Hedgehog Signaling Pathway and Lung Cancer

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Signaling pathways responsible for embryogenesis play a critical role in the maintenance of stem cells in adult life and cellular responses to injury. Dysfunction of the developmental signaling pathways during adult homeostasis leads to various events resulting in the development of neoplasia. We review the biology of the hedgehog signaling pathway and its potential role in the development of lung cancer.

Key Words: Hedgehog signaling, Lung cancer.

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Signaling pathways responsible for embryogenesis appear to play a critical role in the maintenance of stem cells in adult life and cellular responses to injury. Dysregulation of these signaling pathways during adult homeostasis can lead to various events resulting in the development of neoplasia (Figure 1).

Hedgehog (Hh) signaling pathway is one such pathway that is crucial in the embryogenesis. Hh pathway also plays a central role in the repair and regeneration of adult tissue. The Hh signaling pathway was first studied in drosophilae. During embryonic development, drosophilae with a mutation in the Hh gene were covered with pointed denticles, resembling a hedgehog, hence the name. Several studies have recently demonstrated that dysregulation of the Hh signaling plays a role in several cancers including the brain, skin, gastrointestinal tract, pancreas, and lung.[1–6]

Hh SIGNALING

Mammalian Hh signaling pathway (Figure 2) constitutes (a) Hh ligand with three variants: desert (Dhh), Indian (Ihh), and sonic (Shh); (b) a transmembrane receptor-patched homolog 1 and 2 (Ptch1 and 2); (c) smoothened (Smo), a G protein–coupled receptor; and (d) a cytoplasmic complex that regulates the cubitus interruptus (Ci) or glioma-associated oncogene homolog (Gli) family of transcriptional effectors. All the three ligands bind to the same receptors and elicit similar responses. Shh is the most extensively characterized variant and is widely expressed during embryogenesis. Shh acts as a morphogen and plays an important role in the formation of the neural tube, axial skeleton, primitive gut, and the tracheobronchial tree.[7,8]

Autocatalytic cleavage and coupling of cholesterol are the essential posttranslational processes that maintain the signaling capability of the Hh ligands.[9] The secretion of the functional Hh ligand by the Hh-secreting cell is dependent on the availability of dispatched (Disp), a transmembrane protein with homology to patched (Ptch), which is an Hh receptor on the Hh responsive cell.[10,11]

The Ptch 1 and 2 are membrane receptors for Hh ligands. Ptch 1 is more widely expressed and well characterized. Binding of Hh ligand with the Ptch alters the interactions of Ptch with Smo, resulting in the activation of Smo. This initiates a cascade of events resulting in the Ci and Gli entering the nucleus and acting as transcriptional activators. It is unclear how the activation of Smo communicates with the cytoplasmic Ci/Gli transcription factor complex. Gli bind to the DNA through zinc finger domains directed to particular target genes regulating key cell survival and differentiation functions. Gli1 is a transcription activator, and Gli2 and Gli3 are both activators and repressors of transcription. Gli3 and Ci regulate transcription by binding to the CREB-binding protein, which is a transcription coactivator. Cyclin D and cyclin E are known transcriptional targets of Hh signaling, and these proteins are vital in the G1-to-S transition in the cell cycle.[12] Hh signaling activates the mitosis promoting factor by increasing the intranuclear availability of cyclin B.[13] Hh signaling also opposes normal stimuli for epithelial cell cycle arrest (by inhibiting P21) and promotes cell growth.[14] Hh signaling inhibits a well-known regulator of apoptosis, the p53 tumor suppressor gene.[15]

When the Hh ligand is unavailable Ptch1 inhibits the activity of Smo, thus repressing the downstream signaling events. When the Hh signaling is lacking, Gli proteins are bound to microtubules in the cytosol along with a multiprotein complex consisting of Fused (Fu) and suppressor of Fu (SuFu).

