Paracrine Hedgehog Signaling in Cancer

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Abstract
Ligand-dependent and ligand-independent activation of the Hedgehog (Hh) signaling pathway is involved in tumorigenesis. The importance of mutations in Hh pathway components leading to constitutive signaling has been well established in basal cell carcinoma and medulloblastoma. However, the role of ligand-driven Hh pathway activation in cancer remains to be established. Three recent articles support a model in which, in the absence of mutations in the Hh pathway, Hh ligands expressed by a subset of epithelial cancers, including colon, pancreatic, and ovarian cancer, promote tumor growth indirectly by activating Hh signaling in the surrounding stroma, which, in turn, provides a more favorable environment for tumor growth. These data have important implications for the use of Hh pathway inhibitors currently in development and for selection of tumors likely to respond to such inhibitors. [Cancer Res 2009;69(15):6007–10]

The Hedgehog signaling pathway controls cell proliferation and differentiation during embryonic development, and when mutated or misregulated contributes to tumorigenesis (1). Activation of the Hedgehog pathway by mutations has been associated with the development of basal cell carcinoma and medulloblastoma (2). In addition, several studies have suggested that even in the absence of mutations in Hh pathway components, tumor cells produce and respond to Hh ligand in an autocrine-juxtacrine manner (3–6). However, three recent publications support an alternate model in which tumor-produced Hh ligands do not activate this pathway in tumor epithelial cells, but rather trigger signaling in the stromal environment in a paracrine manner (7–9). In response to Hh pathway activation, the stromal compartment contributes to tumor formation and progression via mechanisms that remained to be characterized (Fig. 1).

Paracrine Hh signaling is critical for the development and maintenance of various epithelial structures (1, 10). Upon secretion by epithelial cells, the signaling protein Hh [Sonic Hh (Shh), Indian Hh (Ihh), or Desert Hh (Dhh)] binds to the Patched 1 (PTCH1) 12 trans-membrane domain receptor on adjacent mesenchymal cells, thereby de-repressing the Smothened (SMO) 7 trans-membrane domain protein (1). Activated SMO relocates to primary cilia and initiates an intracellular signaling cascade that ultimately drives the activation and nuclear translocation of the GLI (after glioblastoma) transcription factor family members, resulting in upregulation of Hh target genes, including GLI1 and PTCH1, which respectively trigger positive and negative feedback loops following pathway activation.

The role of Hh signaling in cancer was first identified in patients with Gorlin syndrome, a rare condition caused by loss-of-function mutations in PTCH1. Loss of PTCH1 leads to constitutive activation of the pathway and the development of numerous basal cell carcinomas. These patients are also predisposed to medulloblastoma, a tumor of cerebellar granule neuron progenitor cells, and rhabdomyosarcoma, a connective tissue tumor thought to arise from skeletal muscle progenitors (2). Most sporadic basal cell carcinomas and up to a third of sporadic medulloblastomas show Hh pathway activation, caused most commonly by inactivating mutations in PTCH1 or more rarely by activating mutations in SMO. As the Hh pathway is mutated at the level of PTCH1 or SMO, inhibition of SMO with small molecule drugs such as the natural compound cyclopamine or other small molecule drugs such as the chemically optimized compound Hh-Antag, might provide a way to interfere with tumorigenesis (11, 12). Indeed, tumor regressions have been reported in patients with advanced basal cell carcinoma treated in a phase I clinical trial with GDC-0449, a Hh pathway inhibitor (13) developed by Genentech Inc. and Curis Inc. (Cambridge, MA) under a collaboration agreement between the two companies.

Importantly, inhibition of the Hh pathway might also have a therapeutic effect in tumors in which Hh ligand(s) are overexpressed. In the absence of mutations in components of the Hh pathway, Hh signaling has been described to play a role in the growth of a variety of epithelial cancer types, including small-cell lung cancer, pancreatic and other gastrointestinal tract malignancies, as well as prostate cancer (3–6). These studies reported an autocrine requirement for the pathway in tumor epithelial cells. This role is in contrast with the normal function of the pathway in these tissues during development in which Hh ligand secreted by the epithelium typically functions in a paracrine manner by activating the signaling cascade in the mesenchyme (10).

