Investigating the Role of a Schizophrenia Risk Gene in Protein Degradation

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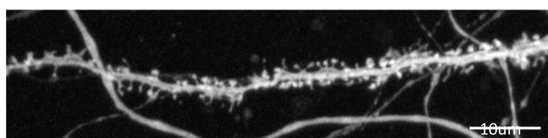
Introduction

14-3-3 proteins
- The 14-3-3 protein family is genetically linked to schizophrenia
  - Mouse models deficient in 14-3-3 proteins have been shown to exhibit schizophrenia-like behaviors and anatomical pathologies
  - Reduced dendritic spine density is also appreciated in 14-3-3 deficient models

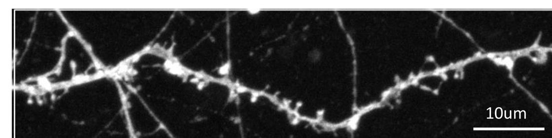
Dendritic spines
- Dendritic spines are small protrusions that extend off the dendrites of neurons and comprise the postsynaptic compartment (Yasuda, 2017)
- Decreased dendritic spine density has also been linked with schizophrenia in humans (Glausier & Lewis, 2013)
- The proper formation and maintenance of dendritic spines is mediated by the actin cytoskeleton (Lei *et al.*, 2016)

δ-Catenin
- δ-Catenin is an adhesive junction-associated protein that is crucial in the modulation of actin dynamics (Kim *et al.*, 2002)
- δ-Catenin also plays an important role in the function of synapses and assists with cellular adhesion and movement (Lu *et al.*, 1999)
- δ-Catenin has been shown to regulate the development of dendritic spines (Arikkath *et al.*, 2009)

14-3-3 proteins and δ-catenin association
- δ-Catenin has been identified as a potential binding target for 14-3-3 proteins (He *et al.*, 2012)
- The interactions between 14-3-3 proteins and δ-catenin has led to research that presents conflicting data on the nature of their relationship
  - One study suggested a synergistic effect between the two (He *et al.*, 2012) while another showed the opposite (Toyo-oka *et al.*, 2014)



- Cultured hippocampal neurons from wild type mice
- Dendritic spines can be seen all along the length of the dendrites



- This sample is from hippocampal neurons when a 14-3-3 protein inhibitor is expressed
- There is a glaring reduction in the number of dendritic spines

Objective

- We aimed to improve upon the methods of other experiments in order to gain more clarity on the exact relationship between 14-3-3 proteins and δ-catenin
- Previous studies used different methods to inhibit protein synthesis during the time course experiment
- Cycloheximide inhibits the synthesis of all proteins, meaning it may prevent other vital cell functions or alter protein degradation (Dai *et al.*, 2013)
- To mitigate the potential harmful effects of cycloheximide, we opted for a doxycycline inducible tet-off system
  - This method allowed us to target and inhibit the synthesis of specific proteins

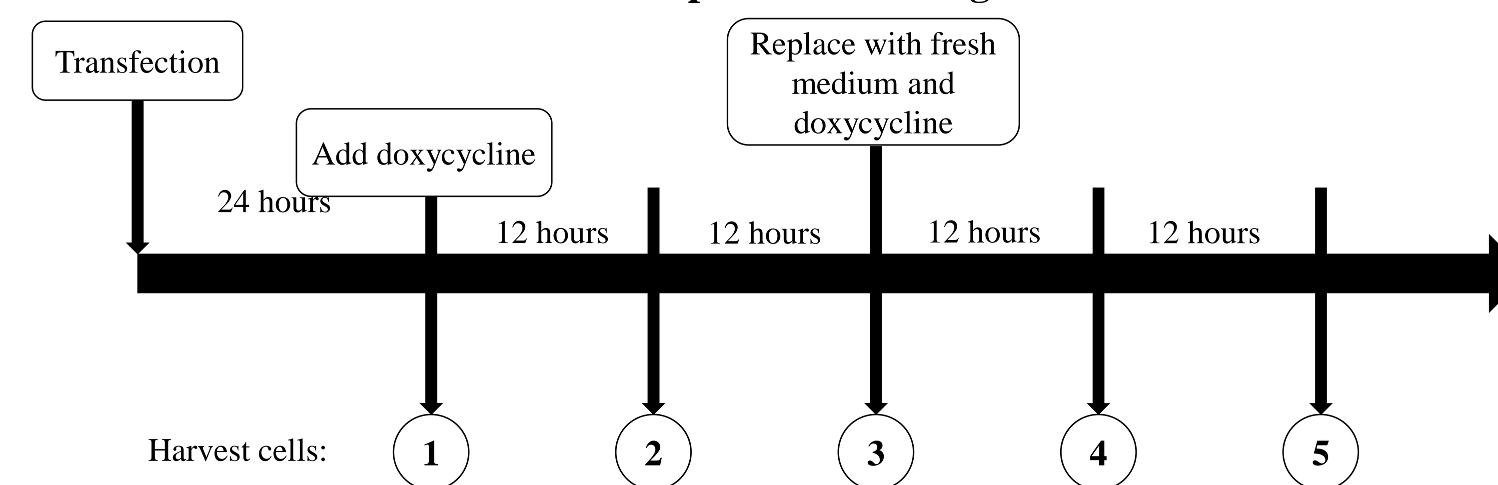
Hypothesis

- We hypothesize that overexpression of 14-3-3 proteins will result in a slower degradation of δ-catenin
- A potential cause for this could be that the abundance of 14-3-3 proteins leads to increased stabilization of δ-catenin

