

Perspective

Translating Cyclooxygenase Signaling in Patch Heterozygote Mice into a Randomized Clinical Trial in Basal Cell Carcinoma

Different perspective on Tang et al., p. 25

Jack L. Arbiser

Abstract

This different perspective on Tang et al. (beginning on p. 25 in this issue of the journal) discusses the pivotal role of cyclooxygenase (COX) signaling in the pathogenesis of basal cell carcinoma (BCC). These investigators conducted elegant experiments showing increased BCC burden in patch heterozygous mice overexpressing COX-2 in the epidermis. Genetic deletion of COX-1 or COX-2 resulted in a robust decrease in BCC burden in patch heterozygote mice. They then studied pharmacologic COX inhibition in mice and humans with loss of patch, finding a trend in humans toward decreased BCC burden. This finding has implications for public health. *Cancer Prev Res*; 3(1); 4–7. ©2010 AACR.

Basal cell carcinoma (BCC) remains the most common form of cancer in humans, with more than 1 million new cases yearly in the United States. Despite the usual nonaggressive course of BCC, it is a major cause of morbidity because it is treated primarily with destructive surgical procedures (1). BCC can progress locally and compromise vital structures, such as the eye and nose, and is occasionally metastatic (2). Furthermore, studies of BCC treatment have been hindered by a paucity of BCC cell lines from human patients, which are far fewer in number than lines derived from far less common (albeit more aggressive) malignancies, such as melanoma (3).

Despite the paucity of BCC lines, much progress has been made in determining the pathogenesis of BCC. A reverse genetic approach has found that dysregulation of the sonic hedgehog pathway, most commonly through deletion of the negative regulator of sonic hedgehog signaling, underlies the majority of BCCs (4). Basal cell nevus syndrome is the major genetic cause of BCCs in humans and itself is caused by hemizygoty for patch. The effect of a small-molecule inhibitor in patients with advanced BCC provided a recent elegant demonstration of the role of the sonic hedgehog pathway in this disease. Dramatic responses were noted, although breakthrough resistance was observed (2).

Overexpression of sonic hedgehog or loss of patch is by itself insufficient to cause the full-blown spectrum of BCC (5, 6). Heterozygoty for patch leads to BCC after a course of radiation in mice and after UV radiation or ar-

senic exposure in humans (7). Mutant *p53* is extremely common in human BCCs, and so the combination of *p53* dysfunction and sonic hedgehog activation accounts for some of the human BCC phenotype. Sonic hedgehog does not require mutant *p53* because it is often activated in medulloblastomas and pancreatic cancers that do not have mutant *p53*, although these cancers do have loss of *p16ink4a* (8).

Two lines of evidence show that sonic hedgehog can signal through a *p16ink4a*/reactive-oxygen pathway or a mutant *p53*/low reactive-oxygen pathway (Fig. 1). First, crossing mice that constitutively express a dominant negative tuberlin with patched heterozygous mice produces a high incidence of medulloblastoma in the offspring, which do not develop BCC, however (9). The dominant negative allele of tuberlin upregulates reactive oxygen, and the total lack of BCC in these crossed mice supports the hypothesis that reactive oxygen may be protective against this disease (10). Second, crossing mice with defective cilia with mice having activated sonic hedgehog signaling prevents the development of BCC, consistent with the presence of cilia in human BCC, but not in medulloblastomas associated with elevated reactive oxygen (11).

In this issue of the journal, Tang et al. now report their elegant study showing the role of the enzymes cyclooxygenase-1 (COX-1) and COX-2 in the development of BCC (12). They show that the lack of either enzyme results in a very significant decrease in the size, but not in the number, of BCCs. Conversely, COX overexpression increased the size of BCCs. Last, the COX-2-selective inhibitor celecoxib caused a trend toward decreased tumor burden in patients at a high BCC risk. These results show that COX plays a role in BCC. Two questions remain to be answered, however. First, why does COX inhibition produce greater BCC suppression in mice than in humans? Second, given the cardiac risk factors of COX-2 inhibition, what is the optimal way to prevent and treat human BCC?

