Online Proceedings

POSTER SESSION 2

Balcony, Easel 86

1:00 PM to 2:30 PM

Efficient Selection of Genetically Modified T Cells for Human Immunotherapy using Methotrexate

Teresa Einhaus, Sophomore, Molecular Sciences, Bellevue College
Mentor: Gita Bangera, RISE Learning Institute, Bellevue College
Mentor: Bish Paul

Genetically modified T cells have the potential for a number of therapeutic uses in anti-cancer immunotherapy, but a current limitation is the low yield of modified cells. Methotrexate is an FDA-approved drug used to destroy rapidly dividing cells by blocking their metabolism of folic acid. With the addition of a construct containing a mutant DHFR (dihydrofolate reductase) for methotrexate resistance, modified cell populations can be selectively expanded. Our mutant DHFR gene, delivered by lentivirus, has a Tyr-22 mutation which confers methotrexate resistance by changing its binding site. The purpose of this study is to determine the optimal concentrations and timing for the addition of methotrexate for selection of our target cell population. First, we grew cultures of Jurkat cells, an immortal T cell line, to find their normal growth and viability curves. Next, we transduced the cells by the addition of lentivirus at 1 or 2 X 10^4 viruses per 10^6 cells. We measured gene expression of a green fluorescent protein reporter gene using flow cytometry, which showed the lower viral concentration produces a peak protein expression of 14% on day two. Finally, we used various concentrations of methotrexate and found the optimal dosage for chemoselection to be 50 nM, with around 90% of the population showing expression on day five. We demonstrate a six-fold increase in gene modified cells in the presence of methotrexate and that the growth rate of modified resistant cells is comparable to non-modified cells. Currently, we are testing this selection protocol in human CD4 T cells. Overall, this study has major implications for the use of gene therapy requiring T cell products in clinical trials.

POSTER SESSION 2

Balcony, Easel 87

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Sequencing and Analyzing *Psuedomonas fluorescens* strain L5.1-96 Genome Segments and Unique Protein

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Mentor: Gita Bangera, RISE Learning Institute, Bellevue College

In order to protect wheat from the aggressive root fungal infection, Take-all, farmers have turned to biological methods. Bacteria that produce 2,4-diacetylphloroglucinol (DAPG) are one of the best methods of controlling it, but some strains work better. *Psuedomonas fluorescens* L5.1-96 is the most effective strain, so 1000 bp long genome segments, from a made genomic library, are being sequenced and analyzed. One segment, a heat shock protein, was found to be unique among strains studied for wheat root colonization. To find whether this protein is necessary for L5.1-96's effectiveness, a proper assay needs to be created for the protein for future analysis. The gene had a 98% DNA match to *Psuedomonas brassicacearum*, so primers for PCR of the gene were created based on its DNA sequence. The amplified gene will be placed into a high efficiency plasmid for transforming competent *E. coli* cells, which will be analyzed for their protein composition, in order to create a Western Blot test for the protein. Once the test is created, the strain can be analyzed to find out whether this gene is important for root colonization.