CUTANEOUS MALIGNANCY

Basal Cell Carcinoma, Hedgehog Signaling, and Targeted Therapeutics: The Long and Winding Road

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Basal cell carcinoma (BCC), the most common and most visible of all human tumors, frequently arises on sun-exposed skin and can produce extensive local damage if left untreated. Targeted therapeutics are now available that interfere with uncontrolled Hedgehog (Hh) signaling, the molecular hallmark of BCC, ushering in a new era in cutaneous oncology. This review describes some of the pivotal work that contributed to our current understanding of BCC and Hh signaling, and ultimately led to the development of drugs targeting the Hh pathway in BCC patients.

NBCCS AND THE GENETIC BASIS OF BCC

The first published report of sporadic skin tumors resembling BCCs dates back to 1827 (Jacob, 1827), and in 1960, Gorlin and Goltz described an autosomal dominant syndrome characterized by an increased predisposition to BCC development (Gorlin and Goltz, 1960). Individuals with Nevoid Basal Cell Carcinoma Syndrome (NBCCS) develop BCCs at an earlier age than the general population and with greater frequency, with some patients developing hundreds of tumors over their lifetime. Other neoplasms also arise with greater frequency in these individuals, particularly the pediatric brain tumor medulloblastoma, and these individuals frequently develop locally destructive jaw cysts as well as other abnormalities involving bone and other tissues (Kimonis *et al.*, 1997). The occurrence of cancer together with clinical findings thought to be the consequence of developmental aberrations suggested that defects in an embryonic signaling pathway were responsible for NBCCS. This was confirmed in 1996, when two groups reported that NBCCS patients harbor loss-of-function mutations in *PTCH1* (Hahn *et al.*, 1996; Johnson *et al.*, 1996) that encodes a receptor for the Hh family of embryonic signaling proteins (Stone *et al.*, 1996). Importantly, sporadic BCCs were also found to have mutations in *PTCH1*, pointing to a role for this gene in BCCs arising in the general population. NBCCS patients carry a mutant *PTCH1* allele in all cells and the remaining normal allele is lost in BCCs, arguing that PTCH1 functions as a classic tumor suppressor.

THE HEDGEHOG PATHWAY IN DEVELOPMENT

The identification of loss-of-function *PTCH1* mutations in the majority of BCCs pointed to a causal role for this genetic defect in tumorigenesis; however, to appreciate the functional consequence of PTCH1 deficiency on Hh signaling, it was important to understand how this pathway is normally regulated. Hh was first described in 1980 by Christiane Nüsslein-Volhard and Eric Wieschaus (Nusslein-Volhard and Wieschaus, 1980), who generated and characterized multiple classes of *Drosophila* mutants with visible alterations in patterning of the early embryo. In one group of embryos, the highly patterned ventral organization of hairs, alternating with hairless stripes, was replaced by a continuous lawn of short, stubby hairs reminiscent of a hedgehog's spines, and hence the moniker for this mutant. In 1995, Nüsslein-Volhard and Wieschaus shared the Nobel Prize with Edward Lewis for their discoveries that provided fundamental insight into the genetic regulation of early embryogenesis—discoveries with great relevance to human biology and disease, as exemplified by the central role of Hh pathway alterations in BCC pathogenesis.

Hh is one of a handful of key signaling pathways that orchestrates embryogenesis by exerting both spatial and temporal control over proliferation, survival, and cell-fate decisions. In the absence of Hh proteins, Ptch1 blocks the function of a key signaling effector in the Hh pathway called Smoothened (Smo) (Figure 1, upper panel). Signaling is initiated when secreted Hh ligands bind Ptch1 on target cells and inhibit its function, leading to derepression of Smo, subsequent activation of downstream Gli transcription factors, and upregulation of Hh target genes. Physiologic signaling is generally paracrine, with different cell populations producing and responding to Hh, and is strictly dependent on the presence of Hh ligand, so that repression of Smo by Ptch1 is restored in the absence of Hh protein.

