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Cholesterol activates the G-protein coupled receptor Smoothened to promote morphogenetic signaling

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The manuscript titled “Cholesterol Activates the G-protein Coupled Receptor Smoothened to Promote Morphogenetic Signaling”, seeks to elucidate whether cholesterol alone was sufficient to stimulate Hedgehog signaling. It is well known that cholesterol is necessary for basic cellular signaling transduction, through receptor stabilization, formation of rafts within the plasma membrane, and production of signaling ligands. These hold true for Hedgehog (Hh) signaling as well. Furthermore, upon elucidating the structure of Smoothened (SMO), cholesterol was found within the extracellular cysteine-rich domain (CRD) grove of SMO. Additionally, mutations found within this site can ablate SMO signaling activity.

To examine whether cholesterol alone is sufficient to stimulate Hh signaling, the authors use Methyl-β-Cyclodextrin (MβCD) sterol complexes to deliver cholesterol, which can increase the cholesterol content of the plasma membrane rapidly. With this delivery method, they show that cholesterol is not just necessary but also sufficient to activate Hh signaling pathway by directly activating SMO through binding to the CRD domain. Additionally, the authors provide evidence that cholesterol can drive differentiation of neural progenitors, similar to that of the Hh ligand Sonic Hedgehog (SHH). In conclusion, the authors suggest that an increase in plasma membrane cholesterol is sufficient to activate Hh signaling.

While the novelty of cholesterol as an activating ligand for SMO furthers our knowledge of cholesterol in an exciting new direction, a few points could aid in our understanding the mechanism in which cholesterol is stimulating Hh signaling.

1. In Figure 1A, it is interesting that it appears that there is a relatively narrow concentration range of MβCD:cholesterol that stimulates GLi1 transcription, as transcription levels drop after 4.8 mM of MβCD:cholesterol. However, in Figure 1B, in examining protein levels of Gli1 and Gli3, cells were not stimulated with 4.8 mM MβCD:cholesterol or above, so it is unclear whether this narrow concentration is also observed in translation of downstream targets of Hh signaling.

2. Another point of interest in Figure 1B is the fact that upon stimulation with MβCD:cholesterol, full-length Gli3 disappears, but expression is still being observed upon SHH stimulation.

3. While one can conclude from Figure 1 that cholesterol is inducing Hh signaling, is it possible that some of the stimulation of Gli1 transcription and Gli3 translation, be due to the production of Hh ligands upon cholesterol stimulation. While the timeline of this experiment yields this explanation unlikely, the concentration of Hh ligands overtime upon cholesterol stimulation would strengthen the conclusion made in this figure.

4. In Figure 2C, desmosterol can also activate Hh signaling. The authors discuss that they cannot exclude the possibility that desmosterol activated signaling because it was rapidly converted to cholesterol in cells. If so, is it possible to check free cholesterol levels upon desmosterol stimulation?

5. The authors conclude that cholesterol acts at SMO to activate Hh signaling. However, in Figure 3B, it seems that vismodegib and cyclopamine can only partially block Hh signaling. This may be due to poor drug efficacy, however, cholesterol may be stimulating Hh signaling through mediators other than SMO. This can also be supported by Figure 3C, MβCD:cholesterol appears to stimulate Gli1 translation to a greater extent than SHH in $SMO^{-/-}$ cells. However, since no error bars or statistics appear to be done for this figure, it is hard to make a conclusion.

6. In supplemental Figure 3, it is perplexing that cholesterol is stimulating Hh signaling, but SMO translocation to the cilia is not observed. Has this phenomenon been observed in other studies examining Hh signaling? Since the negative regulator PTCH is still in the system upon cholesterol treatment, is it possible that PTCH is preventing SMO entering in to primary cilia? Does SMO translocation to the cilia still occur in $Ptch^{-/-}$ cell stimulated with cholesterol?

7. How does cholesterol carried by MβCD compare to that in circulation? Especially, since the MβCD:cholesterol complex stimulates Gli1 transcription in such a narrow range, how do these cholesterol concentrations compare to physiological concentrations?