Methods

- Co-transfection of DNA plasmids expressing target proteins with EndoFectin reagant
  - Given that δ-catenin is only expressed in the brain, it was necessary to artificially express δ-catenin in the cultured HEK tsA 201 cells used for this study
  - pBI-δ-catenin and pCl-tTA plasmids were co-transfected to express δ-catenin in the absence of doxycycline
  - Plasmids encoding 14-3-3 ε/ζ were transfected to overexpress 14-3-3, while a pcDNA plasmid was transfected as their control
- Cell harvesting
  - Cells from each group were harvested sequentially every 12 hours to examine δ-catenin degradation over time
  - Cell pellets from various time intervals were stored in a -20°C freezer until all samples were ready for cell lysing
- Cell lysis
  - Cells were lysed to release the needed proteins and allow for analysis
- Western Blot
  - We chose the western blot technique to analyze the remaining δ-catenin in our samples
  - Anti-δ-catenin primary antibodies and goat anti-mouse IgG secondary antibodies were used for the immunoblotting
  - GAPDH antibodies were used as a loading control antibody to confirm protein loading was consistent throughout the gel
- Scanning
  - The membranes were analyzed with an infrared scanner

Experimental Design:



Results

A

pBI-δ-catenin	+	-	+	+
pCL-tTA	-	+	+	+
pcDNA	+	+	+	+
Doxycycline	-	-	-	+

IB: δ-catenin
IB: GAPDH

To validate the doxycycline inducible tet-off system, we transfected 4 groups of cells with different plasmid combinations. In the absence of doxycycline, δ-catenin is only expressed when both tet-responsive reporter plasmids expressing δ-catenin (pBI) and tetracycline-controlled trans-activator (tTA) are transfected. In the presence of doxycycline, δ-catenin expression is turned off.

B

Control / Doxycycline / Cycloheximide
Time (hrs): 0 12 24 36 48 / 0 12 24 36 48 / 0 12 24 36 48
IB: 14-3-3

In the tet-off system designed for this study, only the expression of δ-catenin is dependent upon the absence of doxycycline. On the other hand, cycloheximide, which was used in previous experiments, inhibits all protein synthesis. To examine the effect of doxycycline and cycloheximide on the expression of 14-3-3 proteins throughout the designed experimental timeline, we treated δ-catenin and 14-3-3 ε/ζ expressing cells with doxycycline or cycloheximide and harvested cells at five different time points after each treatment protocol. We found that 14-3-3 expression is unaffected in the doxycycline group within 48 hours after the treatment, while the level of 14-3-3 decreased following cycloheximide treatment.

C δ-catenin + pcDNA
D δ-catenin + ε + ζ
E δ-catenin + ε
F δ-catenin + ζ

Time (hrs): 0 12 24 36 48 (each panel)
IB: δ-catenin
IB: GAPDH

To evaluate the effect of different 14-3-3 isoforms on the degradation of δ-catenin, we co-transfected cells with δ-catenin and 14-3-3ε/ζ and turned off the expression of δ-catenin 24 hours post-transfection. As a control, an additional group of cells were transfected with δ-catenin and pcDNA to assess the degradation of δ-catenin without 14-3-3 overexpression. Compared with the control (C), overexpression of 14-3-3 ε and/or ζ reduces the rate of δ-catenin degradation (D-F).

Discussion

- The conflicting data in current literature about the relationship between 14-3-3 proteins and δ-catenin presents an intriguing debate that we sought to investigate further in this project
- While some studies reported that 14-3-3 deficiency resulted in increased levels of δ-catenin and depolymerization of F-actin (Toyo-oka *et al.*, 2014), others suggested that 14-3-3 stabilizes δ-catenin and a *decrease* of δ-catenin would be appreciated in 14-3-3 deficient models (He *et al.*, 2012)
- The preliminary results of our more comprehensive approach support that of *He et al.*
  - We found 14-3-3 overexpression led to slower δ-catenin degradation
- An interesting point is that these results oppose the data from mouse model experiments, which show 14-3-3 functional knockout (FKO) mice have increased δ-catenin levels and decreased dendritic spine density
- A limitation of this study is the use of kidney cells rather than neurons
  - Since δ-catenin is only expressed in the brain, we had to transfect genes to code for δ-catenin in our cell line
  - This means that our results may not accurately translate to neurons or animal models
- In future studies, we may examine how 14-3-3 *inhibition* affects the degradation of δ-catenin
- We may also investigate if the direct binding of 14-3-3 to δ-catenin is necessary to alter δ-catenin degradation
  - This would be done by using this same time course design model with a mutant form of δ-catenin that does not bind to 14-3-3

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