Gene deletion studies targeting Sonic hedgehog (Shh), the predominant Hh ligand in mammals, have established a requirement for the Hh pathway in the embryonic development of many tissues and organs. Perhaps most strikingly, Shh-null mice possess severe defects in craniofacial development owing to a requirement for Hh signaling for proper division of the forebrain

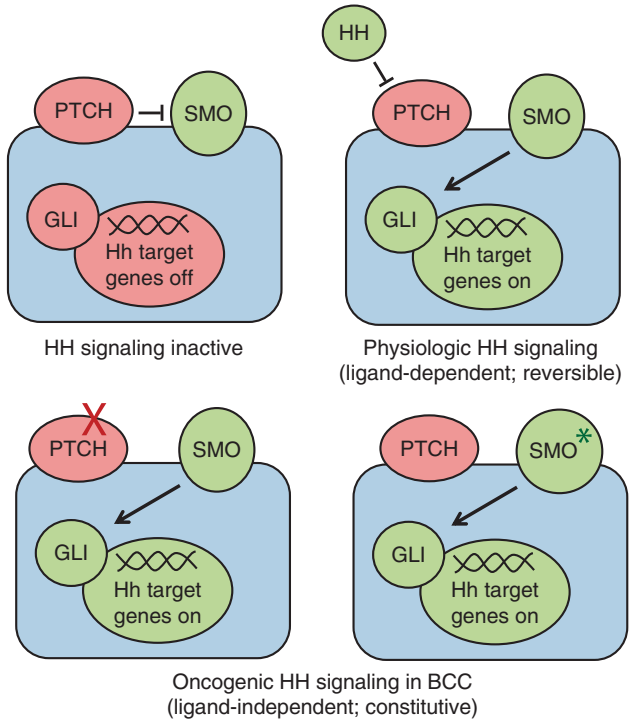

Figure 1. Simplified diagram illustrating physiologic versus oncogenic Hedgehog (Hh) signaling. Red shading indicates negative regulators whereas green indicates positive regulators of Hh signaling. BCC, basal cell carcinoma.

into cerebral hemispheres and the primordial eye field into two eyes. This results in a single, centrally located eye in Shh-deficient embryos (cyclopia) (Chiang *et al.*, 1996), consistent with similar findings in human fetuses carrying mutations in *SHH* (Belloni *et al.*, 1996; Roessler *et al.*, 1996). Cyclopia is thus a highly characteristic phenotype caused by disruption of Hh signaling during embryogenesis, an observation that figured prominently in the discovery of Hh pathway antagonists, as discussed below. Although Hh pathway activity is detected in several adult organs, studies in mice have identified essential functions in just a few. Most notable among these is the hair follicle that is strictly dependent on Hh signaling for growth and hair shaft elongation (Wang *et al.*, 2000).

HEDGEHOG PATHWAY DEREGULATION IN CANCER

In the great majority of BCCs, the Hh pathway is deregulated because of disruption of the signaling repressor PTCH1, but in a minority of tumors, a mutant form of SMO is present (Xie *et al.*, 1998) that is insensitive to inhibition by PTCH1 (Figure 1, lower panel). The result in both cases is constitutive, ligand-independent Hh signaling in epithelial cells attributable to unrestrained SMO activity. Essentially all BCCs have an activated Hh pathway as evidenced by high-level expression of Hh target genes, suggesting that this signaling alteration plays a central role in tumor pathogenesis. This concept has been supported by mouse modeling studies that used either transgenic approaches, in which positive regulators of the Hh pathway (Shh, oncogenic SMO, GLI1, Gli2) were overexpressed in skin (Oro *et al.*, 1997; Xie *et al.*, 1998; Grachtchouk *et al.*, 2000; Nilsson *et al.*, 2000; Grachtchouk *et al.*, 2003), or gene deletion studies, to generate mice with a disrupted *Ptch1* allele mimicking the genetic defect in NBCCS patients (Aszterbaum *et al.*, 1999). These studies have established that uncontrolled Hh signaling is sufficient for promoting BCCs and BCC-like tumors in mice, and that sustained Hh pathway activity is required for BCC maintenance (Hutchin *et al.*, 2005).