The mouse studies indicate that BCC number seems to be similar in mice regardless of whether COX was

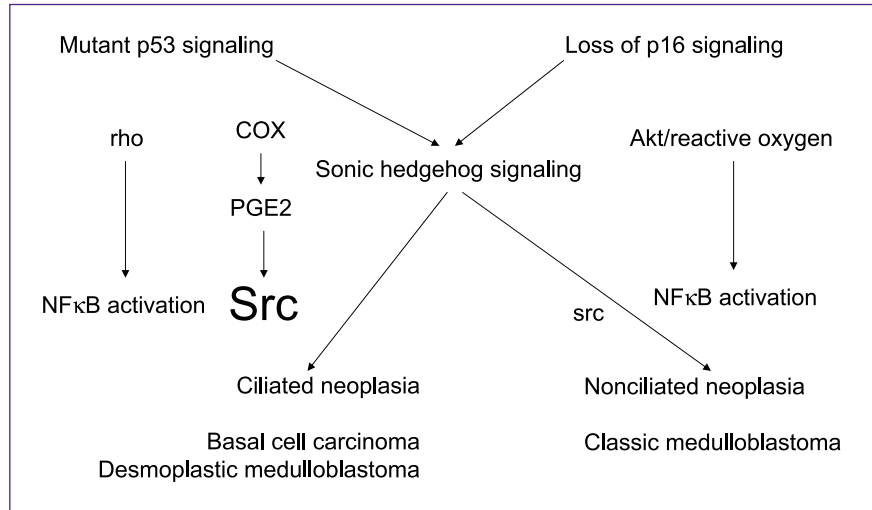
Author's Affiliation: Department of Dermatology, Emory University School of Medicine, and Atlanta Veterans Administration Medical Center, Atlanta, Georgia

Corresponding Author: Jack L. Arbiser, Department of Dermatology, Emory University School of Medicine, and Atlanta Veterans Administration Medical Center, WMB 5309, 1639 Pierce Drive, Atlanta, GA 30322. Phone: 404-727-5063; Fax 404-727-0923; E-mail: jarbise@emory.edu.

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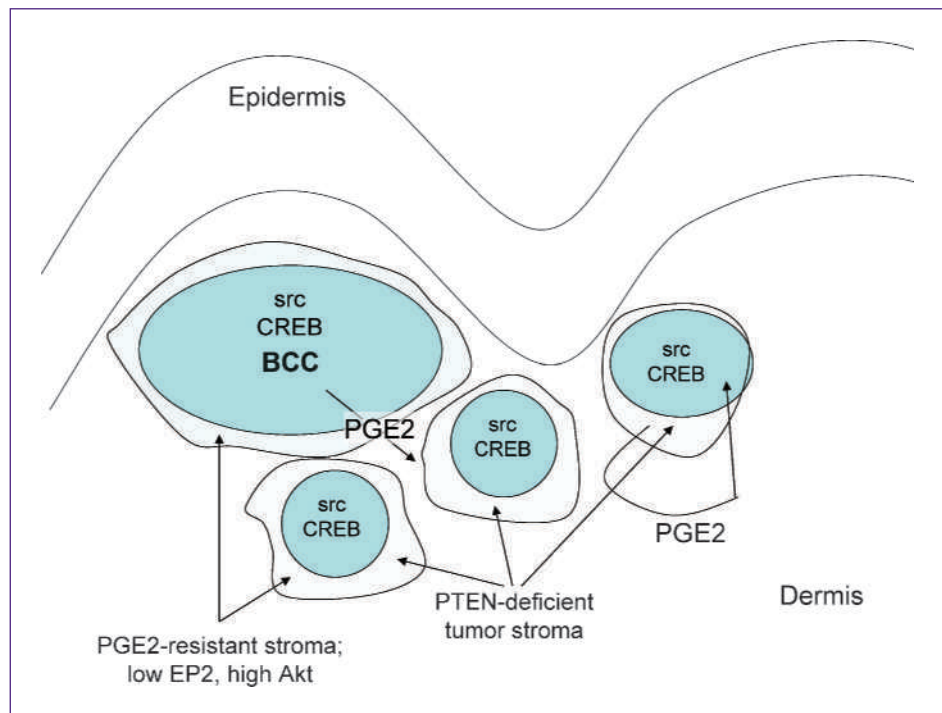
Fig. 1. Differences in signaling between tumors with mutant p53 versus loss of p16. Tumors with mutant p53 preferentially activate src through activation of PGE₂-prostanoid signaling (involving COX), whereas tumors with loss of p16 use reactive-oxygen/NF-κB signaling.



