Skin Deep and Deeper: Multiple Pathways in Basal Cell Carcinogenesis

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Abstract

This perspective places the report by Villani et al. that appears in this issue of the journal (beginning on page 1222) in the context of recent work showing an intersection between two important developmental pathways implicated in oncogenesis: the hedgehog and insulin-like growth factor (IGF) pathways. Villani et al. define a key role for the IGF regulatory protein Igfbp2 in a genetic model of basal cell carcinoma (BCC). This model uses the K14 promoter to target ablation of Ptch1 in the basal cells of the interfollicular epidermis (IFE) and contiguous outer root sheath (ORS) of the hair follicle. Constitutive activation of Hh signaling in this model is indicated by increased Gli1 and Gli2 expression in the IFE and ORS accompanied by hyperkeratosis, increased bromodeoxyuridine labeling, and a failure of hair follicles to progress through anagen (the active growth phase of hair follicles). The IGF signaling pathway is activated by Igf1 and Igf2 peptide ligands. These ligands produce most of their biological effects on growth and differentiation by inducing the autophosphorylation of IGF1 receptor, and disordered IGF signaling contributes to the pathogenesis of epidermal hyperplasia, as seen in BCC (reviewed in ref. 6). Overexpression of Igf1 in the basal layer of the epidermis results in thickened and wrinkled skin in mice and is associated with epidermal hyperplasia, hyperkeratosis, increased proliferation, and hyperphosphorylation of Igf1r (7).

Hedgehog, Insulin-like Growth Factor, and Epithelial Cell Transformation

The hedgehog (Hh) pathway is indispensable for embryonic development, where it plays critical roles in cell proliferation, differentiation, and tissue patterning (1). In adults, Hh signaling is linked to stem/progenitor cell maintenance, normal tissue homeostasis, and wound repair. A growing body of evidence also implicates aberrant Hh pathway activity in the development of a variety of cancers, including basal cell carcinoma (BCC), medulloblastoma, glioblastoma, and cancers of the pancreas, breast, prostate, ovary, lung, and colon. These observations have sparked vigorous interest in the development of novel, small-molecule antagonists of the Hh pathway. Key regulatory components of Hh pathway signaling include Smoothened (SMO), a seven-transmembrane domain cell surface protein essential to pathway activation, and Patched homologue 1 (PTCH1), a cell surface receptor protein that serves as a primary repressor of SMO. Binding of any of three Hh ligands to PTCH1 relieves PTCH1 repression of SMO, leading to downstream pathway activation including modification of the three GLI family transcription factors (GLI1-GLI3), which in turn promote transcription of genes regulating cell growth and differentiation (reviewed in ref. 2).

Identifying the target genes that affect tumorigenesis downstream of aberrant Hh signaling is critical not only to understanding the biological mechanisms of the pathway but also to ascertaining which patients are likely to benefit from therapeutic or preventive inhibition of Hh signaling. Besides enhancing the effective clinical translation of the SMO inhibitors currently in development, this information will provide researchers with alternate Hh-related targets to pursue. This pursuit seems particularly germane in light of recent reports describing a medulloblastoma patient who developed a somatic mutation in SMO that rendered his tumor insensitive to the SMO antagonist it had responded to initially (3, 4).

In this issue of the journal, Villani et al. report work that advances the understanding of the Hh pathway and its roles in oncogenesis by identifying insulin-like growth factor (IGF)–binding protein 2 (IGFBP2) as a key mediator of Hh signaling in a genetic model of basal cell carcinoma (BCC) (5). This model uses the keratin 14 (K14) promoter to target ablation of Ptch1 in the basal cells of the interfollicular epidermis (IFE) and contiguous outer root sheath (ORS) of the hair follicle. Constitutive activation of Hh signaling in this model is indicated by increased Gli1 and Gli2 expression in the IFE and ORS accompanied by hyperkeratosis, increased bromodeoxyuridine labeling, and a failure of hair follicles to progress through anagen (the active growth phase of hair follicles). The IGF signaling pathway is activated by Igf1 and Igf2 peptide ligands. These ligands produce most of their biological effects on growth and differentiation by inducing the autophosphorylation of IGF receptor type I (IGF1R). A second receptor, IGF2R, is thought to primarily regulate IGF2 availability rather than transduce IGF signals. There is considerable evidence that the IGF system is essential for epidermal homeostasis and that disordered IGF signaling contributes to the pathogenesis of epidermal hyperplasia, as seen in BCC (reviewed in ref. 6). Overexpression of Igf1 in the basal layer of the epidermis results in thickened and wrinkled skin in mice and is associated with epidermal hyperplasia, hyperkeratosis, increased proliferation, and hyperphosphorylation of Igf1r (7).
Conversely, mice that survive into maturity with a targeted deletion of *Igf1r* in K14+ basal keratinocytes exhibit epidermal hypoplasia linked to a reduction in epithelial progenitors and colony-forming capacity (8).

