Investigating the functional roles of δ-catenin protein

Celeste Parra Bravo, Elmer Guzman, Ken Kosik, MD

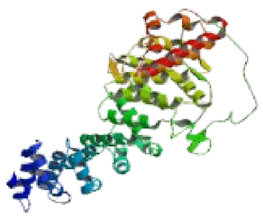
Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara

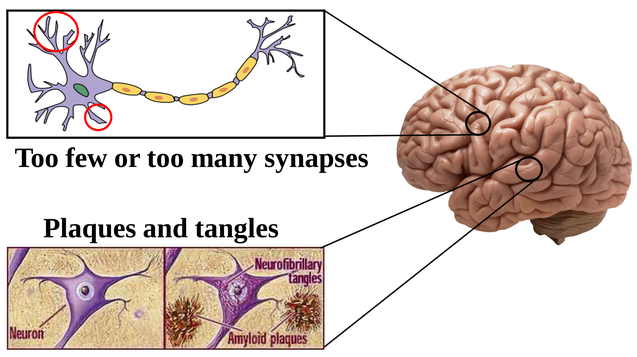
Introduction

There is one thing that incurable and debilitating disorders like Alzheimer's disease, Schizophrenia, and Autism all have in common: simple mutations in the brain. Neurodevelopmental and neurodegenerative diseases are nothing new to the worldwide population, but only in the last 50 years have scientists conducted extensive research in a variety of neurological disorders, focusing primarily on how they develop. There is still so much that we don't know about the brain and scientists must be able to understand the key players that cause the brain to malfunction in order to find treatments stop the incorrect development of the brain, as well as its early and rapid deterioration. One of those key players is delta catenin.

Delta catenin is a synaptic protein that was found to be a key player in the regulation of dendrites, density of the synapse, plasticity and function of neurons, all of which keep the cells in the brain working and interacting properly. Genetic mutations or loss of the delta catenin gene may disrupt normal neuronal wiring. My mentor and I began the investigation into how the regulation of delta-catenin inside human neurons affects its surroundings.

Delta catenin 2





Mutations in the gene of amyloid peptide, a protein that interacts closely with delta-catenin causes plaques tangles within the cells, a process that is directly linked to Alzheimer's disease.

Mutations in the delta-catenin gene itself cause a disruption in dendritic regulation by creating too many or too few synapses, a process directly linked to Autism.

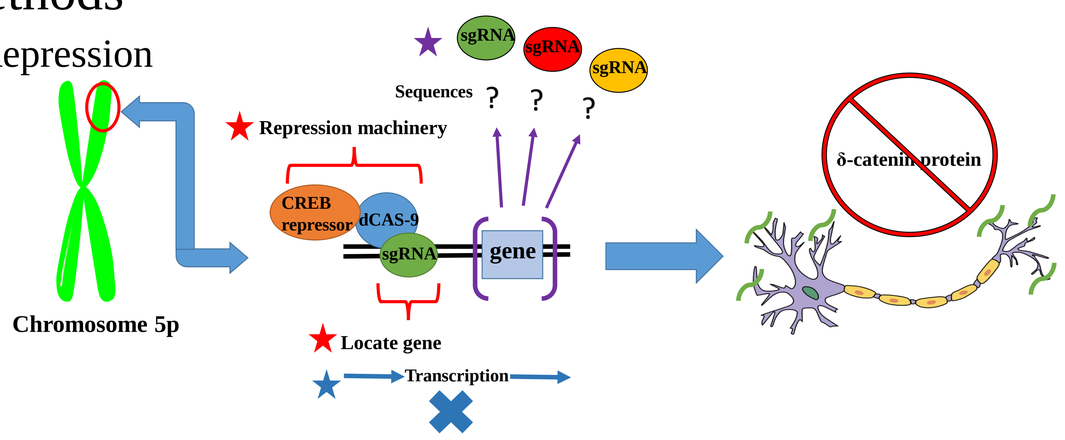
Objective

There has been a significant amount of work done in analyzing what occurs when delta-catenin is knocked down in mouse cells, how the absence of this protein has affected its surroundings. Our project began with the intention of similarly identifying downstream consequences that occur in human induced Pluripotent Stem cell-derived neurons.

My objective for the summer was **to confirm the best knockdown of delta catenin occurring in hiPS-derived neurons.** Three samples each targeting a different sequence within the gene were tested to identify which sequence, when repressed, would stop the most amount of protein from being produced.

