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Melanoma gene knockdown by introduction of engineered RNA

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Relatively new to the scientific community is the RNA interference pathway (RNAi) that can be used to silence post-transcriptional gene expression. Long dsRNA can be introduced into cells and through various mechanisms become a shorter siRNA-RISC complex. This complex targets and hydrolyzes mRNAs with complimentary sequences. This means that theoretically, any gene in which the exon-spliced RNA sequence is known can be targeted by engineered siRNA. The goal of my research project is to develop peptide mediated siRNA targeting of melanoma cells. The cell line used has a plasmid vector that exhibits luminescence when an enzyme is induced (luc+). This luminescence gene is targeted by our siRNA and we can quantify the down regulation of the gene using a light meter. siRNA has been introduced into the melanoma cells using lipofection to investigate the proposed amount of gene down regulation (light activity). Recordings take place at 24h, 48h, and 72h. Continuing research is necessary to validate results. siRNAs are usually introduced into cells through non-specific techniques like poly-cationic transfection or lipofection. However, peptide mediated siRNA delivery is brand new to the field. Wild-type α-MSH is a peptide that is implicated in the regulation of skin pigmentation for which the receptors are present in human melanoma cell lines. However, these α-MSH peptides are broken down and metabolized by enzymes in the body within a few minutes. An α-MSH analog named NDPMSH is able to resist the metabolic processes of the body as well as selectively bind to the melanoma receptors. This molecule targets the Melanocortin 1 receptor on the melanoma cell lines and is internalized (Chen et. Al 688). This peptide, when linked to the siRNA, can theoretically be delivered selectively to melanoma cells and inhibit gene expression. The long -term goal of this research project is to use the NDPMSH peptide to usher the siRNA to melanoma tumor cells in vivo, causing them to undergo apoptosis.