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Laboratory-Clinic Interface

Crosstalk between hedgehog and other signaling pathways as a basis for combination therapies in cancer

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ABSTRACT

The hedgehog (Hh) pathway is aberrantly activated in a number of tumors. In medulloblastoma, basal cell carcinoma, and rhabdomyosarcoma, mutations in Hh pathway genes lead to ligand-independent pathway activation. In many other tumor types, ligand-dependent activation of Hh signaling is potentiated through crosstalk with other critical molecular signaling pathways. Among such pathways, RAS/RAF/MEK/ERK, PI3K/AKT/mTOR, EGFR, and Notch are of particular interest because agents that selectively inhibit these pathways are available and can be readily combined with agents such as vismodegib, sonidegib (LDE225), and BMS-833923, which target smoothened—a key Hh pathway regulator. Numerous preclinical studies have revealed the ways in which Hh intersects with each of these pathways, and combination therapies have resulted in improved antitumor efficacy and survival in animal models. Hh also plays an important role in hematopoiesis and in the maintenance of BCR-ABL-driven leukemic stem cells. Thus, combined inhibition of the Hh pathway and BCR-ABL has emerged as a promising potential therapeutic strategy in chronic myeloid leukemia (CML). A number of clinical trials evaluating combinations of Hh inhibitors with other targeted agents are now underway in CML and a variety of solid tumors. This review highlights these trials and summarizes preclinical evidence of crosstalk between Hh and four other actionable pathways—RAS/RAF/MEK/ERK, PI3K/AKT/mTOR, EGFR, and Notch—as well as the role of Hh in the maintenance of BCR-ABL-driven leukemic stem cells.

Introduction

The hedgehog (Hh) pathway is critical for embryonic development and adult homeostasis, but aberrant activation of the Hh pathway is implicated in tumorigenesis [1]. The Hh pathway is therefore an attractive target for anticancer therapy. A number of agents that inhibit smoothened (SMO), a key regulator of this pathway, have been introduced in the clinic [2]. Tumors with activating mutations in this pathway have shown sensitivity to Hh inhibitors, whereas the activity of Hh inhibitors in other tumors has been minimal [2,3]. Thus, simultaneously targeting Hh and other signaling pathways may prove to be a more effective method for impeding tumor growth. This review discusses the role of the Hh pathway in cancer (alone and in concert with other pathways), the existing preclinical evidence of synergy between Hh inhibitors and other targeted agents in cancer, and ongoing clinical trials investigating these multitargeted combinations.

The role of Hh signaling in cancer

The Hh pathway plays a key role in proliferation and differentiation during embryogenesis [1] and in the regulation of stem cell renewal and tissue homeostasis in the adult [2,4,5]. During canonical Hh signaling, three mammalian Hh ligands—sonic hedgehog (SHH), desert hedgehog, and Indian hedgehog—each with distinct spatial and temporal expression patterns, activate Hh signaling by binding to the trans-membrane receptor patched (PTCH), which resides at the base of the primary cilium [6]. Upon binding, PTCH-mediated inhibition of SMO, a G-protein coupled receptor-like protein, is relieved. SMO then translocates to the tip of the primary cilium and initiates downstream Hh signaling, leading to activation of the glioma-associated oncogene (GLI) family of transcription

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factors and their target genes [6]. GLI transcription factors are inhibited in part by suppressor of fused (SUFU), which controls their nuclear entry and transcriptional effects [6].

Multiple mechanisms of Hh activation in cancer have been proposed [7]. Ligand-independent activation is caused by loss-of-function mutations in the negative regulators *PTCH* and *SUFU*, activating mutations in the positive regulator *SMO*, or amplification of GLIs. Ligand-independent Hh activation occurs in basal cell carcinoma (BCC) [8], medulloblastoma (MB) [9], and rhabdomyosarcoma [10]. Ligand-dependent Hh activation involving autocrine (tumor cells both secrete and respond to Hh ligand), or paracrine (tumor cells secrete Hh ligand and stimulate the growth of stromal cells or stromal cells secrete Hh ligand and stimulate the growth of surrounding tumor cells) signaling is observed in numerous tumors [7]. Autocrine Hh signaling has been implicated in chronic myeloid leukemia (CML) [11] and in pancreatic, lung, breast, prostate, upper gastrointestinal tract, and colorectal cancers [7]. Paracrine Hh signaling has been implicated in glioma, B-cell malignancies, and pancreatic, colon, ovarian, breast, and prostate cancers [7,12–20]. The mechanisms by which the Hh signaling pathway and the tumor stroma interact during paracrine signaling are not completely understood; however, activated Hh signaling in stromal cells may lead to the production of growth factors (e.g., vascular endothelial growth factor, insulin-like growth factor) and stimulation of signaling pathways (e.g., Wnt, interleukin 6 [IL-6])—that in turn stimulate tumor growth [18,19,21,22].

The Hh signaling pathway has also been implicated in the regulation of cancer stem cells (CSCs) by promoting their self-renewal [23]. Activated Hh signaling has been identified in the CSCs of numerous solid tumors (glioblastoma, breast, colon, pancreatic, hepatocellular) and hematologic malignancies (CML, multiple myeloma), and has been shown to increase tumor-initiating populations and contribute to cell migration, clonogenecity, growth, and survival. These CSC-promoting effects can be abrogated by treatment with SMO inhibitors [24–33]. Hh signaling has also been shown to promote tumor metastasis and recurrence, likely through its induction of genes involved in the epithelial-to-mesenchymal transition (EMT) [23,27,34,35].

