Potential Gene Therapy of Human Liver Cancer with Recombinant AAV3 Flp-FRT Vectors

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The survival rate for metastatic liver cancer, such as hepatocellular carcinoma (HCC), is less than 3% over a 5 year span, so the need for a cure has never been more urgent. The use of a nonpathogenic adeno-associated virus (AAV)-based vector in order to deliver a therapeutic gene to HCC cells is a promising form of treatment. The serotype AAV3 is able to specifically target liver cancer cells through the use of hepatocyte growth factor receptor, which is only overexpressed in cancerous hepatocytes. Through the use of AAV3, a form of a recombinase protein, Flippase, which specifically cleaves its target sequence, and a mutated Flippase Recognition Target (FRT) sequence, it is anticipated that selective breakage in the liver cancer cell DNA can be achieved, which would then be expected to result in cell death. A human HCC cell line, Huh7, was transfected with plasmids containing combinations of Rep (binding protein), FRT gene sequence, and EGFP (reporter gene). The initial experiment compared these transfected cells to investigate integration efficiency of FRT. A qPCR was performed to establish integration efficiency of transfected cells. Flippase vector infected cells were monitored for Flp-FRT induced cytotoxicity by monitoring EGFP levels. In further experimentation, different mutations on FRT sites will be evaluated in order to find the most efficient way to eradicate cancer cell growth.

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