Three recent reports showed that Hh ligand-expressing cancer cells of epithelial origin are refractory to ligand, whereas surrounding stroma cells are ligand responsive (7–9). Yauch and colleagues made the key observation that Smo-antagonist-mediated growth inhibition of a large number of cancer cell lines does not correlate with Hh target gene expression in these cells, suggesting that the observed in vitro growth repression might be due to off-target effects when these compounds are used at high concentrations. The half-maximal inhibitory concentration (IC50) of Hh-Antag to inhibit the Hh pathway in a mesenchymal cell line was more than two orders of magnitude lower than the IC50 required to inhibit cell growth in the most sensitive cancer cell line tested. Furthermore, the effect of high drug concentrations on the viability of these cells does not correlate with the regulation of bona fide Hh target genes such as GLI1 and PTCH1. Although the existence of a potential autocrine role for Hh signaling in some tumor types cannot be ruled out, these results imply that studies using high concentrations of some of the SMO-inhibitors need to be interpreted with caution. Despite these potential off-target effects, the pathway seems to be involved in cancer. A subset of human
colorectal, pancreatic, and ovarian tumors expressed substantial levels of Hh ligand, in particular SHH and or IHH. Strikingly, analysis of a large number of human tumor xenograft experiments showed that the levels of Hh ligand mRNA expression in tumor cells correlated with increased Gli1 and Ptch1 mRNA levels in the stroma and not the tumor compartment, indicating that the Hh ligands activate Hh signaling in the surrounding stroma and not the tumor epithelium. Inhibition of this paracrine signal may be of therapeutic value, because specific inhibition of Hh signaling in the stroma with either Hh-Antag or a neutralizing anti-Hh antibody resulted in growth inhibition of Hh ligand-expressing xenografts. Furthermore, genetic ablation of Smo in the murine stroma led to reduced growth of human tumor xenografts, corroborating the contribution of Hh-activated stroma in tumorigenesis. Monitoring Hh ligand expression in tumor cells or Hh pathway activation in the stroma might provide an important clinical biomarker for selection of Hh-inhibitor responsive tumors, as xenografts of cancer cell lines that express these ligands are inhibited by Hh-Antag, whereas xenografts that lack Hh ligand fail to respond to this inhibitor.

Additional evidence for a paracine model of Hh signaling in cancer came from studies on mouse models of pancreatic ductal adenocarcinoma (PDAC) (refs. 7, 8). Nolan-Stevaux and colleagues recently showed that genetic deletion of Smo from pancreatic
epithelial cells did not affect the development and or progression of KrasG12D-driven PDAC (7). In addition to the fact that Smo expression was dispensable for the initiation and progression of PDAC, Ptc1 and GlI1 expression levels were not substantially affected by genetic ablation of Smo in neoplastic ductal cells. The opposite experiment done by Tian and colleagues showed that epithelial expression of an oncogenic allele of Smoothened (SmoM2), which triggers constitutive, ligand-independent activation of the Hh pathway (14), was not able to induce neoplastic transformation in murine pancreatic epithelium nor to affect tumor development and progression in KrasG12D-driven PDAC models. Important, SmoM2 failed to activate the canonical Hh pathway in epithelial cells as measured by the introduction of a Ptc1-LacZ reporter allele or by measuring Gli1 mRNA levels using quantitative PCR following laser capture microdissection of the tumor and stromal compartments.

Interestingly, epithelial expression of activated Gli2 has previously been shown to cooperate with activated KrasG12D to induce undifferentiated pancreatic tumors (15). Although the origin of these tumors has not been fully described, it is important to note that expression of this truncated form of Gli2 is resistant to key posttranscriptional regulatory mechanism and does not reflect the ability of the cell to transduce a signal initiated at the cell surface by Hh ligand. Additional evidence of Gli signaling in pancreatic
tumor cells came from the recent identification of mutations in the Gli transcription factors in human pancreatic cancer cell lines (16). However, the effect of these mutations on Gli activity needs to be determined before concluding that these downstream Hh signaling components can contribute cell autonomously to adenocarcinoma formation. Finally, Nolan-Stevaux and colleagues showed that Gli activity in pancreatic tumor cells could be stimulated in an Hh-independent manner by TGF-β and KRAS. Regardless of the Smo expression status in PDAC cells, TGF-β treatment resulted in upregulation of Gli1 and Gli3, whereas depletion of Kras expression downregulated Gli1 and Ptc1 mRNA. In addition, Gli1 contributed to PDAC cancer cell survival and the KRAS-dependent malignant cellular phenotype. However, these observations are currently limited to in vitro cell culture, and the levels of Gli activity in the tumor cell compartment seem to be much lower than in adjacent stromal cells as shown by the Ptc1-LacZ reporter or by quantitative mRNA analysis of microdissected cells from mouse or human PDAC (8). It will be interesting to extend these results to in vivo models, for example by specific deletion of Gli1 from the epithelial compartment in the mouse PDAC model.

