Role of Interferon Gamma in BMP7 Mediated Gliosis

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Introduction: The retina consists of neuronal and non-neuronal cells known as glial cells. The glial cells in the retina include the Müller glia, retinal astrocytes, and microglia. Muller glia and retinal astrocytes are known to undergo gliosis, a protective response, in reaction to diseases or damaging effects such as high blood sugar concentrations or physical injury of the retina. The Belecky-Adams lab has shown that a growth factor, bone morphogenetic protein 7 (BMP7) is capable of triggering reactive gliosis indirectly, by activating secretion of interferon gamma (IFNγ) from microglial cells. The aim of this study is to determine if fludarabine, an inhibitor of IFNγ mediated signaling, is effective in blocking activation by IFNγ in retinal astrocytes. This aim is the first step in testing the hypothesis that a blockage of IFNγ signaling in BMP7-treated retinas will reduce gliosis.
Methods: Retinal astrocytes cells *in vitro,* were treated with 50 or 100μM of STAT1 inhibitor, fludarabine, for 2 hours, followed by addition of vehicle or 150ng/ml IFNγ. Protein was extracted from cells 24h after addition of vehicle or IFNγ, and quantified using BCA protein assay. Fifty micrograms of protein was loaded onto a denaturing gel and subsequently transferred to PVDF membrane and probed with an antibody against glial fibrillary acidic protein (GFAP) and β-tubulin. Bands were quantitated using densitometry and GFAP normalized to the β-tubulin loading control.
Results: Retinal astrocytes treated with IFNγ but no inhibitor showed an increase in levels of GFAP, a known marker of gliosis. Cells pre-treated with fludarabine followed by IFNγ showed a reduction in GFAP expression in comparison to those treated with IFNγ alone.
Conclusions: Fludarabine is effective in blocking the effects of IFNγ in retinal astrocytes. The results will allow further investigation of our hypothesis to determine if blocking IFNγ in BMP7-treated retinas reduces gliosis.

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