What about other cancers? Medulloblastomas associated with Hh pathway deregulation are similar to BCCs in that they are driven by ligand-independent Hh signaling, in keeping with their increased incidence in NBCCS patients and Ptch1-deficient mice (Goodrich *et al.*, 1997). A small proportion of medulloblastomas harbor oncogenic mutations in SMO, similar to BCCs. Hh signaling has also been implicated in the pathogenesis of various internal malignancies (e.g., pancreatic cancer, gastric cancer, prostate cancer, small-cell lung cancer). In contrast to BCC and medulloblastoma, in visceral cancers the stromal cells frequently exhibit elevated Hh signaling because of increased production of Hh ligand(s) by tumor cells, mirroring the paracrine mode of Hh pathway activation that is common during development (reviewed in Barakat *et al.*, 2010). The discovery of heightened Hh pathway activity in several life-threatening internal malignancies has generated intense interest in targeting this pathway for the treatment of neoplasms other than BCC and medulloblastoma.

DIFFERENT SUBTYPES OF BCC

Like many tumors, BCCs can be classified into several subtypes based on morphology and differentiation. The superficial and nodular subtypes of BCC are indolent, and are thought to arise from progenitor cells located in the epidermis and within the hair follicle, respectively. In contrast, the cellular origins of the more aggressive variants, including infiltrative, basosquamous, and morpheaform or sclerosing BCCs, are unclear. These aggressive subtypes frequently cause local tissue damage, are often not circumscribed by a basement membrane, and may be associated with fibroplasia, suggesting that stromal communication via paracrine signals may also be especially important in these tumors.

How the seemingly diverse variations of BCC are manifest currently remains unclear. The hair follicle itself comprises at least eight related epithelial cell lineages, and both tumor

morphology and behavior may be influenced by the particular cellular lineage that sustains the initial oncogenic hit to the Hh pathway. BCC-like lesions arising from different experimental mouse models of BCC often exhibit diverse morphologies with varying degrees of resemblance to human BCCs, as will be described in detail below. These findings suggest that the nature of the genetic mutation may also affect BCC subtype. Finally, and perhaps most importantly, the amplitude of Hh pathway activation may underlie many of these variations. Indeed, studies in transgenic mice have suggested that high activation of Hh signaling can elicit BCC-like lesions that more closely resemble classical forms of nodular human BCCs, whereas low pathway activity induces the formation of tumors resembling benign basaloid hamartomas (Grachtchouk *et al.*, 2003, 2011). In turn, the degree of Hh signaling likely impinges upon, and conversely may be affected by, synergistic activation of other pathways such as Wnt, epidermal growth factor receptor, phosphatidylinositol 3 kinase/mammalian target of rapamycin, and p53. Thus, cell of origin, the nature of the genetic mutation, the degree of Hh pathway activation, and synergy with other collaborating pathways are likely all key determinants of BCC morphology and behavior.

CELL OF ORIGIN

Where do BCCs come from? Over the past few years, with the creation of numerous mouse models of cancer as well as the tools to manipulate genes in specific cell populations, much research has been devoted toward filling in our knowledge about the origin story for different cancers—unmasking the identity of the initial cells that, upon sustaining a genetic mutation, can give rise to tumors (Visvader, 2011). For decades, BCCs have been presumed to arise either from the basal layer of the epidermis or from the hair follicle outer root sheath. Evidence for this was indirect, and based primarily on histology and similarities in cytokeratin expression between tumor lesions and their normal cellular counterparts. More recently, in the aforementioned mouse models of BCCs, attempts to elucidate the cellular origin for these lesions were based on somewhat circumstantial observations made at early stages of tumor development, and various reports have claimed that BCCs arise from both epidermis and hair follicles.

Part of the difficulty in identifying the cellular origin for BCC is that skin is a complex organ. This has become especially apparent over the past few years, as genetic lineage tracing has led to the identification of multiple independent stem cell populations in the hair follicle and epidermis that operate independently during homeostasis, but can exhibit plasticity during pathological situations such as wounding. This complexity may, at least in part, underlie the seemingly contradictory results reported recently by multiple groups regarding the cellular origin for BCC (reviewed in Kasper *et al.*, 2012). For instance, BCC-like tumors induced by an oncogenic form of Smo have been reported to arise primarily from the interfollicular epidermis, but not from hair follicle bulge stem cells, except upon wounding (Youssef *et al.*, 2010; Kasper *et al.*, 2011; Wong and Reiter, 2011). In direct contrast, lineage tracing studies have suggested that bulge cells give rise to the majority of BCCs in irradiated Ptch1 heterozygous mice (Wang *et al.*, 2011).

Reconciling these conflicting results may have important implications for understanding why BCCs can exhibit such diverse morphologies. Indeed, additional studies have shown that BCCs induced by a truncated form of GLI2 exhibit high-level pathway activation and can arise from both the epidermis and the hair follicle lower bulge or secondary hair germ (Grachtchouk *et al.*, 2011). Notably, tumors originating from the epidermis resembled superficial BCCs, whereas nodular tumors were associated with the follicle, suggesting that cell of origin may indeed influence tumor subtype.

Thus, although these seemingly conflicting results have provoked some confusion in the field, from a wider standpoint these studies may also suggest that perhaps the cellular origin for BCC may not be fixed and immutable, but rather may depend on the nature of the genetic mutation and the degree of Hh pathway activation as well as on stromal context.

ONE-EYED LAMBS AND HEDGEHOG PATHWAY INHIBITION

The identification of the first pharmacological inhibitor of Hh signaling was based on a series of pivotal observations and discoveries dating back to the 1950s (Figure 2), when up to 25% of lambs on certain Idaho ranches were born with severe craniofacial deformities, including a single eye (cyclopia) (reviewed in Keeler, 1978). The discovery that all pregnant ewes that produced cyclopic lambs grazed in areas with similar flora raised the possibility of an environmental teratogen. This was confirmed in 1963 when it was shown that feeding pregnant ewes the corn lily, *Veratrum californicum*, also produced cyclopic lambs (Binns *et al.*, 1963), and several years later the primary teratogen responsible for this phenotype was identified and named cyclopamine (Keeler and Binns, 1966).

Nearly 30 years later, genetic studies have established that cyclopia in both humans and mice is caused by impaired Hh signaling (Belloni *et al.*, 1996; Chiang *et al.*, 1996; Roessler *et al.*, 1996), raising the exciting possibility that cyclopamine is teratogenic because it blocks the Hh pathway. Subsequent studies confirmed that cyclopamine is a Hh pathway inhibitor (Cooper *et al.*, 1998), physically interacts with the Hh signaling effector Smo (Chen *et al.*, 2002), and can block oncogenic Hh signaling (Taipale *et al.*, 2000). Small-molecule screens identified other Hh pathway inhibitors with improved pharmacological properties that also block Smo, and several of these have yielded promising results in preclinical studies that have led to clinical trials in patients with locally advanced or metastatic BCC and medulloblastoma. Favorable clinical responses to one of these inhibitors, vismodegib (GDC-0449), led to its approval by the Food and Drug

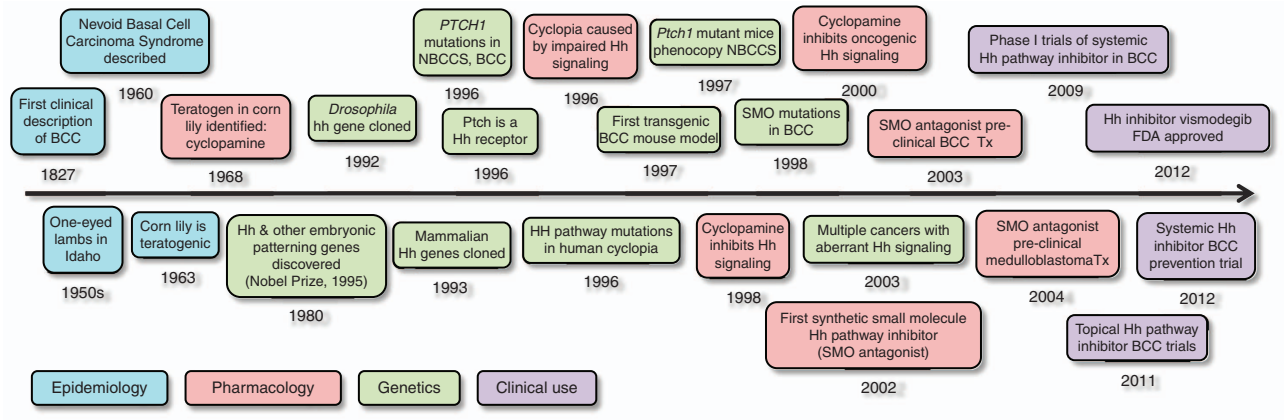

Figure 2. Milestones leading to the identification and targeting of deregulated Hedgehog (Hh) signaling in basal cell carcinoma (BCC). FDA, Food and Drug Administration; NBCCS, nevoid basal cell carcinoma syndrome; Tx, treatment.

