Investigation of the Effects of DNA Concentration on Polyethyleneimine Transfection Success and the Efficacy of the Serp-2 Secretion Signal

Sheng, Cynthia (School: Fort Myers High School)

The serine protease inhibitor-2 (Serp-2) gene is a virulence factor of the myxoma virus, an immunosuppressive disease found in European rabbits. This gene inhibits the proteases caspase-1 and granzyme-B from triggering immune responses. Contrastingly, uveitis is an inflammatory eye disease and the third leading cause of blindness. Advances have been taken to use the anti-inflammatory properties of the Serp-2 gene to develop novel gene therapy treatments for uveitis. In previous research done by the lab, when the Serp-2 transgene was transfected in vivo into mouse retinal cells, the cells did not survive. The researcher postulated that the cell death was due to either a high titer of the vector used in gene delivery or toxicity caused by protein buildup within the cell. To prevent protein buildup, an immunoglobulin kappa (IgK) secretion signal was added to the transgene. This study tested the efficacy of the IgK secretion signal while also researching the effects of DNA concentration of transfection success. The secreted Serp-2 (sSerp-2) DNA was polyethyleneimine transfected into in vitro HEK-293T cells and analyzed for gene expression. A western blot procedure compared the concentration of sSerp-2 protein in the cell lysate to the media. This study demonstrated that the amount of Serp-2 proteins in the media exceeded the amount in the cell lysate, indicating that the IgK secretion signal was effective.

Awards Won: