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COMMENTARY

Progressive accumulation of cytoplasmic aggregates in PRPF31 retinal pigment epithelium cells interferes with cell survival

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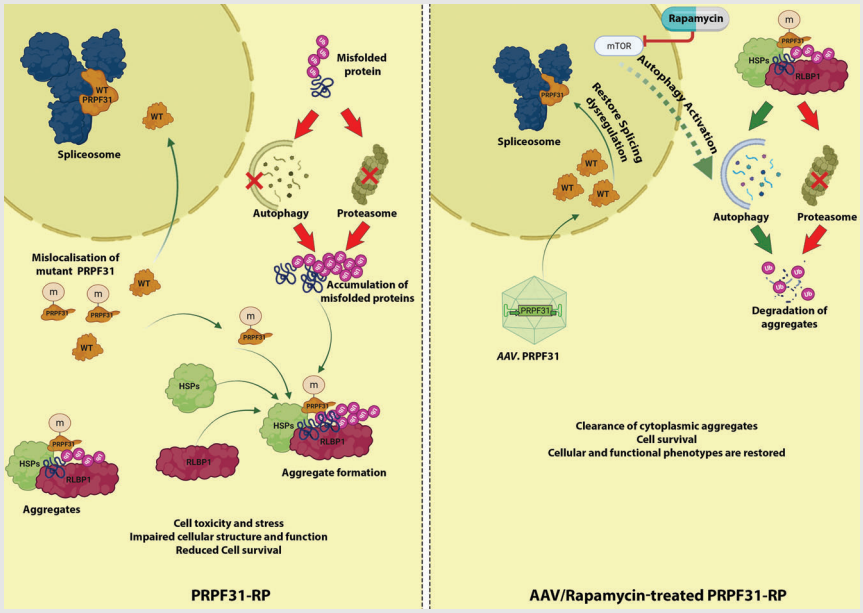
Graphical Abstract



Schematic representation showing mislocalisation of mutant PRPF31 in the cytoplasm of PRPF31-retinitis pigmentosa (RP) cells and accumulation of aggregates containing HSPs, visual cycle and ubiquitin conjugated proteins due to autophagy and proteasome dysfunction. Large cytoplasmic aggregates accumulate gradually in the cytoplasm of PRPF31-RP and cause cell toxicity and stress, impaired cellular structure and function and reduce cell survival (left panel). Administration of Rapamycin activates autophagy and reduces cytoplasmic aggregates. However, to restore PRPF31 expression and splicing activity, gene supplementation using *AAV.PRPF31* is necessary (right panel). HSPs, heat shock proteins; m, mutant PRPF31; Ub, ubiquitin; WT, wild type.

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Abstract

Retinitis Pigmentosa (RP) is a common form of inherited degenerative disease that often leads to blindness. About 10% autosomal dominant RP cases have been associated with mutations in PRPF31 gene, which is involved in pre-mRNA splicing. This commentary summarises the key findings of our recent publication 'Activation of autophagy reverses progressive and deleterious protein aggregation in PRPF31 patient-induced pluripotent stem cell-derived retinal pigment epithelium cells' in the context of large cytoplasmic aggregates which accumulate progressive with time and impair cell function and survival. Understanding the pathomechanism of PRPF31-RP provides invaluable information that can be used to understand other PRPF-RPs, and help to design effective and appropriate therapeutic strategies for the treatment of RP patients with PRPF31 mutations.

KEYWORDS

AAV, aggregate formation, gene therapy, iPSC-RPE, PRPF31, retinal organoids, retinitis pigmentosa, RPE

A recent publication by our group in Clinical and Translational Medicine titled 'Activation of autophagy reverses progressive and deleterious protein aggregation in in pre-mRNA (Messenger RNA) processing factors 31 (PRPF31) patient iPSC-derived retinal pigment epithelium (RPE) cells' provides new insights into why mutations in PRPFs cause retinitis pigmentosa (RP).[1] RP is an inherited retinal disease that affects more than 1 million individuals globally.[2] Approximately 15% of autosomal-dominant RP (adRP) cases are linked to genetic mutations in PRPFs. These core components of the spliceosome catalyse pre-mRNA splicing[3,4] are ubiquitously expressed throughout the body.[5] Given PRPFs are ubiquitously expressed throughout the human body, it is interesting that mutations only cause retinal disease. This retinal cell-specific phenotype remained a mystery until 2018, where a study by our group showed a global dysregulation of spliceosome specifically in RPE cells and photoreceptors but not other patient cells (for example fibroblasts or induced pluripotent stem cells).[5] Notwithstanding the importance of these results, the intricacies by which PRPF mutations lead to photoreceptor and RPE cell death, remained unanswered. In many neurodegenerative diseases, accumulation and deposition of misfolded proteins is the main cause of

neuronal cell death.[6] Similar pathogenic mechanisms have also been observed in retinal cells with the aggregation of visual cycle-related proteins like rhodopsin[7,8] or other aggregated proteins like tau or β-amyloid, linking thus the accumulation of misfolded proteins with retinal diseases[9] and other neurodegenerative diseases.[10,11]

