Biochimica et Biophysica Acta 1856 (2015) 62–72

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Digging a hole under Hedgehog: downstream inhibition as an emerging anticancer strategy☆

Laura Di Magno [b,1], Sonia Coni [a,1], Lucia Di Marcotullio [a], Gianluca Canettieri [a,*]

[a] Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy
[b] Center for Life NanoScience@Sapienza, Italian Institute of Technology, Rome, Italy

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form 4 May 2015
Accepted 11 June 2015
Available online 12 June 2015

Keywords:
Hedgehog
Smo
Gli
Cancer therapy
Metabolism
Epigenetics

ABSTRACT

Hedgehog signaling is a key regulator of development and stem cell fate and its aberrant activation is a leading cause of a number of tumors. Activating germline or somatic mutations of genes encoding Hh pathway components are found in Basal Cell Carcinoma (BCC) and Medulloblastoma (MB). Ligand-dependent Hedgehog hyperactivation, due to autocrine or paracrine mechanisms, is also observed in a large number of malignancies of the breast, colon, skin, bladder, pancreas and other tissues. The key tumorigenic role of Hedgehog has prompted effort aimed at identifying inhibitors of this signaling. To date, only the antagonists of the membrane transducer Smo have been approved for therapy or are under clinical trials in patients with BCC and MB linked to Ptch or Smo mutations. Despite the good initial response, patients treated with Smo antagonists have eventually developed resistance due to the occurrence of compensating mechanisms. Furthermore, Smo antagonists are not effective in tumors where the Hedgehog hyperactivation is due to mutations of pathway components downstream of Smo, or in case of non-canonical, Smo-independent activation of the Gli transcription factors. For all these reasons, the research of Hh inhibitors acting downstream of Smo is becoming an area of intensive investigation. In this review we illustrate the progresses made in the identification of effective Hedgehog inhibitors and their application in cancer, with a special emphasis on the newly identified downstream inhibitors. We describe in detail the Gli inhibitors and illustrate their mode of action and applications in experimental and/or clinical settings.

Contents

1. Overview of the Hedgehog signaling

Hedgehog (Hh) signaling regulates embryonic development, tissue homeostasis and stem cell fate in vertebrate and invertebrate organisms [1]. The pathway is evolutionary conserved and highly active during

☆ All the authors declare that they have no conflicting financial interests.
* Corresponding author.
E-mail address: gianluca.canettieri@uniroma1.it (G. Canettieri).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbcan.2015.06.003
0304-419X/

mammalian development, especially within the neural tube and skeleton, and is subsequently silenced in most adult tissues [2]. In mammals, Hh signaling takes place at the primary cilium, a single antenna-like structure that protrudes from the cell surface of most adherent cell types and functions as a platform that mediates signal transduction.

There are three different Hedgehog ligands, Sonic Hedgehog (Shh), Desert Hedgehog (Dhh) and Indian Hedgehog (Ihh), which are released by specialized secreting cells as lipid-modified polypeptides after proteolytic processing. In analogy with other morphogens, the secreted Hh ligands generate a gradient of extracellular concentration, which mediates a dose-dependent intracellular response in the target cells. The Hh ligands bind the receptor Patched (Ptch), a twelve-pass transmembrane protein, and the interaction turns on the transduction cascade [3] (Fig. 1). Co-receptors Cdo, Boc, and Gas1 facilitate high-affinity binding of mature Hedgehog ligand to Ptch, thereby enhancing Hedgehog signaling strength [4]. Binding of Hh ligand abrogates the Ptch repressive effect on the seven-pass transmembrane protein Smoothened (Smo) [5]. In the absence of ligand, Ptch prevents pathway activation by blocking the entry of Smo into the primary cilium. Upon binding of Hh, Ptch leaves the primary cilium, swapping with Smo that, through unknown activation steps, propagates the Hedgehog signal downstream [6,7] (Fig. 1).

Smo regulates the activity of the Gli transcription factors, which in mammals consist of three different isoforms: Gli1, Gli2 and Gli3. The cascade of events occurring between Smo derepression and Gli activation is still poorly understood. The cytoplasmic transducer Sufu plays a key role in this context, as it controls the dynamic response of Gli to Hh agonists. Sufu binds all three Glis and exerts different functions: it sequesters Gli1 in the cytoplasmic compartment and regulates Gli2 and Gli3 processing [8–10]. Loss of Sufu is associated to increased Gli activity indicating that Sufu acts as inhibitor of the signaling [11].

Gli2 functions as an early activator, while Gli1 is a late activator and a target of itself, thus generating a positive feedback loop [12]. Gli3 shows an exclusive repressor activity in the absence of ligand. When the signaling is off, Gli2 and Gli3 are phosphorylated, ubiquitinated and partially cleaved to generate truncated repressor forms (GliR), which bind the promoters of Hedgehog target genes, thereby preventing transcription. Upon binding of Hh to Ptch, the proteolytic processing is prevented and the ratio between full-length active/truncated repressive Gli isoforms (GliA/GliR ratio) raises with consequent transcriptional activation of Hh target genes. The extracellular Hh concentration affects the ratio between GliA and GliR and thus the strength of the transcriptional response [12].

