

Perspectives

Hedgehog Signaling

Progenitor Phenotype in Small-Cell Lung Cancer

D. Neil Watkins^{1,*}

David M. Berman²

Stephen B. Baylin¹

¹Sidney Kimmel Comprehensive Cancer Center; ²Departments of Pathology; Molecular Biology and Genetics; Johns Hopkins University School of Medicine; Baltimore, Maryland USA

*Correspondence to: Neil Watkins; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins; 1650 Orleans St, RM. 572; Baltimore, Maryland 21231 USA; Email: nwatkins@jhmi.edu

Received 04/03/03; Accepted 04/08/03

Previously published online as a Cell Cycle Paper in Press at:

<http://www.landesbioscience.com/journals/cc/toc.php?volume=2&issue=3>

KEY WORDS

Cancer, Hedgehog signaling, Morphogenesis, Stem cells

ABSTRACT

Recently, we have shown that small cell lung cancer (SCLC) is dependent on activation of Hedgehog signaling, an embryonic pathway implicated in development, morphogenesis and the regulation of stem cell fates. These findings form the framework for an emerging view of cancer as a process of aberrant organogenesis in which progenitor/ stem cells escape dependence on niche signaling through mutation in genes such as *Ptch*, or through persistent activation of progenitor cell pathways. Interestingly, the normally quiescent airway epithelial compartment uses the Hh pathway to repopulate itself when challenged by injury. How Hh signaling works to promote the malignant phenotype promises to be as important biologically as the promise of Hh pathway inhibitors are clinically.

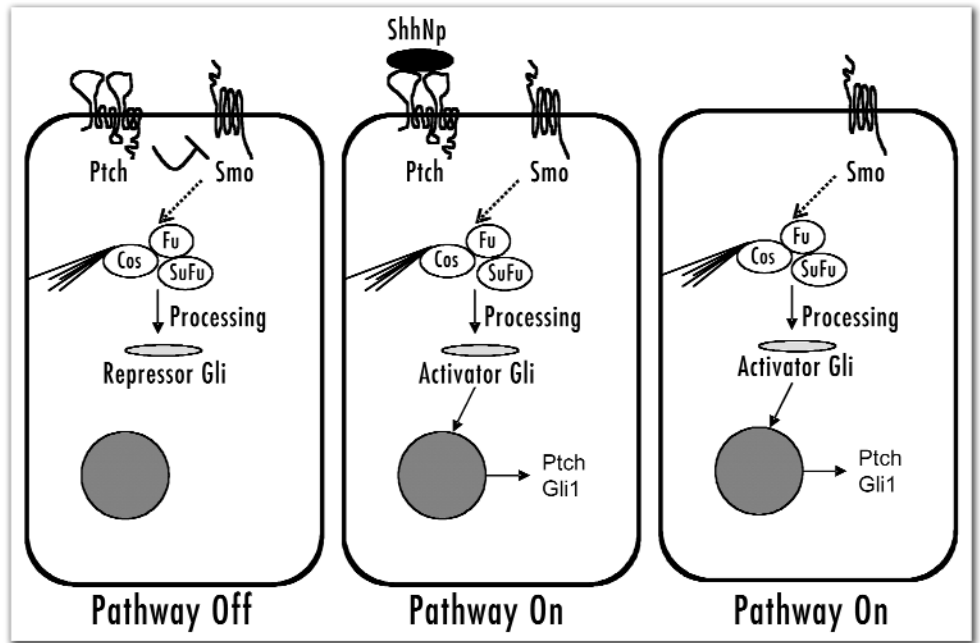
Small cell lung cancer (SCLC) is a highly aggressive, frequently lethal malignancy representing approximately 20-25% of all lung tumors.¹ Despite initial responses to chemotherapy, the lethality of this tumor is illustrated by 5 year survival rates of between 2–8%,² and is reflected in early and frequent dissemination to multiple organs including the brain, liver and adrenal glands.³ Although classified as a neuroendocrine (NE) tumor, the biological origins of this cancer have remained a matter of conjecture.^{1,4} Recently, we have shown that SCLC is dependent on activation of Hedgehog signaling,⁵ an embryonic pathway implicated in development, morphogenesis and the regulation of stem cell fates.⁶ This finding sheds new light on the potential histogenesis of SCLC, and its relationship to airway epithelial development, repair and differentiation.

The Hedgehog (Hh) pathway is a conserved embryonic signaling cascade originally identified a mediator of segment polarity in the fly.⁷ Signaling by the predominant mammalian ortholog of the *Drosophila* gene *Hedgehog*, Sonic Hedgehog (Shh), establishes morphogenic gradients essential for axial patterning of the mammalian embryo.⁸⁻¹⁰ The active Shh signaling peptide is formed by an auto-processing reaction that converts a 45kDa protein into a 19kDa signaling peptide that is doubly lipid modified, with palmitate and cholesterol moieties at its N and C termini, respectively. Efficient generation of the Shh signal requires these modifications of the original protein,¹¹⁻¹² as well as the transporter-like function of the transmembrane protein Dispatched.¹³

The Shh receptor, Patched (*Ptch*), is a twelve-transmembrane protein with homology to the resistance, nodulation, division (RND) bacterial transporter family.¹⁴ *Ptch* catalytically inhibits the seven-transmembrane protein Smoothed (*Smo*), rendering the pathway inactive in the absence of Shh.¹⁰ Binding of the Shh ligand inactivates *Ptch*, leading to *Smo* mediated effects on a cytoplasmic complex tethered to microtubules by the kinesin-like protein Costal2. This complex regulates proteolytic processing of the Hh pathway transcriptional effector Gli proteins and their access to the nucleus,^{6,15} where they activate Hh target genes, including *Ptch*, and the transcriptional activator Gli1.^{6,15-17} A simplified diagram illustrating the mammalian Hh pathway is shown in Figure 1.

More recent studies have illustrated the capacity of Hh signaling to regulate stem cell fates.^{6,18} This is nicely illustrated in cerebellar development, where a Shh gradient established by Purkinje cells regulates expansion and proliferation of granule cell precursors.^{19,20} Inactivating mutations in *Ptch* result in aberrant Hh pathway activation, and are associated with medulloblastoma, a pediatric brain malignancy arising in the cerebellum.^{19,21-23} Moreover, Hh pathway activation is essential for the growth of medulloblastoma,²⁴ suggesting that the specification of progenitor cell fates by Hh signaling occupies a critical, “master-regulator” role in controlling the malignant behavior of cells. These findings form the framework for an emerging view of cancer as a process of aberrant organogenesis in which progenitor/stem cells escape dependence on niche signaling through mutation in genes such as *Ptch*, or through persistent activation of progenitor cell pathways.^{6,18} The

Figure 1. A Simplified model of hedgehog (Hh) signaling. In the absence of the ligand Sonic Hedgehog (Shh), the receptor Patched (Ptch) constitutively inhibits Smoothened (Smo). This promotes cytoplasmic tethering and processing of the Gli transcription factors by a multi-protein, microtubule associated complex consisting of the kinase Fused (Fu), the novel protein Suppressor of Fused (SuFu) and Costal2 (Cos). Processing favors repressor forms of Gli proteins, which silence the Hh transcriptional program. Binding of Shh inhibits Ptch. This in turn de-represses Smo, releasing unprocessed Gli activators from the complex to promote transcription of Hh targets in the nucleus, including Gli1 and Ptch. Similar pathway activation is achieved in the absence of Ptch function through mutation or epigenetic silencing.



resultant tumor may therefore be viewed as more than the sum of a cancer cell's mutations, but rather a complex genetic and epigenetic perversion of organ development and regeneration.

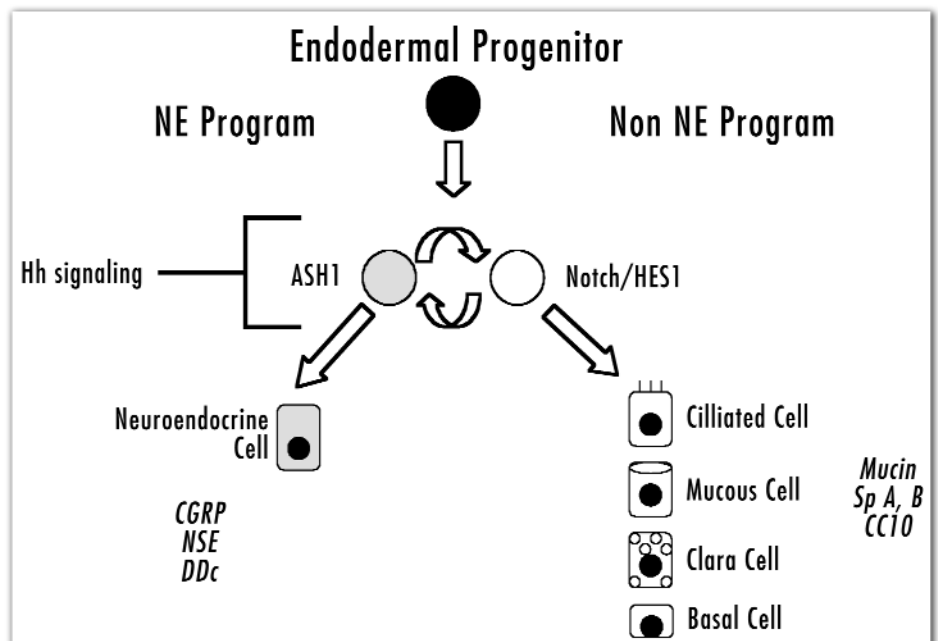
How might we integrate these ideas in the context of lung cancer? Based on the hypothesis that cancer may relate to abnormal repair processes, we investigated Hh signaling in an airway epithelial injury/regeneration model developed in Barry Stripp's laboratory.^{25,26} In this model, we observed intra-epithelial Hh signaling during regeneration of the airway epithelium, where subsets of regenerating airway cells expressed Shh, or the pathway target and transcriptional effector Gli1.⁵ This finding was a surprise on two levels. First, it suggested that the normally quiescent airway epithelial compartment uses the Hh pathway to repopulate itself when challenged by injury. Second, it contrasts with the known role of Hh signaling in early lung development, in which Shh synthesized in endodermally derived lung buds signals to adjacent mesenchyme to pattern branching morphogenesis.²⁷⁻³⁰

