THE UNIVERSITY of EDINBURGH
Edinburgh Medical School

Biomedical Sciences

Course Name: Biomedical Sciences 2

Title of Assessment: How can defects in primary cilia cause birth defects?

Topic 4: How can defects in primary cilia cause birth defects?

Primary cilia are usually non-motile, single organelles extending from the cell surface, which function as sensory molecules crucial for many signalling pathways (Satir, Pedersen and Christensen, 2010).

Their importance remained largely unknown until the beginning of the current century, when structural and functional defects in cilia were linked with improper development of a wide range of tissues (Youn and Han, 2017). 'Ciliopathies' is the collective name for all diseases caused by dysfunctional cilia and in order to fully understand the mechanism behind them, it is necessary to know how primary cilia control the development of a healthy tissue.

One of the signal transduction pathways requiring primary cilia for a proper functioning is Hedgehog signalling (Hhs) pathway (Bay and Caspary, 2012) and, as it will be described, it can lead to birth defects in nervous tissue.

Hedgehog signalling pathway (Hhs) – Introduction

In mammals, there are three main types of Hhs proteins, namely, Sonic (SHH), Indian (IHH) and Desert (DHH) and in many instances, their functions overlap (Bangs and Anderson, 2016). The best-studied protein and hence type of signalling is SHH and mechanisms described here refer particularly to SHH.

Sonic hedgehog signalling (Shh) involves proteins embedded in cilia. When Shh is inactivated, one of them - a 12-transmembrane protein Patched 1 (Ptch1) inhibits the action of another protein called Smoothened (Smo) found within the cell (Youn and Han, 2017). Inactivated Smo cannot enter the cell surface nor primary cilium, which in turn is correlated with transcription factors – Gli2 and Gli3 – being phosphorylated (Youn and Han, 2017). Phosphorylated Gli2/3 bind to the SuFu (Suppressor of Fused) protein, which maintains Gli2/3 in an inactive form (Lee, Zhao and Ingham, 2016). Gli2/3 are cleaved by proteasome into a repressor form $Gli2/3^{R}$ (Dennis and Bradshaw, 2011). In this form, $Gli2/3^{R}$ enter the nucleus and inhibit transcription activation of Shh mediated genes. See Fig1a and Fig2A.

Once Shh is activated, Ptch1 leaves primary cilia and enters the cell, where it is degraded (Dennis and Bradshaw, 2011). This, in turn, means that Smo is no longer inhibited and can enter primary cilium. Activated Smo induces downstream reactions upon which Gli2/3

accumulate in primary cilia, dissociate from SuFu, are stabilised and as a result, they are not cleaved anymore (Bay and Caspary, 2012). In this full form, Gli2/3^{A} enter the nucleus and induces expression of Hh mediated genes (Dennis and Bradshaw, 2011). See Fig1b and Fig2B.

