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Hot Topic

Inhibition of Hedgehog signaling in the gastrointestinal tract: Targeting the cancer microenvironment

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ABSTRACT

This review summarizes emerging information regarding the Hedgehog (Hh) signaling pathway during neoplastic transformation in the gastrointestinal tract. Although there is a role for the well-established canonical pathway in which Hedgehog ligands interact with their receptor Patched, there is sufficient evidence that downstream components of the Hh pathway, e.g., Gli1, are hijacked by non-Hh signaling pathways to promote the conversion of the epithelium to dysplasia and carcinoma. We review the canonical pathway and involvement of primary cilia, and then focus on current evidence for Hh signaling in luminal bowel cancers as well as accessory organs, i.e., liver, pancreas and biliary ducts. We conclude that targeting the Hh pathway with small molecules, nutriceuticals and other mechanisms will likely require a combination of inhibitors that target Gli transcription factors in addition to canonical modulators such as Smoothened.

Introduction

During the 1970s, mutagenesis screens in *Drosophila* uncovered a number of important developmental signaling pathways that have since been applicable to mechanisms for both mammalian development and cancer [1]. The Hedgehog (Hh) signal transduction pathway is one such pathway. The initial discovery uncovered a gene locus that when mutated induced the abnormal development of hair-like projections on flies (denticles) such that its physical appearance was reminiscent of the porcupine-like mammal called hedgehog. The Hh locus was found to encode a 50 kDa precursor protein autocatalytically processed to the 19–20 kDa functional protein that was later discovered to be the ligand for a receptor encoded by another locus called *Patched* (*Ptch*). Hh ligand binding to its receptor Ptch relieves the inhibition exerted on a vast signaling cascade, which includes *Smoothened* (Smo), and a repressor complex (Costal 2, Fused kinase, Suppressor of Fused) that regulates the availability of the transcriptional regulator *Cubitus Interruptus* (*Ci*) [2]. Details of the *Drosophila* pathway have been extensively reviewed elsewhere [3–5]. Moreover since this review focuses on Hh signaling in the mammalian gastrointestinal tract during transformation, key components of the mammalian Hh pathway and what is known regarding canonical Hh signal transduction will be briefly summarized first for several tissue types [6–9]. Second, the role of Hh signaling during neoplastic transformation for individual cancers of the gastrointestinal (GI) tract will be described with an emphasis on the response by stromal cells. Stromal cells have typically been the targets of small molecule development and natural product inhibitors (nutraceuticals) with the expectation that they will serve as adjuvant therapies for Hh responsive cancers [10,11]. The final section will discuss specific examples of how Hh signaling contributes to transformation by activating tumor associated mesenchymal and immune cell types. Investigations into the mechanism of Hh signaling has led to the discovery that some cancers are ligand independent (non-canonical signaling) [12] and will require examination of therapies that inhibit downstream signaling components such as the mammalian homolog of Ci called glioma-associated protein 1 (Gli1) [13,14]. In addition, one might query whether components of the Hh signaling pathway can be used as biomarkers [15]. Interestingly, it has recently been reported that Shh circulates in plasma raising the possibility of using the ligand as a biomarker in some cancers [16].

Overview of the Hh signaling pathway in mammalian cells

Canonical Hh signaling involves *epithelial* expression of the ligands, which subsequently bind the 12-pass transmembrane receptors Ptch1 and Ptch2 to relieve their inhibitory influence on an adjacent 7-pass transmembrane Hh activator called Smo. Smo is located on ectodermal (neural) or mesodermal-derived cell types that respond to the Hh ligands (Fig. 1). Co-receptors that bind ligand and cooperate with Ptch to modulate the cellular response such as proliferation include members of the immunoglobulin

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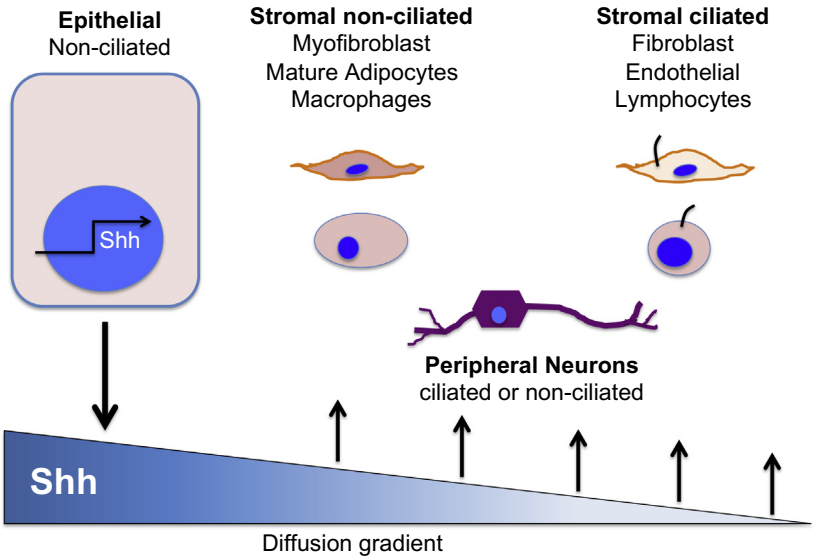

Fig. 1. Canonical Hedgehog Signal Transduction. Canonical Hedgehog signal transduction implies that the ligand, e.g., sonic hedgehog (Shh) is secreted by an epithelial cell and forms a gradient as it diffuses away from the cell. The ligand is sensed by cells in the stroma, which express Hh signaling components and primary cilia (ciliated versus non-ciliated).

superfamily, Growth arrest specific 1 (Gas1), CAM-related/down-regulated by oncogenes (Cdo) and brother of Cdo (Boc) [17–19]. Mammalian cells express three Hh ligands – Sonic Hh (Shh), Indian Hh (Ihh) and Desert Hh (Dhh) that bind the Ptch receptor with apparently the same affinity [6] (Table 1). In the gastrointestinal tract, the major ligands expressed are Shh in the proximal gut (esophagus, stomach, liver and pancreas) and Ihh in the midgut and hindgut (small intestine and colon) [20]. However, Dhh expression appears to be tissue-restricted to the nervous system and testes [21]. Some tissues exhibit differential potency amongst the ligand paralogs with respect to Hh signaling (Shh >>> Ihh > Dhh). Although the luminal GI tract (stomach, small intestine and colon) constitutively expresses Hh ligands during development and after birth, parenchymal organs (liver and pancreas) express the ligands only in mature tissues and when injured [22–26].

Unlike Ptch, Smo is a heterotrimeric guanine nucleotide binding-protein coupled receptor (GPCR) that can activate at least two intracellular signaling cascades – a non-canonical, ligand-independent pathway that ultimately modulates the actin cytoskeleton by activating Rac1 and Rho1 GTPases [27,28] or a canonical, ligand-dependent pathway via Gli2 activation. Canonical signaling through Smo involves intracellular activation of Gli2 by limited proteolysis. Full-length Gli2 resides in the cell cytoplasm coupled to a suppressor complex comprised of Fused kinase (Fu), Suppressor of Fused (SuFu) and Costal2 (Cos2). Gli2 repression of target genes occurs after a series of phosphorylation steps by protein kinase A (PKA), glycogen synthase 3β (GSK3β) and casein kinase I (CK1), which directs Gli2 to the proteasome for limited protein degradation after ubiquitination by the Skip–Cullin–F-box (SCF) protein/β–Transducin repeat Containing Protein (TrCP) complex (Fig. 2). Limited degradation of phosphorylated Gli2 removes the C-terminal activation domain uncovering the N-terminal repressor domain. The partially degraded DNA binding protein then translocates to the nucleus and forms a complex with co-repressors such as HDAC-Sin3A to block target gene activation [29,30]. Smo activation releases Gli2 from the suppressor complex and the transcription factor preventing phosphorylation by PKA, GSK3 and CK1. Limited proteasomal degradation in the absence of specific phosphorylation removes the N-terminal repressor domain producing the Gli2 activator version of the protein, which translocates to the nucleus to bind the promoters of genes induced by Hh signaling. Examples of genes induced by Gli2-mediated Hh signaling include its receptor *Ptch*, *Hedgehog interacting protein* (*Hhip*) and the transcription factor *Gli1* [29]. Thus *Gli1, Ptch* and *Hhip* are general transcriptional targets of canonical Hh signaling activity. Gli1 mediates transcriptional activation; whereas Ptch and Hhip are transmembrane receptors that block the pathway, albeit by different mechanisms. Hhip binds and sequesters ligand; whereas, ligand binding to Ptch relieves its inhibition on Smo de-repressing Hh signaling. Examples of numerous tissue-restricted genes regulated by Hh signaling include *Vascular Endothelial Growth Factors* (*VEGF*), *Angiopoietin-1* and *Angiopoietin-2* in endothelial cells [31,32]. *Snail, Zeb1,2, Twist2, FoxF1* in fibroblasts [33–35] α-smooth muscle actin, vimentin and IL-6 in myofibroblasts [36,37], *N-myc* in neurons regulating development and myelination [38,39], *BMI, Sox2, Nanog* in cancer stem cells [7,40] (Table 1).

The Hh pathway has evolved to represent crosstalk between the endodermally-derived epithelium that produces the ligand and responding cells derived from ectoderm (neural cells) and mesoderm (fibroblasts, immune cells, myocytes) (Fig. 1). In the adult gastrointestinal tract, it is the stromal cells derived from meso- or ectodermal cell layers that typically express the Hh signaling receptor and transcription factors. However, immunohistochemical staining of adenocarcinomas from the gut and other cancer tissue types suggest that these epithelial-derived human tumors are capable of both ligand and receptor expression [26]. This observation implies autocrine regulation of neoplastic cell growth that emerges during transformation since *Ptch, Smo* and *Gli1, Gli2, Gli3* mRNA expression is essentially never observed in the normal gut epithelium [41].

In addition to expression of specific signaling components, some Hh responding cells also produce an extracellular organelle called the primary cilium, which is required to properly transduce canonical Hh signaling [42,43]. Primary cilia are collections of acetylated α-tubulin arranged as nine dimers in a cylindrical configuration with (9 + 2) or without a central dimer (9 + 0) encased within a plasma membrane sheath. Regardless of the arrangement, primary cilia are motile or non-motile sensory organelles. Although some chemosensory cells such as tuft cells contain bundles of acetylated α-tubulin at the cell apex, these bundles do not associate with basal bodies and therefore are not considered primary cilia.