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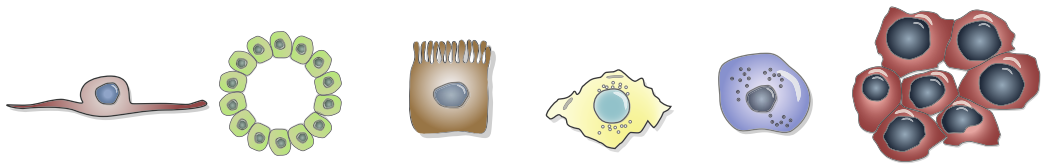
The many faces of Hedgehog signalling in the liver: Recent progress reveals striking cellular diversity and the importance of microenvironments

To the Editor:

We have read with great interest the work of Grzelak and colleagues. The authors report the occurrence of primary cilia-positive cell populations and have characterised detailed features of Hedgehog (Hh) signalling during chronic liver injury, induced by thioacetamide (TAA) [1]. They describe an attractive scenario for driving fibrosis and repair in response to TAA treatment based on local niches and two signalling routes: (i) the canonical pathway involving primary cilia and SMO and (ii) an apparently SMO- and primary cilium-independent pathway.

The question of whether and when primary cilia-positive cells exist or occur in the liver, is important because cellular Hh signalling may qualitatively differ in cells equipped with or bare of primary cilia at their surface. Grzelak and colleagues properly approached this question by using specific staining methods for detecting this organelle. They demonstrate that the majority, if not all hepatocytes in normal and TAA-treated livers, do not express a primary cilium, whereas primary cilia-positive cell populations, identified as liver progenitor cells (LPCs), occur in injured liver. Thus, they confirm earlier results from various



Structure or function	Endothelial cell	Cholangiocyte	Hepatocyte	Hepatic stellate cell (HSC) - quiescent	Kupffer cell	Liver progenitor cell (LPC)
Primary cilium (Pc)	Yes	Yes	No	~5%	No	Yes
Pc positive/Smo-dependent signaling	Yes	Yes	No	No/~5% Yes	No	Yes
Pc negative/Smo-dependent signaling	?	?	Yes	Yes	?	No
Smo-independent signaling	?	?	Yes	?	Yes	Yes
Hh producing and/or responding cell	P/R	P/R	P: IHH, pericentral cells /R: all cells	P: activated HSC/R: all cells	P/R	P/R
Cyclopamine responsive	Yes	Yes	Yes	Yes	Yes	Yes
Hhip expression	+++	?	+	+++	?	+++
Strength of Hh signaling (Gli response)	++	++	+	+++	?	+++
Major GLI factors investigated	Gli1, **Gli2**, Gli3	Gli1, **Gli2**, Gli3	Gli1, **Gli2**, Gli3	Gli1, **Gli2**, Gli3	?	**Gli2**
Known Hh-dependent functions	• Regulates capillarisation	• Influences viability and differentiation • EMT • Chemokines	• IGF1 • IGFBP1 • Metabolism	• EMT	• Chemotaxis	• EMT
[Ref.]	[7]	[3]	[6]	[4,7]	[8]	[1,7,5]

Fig. 1. Diversity of Hedgehog signalling among major liver cell types. The information listed is based on published features of Hedgehog signalling in different cell populations of healthy (hepatocytes, cholangiocytes, endothelial cells, HSC) or diseased liver (all other cell types). The liver progenitor cells (LPCs) shown here refer to those occurring after exposure to thioacetamide [1]. Because this cell population as well as HSC and Kupffer cells vary considerably depending on the type and severity of the disease, the spectrum of their Hedgehog signalling may vary as well. ?, not yet known; P/R, producing and responding; +, weak; ++, medium; +++, strong; EMT, epithelial-mesenchymal transition.

groups, inferred from electron and confocal microscopic images (for discussion see [2]). Indeed, the presence of primary cilia may considerably enhance the strength of GLI factor activation as evidenced by Grzelak and colleagues using the BMOL1.2 LPC cell line.

However, the generalisation of the results on the contribution of SMO-dependent and -independent GLI activation to the functions of this cell line should be made with due caution, because the spectrum of Hh-related signalling appears to be rather broad in different (liver) cell types as depicted in Fig. 1. Besides canonical (cilia- and SMO-dependent) signalling in endothelial cells, cholangiocytes [3], activated hepatic stellate cells (HSC) [4] and progenitor cells [1,5], there is obviously cilia-independent but SMO-dependent signalling in mature healthy hepatocytes [6], Kupffer cells and, most probably, in quiescent HSC. The latter cells, at least their majority, do not express cilia [1], in contrast to activated HSC. The existence of this unusual type of Hh signalling (cyclopamine responsive) is most evident for healthy hepatocytes [6]. In addition, SMO-independent, truly non-canonical signalling, has been observed at least in part of the cell types (Fig. 1). Thus, each major cell type in the liver appears to exhibit its own, specific combination of different types of Hh signalling (Fig. 1) [1,3–8]. These differences may be highly flexible due to various activated states of these cells under pathological conditions. Since most of these cell types are not only Hh responding but also secreting cells, depending on those activated states, the concept of intrahepatic signalling niches of Hedgehog suggested by Anna Mae Diehl's group [cf. [5]] and Grzelak and colleagues [1] acquires more attention. Thus, even the normal sinusoid (or possibly the space of Dissé) must be considered as a Hh signalling niche of high complexity, forcing us in the future to focus on the microanatomical environments of cells, local sources and concentrations or gradients of morphogens, and on the different sensitivities of Hh-responsive cells to understand their physiological impact. Given the cellular diversity, questions regarding which type of signals (Hh ligands and other factors modulating Hh signalling) are transmitted within different niches and what type of message they are transmitting become important. To address these questions, comparison with other organs may be helpful [9].

Concerning liver disease, another aspect of great importance is to elucidate how the normal Hh niche becomes transformed into (locally or globally) a disease-associated niche. With respect to TAA, Grzelak and colleagues have provided us with valuable insights of what occurs in terms of the Hh-driven transient expansion of cellular diversity and the local reconstruction of niches [1]. Concerning the signals involved and their proper cellular origin, however, their paper is less clear. Because *in situ* hybridisation was performed only for *Shh* [1], it remains unclear whether and where *Ihh* is also expressed in higher amounts in response to TAA. At the protein level the use of pan Hh antibodies leaves us with a similar uncertainty: IHH immunostaining in hepatocytes was shown in disturbed areas in close proximity of LPCs only in a few pictures [1]. Although the authors refer to the IHH-positive hepatocytes as "injured cells" [1], proof of the actual injury is lacking. Within this context, it should be emphasised that *Ihh* in normal liver has been recognised as a target gene of Wnt/ß-catenin signalling [10], and, correspondingly, expression could be demonstrated in healthy hepatocytes around the central veins [6,10] where Wnt signalling is highest (cf. [2]). Thus, it would be informative if more details of Hh ligand expression in control livers and in response to TAA were available.

In summary, (intra)hepatic Hh signalling emerges as a major player in health and disease, the proper impact of which can only be elucidated if we manage to narrow down our research to microenvironments and mutual cell-cell interactions.

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Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflicts of interest with respect to this manuscript.

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