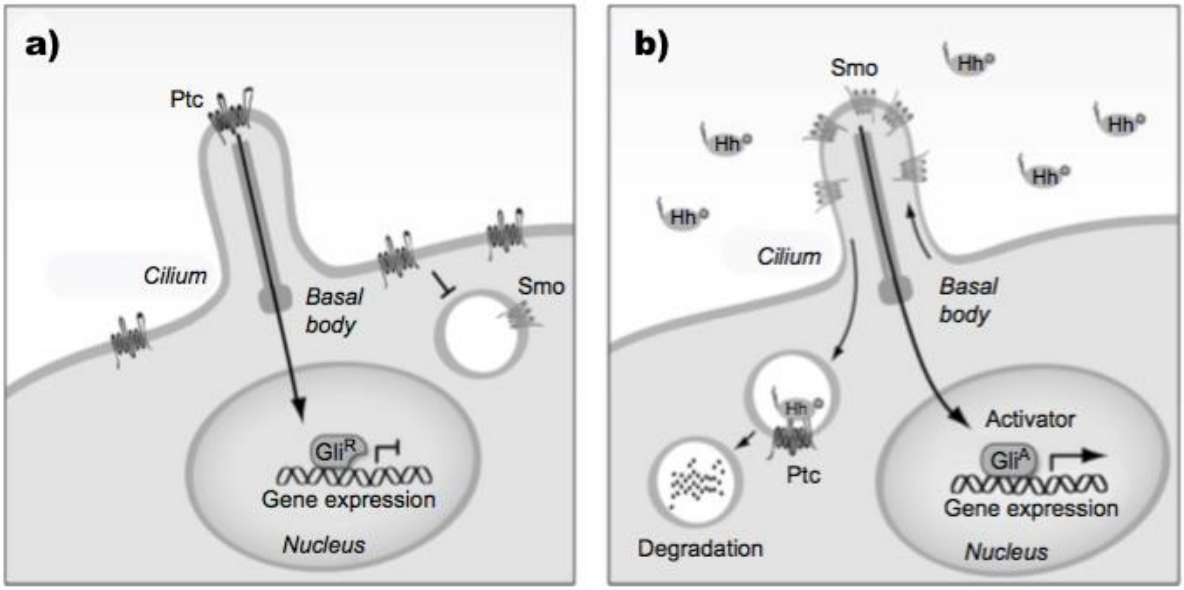

Figure 1. The Hedgehog signalling pathway (a) inactivated; (b) activated.

Ptc = Ptch1
Source: (Dennis and Bradshaw, 2011).

It can be hypothesised that regardless of whether the Shh is activated or not, in both states, cilia play important role in mediating expression of Shh target genes as switching between repressor and active form of Gli2/3 takes place within primary cilia and requires other proteins located there (e.g. SuFu). This claim can be supported by studies on cell trafficking of proteins involved in Shh within primary cilia.

Intraflagellar Transport (IFT) as one of the trafficking mechanisms within primary cilia

IFT is a bidirectional microtubule-based transport system that enables movement of particles and molecules from the ciliary base to its tip – anterograde transport – and vice versa – retrograde transport (Eguether, Cordelieres and Pazour, 2018). Firstly, IFT is required for ciliogenesis itself (Eguether, Cordelieres and Pazour, 2018). Secondly, it was shown that Gli3 and SuFu proteins are located at the tip of a cilium and that IFT plays role in the regulation of their position within a cilium as well as enable Gli3 activation (Haycraft et al., 2005).
The following mechanism was proposed, see Fig2:

