Investigation into the role of ADAMTS9 in primary cilia structure/function

Antony Rose, Elisa Molinari, John A Sayer
Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction

A leading cause of kidney damage in children is due to an inherited cystic kidney disease named nephronophthisis (NPHP). NPHP is classified as a type of ciliopathy as it affects the structure and function of primary cilia which are essential for maintaining the kidney's environment; allowing the kidney to function efficiently. It has been reported by other researchers that the loss of ADAMTS9 can result in NPHP[1]. ADAMTS9 is a member of the ADAMTS family of proteins which are known to be involved in the cleavage of proteoglycans, control of organ shape during development and inhibiting angiogenesis.
In this study, based off the information in other studies[2], the aim was to investigate what role ADAMTS9 played in the structure of primary cilia; causing them to experience stunted growth which in turn diminished their function. Mouse NIH3T3 cell line was chosen to experiment on as they undergo ciliogenesis and were shown to express ADAMTS9[2].

Method

- NIH3T3 cells (mouse cell line) were cultured in a 12 well-plate and 6 well-plate with the cells grown on coverslips in the 6 well-plate. Half the wells in each plate respectively were treated with an ADAMTS9 siRNA, while the other half treated with a silencer negative control siRNA to act as a control.

- Coverslips from the 6 well plate were stained and fixed to microscope slides to allow immunofluorescent images to be generated using an Axioimager. This can be seen in figure 1.

- RNA was extracted by cell lysis from the 12 well plate cultures and then purified through using a RNeasy mini kit. RNA quality and concentration was then checked using a nano-drop spectrophotometer and converted into cDNA through reverse transcription.

- qPCR was then performed using the generated cDNA and C57BL/6 mouse kidney cDNA as a positive control.

1.

