248 Retinal Degeneration: Animal Models
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Exhibit/Poster Hall Poster Session
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Program Number: 2249 **Poster Board Number:** D0239
Presentation Time: 11:00 AM–12:45 PM
Comparative proteomic analysis between the degenerated human and zebrafish retina
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Purpose: Müller glia with stem cell characteristics are responsible for the spontaneous regeneration of the zebrafish retina after injury. These cells are also present in the human retina, however there is no evidence for regeneration. It is thought that factors either expressed in the mature human retina or produced as a result of injury, may prevent endogenous regeneration in humans. To identify these factors, this study compared the protein expression profile of normal and gliotic human retina with that of the degenerated and regenerating zebrafish retina
Methods: Retinal samples were obtained from patients undergoing retinectomy for PVR in accordance with the tenets of the Declaration of Helsinki. Normal cadaveric retina was obtained from Moorfields Eye Bank by approval of the local Ethics Committee. Zebrafish eyes were injected with 200µM Ouabain, and retina was excised at days 3 and 18 post injection. The use of animals was in accordance with the U.K. Home Office regulations for the use of laboratory animals. Differential protein profile expression was performed on retinal lysates using Label-free proteomics by LC-MS/MS and 2D DIGE methods. Protein identification and bioinformatics were performed using Protein Lynx Global Server (PLGS), and Non-Linear dynamics Progenesis software
Results: Mass spectrometry identified 473 proteins in the human retina and 328 proteins in the zebrafish retina. Orthological protein comparison showed that 80 proteins were present in both zebrafish and human retinae. Of these proteins, 6 were upregulated >2-fold in the gliotic human retina, but only one, identified as galectin, was upregulated >2-fold in the degenerated zebrafish retina. In addition, 36 of these 80 proteins were <0.5-fold downregulated in the gliotic human retina, whilst 26 were also downregulated in the degenerated zebrafish retina. Only one protein, known as adenylate kinase, was upregulated >2-fold in the degenerated zebrafish retina. Many protein changes observed in both species were associated with heat shock proteins, histones and the extracellular matrix
Conclusions: This proteomic-based study has identified differences in the protein expression profile of the degenerated human and zebrafish retinae. Further investigations of the role of differentially expressed proteins in both species during retinal degeneration may help to design novel approaches to promote endogenous repair mechanisms in the human retina
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Effects of Targeted Cone Ablation on the Integrity of Neighbouring Photoreceptor Subtypes
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Purpose: Zebrafish are optimal for the genetic investigation of cone photoreceptor physiology, regeneration, and functional integration. We have engineered a pair of novel transgenic zebrafish models enabling targeted ablation of specific cone subtypes. This model allows us to determine whether specifically ablating cone subtypes results in a bystander effect on neighbouring photoreceptors, toxic or otherwise, and ultimately provides opportunity to explore other retinal responses to specific cone loss, such as regeneration, functional integration, and connectivity changes.
Methods: We engineered two transgenic zebrafish lines to express nitroreductase (NTR) in blue- or UV-sensitive cones, allowing conditional cone ablation upon the introduction of an otherwise inert prodrug, metronidazole (MTZ). A cell death assay was performed on MTZ-treated and DMSO (vehicle control)-treated larvae, and the number of apoptotic cells counted. Co-localization of apoptotic markers with a NTR-mCherry expressing blue or UV cone demonstrated the death of a target. Abundance of remaining cone types was assessed via antibody labeling.
Results: Increased cell death was not detected amongst adjacent cells when blue ($p>0.85$, n=11 for MTZ, n=12 for DMSO) or UV cones were ablated ($p>0.99$, n=9 for MTZ, n=5 for DMSO). The predicted increase in apoptosis for target cell types upon introduction of MTZ was observed ($p<0.001$ for blue ablation, $p<0.05$ for UV ablation). Similarly, the relative abundance of non-target cone types did not change between MTZ- or DMSO-treated fish for blue ($p>0.97$, n=12 for both treatments) or UV ablation ($p>0.78$, n=5 for MTZ & n=6 for DMSO).
Conclusions: We investigated whether a bystander effect was elicited when specifically ablating blue- or UV-sensitive cones using the NTR method. There was no significant increase in apoptosis of non-target (non-mCherry expressing) cone cells when treated with MTZ for either cone ablation model. As expected, both lines showed an increase in the number of mCherry-expressing apoptotic cells, demonstrating that the cells expressing NTR-mCherry are ablated when exposed to MTZ. This model of conditional targeted cone ablation thus does not appear to cause off-target, deleterious effects to surrounding photoreceptors, and can be used to explore changes in retinal physiology, cone connectivity, and regenerative responses after only one cone subtype is lost.
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