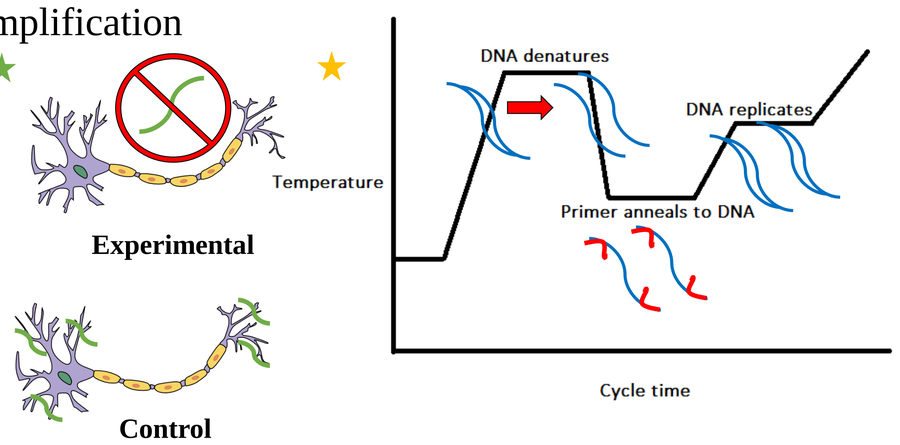
Methods

1 Repression



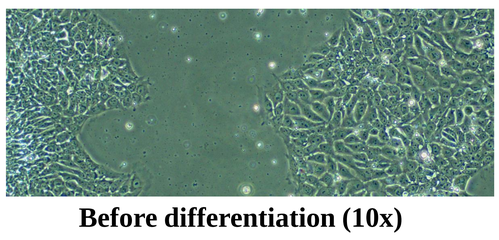
- We wanted to find the sequence within the gene that coded for delta catenin the best, so when the gene was repressed, the protein would stop being produced as much as possible. For this, we designed **3 guide RNAs** that served to localize each sequence and bind accordingly. Whichever guide RNA sample represses the most, we will know that this sequence codes for delta catenin the best.
- A **CREB repressor** and a **CRISPRi** system fuse together to make up the repression machinery for the gene. With the guide RNA localizing the gene, the complex formed binds to the target.
- Once the complex is on the gene, transcription is inhibited and production of the protein halts.

2 Amplification

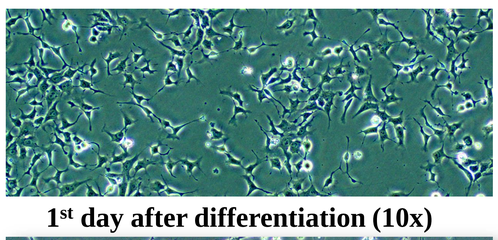


- Once the protein is knocked down, the next step is to measure the relative expression of delta catenin mRNA. The amount of mRNA has a strong correlation to the amount of protein, so it is used as a tool to measure the protein's expression. We used a method called **qPCR** which amplifies a targeted DNA by copying. Our experimental samples were the cells that had delta catenin knocked down, and our control samples did not have delta catenin knocked down.
- First, it denatured the DNA that coded for delta catenin, meaning it separated the two strands of the DNA into 2 single ones; then, a primer attached to the DNA which, lastly, helped copy it many times over, amplifying the amount of DNA there was. It was expected that the experimental samples would have a smaller amount of the amplified gene, compared to the control. To determine if this is true, we used a data analysis technique to measure its relative expression.

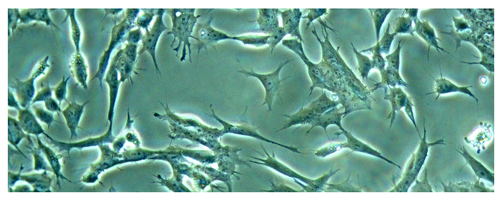
Induced Pluripotent Stem cell differentiation into human neurons



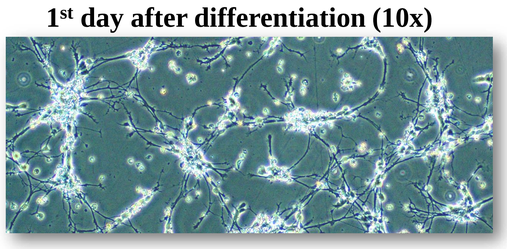
Before differentiation (10x)



1st day after differentiation (10x)



2nd day after differentiation (20x)