The Hh operates through a series of inhibitory steps. The availability of the Hh ligand for signaling is regulated by the expression of Hh interacting protein (HIP) on the cell surface of Hh responsive cell. The HIP is a membrane glycoprotein that binds to Hh ligands with an affinity similar to that of the membrane protein Ptch1. HIP lacks signal transduction capacity and acts to internalize and degrade the Hh ligand.[16] Activation of the Hh pathway causes an increased expression of the HIP via a negative feedback mech-

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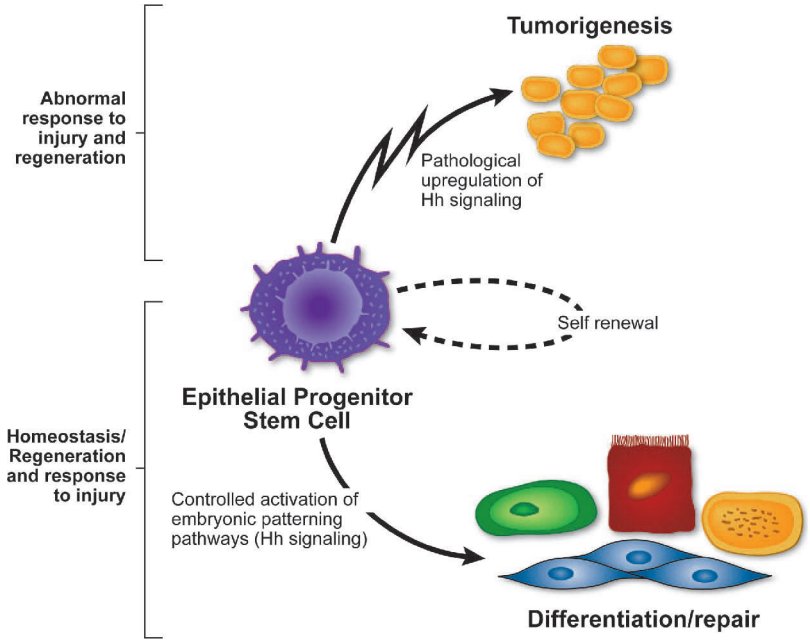

FIGURE 1. Role of developmental signaling pathways in the Tumorigenesis.

