

Overall summary

Cilia are highly conserved organelles that provide important motility and sensory functions in eukaryotic cell biology and metazoan development. Ciliary defects cause human inherited diseases called ciliopathies. The ciliary tip is an understudied ciliary domain. Little is known about its biochemical composition, and it is likely to have important functions in assembling and maintaining cilia, and potentially in other ciliary functions, such as their sensing.

In his PhD study, Mr Gorilák sets out to identify and characterise new ciliary tip proteins. To this end, genes encoding candidate ciliary tip proteins were cloned into mammalian cell expression vectors and used to generate stable human cell lines for over-expression. Localisation of candidate proteins to the ciliary tip was determined by immunofluorescence analysis using markers for the ciliary base and ciliary shaft to aid interpretation. The approach was carefully validated using known ciliary tip proteins, demonstrating that the approach was viable and controlling, at least to some extent, for artefacts due to potential over-expression and use of a C-terminal tag. Two ciliary tip proteins were initially identified using this approach.

Mr Gorilák then focussed on one of these, a Zinc finger containing protein called here ZNF1C, for further analysis. Fluorescence microscopy demonstrated that ZNF1C associates with moving IFT and at least to some extent with stationary IFT that might indicate the transition from doublets to singlet microtubules along the axoneme. Proteomics analysis suggested potential interacting proteins, highlighting ARM9C and CFAP58. Ablation of ZNF1C appears to cause aberrant extension of the primary cilia in cultured cells.

Over-expression carries with it some caveats, which are well discussed and realised by the candidate. In particular, expression of proteins in cells that do naturally express the protein may cause false-negatives, especially if the cilia are of different types. One such protein is ULK4, a protein that did not localise to the tips of primary cilia in this screen but is associated pathology associated with motile cilia defects. Therefore, the candidate over-expressed ULK4 in ependymal cells that make motile cilia, and demonstrated that ULK4 localises to the tips of motile cilia in these cells.

During the course of his studies, Mr Gorilák developed and published the ExM method for use in kinetoplastid parasites, an important contribution to kinetoplastid research methods, and also contributed to several other publications.

Introduction and references

The introduction is clearly written and is well researched and referenced.

The introduction would benefit from a brief discussion on trypanosomes with respect to their evolutionary divergence, the role of the flagellum through the lifecycle, and key differences in flagellar structure between trypanosomes and mammals.

Methods

These are well explained and referenced and are associated with a good methods publication that will be valuable to the field.

Although I am cognisant much of this was done by the Wickstead lab, more detail should be given to how the 19 candidate proteins were selected for screening.

Figures and results

The data is uniformly high quality and PhD worthy and the ExM is especially beautiful.

The fluorescence microscopy is well presented and is properly annotated with scale bars and channels. Where relevant, the microscopy Figures would benefit from including the tag terminus (e.g. in the legend). Where a magnified inset of a larger field of view (FOV) is included, it would be helpful to indicate from where in the FOV the inset comes from (e.g. by a rectangle). Where insets display only selected channels, this should be explicitly indicated.

In Figure 20, the candidate notes that cilia are longer when ZNF1C is ablated, but the P value is 0.15, which is not plausibly significantly different. Given the differences are clear “by eye”, I would suggest that the statistics were not done correctly. For example, doing the T test on the single-value averages for each of the 3 experiments will have lost the statistical power of measuring 300 individual cilia. This figure would also benefit from an illustrative micrograph of an elongated cilia.

Although the microscopy is compelling, statements in the text are sometimes unsupported by numerical data. For example, what proportion of ZNF1C and IFT foci are moving vs stationary (p75/76/77)? What proportion of stationary ZNF1C colocalise / don't colocalise / are offset from the IFT marker? What proportion of long and short ependymal cilia are positive for ULK4 (p88)? The increase in EV release in ZNF1C is mentioned only in passing and should be backed up by hard data.

Conclusion

This PhD study identifies three new mammalian ciliary tip components and made important functional insights. Moreover, Mr Gorilák has developed and established an important new research technique for kinetoplastid cell biologists and has contributed to several other manuscripts. The data is consistently high quality and this study fulfils all the relevant PhD criteria. I therefore unreservedly recommend this thesis for defence.

Questions for the defence:

Could the candidate speculate on the functional significance of the doublet – singlet transition in some types of cilia, and why it might be that some cells (e.g. trypanosomes) do not exhibit this?

Could the candidate elaborate on how these 16 proteins were chosen for the over-expression screen? For example, how was the bioinformatics done, what was the total number of candidates, by what criteria were these 16 proteins specifically selected over other proteins?

Could the candidate elaborate on why siRNA of ZNF1C gives rise to longer cilia (Figure 20) and how this might be tested?

Given your findings, what do you hypothesize is the potential function of ZFN1C and how would you investigate it further?

What do you think is the most important output from your PhD study?