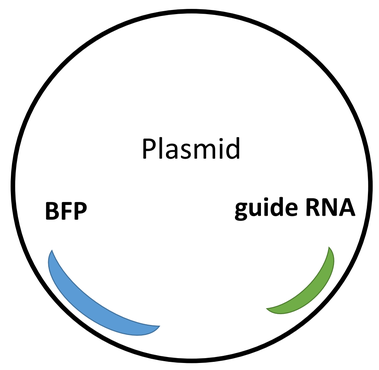


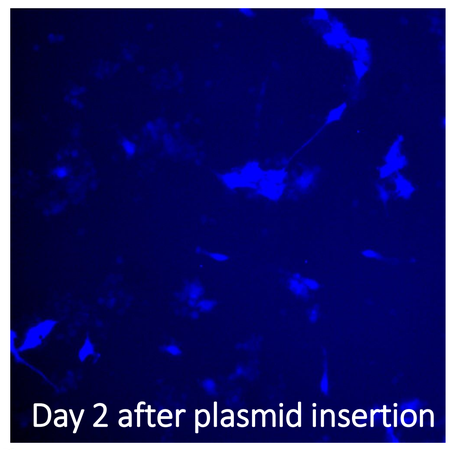
7th day after differentiation (20x)

Confirming presence of guide RNA in H4 cells

H4 cells to were used to test repression with guide RNAs before neurons were used, primarily because H4 cells are easier to work with. The expression of guide RNA was confirmed in these as well.

A plasmid coding for Blue Fluorescent Protein and a guide RNA was inserted into the cells.



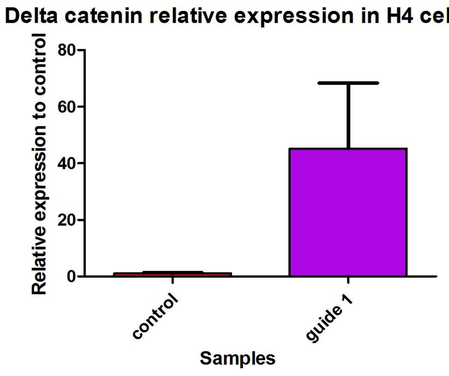


Cells fluoresce = plasmid taken up by cell = guide RNA expression

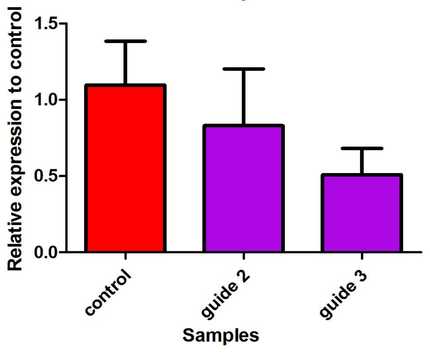
Results

qPCR lower relative expression = lower δ-catenin mRNA levels = knockdown confirmed

Delta catenin relative expression in H4 cells

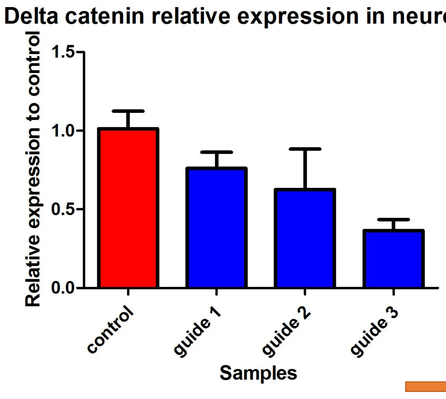


Delta catenin relative expression in H4 cells



The delta catenin knockdown results in the H4 cells were **inconclusive**. The sample containing guide RNA 1 had a higher relative expression that the control, meaning the mRNA levels in guide 1 were much higher than the control. A One-way ANOVA test showed guide 2 and 3 samples also did not show any significant difference.

Delta catenin relative expression in neurons



The delta catenin knockdown results in the neurons showed positive results, as all three experimental samples showed lower relative expression of mRNA compared to the control, **confirming the knockdown of delta catenin**. A T-test showed significant difference between the control samples and guide 3.

Conclusions and Future work

From the qPCR neuron data, the sample with **guide RNA 3 has the lowest relative expression of mRNA compared to the control, meaning it was the most effective in the delta catenin protein knockdown.** Now that the knockdown is confirmed with the best guide RNA, our focus can now shift to studying the differences in morphology and electrophysiological properties between the neuron knockdown samples and the control. In the end, it is important to continue studying delta-catenin's roles within the human brain in order to gain a better understanding of how this protein interacts with its surroundings, and eventually, we will be able to understand enough to manipulate it and move forward to develop ways to ameliorate the effect of genetic mutations that cause incurable and debilitating neurological diseases.

Acknowledgments

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