Administration in early 2012 for the treatment of locally advanced or metastatic BCC. Multiple other SMO antagonists are currently in trials, and additional inhibitors are being examined that target different components of the Hh pathway or interacting pathways (reviewed in Amakye *et al.*, 2013).

CLINICAL USE OF HEDGEHOG PATHWAY INHIBITORS

Results of the initial clinical reports showing efficacy of Hh pathway inhibition using vismodegib in BCC and medulloblastoma were published in 2009. The BCC study demonstrated efficacy in both locally advanced and metastatic BCCs (Von Hoff *et al.*, 2009), and this was subsequently confirmed in a larger cohort (Sekulic *et al.*, 2012). The response rate in the latter study was 43% for locally advanced BCC (21% of patients were clear of disease), and 30% for patients with metastatic BCC that is highly treatment resistant and carries a poor prognosis. The medulloblastoma case report documented a rapid but short-lived therapeutic response in an adult patient with widespread metastatic disease: nearly all tumors regressed within 2 months of treatment, but 1 month later, tumors had regrown despite continued drug treatment (Rudin *et al.*, 2009). As previously shown for other cancer therapeutics targeting pivotal oncogenic drivers, resistant tumors expressed a mutant form of SMO that no longer bound the drug (Yauch *et al.*, 2009). Although resistance also develops in a subset of BCCs during treatment with vismodegib, this is relatively uncommon and observed primarily in advanced tumors from patients who may have been previously exposed to mutagenic treatments.

In a more recent BCC study, essentially all preexisting tumors in NBCCS patients regressed during vismodegib treatment. In addition, the clinical appearance of new BCCs was blocked (Tang *et al.*, 2012), arguing that Hh pathway inhibition may provide an effective prevention strategy for certain high-risk patients. This report also showed that despite striking regression and apparent clearing of preexisting tumors, discontinuation of treatment led to tumor regrowth. These results were anticipated several years earlier based on studies using a conditional mouse model examining BCC regression and recurrence following reversible genetic modulation of Hh signaling (Hutchin *et al.*, 2005).

Treatment with vismodegib and other systemic Hh pathway inhibitors is associated with several side effects, including muscle cramps, alterations in taste perception, weight loss (presumably related to taste alterations), and alopecia (Tang *et al.*, 2012), and these are severe enough to drive some patients to discontinue treatment. Development of alopecia is not surprising given the established role of Hh signaling in hair growth, and the expression of Hh pathway components in taste organs suggests that taste disturbances may also reflect on-target side effects. Understanding the mechanisms underlying Hh pathway inhibitor-associated toxicities may uncover previously unappreciated functions for Hh signaling in adult organs and may lead to approaches to mitigate side effects.

The location of BCCs makes them ideally suited for medical treatment using topical or intralesional therapy that should lessen or eliminate side effects associated with systemic treatment. Two studies using topical Hh pathway antagonists have been reported. In one, the treatment was ineffective probably owing to a lack of Hh pathway blockade, despite the fact that the drug (CUR61414) was effective in preclinical trials in mice (Tang *et al.*, 2011). The second study documented effective inhibition of Hh signaling and either a reduction in tumor size, or clinical clearing, within 4 weeks of treatment with a topical formulation of the Hh pathway inhibitor LDE225 (Skvara *et al.*, 2011).

The availability of vismodegib and other systemic Hh pathway antagonists provides an important new addition to the treatment armamentarium for patients with advanced and metastatic BCCs and, in some cases, is likely extending the lives of these individuals. However, it remains too early to know whether systemic or topical/intralesional treatment will be useful, either as monotherapy or as a neoadjuvant, in less advanced tumors that represent a vast majority of BCCs. Also currently

unclear is whether Hh pathway inhibitors may serve as a preventative in high-risk patients. Regardless, it seems likely that Hh pathway inhibitors of one type or another will play an increasingly important role in the medical management of at least some patients, and perhaps many, with BCC.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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