The three studies discussed here support a model in which Hh ligands secreted by the epithelial tumor cells activate Hh signaling in the surrounding stroma. Hh signaling in stromal cells likely results in the expression of soluble factors and extracellular matrix components that act upon the tumor epithelium or other cell types, ultimately promoting tumor growth (7–9). In agreement with the paracrine model, Shh seems to contribute to fibrotic tissue formation in the tumor microenvironment of an orthotopic model of human pancreatic cancer (17). Shh can also act as an indirect angiogenic factor that induces the expression of vascular endothelial growth factors and angiopoietins in the mesenchyme (18). In Yauch and colleagues inhibition of stromal Hh signaling resulted in decreased expression of components of the Wnt and insulin-like growth factor signaling pathways in the tumor stroma (9). Interestingly, these two pathways are also involved in the progression of cancers driven by mutations in the Hh pathway; insulin-like growth factor 2 is induced as medulloblastoma lesions progress to more advanced tumors (19) and Wnt/β-catenin signaling contributes to the development of skin hamartomas (i.e., benign tumor-like lesions) in mice that express SmoM2 (20). Future studies are required to determine how these and other factors affect tumor growth and which factors can be targeted for therapy.

Recent studies have implicated Hh signaling in the self-renewal and survival of various epithelial and nonepithelial cancer stem cells (21–26), commonly a rare population of cells within the tumor mass that can self-renew and reestablish the tumor of origin when transplanted into immunodeficient mice. For example, Hh signaling seems to regulate the self-renewal of epithelial cancer stem cells in the breast (24) and multiple myeloma stem cells (25), and perhaps more convincingly the maintenance of chronic myelogenous leukemia stem cells (23, 26). Because many of these studies involve the use of high concentrations of cycloamine, it will be important to confirm these observations in vivo by genetic activation or ablation of Smo (as in refs. 23, 26). Although Hh signaling might have a direct role in certain tumor stem cell compartments, paracrine Hh signaling in stromal cells might also be involved in the establishment and maintenance of a cancer stem cell niche.

In conclusion, tumor-stromal interactions have been recognized as critical to tumor establishment and growth, and the development of anti-angiogenic drugs has already yielded successful agents for the treatment of cancer (27). The paracrine signaling mechanism of Hh provides another target for tumor control through inhibition of signaling in the relatively normal stromal cell, which should be less likely to acquire mechanisms of therapeutic resistance. However, demonstrating antitumor efficacy will be challenging in a clinical setting. Currently, several Hh inhibitors are under development and the Hh pathway inhibitor GDC-0449 is being evaluated for efficacy in two tumor types that might have a predominantly paracrine mechanism of Hh signaling (1). For ovarian cancer, patients who have achieved complete remission after a second or third course of chemotherapy will be treated with GDC-0449 or placebo to determine whether Hh pathway inhibition can increase the time until tumor recurrence. Similarly, for metastatic colorectal cancer, GDC-0449 or placebo is given to patients receiving their first course of biochemotherapy to determine if Hh pathway inhibition can improve response to therapy or can increase the time until disease recovers. Both of these trials will determine whether tumors that express high levels of Hh ligand can be inhibited by Hh inhibitors, and potentially provide proof-of-concept for Hh pathway inhibition in paracrine-driven tumors.

In contrast to the better-defined role of Hh signaling in mutation-driven tumors such as basal cell carcinoma, it has been more difficult to characterize the role of paracrine signaling of Hh in cancer, even though this paracrine mechanism is well recognized in developmental biology. However, paracrine Hh signaling may play a critical role in a much larger subset of human cancers than mutation-driven cancers. In addition, it may be possible that both paracrine and autocrine Hedgehog signaling cooperate in some tumors. Increased understanding of the role of this pathway in Hh ligand overexpressing tumors may lead to the development of more effective targeted therapies for a broad range of cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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