overexpressed or knocked out; BCC size and burden, however, did change. These findings suggest that COX inhibition does not repair initial genetic mutations but instead impairs tumor progression. COX inhibition may do this by direct suppression of tumor growth, increased apoptosis, or decreased angiogenesis. It is important to look at events downstream of COX to try to best use this information for prevention and treatment. COX-1 and COX-2 both generate prostaglandin E₂ (PGE₂). PGE₂ binds to any one of four prostanoid receptors, and current data in-

dicates that the prostanoid receptor EP2 might play the most important role (versus other prostanoid receptors) in cutaneous carcinogenesis. PGE₂ signaling through prostanoid receptors activates src, a potentially pro-proliferative pathway, and cyclic AMP (cAMP)/cAMP response element binding (CREB), a potentially antiproliferative pathway (13, 14). The ratio of src signaling to cAMP/CREB signaling may differ between humans and mice and may show tissue specificity. A recent study showed that PGE₂ induced apoptosis in normal fibroblasts, but not

Fig. 2. Model of the role of PGE₂ in BCC pathogenesis. BCC cells produce PGE₂, which selects for stromal cells that decrease expression of PTEN. These PTEN-deficient stromal cells support the BCC. PGE₂ also stimulates protumorigenic src and antitumorigenic cAMP/CREB in the BCC itself.



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in fibroblasts that are deficient in phosphatase and tensin homolog on chromosome 10 (PTEN) and thus have increased activation of Akt (Fig. 2; ref. 15). The implication of these findings is that continued secretion of PGE₂ by BCCs could select for a stromal population having decreased PTEN/increased Akt that might support BCC *in vivo*. Conversely, activation of cAMP/CREB by PGE₂ might limit the growth of neoplasias. Therefore, the greater effect of COX inhibition in mice (versus in humans) may be due to preferential inhibition of src-mediated neoplasia in mice; in humans, on the other hand, PGE₂-mediated cAMP/CREB signaling may play a greater role in EP-receptor-mediated effects (compared with mice).

What is the most rapid way to translate the findings of Tang et al. to the clinic? The increased risk of cardiovascular events in patients taking COX inhibitors makes us cautious about recommending the widespread use of these drugs for patients at a high risk of BCC. The studies of Tang, however, lead us to consider two other possibilities that need further investigation. First, small molecules derived from plants have been shown to inhibit COX without causing cardiac toxicities, which they seem to avoid by inhibiting other pathways that compensate for the cardiac toxicity of pure COX inhibition. Curcumin is one such compound, inhibiting angiogenesis, COX, and NF-κB, among other effects (16, 17). Another natural COX inhibitor is honokiol, which inhibits COX mRNA production (18), is systemically bioavailable, inhibits angiogenesis, and promotes apoptosis by inhibition of ras-mediated phospholipase-D activation and by promotion of mitochondrial apoptosis (19). Honokiol has shown particular efficacy against tumors with mutant p53, including breast, prostate, and lung carcinomas (19).

The second possibility raised by the results of Tang et al. is src inhibition. As mentioned earlier, src likely is the major mediator of PGE₂-mediated promotion of BCC and possibly other tumors. One would predict that knocking out src in the epidermis, preferentially in an inducible fashion, would have a strong inhibitory effect on the development of skin BCC in mice. Src can be

inhibited in two major ways. First, agents can inhibit the kinase activity of src, and they are currently in clinical trials (20, 21). Second, src can be inhibited via targeting the enzyme *N*-myristoyltransferase. Src requires modification by the covalent addition of myristoyl moieties by *N*-myristoyltransferase 1, which permits membrane localization of src. We recently discovered a small molecule, Tris (dibenzylideneacetone) dipalladium, which inhibits *N*-myristoyltransferase (at submicromolar levels) and the growth of melanoma in mice (22). Of interest, mice heterozygous in *N*-myristoyltransferase 1 are viable and fertile, showing that systemic inhibition of this pathway is a feasible target for chemoprevention (23). Indeed, systemic Tris (dibenzylideneacetone) dipalladium was not toxic in mice (22).

BCC remains the most common form of cancer in humans and a significant cause of morbidity, occasionally even causing mortality. Despite the lack of any BCC lines in the National Cancer Institute-60 Developmental Therapeutics Program human tumor cell line screen for traditional *in vitro* drug screening, a large amount of data has been generated toward identifying druggable targets in BCC. The findings of Tang et al. discussed here (12) point toward combinations targeting COX-src activation and sonic hedgehog signaling, combinations that may be synergistic in decreasing the burden of BCC in patients with or at a high risk of this disease.

Disclosure of Potential Conflicts of Interest

J.L. Arbiser and Emory University are pursuing patents on honokiol and Tris (dibenzylideneacetone) palladium.

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