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Review

Metabolites in vertebrate Hedgehog signaling

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ABSTRACT

The Hedgehog (HH) signaling pathway is critical in embryonic development, stem cell biology, tissue homeostasis, chemoattraction and synapse formation. Irregular HH signaling is associated with a number of disease conditions including congenital disorders and cancer. In particular, deregulation of HH signaling has been linked to skin, brain, lung, colon and pancreatic cancers. Key mediators of the HH signaling pathway are the 12-pass membrane protein Patched (PTC), the 7-pass membrane protein Smoothened (SMO) and the GLI transcription factors. PTC shares homology with the RND family of small-molecule transporters and it has been proposed that it interferes with SMO through metabolites. Although a conclusive picture is lacking, substantial efforts are made to identify and understand natural metabolites/sterols, including cholesterol, vitamin D3, oxysterols and glucocorticoides, that may be affected by, or influence the HH signaling cascade at the level of PTC and SMO. In this review we will elaborate the role of metabolites in HH signaling with a focus on oxysterols, and discuss advancements in modern analytical approaches in the field.

Contents

1. Introduction

While the Hedgehog (HH) signaling pathway plays a role in embryonic development, stem cells, cellular metabolism, axon targeting, synapse formation and nociception [1–5], it is also involved in human disorders and diseases, including developmental abnormalities and several forms of cancer [6–12]. The HH signaling cascade is unusual as it appears to involve metabolites while it alters the sub-cellular localization of pathway components that are closely entangled with the primary cilium [2]. Key players in this pathway, the morphogen Hedgehog, the trans-membrane proteins Patched and Smoothened and the zinc finger transcription factor Cubitus interruptus were predominantly identified through genetic screens in Drosophila in the late 1970's and early 1980's [13–17], with the subsequent discovery of the vertebrate homologs Sonic Hedgehog, Indian Hedgehog, Desert Hedgehog (SHH, IHH, DHH), Patched (PTC), Smoothened (SMO) and GLI [18–24]. As the interactions in this pathway were mapped, substantial similarities between Drosophila and vertebrates became apparent, reflecting deep evolutionary roots [2,25–28]. However, also differences exist between species; in particular many of the HH pathway components localize to the cell's primary cilium in vertebrates, an organelle that is not present in Drosophila [28–31].

In cells that produce the HH morphogen, HH undergoes cleavage, and its N-terminal peptide is dual-lipidated by cholesterol and palmitic acid (Fig. 1a). HH is then released by the resistance-nodulation division (RND) protein dispatched (DISP) either as monomeric particles, as multimeric particles or as exo-vesicles [32,33]. The form in which HH is released appears to define its signaling range [2]. It has been proposed that lipidation of HH promotes the association of HH with sterol-rich membrane areas in

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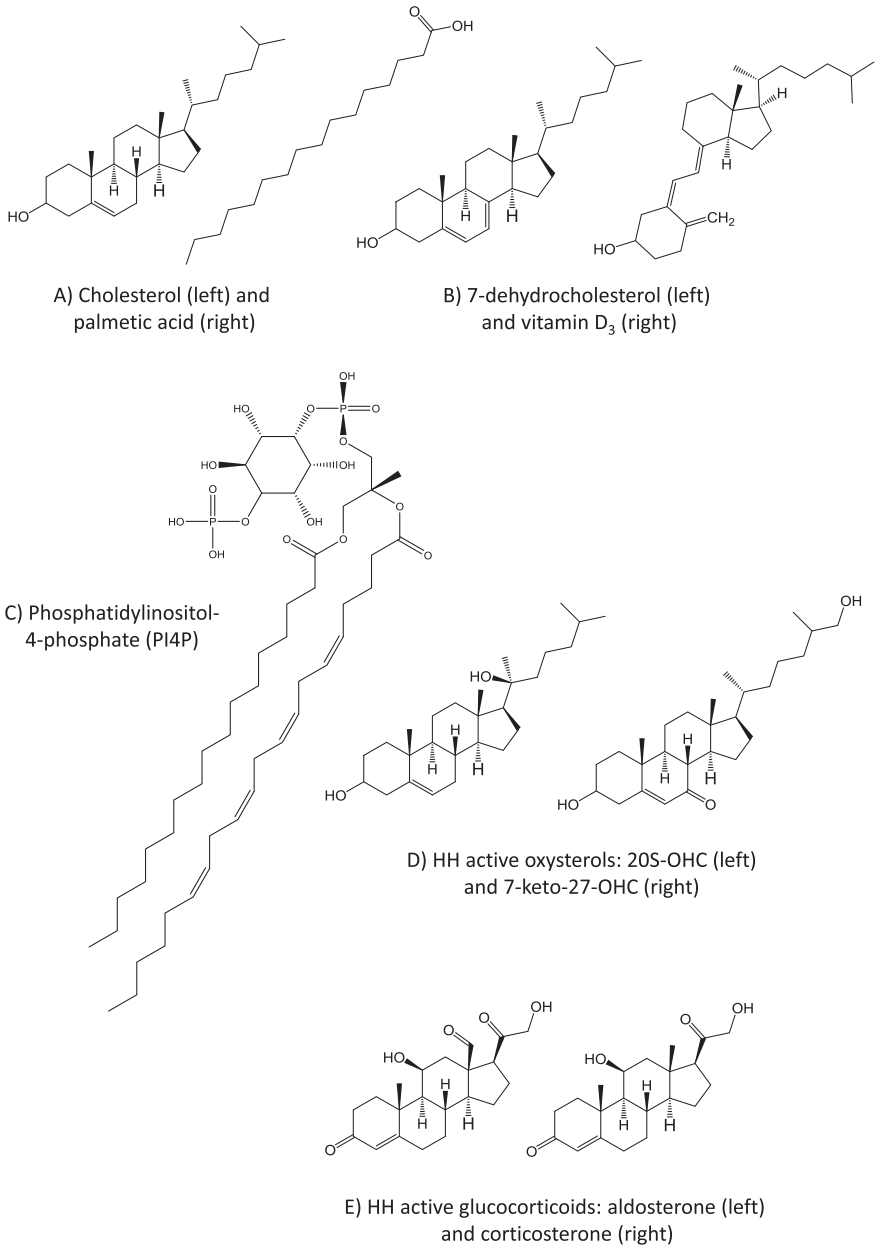

Fig. 1. Structures of selected Hedgehog pathway-modulating natural metabolites.

receiving cells [2,34,35] where HH binds to a number of membrane proteins including its canonic receptor the 12 pass transmembrane protein PTC, that similar to DISP, is a member of the RND family of proteins [21,23,36,37]. The binding of HH to PTC is promoted by the membrane co-receptor proteins CDO (CAM-related/downregulated by oncogens); BOC (brother of CDO) and GAS1 (growth arrest-specific 1) [2,26,38]. In the absence of the HH morphogen, PTC constitutively inhibits the signaling cascade [36,39]. However, upon HH binding, PTC releases the inhibition of the pathway.

How HH signaling is progressed beyond PTC is not entirely settled and there are curious differences between Drosophila and vertebrates [28]. PTC regulates the sub-cellular localization and activity of SMO, but PTC does not appear to directly interact with SMO. When the RND domain of PTC is mutated, it is no longer capable to inhibit SMO, suggesting that metabolites may be involved in the interaction between PTC and SMO [40,41]. What these metabolites are *in vivo* remains to some extent unclear and will be discussed below.

In vertebrates several sub-cellular alterations occur upon activation of the HH signaling pathway. In the absence of HH, the PTC receptor is enriched at the basis and in the primary cilium, a specialized organelle at the cellular surface that depends on the intraflagellar transport system (IFT) and that has been implied in various sensing functions [31]. When HH binds PTC, PTC leaves the primary cilium and enters the endocytotic pathway to be degraded. As PTC exits the primary cilium, also the G-protein coupled receptor GPR161, a rhodopsin family GPCR protein, is transported out of the primary cilium [42]. GPR161 negatively regulates HH signaling in the primary cilium through enhancing PKA activity by increasing cAMP levels. PKA is involved in phosphorylating the HH dependent zinc finger transcription factors GLI2 and GLI3 as described below [42]. Importantly, upon HH binding to PTC, the 7 transmembrane protein SMO, a Frizzled (FZD) class G-protein-coupled receptor with an unusually complex structure, is activated and moves in association with β-arrestin and the microtubule motor KIF3A into the primary cilium [29,30,35,43,44]. This

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