The actions of IGFs can be inhibited or augmented in response to high-affinity binding by different IGFBPs. Each IGFBP is subject to proteolytic cleavage, a modification usually associated with a reduction in ligand affinity and enhanced IGF signaling activity. IGFBP2 generally has been considered to be an inhibitor of IGF-induced proliferation (reviewed in ref. 9). It is therefore surprising that the work of Villani et al. implies that Igfbp2 positively regulates expansion of epidermal progenitors during BCC development in their K14-Cre:Ptch1<sup>lox/lox</sup> mouse model (9). Their data are further supported by studies showing that IGFBP2 from dermal or exogenous sources enhanced the development of BCC cells and keratinocytes of the ORS are morphological-ly similar and in part because the bulge region of the follicle forms a niche for stem cells that, under the appropriate conditions, are capable of giving rise to all the epidermal lineages of the skin. Villani et al. reach a similar conclusion in their model, describing an expansion of progenitor cells under the influence of Igfbp2 upregulation (5). Further studies are required to determine what role, if any, FAK or other nonreceptor tyrosine kinases play in the development of BCC.

The association that Villani et al. observed between elevated Igfbp2 expression and BCC was not limited to their transgenic mouse model; a similar increase occurred in most human BCC samples compared with expression in normal skin controls (5). Indeed, upregulation of IGFBP2 has been reported in a variety of other cancers, including medulloblastoma, glioblastoma, pancreatic, breast, prostate, ovarian, lung, and colon tumors (reviewed in ref. 9), where it has been correlated with more rapid tumor progression. As noted above, many of these cancers have also been linked to aberrant Hh pathway activation (reviewed in ref. 2). Therefore, it is tempting to hypothesize that induction of IGFBP2 downstream of Hh signaling represents a common mechanism for tumorigenesis. This IGFBP2-Hh association is perhaps best exemplified by data in glioblastoma, in which both Hh signaling and IGFBP2 have been shown to have central roles in the maintenance and chemoresistance of the cancer-initiating cell compartment (13, 14). Despite the tantalizing indications of a link between these two pathways, however, direct evidence that IGFBP2 is a downstream target of Hh signaling is still lacking. Treating K14-Cre:Ptch1<sup>lox/lox</sup> mouse skin explants with Hh pathway antagonists, such as cyclopamine, and interrupting Igfbp2 and other pathway components via short hairpin RNA–mediated silencing are two approaches that might distinguish between a direct or bystander role for IGFBP2 in the generation of BCC.

### Controversies in BCC: Multiple Possible Cells of Origin

The recognition that dysregulated Hh signaling plays a critical role in basal cell tumorigenesis led to the use of the “cre-lox” system for engineering numerous mouse models of BCC. These models have targeted multiple distinct levels of the Hh pathway but comprise two basic groups: one in which inactivating mutations are introduced into inhibitory genes, and one in which positive regulators of the pathway are constitutively activated or overexpressed (reviewed in ref. 15). The cell of origin for BCC has long been thought to reside in the hair follicle, in part because BCC cells and keratinocytes of the ORS are morphologically similar and in part because the bulge region of the follicle forms a niche for stem cells that, under the appropriate conditions, are capable of giving rise to all the epidermal lineages of the skin. Villani et al. reach a similar conclusion in their model, describing an expansion of the K15<sup>+</sup> bulge stem cell compartment and of a K14<sup>+</sup> transit-amplifying cell compartment in the adjacent ORS. BCCs seem to form in K14-Cre:Ptch1<sup>lox/lox</sup> mice as a result of the inability of this latter compartment to transition through the normal anagen phase of hair follicle growth and differentiation, which leads to a continued massing of progenitor cells under the influence of Igfbp2 upregulation and Ptc1 inactivation in this cell population (5).

Recently published data from other groups, however, support an alternative etiologic model of BCC. Distinct from the hair follicle, epidermal compartments including the IFE seem to house independent populations of stem/progenitor cells that represent a key resource for normal tissue homeostasis and repair (16). By conditionally targeting Hh pathway activation to distinct subsets of cells within the mouse epidermis, Youssef et al. (17) recently showed that BCCs generated in their model do not originate from the hair follicle but from the IFE and, to a lesser extent, from the upper infundibulum. Even when Hh signaling was specifically activated in hair follicle bulge stem cells and their immediate progeny, BCC formation was not observed and the hair follicle regenerative cycle progressed normally, providing a striking contrast to the results of Villani et al. The differing conclusions of these two studies about the putative cell of origin for BCC are summarized in Fig. 1.

