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Abstract:

The cytosolic sulfotransferases (SULT) are a superfamily of enzymes that catalyze the metabolism of various substrates throughout the body. One member, SULT4A1, has no known substrates and is highly conserved among all vertebrates which is not a shared characteristic among the SULT family. Also unique among the SULTs, SULT4A1 localizes with mitochondria of neurons. Recent reports have suggested that this protein is believed play a protective role against oxidative stress. The goal of this project was to generate a SH-SY5Y cell line with a SULT4A1 gene deletion using CRISPR gene-editing technology. These neuroblastoma cells were used because of their ease of culture in the laboratory and their similarity to neurons. CRISPR uses DNA-cutting enzymes to target genes for a variety of mutations and modifications. These enzymes limit the amount of errors made by previous gene modifying techniques in the past, and they also take less time than previous techniques. A circular piece of DNA (plasmid) was designed to include coding for the gene-editing endonuclease, the target sequence for the gene, and puromycin resistance. The plasmid was introduced to the cells via electroporation, a technique in which an electric shock is used to disrupt the cell membranes and allow entry of the plasmid. Plasmid uptake was verified by selection with puromycin for 24 hours. This ensured that all surviving cells had taken up the plasmid and were expressing the puromycin resistance. Future experiments with these mutant cell lines will elucidate not only the function of SULT4A1 but also why it is highly conserved amongst vertebrate species.