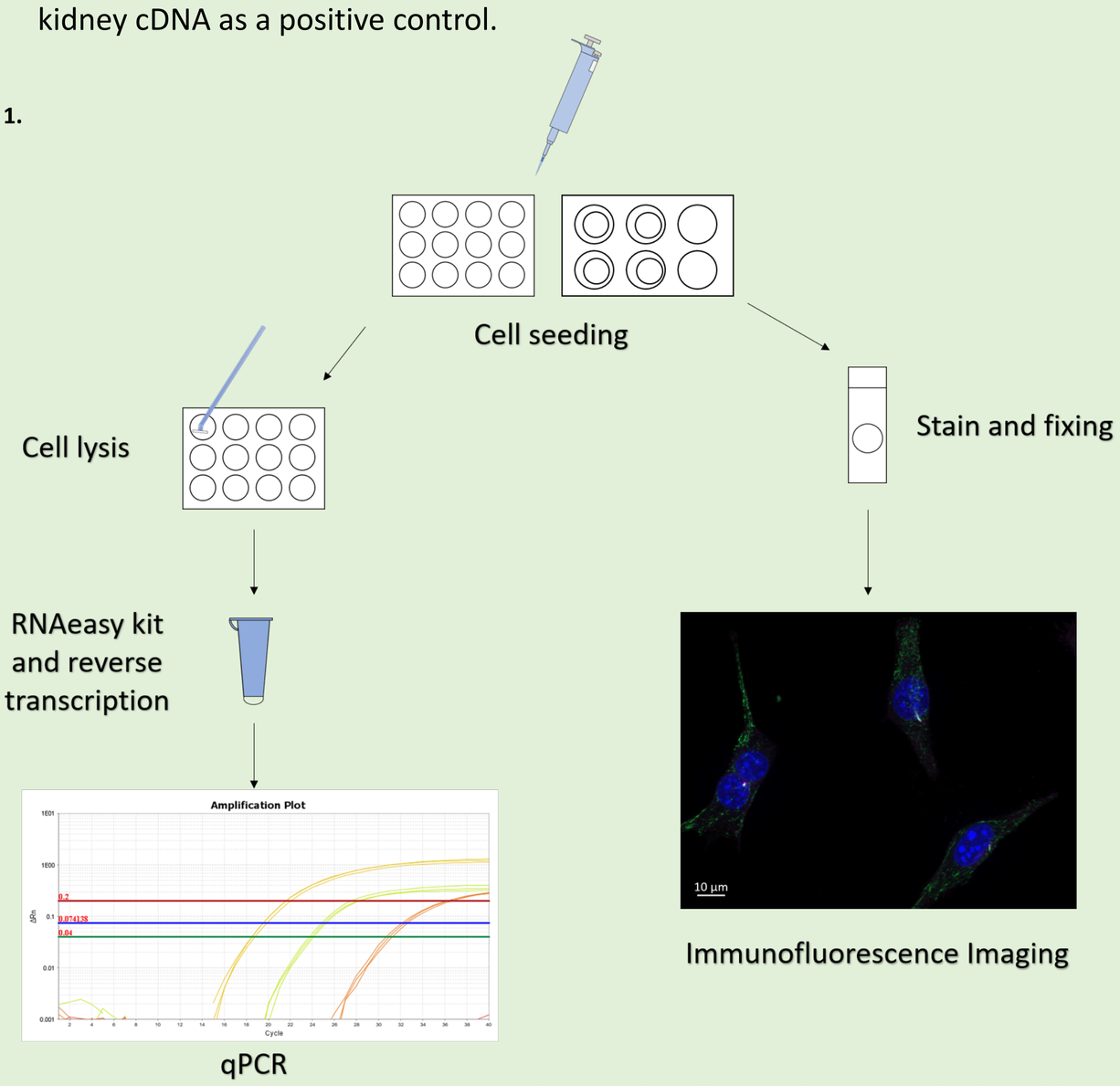


Figure 1. Flow diagram showing method workflow

Result

2.

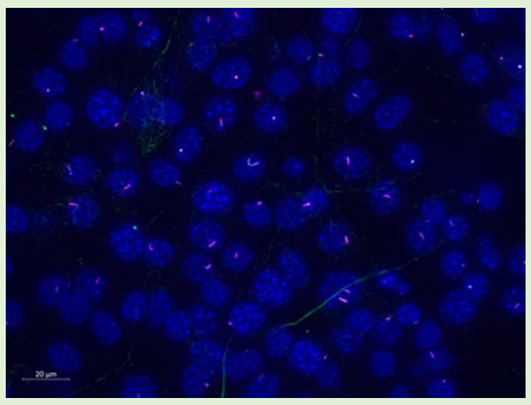


Figure 2. NIH3T3 cells at 40x magnification stained with DAPI (blue), Acetylated tubulin (green) and ARL13B (pink) and imaged using Axioimager.

3.

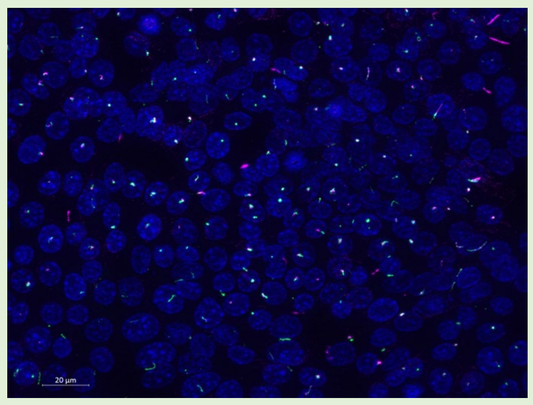
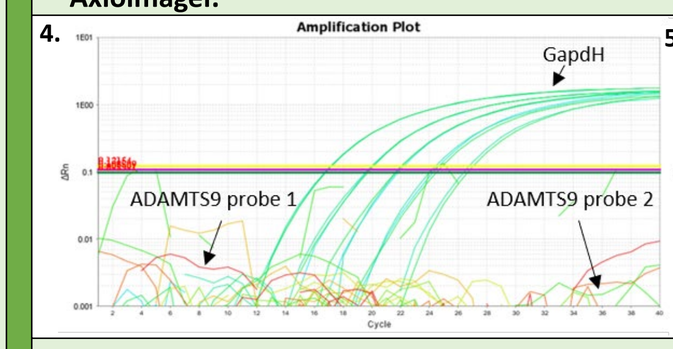


Figure 3. mIMCD3 cells at 40x magnification stained with DAPI (blue), Acetylated tubulin (pink) and ARL13B (green) and imaged using Axioimager.

4.



5.

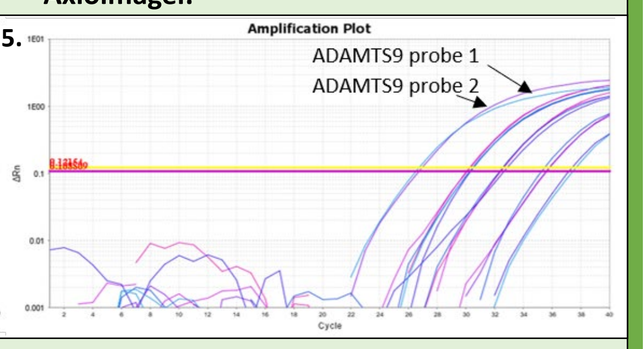
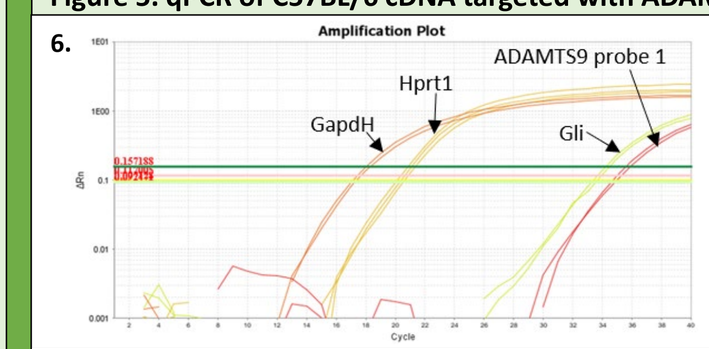