It is clear that the Hh pathway contributes to tumorigenesis at multiple stages—initiation, growth, maintenance, metastasis, and recurrence—however, its role in each is often confounded by signals from other pathways. Indeed, in most cases, tumor eradication will likely require combined inhibition of Hh signaling and other signaling pathways that contribute to the tumor microenvironment.

Crosstalk between Hh and other signaling pathways in cancer

Evidence of crosstalk between Hh and other signaling pathways has been reported in many tumor types (Figs. 1 and 2). Here we describe interactions with 4 pathways—RAS/RAF/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K)/AKT/mTOR, epidermal growth factor receptor (EGFR), and Notch—that are targets of agents currently used in the clinic as well as the role of Hh in the maintenance of leukemic stem cells (LSCs). Hh interacts with many other pathways, including Wnt [36,37], TGFβ [38], stromal cell-derived factor 1 (SDF-1)/chemokine (C-X-C motif) receptor 4 (CXCR4) [39], androgen receptor [40], and thyroid hormone [41]. These interactions, however, are less well characterized and will not be discussed further.

RAS/RAF/MEK/ERK

The RAS/RAF/MEK/ERK pathway is involved in numerous cellular processes including cell proliferation, growth, and survival; its dysregulation, caused by mutations in pathway components (RAS, RAF, MEK), has been identified in several tumor types including colon, lung, pancreatic, and ovarian cancers, melanoma, and leukemia [42,43]. Simultaneous activation of the RAS/RAF/MEK/ERK and Hh pathways is frequently observed in these cancers and numerous studies have suggested crosstalk—both positive and negative—between the two pathways during tumorigenesis [42]. In a transgenic mouse model, simultaneous overexpression of GLI2 and KRAS led to formation of pancreatic adenocarcinomas and increased mortality [44]. In another study, transfection of pancreatic duct cells with SHH and KRAS in orthotopic transplantation experiments led to increased proliferation and tumor growth as compared with transfection with either gene alone [45]. In a mouse model of KRAS-induced pancreatic preneoplastic lesions, loss of Gli1 prevented tumor formation [19]. The role of Hh signaling through Gli1 was further explored in this model—activated Gli1 in the stroma was shown to induce IL-6/signal transducer and activator of transcription 3 (STAT3) signaling, which is thought to play a role in the progression of pancreatic cancer from preneoplastic lesions. Moreover, mutant *KRAS* was shown to enhance SHH expression in pancreatic tumor cells, leading to activation of GLI1 in stromal cells [19,46].

KRAS has also been shown to activate Hh signaling in a non-canonical, ligand-independent manner in pancreatic cancer cells through regulation of *Gli1* expression, GLI1 phosphorylation, and GLI1 protein degradation [42,47–49]. In mouse models of *Kras*-induced pancreatic ductal adenocarcinoma, Smo-independent Gli1 activation was required for survival of tumor cells and Kras-mediated transformation [48,50]. Kras and TGFβ1 were shown to regulate *Gli1* expression in the absence of Smo [48].

Non-canonical activation of Hh signaling through the RAS/RAF pathway was also demonstrated in colon cancer cells [51]. Inhibition of RAS/RAF signaling led to decreased GLI1 mRNA and protein levels. Another example of non-canonical activation of Hh signaling was observed in mouse melanomas—oncogenic RAS and dominant active MEK-1 enhanced the nuclear localization of GLI1, whereas their inhibition led to cytoplasmic accumulation of GLI1 [52]. Similar pathway interactions have been observed in multiple cell lines, including human prostate cancer and glioma [52]. In gastric cell lines, PTCH expression correlated with both ERK1/2 phosphorylation and SHH expression [53]. Moreover, MAPK/ERK signaling increased GLI activity and induced expression of Hh target genes in these cells.

Together, these studies suggest that a positive crosstalk exists between RAS signaling and paracrine Hh signaling. Interestingly, in pancreatic cancer models, KRAS was shown to suppress autocrine Hh signaling—by interfering with GLI2 function and GLI3 processing—while simultaneously activating paracrine signaling [42,46,54]. This negative regulation may be an effort to shift Hh signaling from the tumor to the surrounding stromal environment.

PI3K/AKT/mTOR

The PI3K/AKT/mTOR pathway, which plays a role in growth, metabolism, motility, proliferation, differentiation, survival, and angiogenesis [55], has been shown to regulate GLI activity in both normal and cancerous cells [56–58]. In human cancer cell lines, the phosphatase PP2A, which functions in opposition to mTOR complex 1, was shown to inhibit the nuclear localization of GLI3 as well as GLI3-dependent activation of cyclin D1 [57]. Localization of GLI1 and GLI2 were not affected in this model. In a metastatic melanoma cell line, dominant active AKT1 enhanced the nuclear localization of GLI1, whereas its inhibition led to cytoplasmic accumulation of GLI1 [52]. Moreover, in human prostate cancer and glioma cell lines, inhibition of AKT via PTEN also inhibited GLI1 nuclear localization and activity [52]. Conversely, loss of Pten