA repair enzymes are chemopreventive methods with reported effectiveness in humans (15). Both approaches were effective mainly in patients with a predisposition to develop skin cancer, including xeroderma pigmentosum patients, who have a genetic defect in the repair of UV-damaged DNA, and immunosuppressed organ-transplant recipients. Low-fat diets also have reduced the incidence of NMSCs, but compliance with low-fat diets is not so easily achieved (16).

Research into the pathogenesis of BCC and SCC has stimulated interest in chemopreventive agents that inhibit the activity of cyclooxygenase-2 (COX-2) and ornithine decarboxylase (ODC), both of which contribute to UVB-induced skin cancer development. In murine models as well as in humans, UVB enhances the expression of COX-2-dependent prostaglandin (PG) production, particularly of PGE2 (17–20). COX-2 and PGE2 have important roles in skin carcinogenesis (18), stimulating the proliferation of preneoplastic cells and promoting inflammation during the promotion stage. They also facilitate the epithelial-to-mesenchymal transition, suppress host anti-tumor defense mechanisms, inhibit tumor-cell apoptosis, and

stimulate angiogenesis during the progression stage of skin carcinogenesis. Not expressed in undamaged normal epidermis, COX-2 can be induced in such tissue by acute and chronic exposure to UVB (18) and is overexpressed in the epithelia of actinic keratoses and SCCs (20). COX-2 is also found in BCC, where its distribution differs from that of SCCs. COX-2 is found in the stroma surrounding islands of epithelial BCC cells in mice and primarily in the tissue adjacent to BCC islands in humans (20). Treating experimental animals with COX-2 inhibitors, such as celecoxib, prevents SCCs (reviewed in refs. 18,21) and to a lesser extent BCCs (22).

ODC is the initial rate-limiting enzyme involved in polyamine biosynthesis and is responsible for converting L-ornithine to putrescine (23). The existence of multiple independent mechanisms regulating polyamine levels in cells provides evidence for the importance of polyamines in controlling growth and differentiation signaling. Putrescine, spermidine, and spermine are some of the major polyamine cations in cells. These molecules bind to polyanionic macromolecules such as DNA, RNA, and phospholipids, controlling DNA replication, transcription, and translation. Experiments using specific inhibitors of polyamine biosynthesis have demonstrated the absolute requirement of these biomolecules for cell growth and differentiation. Although certain cellular mechanisms tightly control the expression of ODC in normal cells, ODC regulation is altered in neoplastic cells, yielding constitutively high levels of ODC expression and activity (reviewed in ref. (24). Furthermore, a polymorphism in ODC is associated with colon adenoma recurrence and the survival of colon-cancer patients and modulates the preventive effect of aspirin in colorectal-adenoma patients (25,26).

ODC is transiently induced by a variety of stimuli including mitogens and tumor promoters such as 12-O-tetradecanoyl phorbol-13-acetate (TPA), UVB and hormones and is dramatically elevated in human SCC and BCC (compared with levels in adjacent normal skin tissue). Several transgenic mouse-model studies have shown the essential role of polyamines in the early promotion of skin tumorigenesis. Elevated levels of ODC activity are sufficient to promote skin tumor formation, without the addition of tumor-promoting agents, in the carcinogen-exposed skin of K6/ODC transgenic mice, where ODC is constitutively targeted to the skin with a keratin 6 or keratin 5 promoter (27). In addition, doses of UVB radiation that are insufficient to produce tumors in SKH-1 hairless mice will induce both premalignant papillomas and SCCs in K6/ODC transgenic mice. *De novo* induction of ODC activity in suprabasal epidermal cells causes increased epidermal proliferation, neovascularization, and increased synthesis of extracellular matrix proteins in a manner similar to that of skin responding to a wound. Of interest, ODC overexpression in the suprabasal layer of normal epidermis appears to affect other cell subpopulations in the skin, resulting in increased proliferation of basal cells of the epidermis and increased angiogenesis in the dermis (reviewed in ref. 23).

Experimental evidence of the contribution of ODC to the development of BCC is convincing (27). The growth of BCCs is augmented in *ptch*^{+/-} mice overexpressing ODC in the outer root sheath cells of hair follicles (*Ptch*^{+/-}/ODC TgN). These *Ptch*^{+/-}/ODC TgN mice mimic the phenotype of basal cell nevus syndrome, which predisposes patients to BCC. The characteristic histologic features of BCC, including palisading basaloid cells in the periphery of tumor nests, the appearance of retraction spaces, the lack of keratinizing centers, and the characteristic expression pattern of keratin 17, Gli1, Gli13, *Ptch1*, and *Ptch2*, closely resemble characteristics of human BCC (27).

