Using a Crispr-Cas9 Method to Knockout AURKA in Pancreatic Cancer Cells

Vudatha, Kavitha (School: Stanton College Preparatory School)

Aurora Kinase A, a serine-threonine protein kinase, is primarily responsible for regulation of cell proliferation. When overexpressed, it can cause an uncontrollable increase in cellular proliferation. The research primarily focuses on the effects of suppressing AURKA gene expression using the CRISPR-Cas9 technology to edit the gene and observe its effect on the growth of pancreatic cancer cells. The previous year project concentrated on AURKA overexpression and its effect on cell culture. This year, the focus is finding an alternative design to silence the AURKA gene using different processes and vectors. Three single-guide RNA were constructed, using the UCSC Genome browser, for the px330 vector. One reporter gene was designed for the sgRNA and another was designed for the sgRNA and Gibson Assembly process. Through ligation and transformation into DH5α e.coli, the sgRNA was combined with px330 and the reporters were inserted into GFP vectors. The sgRNA and the GFP plasmids were co-transfected into HEK293T cells. Pictures were taken to observe the cells, and the cells indicated that the third sgRNA expressed GFP. The presence of AURKA was further validated by a Western blot through protein levels. The procedures were repeated with a lentiCrispr-V2 vector and px330. The sgRNA and GFP plasmids were co-transfected into 293T cells and co-transfected into MIA PaCa-2cells. The 293T and MIA PaCa-2 expressed GFP and AURKA and MIA PaCa-2 is expected to exhibit decreased cell growth and increased cell apoptosis, through biological assay testing.