Knockdown of BETA2 Microglobulin in MHC 1 Molecule to Prevent Transplant Rejection in RPE Cells

Garg, Shreya (School: Logan High School)

Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world, affecting 20 million in the US alone. Retinal Pigment Epithelial (RPE) cells between the retina and blood vessels maintain nutrition to the retina and are essential for normal vision. RPE dysfunction is implicated in AMD. Advanced disease may necessitate an RPE transplant. A major limitation to this therapy is rejection resulting in death of transplanted cells and advanced AMD. Rejection can be prevented using immunosuppressive drugs, which cause serious adverse reactions. B2-microglobulin (B2M) is a key protein in the major histocompatibility complex (MHC) Class I molecules, responsible for presenting antigens to T-cells and mediating rejection. I hypothesized that siRNA (small interfering RNA) gene knockdown of B2M in ARPE-19 cell line will reduce its expression, maintain cell viability, and could prevent MHC mediated rejection of transplanted cells. Optimal siRNA transfection was confirmed using ELISA for GAPDH suppression (expressed in most cells) with various concentrations of cells, reagents, and siRNAs. To test reducing expression of MHC-I molecules, ARPE-19 cells were treated with an siRNA that targets the B2M gene. Cells were assessed for the expression of MHC-I complex proteins using flow cytometry. The percent expression of MHC-I cells was compared to control conditions. I concluded that siRNA knockdown of B2M significantly lowered the expression of MHC-I while maintaining cell viability. Although knockdown is temporary, early prevention of rejection could improve long- term survivability and reduce need for immunosuppressives. Studies would need to be replicated in animal models to confirm in-vitro results.

Awards Won: