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Making a leaner Hedgehog

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Abstract

Secreted Hedgehog (Hh) proteins are essential in development, and their aberrant activity contributes to certain cancers. Chemically targeting a lipid modification of Hh proteins results in loss of their cellular activity, revealing new strategies for cancer intervention and elucidation of the role of lipids in signal transduction.

The secreted Hh proteins are dually lipid-modified cell-cell communication molecules with cholesterol and palmitate covalently attached at their C and N termini, respectively¹ (Fig. 1a). The activation of the Hh pathway entails binding of Hh to its receptor Patched (Ptch), thereby relinquishing the suppressive action of Ptch on Smoothened (Smo), a seven-transmembrane effector protein (reviewed in ref. 2). Hh-induced accumulation of Smo in the primary cilium, an antenna-like organelle found in most cells, initiates a series of biochemical events that culminate in the activation of the Gli family of DNA-binding proteins. First established as a developmental signaling system, the Hh pathway is now recognized as a platform used in cancerous cells for achieving deviant cell growth³. Using an *in vitro* screening strategy, Petrova *et al.*⁴ identify small molecules, including RU-SKI 43, that disable the palmitoylation of Hh, thus introducing chemical probes that can be applied to understand the role of Hh lipidation in signal transduction and defining a new chemical strategy for targeting Hh signaling in disease.

The cholesterol modification in Hh is an autocatalytic event mediated by the C-terminal domain. The addition of the palmitoyl adduct to the thiol group in the N-terminal cysteine residue is catalyzed by an enzyme known as Hedgehog acyltransferase (Hhat)¹. A chemical rearrangement transfers the fatty-acyl adduct onto the N terminus, thereby yielding a free thiol side chain. Hhat belongs to a superfamily of multitransmembrane proteins termed membrane-bound O-acyl transferases (MBOATs) that link fatty acids to membrane-embedded targets, including lipids and proteins⁴. In addition to Hh, two other protein substrates for MBOATs have been identified: the secreted Wnt signaling molecules and the

orexigenic ghrelin hormone (Fig. 1b). Many studies have been devoted to reconciling how the hydrophobic nature of dually lipidated Hh protein can be compatible with their ability to engage cells distant from the cells that produce them. These studies have provided evidence that the palmitoyl adduct facilitates the membrane release of Hh by Dispatched, a protein similar to Ptch, and engages the extracellular chaperone molecule Scube (Fig. 1a). Although the lipid modification status of Hh is not thought to influence its binding to Ptch, Hh proteins that have atypical fatty-acyl adducts or lack an N-terminal cysteine show altered activity, suggesting that this post-translational modification may affect its interaction with one or more Hh co-receptors^{2,5}.

Basal cell carcinoma, medulloblastoma and rhabdomyosarcoma are cancers that can be driven by either inactivating or activating mutations in Ptch and Smo, respectively. So far, disabling Smo seems to be the most chemically tractable approach to crippling these tumors. One compound (vismodegib) received US Food and Drug Administration approval in 2012 for treating locally advanced and metastatic basal cell carcinoma and is in phase 2 testing for medulloblastoma.

Aberrant Hh activation can contribute to cancer by multiple mechanisms. So-called Hh ligand-dependent cancers do not harbor mutations in pathway components, such as Smo, but instead are thought to produce excess amounts of Hh ligand and thereby promote a tumor-supporting environment⁶. To date, targeting canonical Hh responses in this context does not seem to be a promising therapeutic approach. Clinical testing of vismodegib in patients with two such cancers, advanced ovarian and colorectal cancer, failed to show therapeutic efficacy⁷. Another Smo antagonist, IPI-926, failed to show survival benefit in patients with metastatic pancreatic cancer⁷. On the basis of these completed clinical studies, the anticancer utility of Hh inhibitors in this specific context would seem limited. However, contributions of Hh signals that are not dependent upon Smo in cancerous or cancer-supporting cells may exist and could be thwarted by Hh antagonists.

In the meantime, these Hh antagonists should be a boon for cell biology efforts focused on understanding the role of extracellular lipidation events in signal transduction. Hh inhibitors such as RU-SKI 43 will enable us to examine how palmitoylation contributes to Hh biosynthesis and signaling without relying on mutagenesis-based strategies that may not model native effects stemming from nonacylation of the ligand. For example, the consequences of chemically inducing a nonlipidated cysteine with a nucleophilic thiol group on Hh biosynthesis are unknown. Possibly, this non-disulfide-bonded cysteine may result in a misfolded Hh protein that cannot exit the secretory pathway. Thus, Hh activity may control a 'cysteine switch' that ensures the production of functional Hh molecules. This scenario may not be too improbable given that fatty acylation of Wnt proteins is well established to be a quality control mechanism for Wnt production⁸.

The MBOATs have emerged as highly druggable enzymes that function at the apex of several disease-associated signaling systems^{9–11}. The Hh inhibitors described in Petrova *et al.*⁴ reinforce the notion that druggable space in human cells is vast and remains mostly unexplored.

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Figure 1.

Hh pathway response in vertebrates. **(a)** The signal sequence (SS) is removed from the Hh precursor protein, and the C-terminal domain autocatalytically adds a cholesterol adduct. Hhat, the target of RU-SKI 43, mediates the palmitoylation of the N-terminal signaling domain. Fully processed Hh (HhNp) is released from the membrane by Dispatched (Disp) and chaperoned to other cells by Scube. HhNp engages a receptor complex that includes Ptch, resulting in inactivation of Ptch and activation of Smo in the primary cilium. The Gli DNA-binding proteins in turn initiate transcription of target genes in response to Smo activation. Loss of Hh palmitoylation has been shown to affect Hh release from the producing cell membrane, distribution to neighboring cells and activity in responsive cells. **(b)** The MBOATs constitute a large family of extracellular acyltransferases that mediate diverse fatty acylation events, including those important to Wnt, Hh and Ghrelin signaling. RU-SKI 43 joins a growing portfolio of MBOAT inhibitors that may be therapeutically useful.