Ciliary Flow Response - Identification and Functional Analysis of Key Proteins

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Cilia are present on almost every cell type in the human body. The cilium is a complex organelle composed of axoneme and basal body. Fluid flow has many important functions in the body such as endocrine signaling; sensing this flow can be critical for cellular responses. Cells can sense the fluid flow using primary cilia. We want to investigate the mechanisms regulating flow sensitivity of primary cilia via two properties: axonemal stiffness, and the anchoring of cilia to the cell through the basal body. By achieving this, we will know more about the mechanosensory function of primary cilia and what flow conditions are required to activate pathways that generate cellular responses. To find how axonemal stiffness contributes to flow sensing, we propose to alter microtubule flexibility by knocking down the gene expressing CFAP20. This is an inner junction protein that connects A and B microtubules together. We will also look at basal body mechanics that contribute to flow sensing through Rootletin. This is the target protein that anchors the cilium to the centrosome. We propose to use siRNA to reduce the expression of these two ciliary associated proteins and verify the knockdown using Western Blot analysis. By applying fluid flow to a perfused tissue culture, we can characterize ciliary bending using an optical trap that applies a mechanical stimulus directly onto the primary cilium. We can also use Fluo-4 imaging to detect acute changes in intracellular Ca^{2+} levels, giving us more information about the threshold flow. Ultimately, using the data gained through these experiments, we can better understand the deformities in primary cilia that lead to disease and find ways to treat them.

Introduction

We want to investigate how changes in ciliary structure influence signaling pathways based on a mechanical stimulus. We want to manipulate certain proteins under fluid flow and evaluate responses of cilia. This will lead to a greater understanding of the mechanosensory function of cilia. Overall, these functions can help us understand how to prevent and treat ciliopathies.

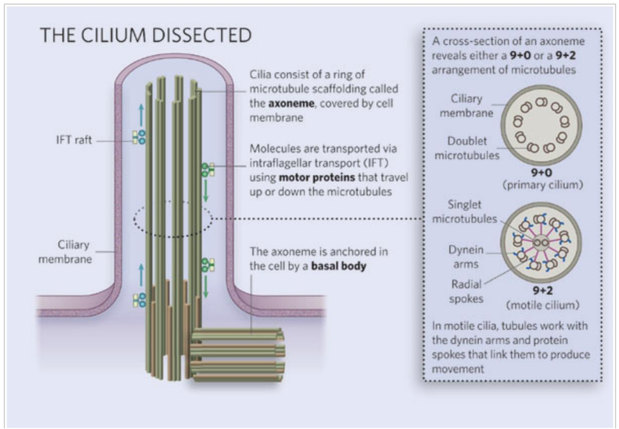

Figure 1: Schematic diagram of a cilium with differences in primary and motile cilia

Background

Cilia are mechanosensory organelles found on many cells in the body. There are two types of cilia- motile and nonmotile cilia. We will focus on nonmotile or primary cilia, which has a greater affect in sensory functions. Cells use primary cilia to detect fluid flow. This can lead to activation of certain pathways such as signal transduction using calcium signaling. The two main structural components of a cilium are the axoneme and basal body. We want to investigate the effects of knocking down two structural proteins in these regions, CFAP20 and Rootletin, and observe differences in ciliary responses that give more information about changes in function.

Methods

We propose to use siRNA, a method for gene knockdown, to reduce the expression of CFAP20 and Rootletin. The knockdown will be verified using Western Blot analysis. Then, we will apply fluid flow to a cell culture containing the knockdown cells and normal cells. To characterize changes in ciliary bending, we can use an optical trap. We can monitor changes in ciliary responses to flow via calcium imaging. This will show us differences in how cilia respond to flow with and without the presence of these proteins.

Results

In one study, targeted disruption of the Rootletin gene was carried out in mice. Figures 3a and 3b show the loss of the Rootletin protein and a changed transcript in the mutant where it was knocked down. (+/+ is WT; -/-is homozygous mutant; +/- is heterozygote). Another study (Figure 3c) used an optical trap to measure the bending stiffness of cilia. They found that under external forces, cilium deflection involves axoneme bending and base pivoting. A third study monitored cilia response to fluid shear stress through increases in cystolic calcium. Figures 2a and 2b show increases in calcium for wild type cells but not in mutants.

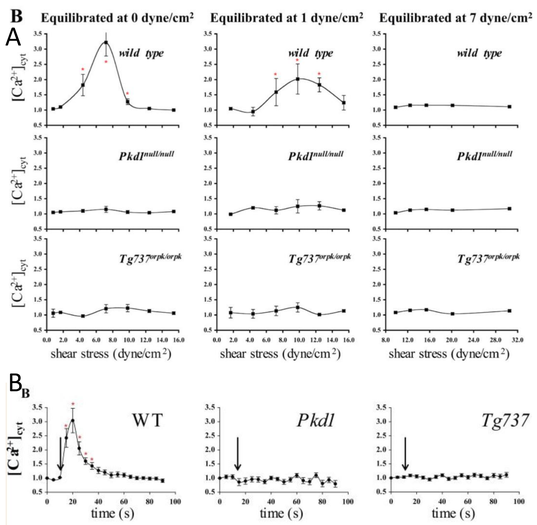

Figure 2a: These graphs show changes in cystolic calcium as a measure of shear stress in 3 types of cells
Figure 2b: These graphs show changes in cystolic calcium as a measure of time in 3 types of cells

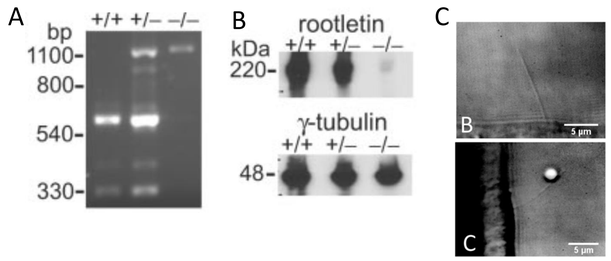

Figure 3a: Rootletin transcript from 3 different cell types
Figure 3b: Immunoblot of Rootletin and gamma tubulin (control)
Figure 3c: Differential interference contrast of deflected cilium

Discussion

The studies collectively show that certain ciliary proteins are required to sense fluid shear stress, and this can lead to signal transduction, measured through increases in cystolic calcium. In mutant cells lines missing certain proteins, there was no clear increase in cystolic calcium whereas in wild types there was a visible increase in cystolic calcium when challenged under varying degrees of fluid stress. This shows that proteins such as CFAP20 and Rootletin can also be potentially necessary in cilium mechanosensory functions. Manipulating the cilium structure can also give us clues on how fluid flow can alter signal transduction. Other studies have shown that the absence or abnormality of ciliary proteins could potentially lead to diseases such as polycystic kidney disease and hypertension. Overall, this shows that cilia are important mechanosensory organelles, and the lack of them can lead to disease when certain pathways do not function properly.

Conclusion

We proposed an experiment to find out if certain changes in ciliary structure will affect downstream signaling functions. In the studies reviewed, there was a lot of evidence highlighting the importance of properly functioning ciliary proteins. In the future we would apply this towards the link of ciliary function and how it impacts disease.

References

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