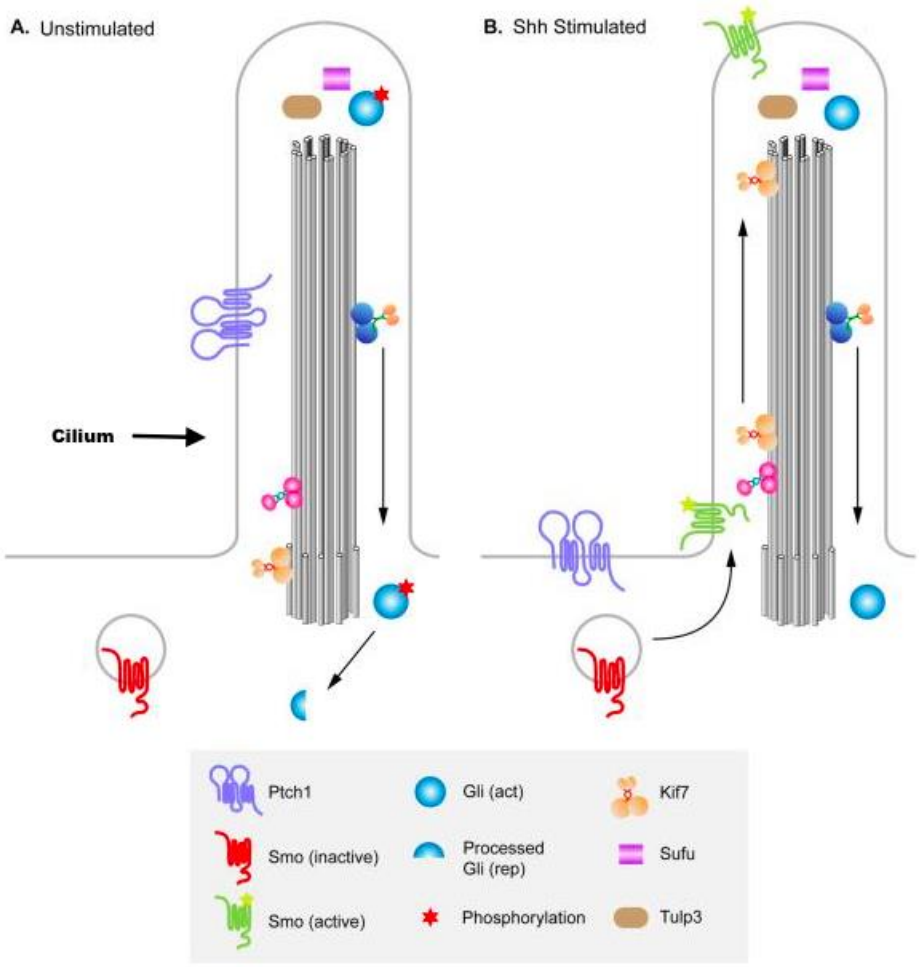

Figure 2. IFT in trafficking primary cilium components when (A) unstimulated and (B) stimulated by Shh.

(A) Primary cilium in absence of Shh: Ptch1 embedded in primary cilium; inactive Smo freely within the cytoplasm; SuFu and Gli proteins are located at the cilium tip; Gli3 becomes phosphorylated, cleaved to its repressor form $Gli3^R$ and retrogradely transported within the cilium; Kif7 at the cilium basal end.

(B) Primary cilium in presence of Shh: Ptch1 leaves primary cilium; Smo enriched in primary cilium membrane and activated; SuFu and Gli proteins located at the cilium tip; Gli3 in its full, active form retrogradely transported within the cilium; Kif7 anterogradely transported within the cilium to its tip.

Source: (Goetz, Ocbina and Anderson, 2009)

It should be noted that the mechanism presented in the Fig2 is consistent with the mechanism showed in the Fig1.

Kif7 is a motor protein. When located at the basal end of cilium helps to prevent activation of the Shh pathway within primary cilium (Goetz, Ocbina and Anderson, 2009). However, when the pathway is already activated, Kif7 moves up to the cilium tip and plays some role in Gli proteins activation (Haycraft et al., 2005). The exact Gli activation mechanism is not yet known. Moreover, the analysis of these two figures, raises other questions, e.g. whether Kif7 in the mechanism in Fig2A inhibits Gli activation or maybe it is just too far away from Gli to activate it and first Kif7 needs to be activated itself to move towards Gli; whether Kif7 transport is required for Gli activation or maybe it just enhances or accelerates this process; whether Kif7 activates Gli proteins directly or inhibits Gli phosphorylation pathway thus preventing formation of Gli^R; does Kif7 affect Gli-SuFu complex at the tip of cilium in a way that leads to the switch Gli^R <-> Gli^A; or maybe Kif7 does not affect the Shh directly at all and instead it regulates cilium architecture thereby influencing Shh? These speculations can be multiplied and although studies addressing these and similar questions have started, more research is needed to provide an answer to the mechanism behind Shh and specific cilia role in it.
Having this said, the importance of cilia in the Shh can be proven through genetic studies, where the knockout of genes involved in IFT results in an invalid Shh pathway (He et al., 2014) thence leading to developmental diseases.