The spatiotemporal function and distribution differ among the three Glis and between their "A" and "R" isoforms to generate an orchestrated network of transcriptional effectors that is collectively named "Gli code" [13].

As a further level of control, an acetylation/deacetylation balance regulates Gli1 and Gli2 activity. Activation of Hh signaling increases HDAC1 and HDAC2 levels and activity, which promotes Gli1/2 deacetylation, thereby increasing their chromatin occupancy over target promoters [14].

Therefore, a fine-tuned balance between full length and truncated, ubiquitinated, phosphorylated and acetylated isoforms is in charge of modulating the graded response to Hedgehog ligands, overall representing the "canonical" signaling.

Hedgehog pathway may also be activated through non-canonical mechanisms, which can be divided in:

i) Smo-independent Gli activation, where Gli can be activated via MAP kinases, Pi3K/AKT and mTor dependent pathways [15].
ii) Gli independent activation, which is in turn divided into [16]:
   a. Smo-dependent mechanisms: they may recruit RhoA, Rac1, Src, PI3K/PLCγ or AMP Kinase (AMPK) and regulate cell migration, axon guidance and metabolic reprogramming.
   b. Ptch-dependent, Smo-independent mechanism: it regulates apoptosis and cell viability.

1.1. Hedgehog signaling in cancer

Hh controls a number of genes involved in cellular proliferation, migration, metabolism, cell fate determination and stemness [17–20]. In

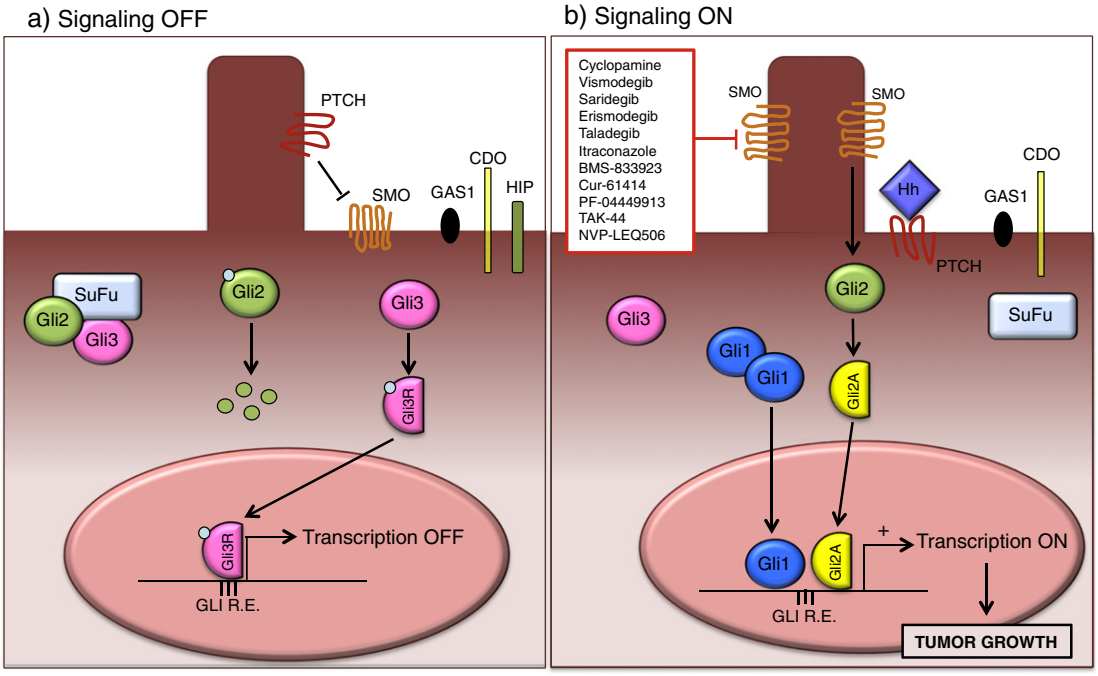

Fig. 1. Canonical Hh signaling. (a) In the absence of ligand, Ptch prevents the localization of Smo in the cilium. Gli2 and Gli3 proteins are phosphorylated, cleaved to generate truncated, repressive isoforms (R) and transcription is repressed. (b) When Hh binds to Ptch, Smo inhibition is released. Proteolytic processing of Gli is prevented with generation of full length, active forms, to allow transcription of target genes. These genes mediate cell growth and are aberrantly expressed in tumors. Pharmacological upstream inhibition of Smo can be achieved with different compounds (see the text).