α -Difluoromethyl-dl-ornithine (DFMO) is an ornithine analog that irreversibly inhibits ODC activity (28). The mechanism of this inhibition involves enzymatic decarboxylation of DFMO to a carbonionic intermediate, which, following the loss of fluorine, alkylates a nucleophilic residue near or at the active site of ODC. This covalent modification of the active site of ODC

leads to its suicidal inactivation. DFMO depletes polyamines in various organs including the skin. ODC inhibition reduces phosphorylation of the retinoblastoma tumor suppressor protein and invokes G1 arrest through upregulation of p21 and/or abolition of p27Kip1 repression (29). DFMO treatment inhibits tumorigenesis in various murine organ models, effectively reducing tumor development in a two-stage, initiation-promotion murine model of chemically induced skin carcinogenesis. It also has been found to reduce UVB-induced tumorigenesis in BALB/c (30) and K5/ODC-transgenic mice (31), to reduce UVB-induced immune suppression in BALB/c mice (30), and to inhibit epidermal carcinogenesis in ODC/Ras transgenic mice (32).

DFMO is a relatively nontoxic compound and thus has strong promise for cancer chemoprevention in humans. As reported by Bailey et al. in this issue of the journal, a randomized, double-blind, placebo-controlled phase-III trial of DFMO (0.5 g/m²/day) versus placebo for up to five years produced promising results in men and women with a previous history of skin cancer (33). The study arms were well-matched for all other important categories including use of sunscreen and nonsteroidal anti-inflammatory drugs (NSAIDs). Although DFMO did not achieve the primary objective of a statistically significant reduction in new NMSC (260 versus 363 cancers, $P = 0.069$) or inhibit the development of SCC, it decreased BCCs by 30%. The BCC result was significant not only from a statistical standpoint ($P = 0.03$), but also because it is the first time that a chemopreventive agent other than sunscreens has prevented BCCs in subjects who do not have conditions that predispose them to develop this cancer (e.g., xeroderma pigmentosum and organ transplantation). Daily oral DFMO at the dose employed in this study (500 mg/m²) was quite safe and well-tolerated except for mild, reversible ototoxicity, which is one of the major toxicities noted in other human clinical trials with the drug (34). Wang et al. recently showed that polyamines regulate Kir channels that maintain the endochochlear potential, which is necessary for adequate hearing, and that a deficiency of spermine synthase is ototoxic (35).

The study of Bailey et al. provides evidence that ODC inhibition represents an important molecular target-based approach for preventing skin cancer and validates the utility of DFMO as a cancer chemopreventive agent in another human organ system besides the colon, where Meyskens et al. showed that DFMO plus the NSAID sulindac reduced advanced or multiple adenomatous polyps by more than 90% (36). This important trial raises issues that will serve as the basis for future studies. First, it will be important to determine the mechanisms by which DFMO exerts its chemopreventive effects against BCC. The current trial showed that DFMO at the dose employed effectively inhibited ODC activity and putrescine concentration, but DFMO effects on the expression of Shh signaling-related proteins and on anti-tumor immune response are also relevant and need to be explored. Second, Fischer et al. showed that treatment with a combination of DFMO and the NSAID celecoxib was more effective in diminishing UVB-induced SCCs in murine skin than was treatment with either agent alone (37). These findings coincide nicely with the findings of Meyskens et al. in colorectal adenomatous polyps (36). Therefore, adding an oral COX inhibitor to oral DFMO may have additive or synergistic effects in BCC and may allow chemopreventive efficacy with a lower dose of DFMO and/or the COX inhibitor, thus potentially reducing the toxicity either single agent might cause. Last, a topical formulation of DFMO (13.9%) has been approved by the U.S. Food and Drug Administration (FDA) for the removal of unwanted hair. Topical DFMO also reduced the number of actinic keratoses in a small randomized controlled trial (38). This medication is applied chronically, and clinical studies have shown that it has a favorable human dermal safety profile with no adverse side effects (39). A topical formulation of the NSAID diclofenac also is FDA-approved for the treatment of actinic keratoses. Therefore, topical DFMO alone or with topical diclofenac could be investigated for chemopreventive effects against BCCs.

By identifying BCCs as a clinically relevant target for DFMO, the analyses by Bailey et al. (33) are a positive step forward for cancer chemoprevention. These investigators have taken basic observations about the pathogenesis of BCC and transformed them into something that has clinical applicability for individuals who are at risk for this too-common neoplasm.

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