Molecular event in HRP Apoptosis

Laura Valdez[1], Lili Guerra[2], Richard LeBaron[3], Andrew Tsin[3]

Background: Human retinal pericytes (HRP) are contractile cells adjacent to and provide support for endothelial cells (EC) of capillaries, which are essential in the regulation of retinal vasculature. Early stages of DR are characterized by the loss of HRP, which leads to the development of advanced-stage pathology including angiogenesis. Although much is known about the etiology of DR, the apoptotic pathway that incites HRP loss remains unclear. Our preliminary studies reveal that monocyte-derived macrophages secrete TGF-β1, which induces the expression and secretion of a TGF-β1-Induced, pro-apoptotic BIGH3 protein (TGF-β–Induced Gene Human Clone 3) leading to apoptosis of HRP *Eye*, *30*(12), 1639-1647 Based on a preliminary study in renal cells (unpublished data), CTP (c-terminal peptide) with an RGD domain is released from BIGH3 by proteolysis leading to renal cell apoptosis. In the present study, we employed Western Blots to determine if a similar molecular event also takes place in the BIGH3 protein to induce HRP apoptosis.

Methods: HRP cells were obtained from Cell Systems and were cultured in complete media with 10% FBS, 1% penicillin/streptomycin, in a humidified 5% CO2 incubator with a temperature of 37°C. Cells were harvested from passages 5-8 until reaching confluency. HRP were starved for 24hrs with media composed of only DMEM and 1% penicillin/streptomycin prior to getting treated 24 hours with and without 15ug/mL of TGF-β, 50ug/mL of Leupeptin (a protease inhibitor), and with both TGF-β and Leupeptin. Conditioned media was collected after 24hrs and stored in -80C until protein concentration assay and probed with an anti-BIGH3 polyclonal antibody, an in-house generated rabbit antibody generated against full-length recombinant BIGH3. Western blot analyses of the cleaved BIGH3 protein band were quantified using ImageJ.

Results/Conclusion:

Consistent with our previous observation, culture HPR secret BIGH3 protein. Western blots show two BIGH3 protein bands: un-cleaved protein (60kD) and cleaved (or truncated). Image analyses of band intensity of the cleaved proteins was significantly reduced (by five-fold) in the presence of Leupeptin (a protease inhibitor). Similarly, a two-fold reduction of the cleaved protein was also observed when Leupeptin was added to the cell media in conjunction with TGF-β. Thus, our results are consistent with prior observations in renal cells that CTP release (by proteolysis) from BIGH3 is a molecular event in the HRP apoptosis. A growth curve was conducted in order to determine the different cell growth phases for the appropriate time of treatment.