Figure 4. qPCR of NIH3T3 cDNA targeted with ADAMTS9 probe 1, ADAMTS9 probe 2 and Gapdh

Figure 5. qPCR of C57BL/6 cDNA targeted with ADAMTS9 probe 1 and ADAMTS9 probe 2

6.



7.

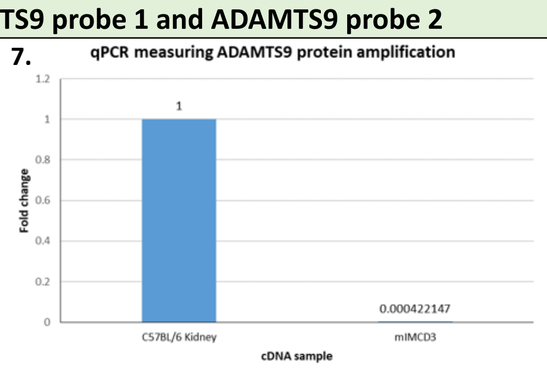


Figure 6. qPCR of mIMCD3 cDNA targeted with ADAMTS9 probe 1, Gapdh, Hprt1 and Gli1

Figure 7. Graph representing the fold change of ADAMTS9 protein amplified from qPCR for both mIMCD3 and C57BL/6 cDNA

NIH3T3 cells were checked for ciliogenesis using Immunofluorescent staining technique with an Axioimager which can be seen in figure 2. From first visual appearance it looked like most of the cell culture had indeed generated cilia, assisting the claim that NIH3T3 cells can be a good model for ciliogenesis.

cDNA generated from NIH3T3 cell line from both a control NIH3T3 sample and transfected ADAMTS9 siRNA knockdown NIH3T3 sample was used to perform a qPCR. This was to validate that the siRNA transfection had successfully knocked down the ADAMTS9 gene ensuring any affect on ciliogenesis was due to ADAMTS9 knockdown. This qPCR was also done with housekeeping genes to ensure qPCR was performed successfully and black6 (mouse kidney cDNA-C57BL/6) acting as a control for comparison and to check the commercial ADAMTS9 probes worked. From the qPCR seen in figure 4 it was concluded that there were no detectable levels of ADAMTS9 expression/amplification in the NIH3T3 cells.

To overcome the hurdle, cryogenically stored mIMCD3 cells were defrosted and seeded in the same way as NIH3T3 cells. These cells were chosen as like the NIH3T3 cells; a previous study had also stated these cells to show ADAMTS9 expression and be a good model for ciliogenesis[2]. Again, from staining the cells and viewing using an Axioimager, these cells can be seen to experience ciliogenesis which is seen in figure 3. When checking for ADAMTS9 expression in these cells, ADAMTS9 was not expressed. This can be seen in figure 7 which compares the concentration of ADAMTS9 protein in black6 cDNA amplified in qPCR and mIMCD3.

Discussion

Both cell lines used in this study NIH3T3 and mIMCD3 are good models for studying ciliogenesis however, when testing for ADAMTS9 expression, neither cell line expressed the gene of interest which contrasts another study[2]. This discrepancy in results could be due to various differences between experiments, despite trying to replicate conditions as best as possible. Examples of this include: different negative control siRNA; this study serum starved at the same time as siRNA transfection whereas the other study transfected for 48 hours and then serum starved for an additional 24 hours and this study treated the NIH3T3 and mIMCD3 cells with SAG 24 hours after serum starvation. Due to limited time and results generated, no definitive outcome about the effect of knocking down ADAMTS9 on ciliogenesis can be made.

References

1. Choi, Y. J., et al. "Mutations of ADAMTS9 Cause Nephronophthisis-Related Ciliopathy." Am J Hum Genet 2019;**104**(1): 45-54.

2. Nandadasa, S., et al. "Secreted metalloproteases ADAMTS9 and ADAMTS20 have a non-canonical role in ciliary vesicle growth during ciliogenesis." Nat Commun 2019; **10**(1): 953.