Characterisation of IFT140 Protein Defects in Urine-Derived Renal Cells: Mechanisms and Therapeutics

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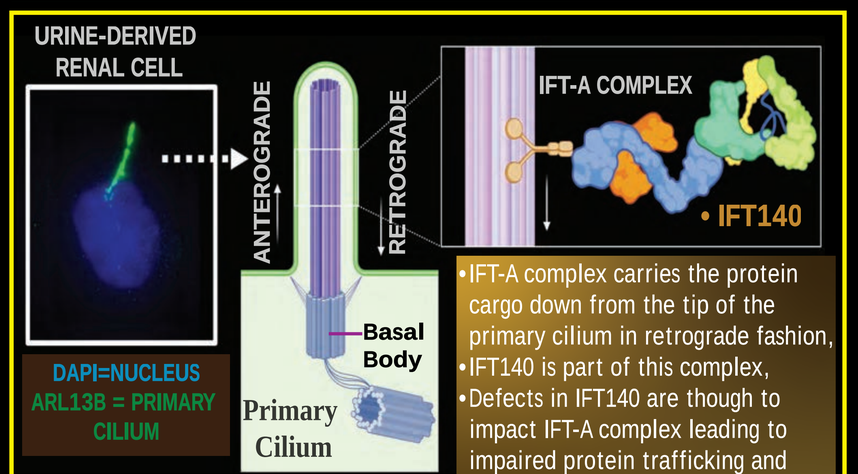
AIMS

- **Investigate a gene mutation affecting a number of Newcastle patients suffering from Autosomal Dominant Polycystic Kidney Disease using kidney cells extracted from patient's urine,**
- **Develop and test kidney cell based models to study the function of this gene and investigate cellular pathways that are disrupted in mutated cells,**
- **Investigate whether this mutation can be alleviated using combined antisense oligonucleotide therapy.**

INTRODUCTION

- **To function properly, all cells need to be able to respond to external stimuli. They do this via an "antenna" known as the primary cilium that extends out of the cell from basal body.**
- **The primary cilium requires more than 950 genes to function properly and mutations in these genes can lead to various syndromes and conditions. One of them is Autosomal Dominant Polycystic Kidney Disease (ADPKD) which can result in end stage renal failure.**
- **Approximately 6% of all ADPKD cases are caused by mutations in a gene called IFT140 that is important for moving proteins along the primary cilium by a process known as intraflagellar transport.**
- **ADPKD is currently incurable, however, small synthetic DNA molecules called antisense oligonucleotides (ASO) could be used to treat ADPKD in the future. ASO are currently used to treat diseases such as Duchenne Muscular Dystrophy, Spinal Muscular Atrophy and are in clinical trials for Cystic Fibrosis.**



Structure of IFT-A complex adapted from Jiang, M *et al.* 2023

- IFT-A complex carries the protein cargo down from the tip of the primary cilium in retrograde fashion,
- IFT140 is part of this complex,
- Defects in IFT140 are though to impact IFT-A complex leading to impaired protein trafficking and disrupted signalling resulting in ADPKD.

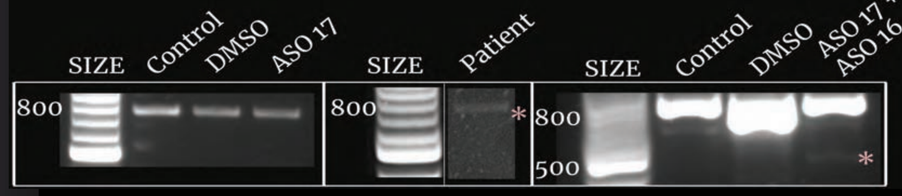
METHODS AND RESULTS

How are proteins created?

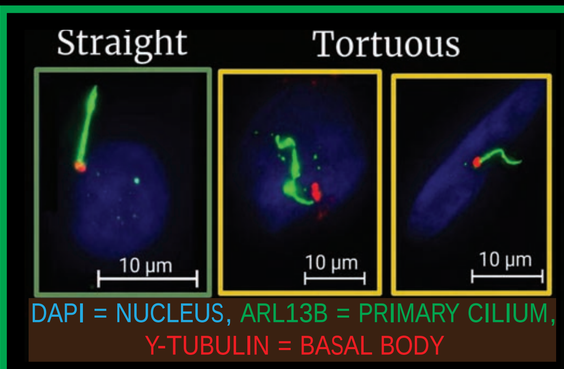
DNA is transcribed into mRNA which will tell cells how to make proteins. There is a step in mRNA maturation where some of its parts need to be removed. One common mutation in IFT140 "hides" part of the mRNA which should not be removed (Exon 17) and as a consequence a STOP signal is formed targetting the mRNA for degradation so no protein is formed (Figure on the left). However, when another part of the mRNA is left out as Well (Exon 16), the STOP signal disappears and partly functional form of protein is formed. To "hide" these parts of mRNA, synthetic DNA molecules called antisense oligonucleotides (ASO) are used.

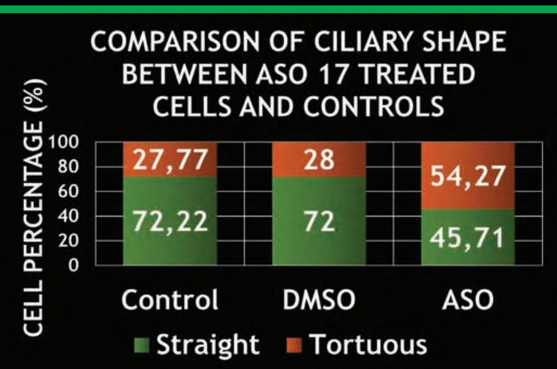
Did ASO Skipping Work?

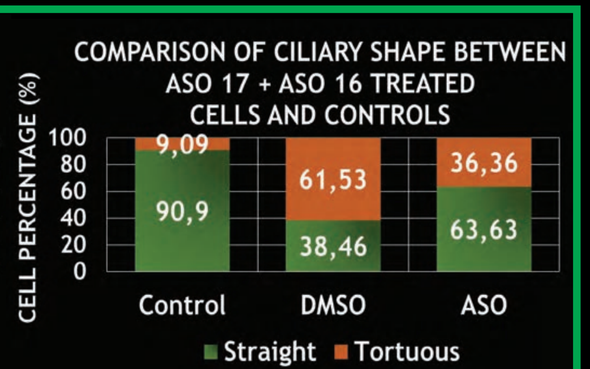
When a part of mRNA is skipped, it becomes smaller. We can analyse the size of the mRNA using technique called RT-PCR. During IFT140 analysis, only normal size IFT140 was observed in patient and ASO 17 treated cells, suggesting that the Exon 17 skipped version is degraded. When both exon 17 and 16 were skipped, a smaller version of IFT140 was observed (last picture), suggesting that the mRNA is no longer degraded.



Size is measured in base pairs, DMSO is an agent used to get ASO in the cells, * indicates bands of interest.







What Impacts Does IFT140 E17 Skipping Have on Gene Expression?

Some key signalling pathways are located in the primary cilium. RT-PCR analysis revealed that mainly Sonic Hedgehog Signalling (Shh) is dysregulated in IFT140 absent cells. Shh dysregulation was linked to cystic kidney disease and kidney fibrosis before.

What Effect Did ASO 17 Have on Primary Cilia?

Shape of primary cilium is thought to be an indicator of its funcionality. Straight and tortuous cilia were observed (top left). Higher ciliary tortuosity was observed in the ASO 17 treated cells compared to the controls (top middle). This effect was was partly ameliorated in combined treatment (top right). This could suggest that protein trafficking is impaired in IFT140 absent cells and that combined treatment restores some of its function.

SUMMARY

- **The IFT140 mutation seems to impact the ciliary shape suggesting an impairment in protein trafficking.**
- **The kidney cell model treated with the antisense oligonucleotides mimicked the patient mutation.**
- **The effects of the mutation were partly ameliorated when treated with combined antisense oligonucleotides.**

References: Jiang, M *et al*. 2023, Bangs, F *et al.* 2017, Watanabe, N *et al*. 2018, Igreja, S *et al.* 2016, Chiriboga, C 2017, Senum, SR *et al.* 2022, Biorender.com, Excel