What factors could help to explain the different conclusions reached by these two research groups in their
respective model systems (5, 17)? Perhaps the most obvious difference between the two models is the mechanism by which the Hh pathway was constitutively activated in mouse epidermal cells: by deleting both copies of the tumor suppressor Ptch1 (Villani et al.) or by forced expression of an activating mutant of Smo (SmoM2, K14-CreER: SmoM2; Youssef et al.). The different phenotypes and locations of the tumors generated in the two models perhaps were due to differential strengths of activation of Hh signaling in distinct progenitor populations or to overall weaker pathway activation in the Villani et al. model. Stronger activation of Hh signaling is associated with the development of tumors that closely resemble human BCC, and weaker signaling is associated with follicular hamartomas, which histologically resemble hair follicles (18).

A second explanation for the seemingly conflicting results observed in the two models is that loss of PTCH1 and constitutive activation of SMO may not be biologically equivalent. In addition to the role of PTCH1 in repressing SMO, there is growing evidence that PTCH1 has biological activities that are not transduced through the canonical Hh pathway. These activities include a capacity to bind directly to cyclin B1, which prevents translocation of cyclin B1 to the nucleus and thus inhibits cell cycle progression (19). A previous study of the Wainwright laboratory in a similar mouse model to that described here showed that ablation of Ptc1 in epidermal basal cells resulted in a significant increase in cyclin B1–positive nuclei (20). Other data show that PTCH1 can inhibit the protranscriptional activity of GLI1 through mechanisms independent of the canonical Hh signaling cascade (21). The significance of these alternative PTCH1 signaling pathways to the increased epidermal proliferation and Gli1 expression reported in the K14-Cre:Ptch1lox/lox mouse model (5) remains to be evaluated, perhaps first by evaluating the effect of a SMO antagonist on these parameters, as a means of determining canonical pathway involvement.

Last, temporal differences in Hh pathway activation may explain the conflicting conclusions of Villani et al. and Youssef et al. Although both groups used a K14 promoter to drive Hh pathway activation in their successful attempts to model BCC development, the two models differ in the age of the mice at the time of reception of this oncogenic signal. Ptch1 disruption and Hh pathway activation in the K14-Cre:Ptch1lox/lox model occur from the perinatal period onward as a result of the constitutive promoter used by Villani et al. (5). In contrast, the K14-CreER:SmoM2 model uses a tamoxifen-inducible Cre recombinase construct, which Youssef et al. activated at postnatal day 28, close to the time of sexual maturity in the mouse. Consequently, activation of Hh pathway signaling in the two models occurs first in K14-expressing cells that may represent stages of skin or hair follicle development and biological niches in one model that are distinct from those of the other model. Human BCC is of course almost exclusively a disease of adults. The hypothesis that accurately modeling
human basal cell oncogenesis requires that the initiating genetic event(s) take place in basal keratinocytes from mature mice remains to be tested, but could be readily addressed in the inducible K14-Cre;SmoM2 model.

Several important questions in BCC oncogenesis could be addressed by transposing the models of Villani et al. and Youssef et al. through a sort of promoter swap, repeating some of the key studies of both laboratories in constitutively expressed K14-Cre:SmoM2 and tamoxifen-inducible K14-CreER;PtcH1lox/lox mice. For example, how does the mechanism of Hh pathway activation influence the skin phenotypes originally reported by these groups? Do the hair follicle defects reported by Villani et al. reflect the early and constitutive nature of pathway activation inherent in this model? What are the relative contributions of canonical and noncanonical Hh signaling pathways on the observed phenotypes? Do the basal cells of the IFE remain the principal cells of tumor origin when PtcH1 disruption is used to drive BCC development? Is IGFBP2 activation implicated in models of BCC driven by Smo activation as well as by PtcH1 loss?

These are exciting times in BCC research, in the laboratory and the clinic. The targeted inhibition of Hh signaling with an orally bioavailable, high-specificity SMO inhibitor produces remarkable clinical responses in patients with advanced and metastatic BCC (22). Of course, not all patients respond and not all responses are durable, prompting several groups to actively explore mechanisms of primary and acquired resistance to SMO inhibitors. Defining key downstream targets will be an important component of this work. In parallel, multiple targeted inhibitors of IGF1R are in active early-phase clinical testing in a wide variety of clinical contexts (23). The work of Villani et al. suggests cross talk between the Hh and IGF pathways and, in particular, a possible role for IGFBP2 in Hh pathway–mediated oncogenesis. Defining whether IGFBP2 activation represents a node for targeted inhibition has implications for both cancer prevention and treatment. Despite how we may draw our diagrams, it is clear that the pathways driving basal cell carcinogenesis are neither simple nor linear. Identifying the most critical factors, through comparative analyses of multiple models of BCC development and parallel exploration in humans, should continue to offer new therapeutic avenues for the many patients with BCC, as well as new approaches to BCC prevention.

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