Investigating the role of alternative splicing in autosomal dominant retinitis pigmentosa using a PRPF31 patient-specific induced pluripotent stem cell disease model

Professor Majlinda Lako, Newcastle University

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Background:

Professor Lako is investigating a gene which causes retinitis pigmentosa (RP) called PRPF31. This gene normally provides instructions for making a protein which is part of the machinery used by cells to edit out unwanted or nonsensical sections of genetic code. This editing process is known as splicing, and the editing machinery is called the spliceosome.

Problems with genes that regulate splicing are a common cause of RP. However, until now, scientists have been puzzled as to why these mutations only cause faulty splicing in the retina, despite the underlying genetic defect residing in all cells in the body of an affected person.

Professor Lako and her team therefore undertook this project to better understand how the spliceosome edits genetic instructions, why the retina is so severely affected when splicing goes wrong, and how these problems might be addressed.

The project has made extensive use of living cell systems, derived from the skin cells of people with PRPF31-related RP, which closely mimic human retinal tissue and provide an excellent model of disease. At the start of the first pandemic lock down, the team were forced to discard these painstakingly developed cell cultures due to a lack of viable storage options, but a grant extension from Retina UK enabled them to be replaced when the researchers could get back into the lab.

Progress and achievements:

Investigating how PRPF31 mutations affect the stability and function of the spliceosome in retinal cells:

By studying their cell models, the team discovered that a truncated form of PRPF31 is produced in significant amounts in patient retinal cells and the adjacent retinal pigment epithelium (RPE) cells. (RPE plays a vital role in supporting and nourishing the light-

sensitive cells of the retina.) Strikingly, in contrast to the normal form of PRPF31 protein, which is imported into the cell's nucleus to play its part in splicing, the truncated PRPF31 protein is retained in the cytoplasm (the fluid filling most of the cell). The abundance of the normal PRPF31 protein is also significantly reduced in the patient retinal and RPE cells compared to healthy controls.

To analyse the effect of the presence of mutant PRPF31 on splicing, the researchers made use of various biochemical and cell biology methods to visualise the activity of the spliceosome and the correct formation of its building blocks in retinal cells. They found that *PRPF31* mutations reduce active spliceosomes and alter the structure of the cell's splicing machinery, which has a knock-on effect on the splicing needed to maintain normal structure and function of the retinal cells and RPE.

Investigating how protein expression is affected in PRPF31 patient derived retinal cells:

In the team's first project-related publication in 2018, they showed that *PRPF31* mutations cause significant changes in the way the genes are edited to give rise to proteins; however, these changes were specific to retinal and RPE cells only and were not observed in other cell types (for example skin cells). To identify which proteins were affected as result of *PRPF31* mutations, the researchers used a method called "mass spectrometry", which enabled them to measure the abundance of different proteins in retinal and RPE cells made from PRPF31 patients and then compare them to healthy control cells.

This analysis identified that a large number of proteins were altered in patient retinal cells. A substantial number of the changed proteins fell within the spliceosome category, which backs up some of Prof Lako's previously published data suggesting a vicious cycle of spliceosome dysregulation in the retina.

Several other categories of protein were also altered in patient specific retinal cells, including some that play important roles in protein degradation and waste disposal. Using special microscopy techniques, the team observed that patient retinal and RPE cells are characterised by the presence of large protein aggregates (clumps), which are not seen in control cells.

RPE cells play a vital role in disposing of worn out photoreceptor machinery. To better understand the impact of the altered protein levels in patient cells, the researchers fed the mutant RPE cells with parts of photoreceptor cells to see how the RPE coped with disposing of these. They found that this daily feeding accelerated the accumulation of debris. They then looked at cell survival and found that this build up of debris in the mutant RPE cells was having a probable toxic effect.

These findings suggest that helping cells to eliminate debris could be a potential approach to therapy, as is already being investigated by researchers looking at dry age related macular degeneration. Prof Lako's team investigated various existing drug compounds to see if any might be useful and found that rapamycin, a cancer and immunosuppressant drug, led to a significant reduction in clumps of debris.

Identifying genes which experience altered editing as a result of PRPF31 mutations

The consequences of PRPF31 mutations are driven by significant changes in the way that genes are edited during the splicing process. Splicing produces different edited versions (isoforms) of mRNA, the molecule that copies DNA and carries the genetic code to the cell's protein building machinery.

Prof Lako has already shown that PRPF31 mutations result in splicing that generates several isoforms of mRNA for each gene. Using a method called RNA-Seq, which involves sequencing and analysing all of the mRNAs in cell, she and her team have identified genes that are incorrectly spliced in various cell types, including photoreceptors and RPE, and have investigated the exact nature of the mis-splicing.

This analysis has shown that RPE cells are especially affected in PRPF31-mediated disease, with the proportions of the different mRNA isoforms per gene dysregulated more severely than in skin cells. The team has investigated the factors driving this preferential splicing and what might be contributing to aggregate (debris) accumulation, identifying >100 genes as part of this approach.

Overall, Prof Lako and her team have made enormous progress, despite the challenges posed by the pandemic. They have shed significant light on the mechanisms of disease in PRPF31-mediated RP, which could lead to potential targets for therapy; indeed, they have already highlighted the potential of certain pharmacological compounds. The team has produced eight publications as a result of this project. Prof Lako has also submitted a new PRPF31-related grant application to Retina UK as part of the 2021-22 funding round.

If you would like further information about medical research please contact Kate Arkell on kate.arkell@RetinaUK.org.uk. If you would like information about fundraising or supporting our work, please contact fundraising@RetinaUK.org.uk or call 01280 815900.