Increasing evidence suggests that PRPF mutations cause protein aggregation in retinal cell types.[1] This has been shown in both immortalised rod photoreceptor cells (661W cells) harbouring *PRPF3* mutations[12] and ARPE19 cells harbouring PRPF31 mutations.[13] The Prpf31 $^{p.A216P/+}$ mouse model also displays cytoplasmic aggregates in the RPE cells: those contain the mutant Prfp31 protein colocalised with the Hspa4l chaperone.[14] Although most studies have shown PRPFs to be associated with retinal diseases, some evidence has linked other splicing factors with protein aggregation in Alzheimer disease.[10] How do these aggregates form in cells with *PRPF* mutations? Is the dysregulated spliceosome function solely accountable for their formation? Do these aggregates accumulate slowly, suffocating the retinal cells and preventing their normal function and survival?

To shed light on these unresolved questions, our group generated retinal organoids and RPE cells from PRPF31 patient iPSCs and unaffected controls and then performed a large-scale tandem mass tags-based quantitative proteomic profiling to identify the most affected biological pathways. Our proteomic analysis demonstrated that RNA splicing, autophagy and lysosome, unfolded protein response and visual cycle-related pathways were highly affected in PRPF31-patient cells compared to control cells.[1] Using a combination of immunofluorescent microscopy (IF) and biochemical analyses, we discovered that highly expressed proteins belonging to these affected pathways such as the mutant PRPF31 protein, misfolded, ubiquitin-conjugated proteins including key visual cycle and other RP-linked splicing proteins, were accumulating in large amounts together with chaperone proteins in the cytoplasm of patient-derived RPE and retinal cells forming cytoplasmic aggregates.[1] The presence of cytoplasmic aggregates was subsequently validated by transmission electron microscopy.

Under normal conditions, misfolded proteins are tagged with ubiquitin molecules and are then degraded by either the ubiquitin-proteasome system or through autophagy.[15] So, why do cytoplasmic aggregates accumulate in patient RPE cells? To gain a better understanding, our group evaluated the activity of autophagy and the proteasome degradation system using multiple assays. Strikingly, our findings indicated that these waste disposal mechanisms are impaired, and hence cytoplasmic aggregates accumulate over time.[1] Similar observations were reported in other neurodegenerative diseases, where cytoplasmic accumulation of misfolded proteins have been associated with reduced or blocked activity of the waste disposal mechanisms.[16,17] To further identify the impact of cytoplasmic aggregates on cell survival, we used immunoblotting and IF analysis to assess the expression and localisation of the apoptosis regulator Caspase-3 in control and patient RPE cells. The predominant expression of Caspase-3 in the nucleus of patient RPE cells and disruption of tight junctions in areas with cytoplasmic aggregates suggests that progressive accumulation of cytoplasmic aggregates leads to cell death.[1]

We next hypothesised that these aggregates could be eliminated by promoting the waste disposal pathway. By testing various pharmacological drugs up-regulating this pathway, we showed that activating autophagy using Rapamycin, effectively reduced cytoplasmic aggregates and improved cell survival in patient RPE cells. Several molecules have been identified that activate autophagy, although cytotoxicity is one of the main downsides for these drugs. Recent studies have reported the effects of Flubendazole, an Food and Drug Administration approved compound, which stimulates autophagy flux without affecting the health of RPE cells.[18] Considering these findings, future studies should focus on assessing the effects of this drug in our model to explore whether it performs similarly to Rapamycin. Although administration of Rapamycin has been shown to improve the clearance of cytoplasmic aggregates in our model,[1] the reduced expression of PRPF31 protein in the nucleus of RPE cells, which leads to reduced spliceosome activity and triggers the accumulation of aggregates, is still not resolved. Advances in gene therapy for the treatment of patients with retinal dystrophies have been made with the approval of Luxturna, demonstrating that gene therapy can efficiently restore gene and protein expression in patient RPE cells.[19] This suggests gene therapy using Adeno-associated virus (AAV). PRPF31 gene augmentation as an optimal approach to restore the PRPF31 protein expression. This approach will be useful at the early stages of the disease, where aggregate accumulation has not progressed. For late stages, a combination of gene therapy with autophagy activation may be needed to restore the reduced expression of PRPF31 and to eliminate accumulated aggregates.

Treatment of the mutant PRPF31$^{+/-}$ iPSC-RPE cells with AAV-PRPF31 has been tested with promising results.[20] We suggest that targeting RPE cells using AAV-based gene therapy in combination with autophagy activation holds a great therapeutic promise for patients with mutations that result in accumulation of toxic cytoplasmic aggregates.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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