Mutations in genes encoding proteins involved in Shh within cilia - KIF7 and GLI3 – linked to brain abnormalities

In one of the studies, He and Subramanian showed that in a mouse with a mutation $Kif^{7-/-}$, microtubules forming the core of primary cilium in neural epithelium grew unevenly – they had a different length (He et al., 2014). In the same study, they proposed that Kif7 is needed for uniform growth of microtubules and that this creates a compartment in a cilium tip where one of the IFT proteins and Gli-SuFu complexes are enriched in a membrane (He et al., 2014). See Fig3.

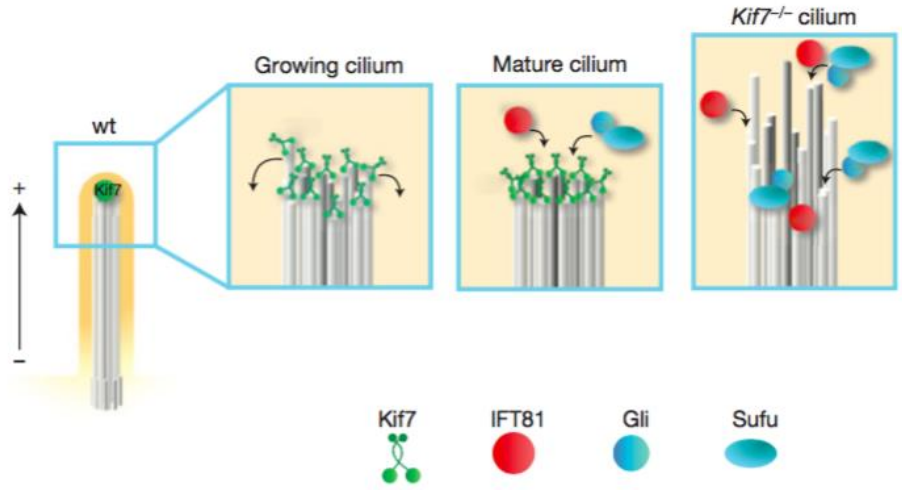

Figure 3. Kif7 regulation of microtubule growth within primary cilium.

wt – wild type cilia, control sample without mutation
Source: (He et al., 2014)

Microtubule disassembly pattern expressed in mutated neural cells may suggest that IFT trafficking within these cells is likely to be disrupted.

$Kif^{7-/-}$ mutation was linked with the development of exencephalic embryos (Cheung et al., 2009) – embryos with brain growing outside of skull – or in less severe conditions with Acrocallosal syndrome (Genetics Home Reference, 2018a). This syndrome is characterised by intellectual disabilities and Corpus callosum agenesis (Valente et al., 2013) – partial or complete absence of this structure, which means the nerve connections between hemispheres are missing. What is interesting, mutation GLI3 is also connected to Acrocallosal syndrome (Genetics Home Reference, 2018b) and the molecular reason for that can be potentially found in the model from the Figure 2 and 3, e.g. mutated Gli3 cannot be enriched in primary cilium tip, which in turn may affect $Gli3^{R}$<->$Gli3^{A}$ switch and therefore the expression of Shh target genes within the target cell.

Final thoughts

It has to be taken under consideration that the experiments used mouse models and the mechanisms observed may differ in humans. Especially since we know that there is a variance in the Hhs mechanisms across metazoa, where on the other end of the spectrum there is well studied *Drosophila melanogaster*, which does not require cilia for Hhs at all (Ingham, Nakano and Seger, 2011). Furthermore, Shh pathway is incomparably more complex than its part presented here and most of the mechanisms are yet to be established. Although it is

considered as a limitation, in fact, it highlights how significant are step-by-step discoveries as even they give us insights allowing more precise pathomechanism targeting.

In conclusion, better understanding of how primary cilia control the development of tissues can help in the treatment of ciliopathies, on the other hand, many cues about cilia role come from analysis of tissue defects or from genetics studies. For that reason, these two approaches must complement each other.

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