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Review

Crosstalk between TGF-β and hedgehog signaling in cancer

Delphine Javelaud, Marie-Jeanne Pierrat, Alain Mauviel *

Institut Curie, Centre de Recherche, 91400 Orsay, France
INSERM U1021, 91400 Orsay, France
CNRS UMR3347, 91400 Orsay, France
Université Paris XI, 91400 Orsay, France

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ABSTRACT

Hedgehog (HH) and TGF-β signals control various aspects of embryonic development and cancer progression. While their canonical signal transduction cascades have been well characterized, there is increasing evidence that these pathways are able to exert overlapping activities that challenge efficient therapeutic targeting. We herein review the current knowledge on HH signaling and summarize the recent findings on the crosstalks between the HH and TGF-β pathways in cancer.

1. Canonical Hedgehog signaling: components and mechanistics

The Hedgehog (HH) pathway plays a critical role during embryogenesis, in particular in directing limb digit and skeletal polarity, and in the patterned formation of cells within the ventral portion of the central nervous system [1,2]. In adulthood, the SHH pathway is involved in the maintenance of tissue homeostasis and repair after severe injury [3]. This pathway regulates numerous important cellular responses such as cell proliferation, survival, differentiation, self-renewal ability, migration and epithelial to mesenchymal transition (EMT) [4]. Disruption of the HH pathway in the mouse embryo result in congenital anomalies affecting the central nervous system, axial skeleton limbs and other organs, which are found in human pathology such as holoprosencephaly [5,6]. Conversely, aberrant activations of the HH pathway have been described in a wide variety of human cancers, including basal cell carcinoma and medulloblastoma [7–9].

1.1. Hedgehog ligands and receptors

In mammals, there are three secreted HH ligands: Sonic HH, Indian HH and Desert HH. They are cholesterol- and palmitoyl-modified proteins expressed by a wide range of cell types, and bind the 12-transmembrane receptor PATCHED-1 (PTCH-1). The functional specificity of HH proteins is governed in part by their expression patterns and by regulatory mechanisms in a given cell type [10–12]. For instance, several HH binding and/or sequestrating proteins such as PTCH-2, CDO, BOC or HIP and GAS1 have been characterized. These proteins are considered co-receptors that modulate ligand presentation to PTCH-1, and positively or negatively influence cellular responses to HH ligands [13].

The current model for HH signaling is as follows: in the absence of HH ligand, PTCH-1 localizes to the primary cilium and inhibits the activity of the 7-transmembrane G-protein coupled receptor-like SMOOTHENED (SMO). The primary cilium is a microtubule-based antenna-like structure that emanates from the surface of a wide variety of cells in mammals [14]. Signals from the primary cilium are ultimately involved in regulating the cell cycle, cytoskeletal organization, intra-flagellar transport and various signaling pathways such as HH and WNT [15]. In the absence of HH ligand, only repressor forms of GLI proteins are translocated into the nucleus (Fig. 1). Binding of HH ligands to PTCH-1 results in its

* Corresponding author. Address: Institut Curie – Centre de Recherche, Team "TGF-β and Oncogenesis", INSERM U1021/CNRS UMR3347, University Center, Building 110, 91400 Orsay, France.
E-mail address: alain.mauviel@curie.fr (A. Mauviel).

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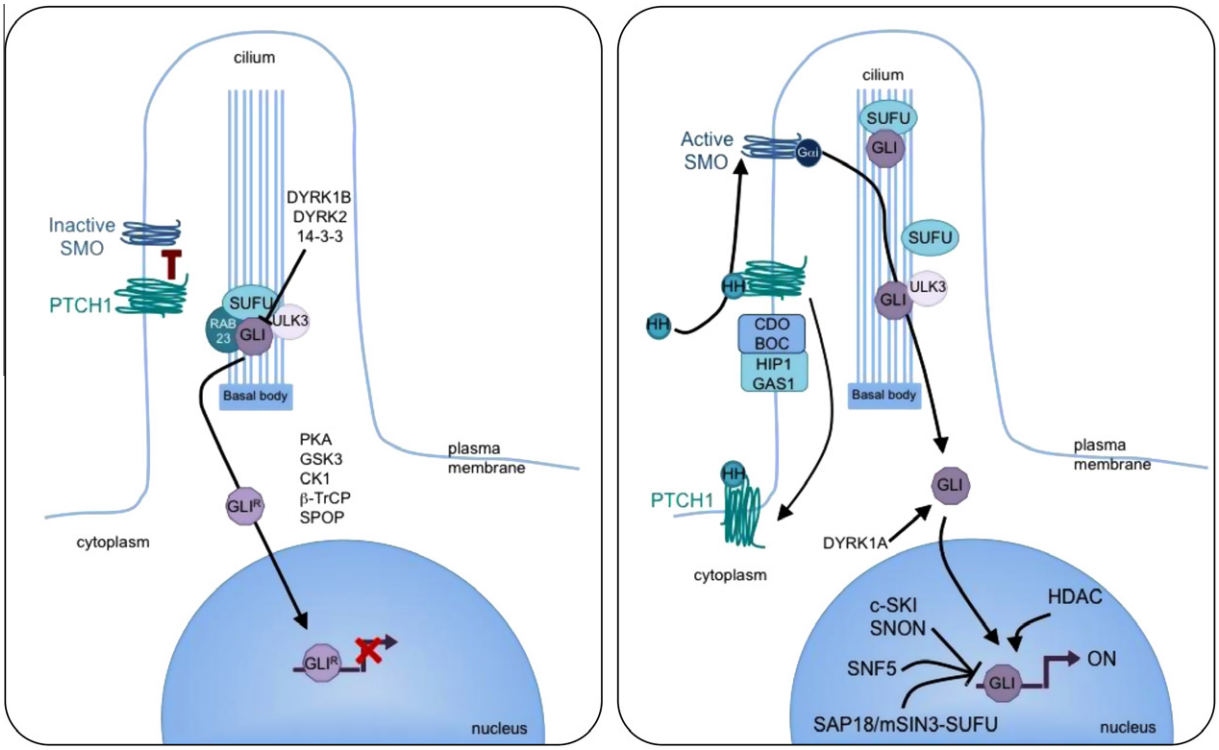