We went on to confirm these studies in a *Ptch-LacZ* reporter mouse in which β -galactosidase activity indicates the activation of the endogenous *Ptch* promoter and therefore reception of Hh signaling.²¹ In this model, we made two key observations relating to the elusive concept of an airway epithelial progenitor cell. First, Hh

pathway activation is seen during segregation of airway epithelial progenitors into NE and non-NE fates during development.⁵ Second, the adult mouse airway contains small numbers of basally located epithelial cells which constitutively demonstrate low-level pathway activation.⁵ One possible conclusion from these data is that the stem cell niche of the conducting airway epithelium, and by inference the locus of tumor initiation, is regulated by Hh signaling during the specification of NE and non-NE fates, as depicted in Figure 2.

Escape from niche dependent signals in adult epithelia may turn out to be an important mechanism in the initiation of cancer.^{6,18} If the stem cell niche of the conducting airway resides at the Clara/NE cell interface, then our findings of Hh pathway activation in the regeneration of this compartment suggest a potential mechanism for tumor initiation through aberrant Hh signaling. SCLC is well known for the paracrine expression of ligand/receptor combinations such as stem cell factor (SCF)/cKit.³²⁻³⁴ We showed that SCLC

Figure 2. Proposed model of Hedgehog signaling in airway epithelial development. Expansion of a common endodermal progenitor occurs during airway development and repair. Bipotential notch signaling segregates cells into neuroendocrine (NE) and non NE fates through control of achaete scute homolog-1 (ASH1). Mature NE cells are characterized by expression of calcitonin gene related peptide (CGRP), neuron specific enolase (NSE) and Dopa decarboxylase (DDC). Non-NE cells express a variety of markers including mucins, Surfactant proteins (Sp A,B) and clara cell specific protein (CC10). We propose that Hh signaling acts to expand and maintain the bi-potential pool at or immediately above the level of Notch/ASH1 mediated cell fate determination. During repair and development, such events allow appropriate segregation of airway epithelial lineages. Pathologic activation of Hh signaling may maintain cells at this point, contributing to the malignant phenotype of SCLC.



manifests paracrine Hh signaling through expression of both the ligand, the receptor *Ptch*, and the pathway target and transcriptional effector *Gli1*.⁵ Strikingly, cells within SCLC tumors in vivo demonstrate apparent compartmental segregation of cells which send and receive the Shh signal in an apparent duplication of the process seen in airway development and repair.⁵ This situation contrasts with ligand independent signaling seen in medulloblastoma cells harboring inactivating mutations in *Ptch*.^{21,24} It is tempting to speculate that SCLC achieves a niche independent stem cell fate through activation and maintenance of paracrine Hh signaling.

The ability to specifically inhibit Hh signaling represents an elegant experimental tool, as well as a potentially exciting therapeutic strategy. Pregnant ewes which consume the plant *Veratrum californicum* give birth to lambs with holoprosencephaly, a malformation syndrome which includes cyclopia as one of its most striking manifestations. *Veratrum* teratogenesis dramatically phenocopies the Shh knockout mouse,^{9,35} prompting investigations in the Beachy laboratory demonstrating that the *Veratrum* alkaloid, cyclopamine can reproduce this phenotype.³⁶ Cyclopamine specifically silences the Hh pathway when activated by Shh ligand, or by mutations in *Ptch*.^{6,37} Cyclopamine, and a series of novel compounds do this by binding to the heptahelical bundle of the Smo molecule,^{38,39} demonstrating the capacity of mechanism-based drug discovery and targeted drug design to specifically disable Hh signaling with compounds of increasing potency.^{38,39}

The therapeutic potential of this approach was initially shown in medulloblastoma, the first demonstration that aberrant Hh signaling is essential for the maintenance of a malignant phenotype.²⁴ Despite mutations in both p53 and Rb, and the paracrine activation of numerous growth promoting pathways, SCLC is no less dependent on Hh for growth both in vitro and in vivo.⁵ Moreover, SCLC express wild type *Ptch*, and are growth inhibited by inactivation of the Shh ligand demonstrating that growth effects can be mediated by Shh itself.⁵ As the downstream effectors of Hh signaling are not well characterized, we can only speculate on how such growth arrest might be achieved in these cells. In the context of SCLC, and perhaps other tumors, the mechanism by which Shh may regulate progenitor cell biology and its relationship to cancer. The emphasis in cancer therapy has switched from cytotoxic compounds to "mechanism based" therapies aimed at disabling specific growth promoting pathways. The remarkable vulnerability of medulloblastoma, SCLC, and perhaps other cancers to specific Hh pathway inhibitors is a dramatic illustration of such an approach. How Hh signaling works to promote the malignant phenotype promises to be as important biologically as the promise of Hh pathway inhibitors are clinically.

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