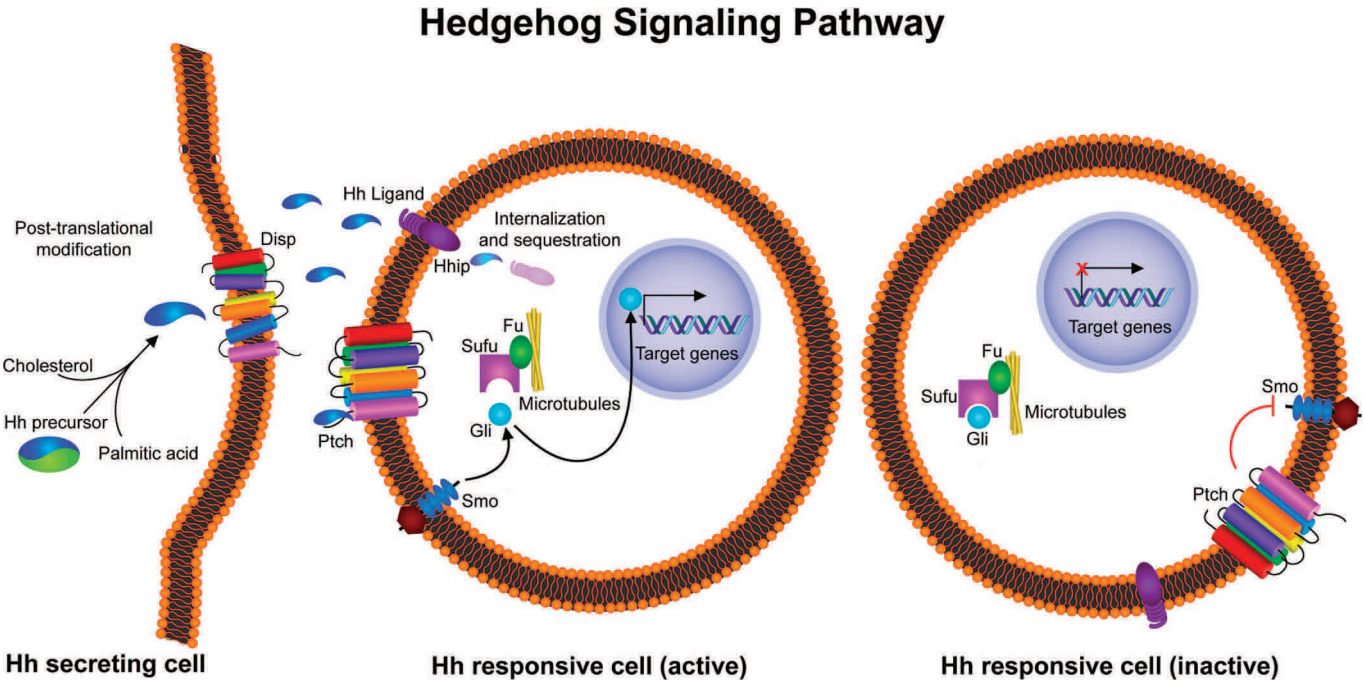

FIGURE 2. Hedgehog signaling pathway.

anism, thus serving as an inducible antagonist of Shh signaling.[16] The Hh signaling is regulated at various levels, indicating the importance of tight control of Hh signaling. Several inhibitors of the pathway like Ptch and HIP are transcriptional target genes and Hh activation induces negative feedback, reducing the intensity of Hh signaling. Gli genes are regulated by complex mechanisms at both the posttranslational and transcriptional level. Hh signaling up-regulates Gli1 expression while repressing Gli3 expression.[5]

Dysregulation of the Hh signaling can occur from ligand-dependent and -independent mechanisms (Table 1).

Hh SIGNALING IN THE DEVELOPMENT OF NORMAL LUNG

The lungs develop from an outpouching of the primitive endodermal tube into the surrounding mesenchyme. In the developing lung in mouse models, an elevated Shh expression was detected in the tracheal diverticulum and in

TABLE 1. Dysregulation of Hh Pathway in Cancer

Pathway Component	Type of Cancer
Increased Hh ligand	Basal cell carcinoma,[1,31] medulloblastoma, small cell lung cancer,[6] digestive tract tumors,[32–34] ovarian tumors,[35] prostate cancer[36]
Reduced expression of Hh interacting protein	Pancreatic cancer,[37] liver,[23] lung,[23] digestive tract tumors,[23] prostate cancer[36]
Inactivating mutations in the Ptch	Medulloblastoma,[1] basal cell carcinoma,[38] rhabdomyosarcoma[39]
Activating mutations in the transmembrane helices of Smo	Basal cell carcinoma,[40] ovarian tumors[35]
Overexpression of the Gli proteins	Basal cell carcinoma,[41] small cell lung cancer,[6] esophageal cancers,[33] gastric cancers[34]
Loss of function mutations of SuFu	Medulloblastoma,[42] rhabdomyosarcoma,[39] prostate cancer[36]

Hh, hedgehog; Ptch, transmembrane receptor-patched homolog; Smo, smoothened a G protein–coupled receptor; Gli, glioma-associated oncogene homolog; SuFu, suppressor of fused.

the trachea and lung endoderm.[17] Studies indicate that the Hh signaling pathway is essential for the growth and differentiation of the trachea and lung, and aberrations in the signaling components may be involved in abnormal development of the lung.[7,8,17] Natural teratogens like cyclopamine and jervine (extracted from of corn lilies) are inhibitors of Hh signaling. Pregnant animals treated with these inhibitors of Hh signaling at an early gestation period results in multiple developmental anomalies including abnormal lung development.[18]

The airway epithelial progenitor (stem) cells play an important role in the development of the respiratory epithelium. The differentiation of these progenitor cells to form the neuroendocrine or non-neuroendocrine (ciliated, mucous, clara, or basal cells) component of the respiratory epithelium is tightly regulated by a complex bipotential notch signaling.[6,19] Notch and Wnt signaling are evolutionary conserved signaling pathways tightly regulating cell death, cell movement, and cell division and differentiation during embryogenesis. During development and repair of the lung epithelium, Hh signaling maintains this bipotential notch signaling.[6,19] Hh and Wnt pathways possibly play an important role in the maintenance and the expansion of the progenitor (stem) cells during development and can mediate lung growth by signaling to adjacent lung mesenchyme.[20]