Fig. 1. Canonical Hedgehog signaling. In the absence of ligand, the HH pathway is maintained inactive by several mechanisms. In the cilium, PTCH-1 blocks signal transduction by SMO. Phosphorylation of GLI proteins by PKA, GSK3 or CK1 results in their proteasomal processing into transcription repressor forms through the recruitment of β-TRCP and SPOP. DYRK2 and DYRK1B phosphorylate GLI proteins and promote repressor form processing. GLI proteins are sequestered in the cytoplasm by their interaction with 14-3-3 and SUFU. RAB23 and ULK3 promote GLI inhibition by SUFU. In the presence of HH, PTCH-1 is excluded from the cilium and SMO, through Gαi, induces nuclear translocation of GLI proteins. HH-transduced signal is modulated positively or negatively by the interaction of PTCH-1 with co-receptors such as CDO, BOC, HIP1 and GAS1. In the nucleus, GLI proteins act as transcription factors. Phosphorylation by DYRK1A and ULK3 and acetylation by HDACs of GLI proteins increase their transcriptional activity. At the chromatin level, GLI activity may be inhibited by the recruitment c-SKI/SNON, SNF5 or SAP18/mSIN3-SUFU. (For more details, refer to text and reviews in [1,4,8,12,14–16])

exclusion from the primary cilium, release and activation of SMO, and signal transduction leading to activation and nuclear translocation of GLI transcription factors, and subsequent target gene transactivation.

1.2. Hedgehog effector molecules: GLI transcription factors

GLI proteins belong to the family of Kruppel-like factors, transcription factors with highly conserved C2H2-Zn finger DNA-binding domains. There are three mammalian GLI proteins, GLI1, GLI2, and GLI3, each encoded by distinct genes. In contrast, their Drosophila homolog, Cubitus interruptus (Ci) is unique [4,16]. GLI proteins exhibit distinct regulations, biochemical properties and target genes. One study based on the overexpression of either GLI1 or a dominant-active mutant form of GLI2 in keratinocytes determined both overlapping and distinct transcriptional programs for these two proteins [17]. For example, while *PTCH-1* and *TNC* are induced by both GLI1 and GLI2, *VGLL4* is exclusively regulated by GLI1 while *LITAF* is a GLI2-specific target. Other studies have identified *OPN* (osteopontin) [18] and *MUC5A* [19] as GLI1 targets, while functional GLI2 binding sites have been characterized on *BCL2* [20] *PTHrP* [21] *Follistatin* [22] and *BMP2* [23] promoters.

The intrinsic molecular nature of GLI proteins largely contributes to their functional specificity. Processing of GLI2 and GLI3 N-terminus represents a critical mechanism allowing regulation of target genes downstream of HH signaling. In the absence of HH ligands, GLI3 is sequentially phosphorylated by PKA, GSK3 and CK1 on multiple sites in its C-terminal region. This allows the recruitment of the F-box protein β-TrCP, an E3 ubiquitin ligase that targets GLI3 to a limited proteolysis by the proteasome to generate a repressor form [24] that does not utilize HDACs for transcriptional repression [25]. HH signaling inhibits GLI2 and GLI3 processing, thereby elevating full-length GLI2/3 levels [26,27]. As GLI3 exhibits only a weak transcriptional activity, it is mainly considered an inhibitor of HH activity while GLI2 serves as the main transducer of HH gene responses. GLI2 has a composite structure, comprising an activator domain in its C-terminus and a repression domain in its N-terminus, flanking a central five zinc finger DNA-binding domain [26]. Cleavage of the latter (GLI2ΔN) unmasks the strong transactivating potential of GLI2. GLI2 is thought to be the primary transcriptional activator downstream of HH signaling [28]. GLI1 lacks a repressor domain and is also a potent transcriptional activator. It is not processed proteolytically and is directly regulated by HH signaling [29], contributing to the amplification of HH responses. It is a direct transcriptional target of GLI2 [30] and is able to rescue some of GLI2 functions [31].

1.3. Suppressor of Fused, master inhibitor of HH signaling

When SMO is inactivated by PTCH-1, GLI2 and GLI3 interact with the inhibitory protein SUFU (Suppressor of Fused), and remain sequestered in the cytoplasm so that only the repressor forms can be translocated to the nucleus [32]. SUFU is able to regulate GLI levels by antagonizing the ubiquitin ligase SPOP, which targets GLI proteins for complete degradation by the proteasome [33,34]. SUFU may also repress GLI-dependent transcription by recruiting the histone deacetylase complex SAP18–mSin3 at the nuclear level [35]. Negative regulations of GLI protein such as proteasomal degradation or sequestration by SUFU are relieved in presence of HH