

ORIGINAL RESEARCH

16. Characterisation of Morphological Changes and Retinal Decay in a Retinal Degeneration Murine Model of Retinitis Pigmentosa

Harriet Fallon¹,¹ South Tyneside and Sunderland NHS Foundation TRUST, UK

https://www.youtube.com/watch?v=hJicU1w8oM&list=P_LhqNg3xJC1bafO0Y5bvBcgMmXpgzJxd44&index=5&t=8104s

Retinitis pigmentosa (RP) is a complex, incurable, polygenic retinal dystrophy that initially presents as loss of night vision in childhood, and progresses to severe, irreversible loss of vision in adult patients. The *Pde6rd10* mouse models a progressive retinal degeneration akin to RP seen in humans. These mice carry a spontaneous mutation in the rod-phosphodiesterase gene that leads to photoreceptor degeneration peaking between (P)20-25 postnatal days, with degenerative plastic changes of second- and third- order neurones becoming increasingly severe following this. RP is currently without cure, however one such idea for successful vision restoration relies on the intraocular injection of haematopoietic stem cells as rod and cone photoreceptor precursor cells. There is a requirement to determine at which points there is sufficient retinal degeneration to for there to be a treatment need, without compromising the integrity of the scaffolding neurones in the remaining retina as retinal disorganisation worsens. Early transplantation of differentiated photoreceptors could delay or even prevent remodelling of the second-order neurones and cell death by providing synaptic input to those circuits.

The study aim was to determine the peak range of photoreceptor cell death and evaluate the structural modifications to retinal neurones following photoreceptor degeneration in the *Pde6rd10* mouse model, documenting the sequence of changes of second- and third-

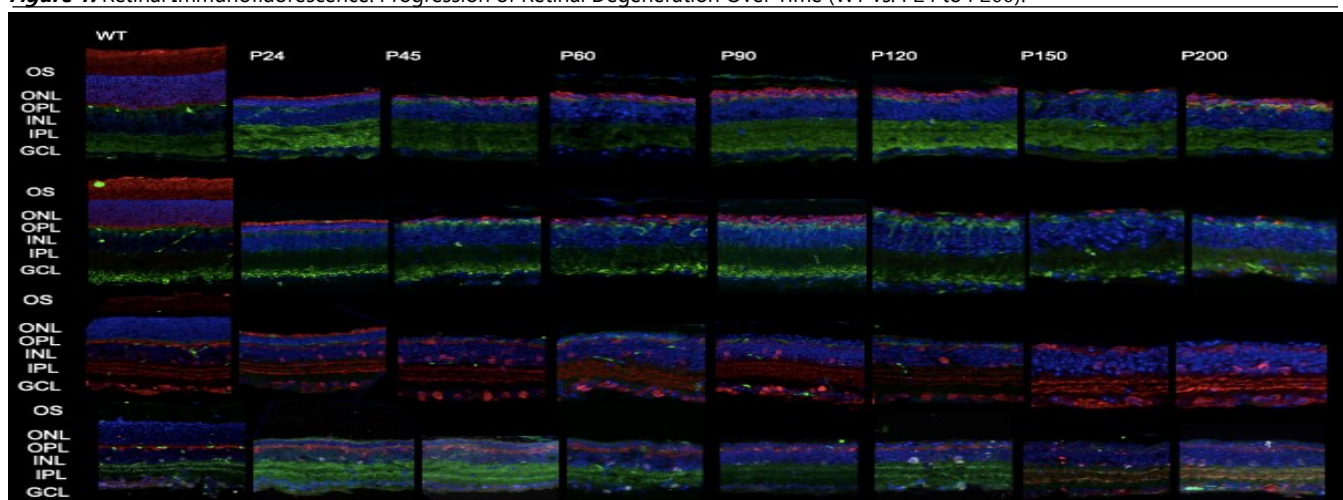
order retinal cells and their synaptic terminals to an advanced stage of degeneration. This data can be used to inform the timespan within which transplanted photoreceptor precursor stem cells could effectively integrate with the retinal cells in the *rd10* retina and function as normal, restoring normal vision.

Immunohistochemistry and qualitative immunofluorescence microscopy were performed on retinal cryosections of *rd10* mice to detect and assess the presence of specific cellular markers across of *wild-type* and postnatal days 21-200 (P21-200) of the *rd10* mouse retina. Antibodies against cell and synapse-specific markers were used for single- and multiple-labelling studies. Histological staining was performed to quantify the degradation of the photoreceptor-containing outer nuclear layer (ONL) of the retina.

Immunofluorescence microscopy demonstrated a remarkable deterioration in the number of rods in the ONL and subsequent changes in the morphology of the retinal cells that synapse with the photoreceptors. No outer segments were distinguishable beyond postnatal day (P24). Synaptic connections were reduced in number, and second order amacrine cells and horizontal cells were reduced in size and complexity throughout the retinal layers in all *rd10* postnatal day samples. Haematoxylin and eosin staining showed a statistically significant ($p < 0.0001$) reduction in the depth of the photoreceptor-containing ONL in all measured intervals from the wild-type to P200, most significantly between the WT and P23; 60.43um and 15.24um, respectively (more about P23-P24). Peak interval deterioration of photoreceptors was determined at P23-24 in addition to important findings about alterations in the surrounding retinal architecture.

This study helps to inform the optimum timeline for the intraocular injection and transplantation of haematopoietic stem cells, and thus the replacement of degenerated photoreceptors ultimately for the potential restoration of vision in RP.

Figure 1. Retinal Immunofluorescence: Progression of Retinal Degeneration Over Time (WT vs. P24 to P200).



Legend: single, double, and triple-stained immunofluorescence cryosections of wild type, and *rd10* postnatal day 24-200 mouse retinas showing progressive retinal cell - including photoreceptor cell - and synapse degeneration through all cell layers.

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ISSN 2076-6327

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