Hh SIGNALING AND LUNG CANCER

Hh signaling is possibly inactive in the human adult lung epithelium except in the epithelial progenitor (stem) cells. This persistence of Hh signaling in the epithelial progenitor (stem) cells could help maintain these cells and play a critical role in the response to airway epithelial injury.[6,19,21] Studies on animal lung airway epithelial injury/regeneration model suggest that persistent injury to the airway is a potent stimulus for the activation of the Hh signaling, and this helps the expansion of airway epithelial progenitor cells.[6,19,21] Shh and Gli1 are expressed in the regenerating lung airway epithelium.[6] Studies on cell lines showed that all the seven small cell lung cancer (SCLC) and seven non-small cell lung cancer (NSCLC) cell lines expressed Shh protein. Five of seven SCLC cell lines expressed both Shh and Gli1 in contrast to NSCLC, which expressed only Shh but not Gli1.[6] Analysis of clinical samples of human lung cancer tissue demonstrated 50% (five of 10) of SCLC expressed both Shh and Gli1 compared to only 10% (four of 40) of NSCLC.[6] Another study investigating the expression of Gli1 in SCLC tissue reported that 85% (34 of 40) of SCLC express Gli1 and more than 60% have a medium to strong expression correlating with increased Hh signaling.[22] It thus appears that lung cancer cells retain aspects of the Hh signaling seen in the primitive lung endodermal cells. However, the degree of dependence on this signaling varies ahmong the subtypes of lung cancer. Inhibition of Shh ligand activity using monoclonal antibody and cyclopamine resulted in the significant growth inhibition in SCLC cell lines expressing both Shh and Gli but not NSCLC cell lines (which do not express both Shh and Gli).[6] Similar results were found in in vivo studies on lung cancer xenografts in nude mice.[6]

HIP is a natural antagonist of Hh signaling as discussed above. Reduced expression of HIP as been reported in lung cancer A549 cell line xenograft in nude mice and a decrease in the expression of HIP was seen in five of 10 human NSCLC tissues.[23] Experimental models studying HIP knockout mice confirmed increased Hh signaling.[24,25] There appears to be down-regulation of HIP in endothelial cells during angiogenesis.[23] These findings suggest that reduced expression of HIP could potentially enhance the Hh signaling and possibly facilitate angiogenesis. Hh signaling thus appears to play a role in proliferation of malignant cells and promote angiogenesis.

Hh SIGNALING PATHWAY AS A THERAPEUTIC TARGET

Therapeutic inactivation of the Hh signaling offers a potential treatment for cancer. Inactivation of the Hh signaling can be done at various levels, mainly (1) extracellular blocking of the Shh ligands using Shh antibodies, (2) activation of Smo in the cell membrane, (3) modulating intracytoplasmic regulators of Hh signaling like protein kinase A and SuFu, and (4) altering the intranuclear functioning of Gli.

Curis Inc. developed a Shh antagonist that showed promising results in preclinical models.[26,27] Antibodies to Shh are currently being evaluated in phase I clinical trials for basal cell carcinoma. Cyclopamine is another potential molecule of interest for the inhibition of Hh signaling.[26,27] Cyclopamine has demonstrated a good safety profile in mice.[2,4,6] Several other compounds that bind to Smo and inhibit the downstream events have been identified (KAAD-cyclop, SANT1-4, CUR61414).[28] However, patients with downstream alterations in the Hh signaling could be resistant to the treatment with Shh antagonist and

Smo targeted therapies. Another potential mechanism for blocking Gli activity is the use of protein kinase A agonists like forskolin, which maintain the Gli in inactive state.[29] Antisense oligonucleotides targeting Gli RNA also provide a viable option to prevent Gli-mediated activation of target genes.[3,30]

It is important to clarify the role of activation of Hh pathway in the process of carcinogenesis and progression in lung cancer. Numerous mechanisms have been implicated in the development, proliferation, and progression of lung cancer; it is critical to understand how the Hh pathway interacts with the other pathways implicated in lung cancer. There have been virtually no significant advances in the systemic therapy of SCLC in the past three decades. With the current trend toward developing targeted therapies, the Hh pathway modulators offer a potential new avenue in the treatment of lung cancer.

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