The affects on spetin7 expression in multiple cell culture techniques

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ABSTRACT

Septins are primarily known for acting as scaffolding molecules that recruit proteins and form the ring-like structures necessary for budding during cell division, and may act in a similar way to regulate the structure of dendritic spine necks. This study focuses on testing septin7 expression in HEK (Human Embryonic Kidney) 293A cells by overexpressing eGFP-septin7 and performing a Western Blot with septin7 antibody to analyze protein. The methods used during this study were plating HEK293A cells until the confluency was at 50% to continue with the transfection eGFP-septin7. After transfection, the lysing process is started in order for the proteins to stabilize so they can migrate through the gel to continue on with the next step of gel electrophoresis. Once finished with the lysis step, gel electrophoresis is used to achieve the septin protein pattern within the HEK293A cells. Finally, the Western Blot technique is used in order to achieve an exact replica of the gel's protein pattern within the process of being stained with antibodies. In addition, we co-transfected cultured hippocampal neuronal slices with dsRed and septin7 tagged with GFP. To test the role of septins within the spine neck we over-expressed GFP-tagged septin7 in cultured hippocampal neuronal slices. I hypothesis this study will contribute to a better understanding of how dendritic spines are dynamically regulated to manipulate signaling between the spine head and dendritic branch.

Background

There are fourteen known septin proteins found in mammals, however, our project focused exclusively on septin7. Septins are primarily known for acting as scaffolding molecules that recruit proteins and form the ring-like structures necessary for budding during cell division, and may act in a similar way to regulate the structure of dendritic spine necks. Septins have been shown to be robustly expressed in dendritic spines of the hippocampus and to form complexes with each other.

A dendritic spine is a small membranous protrusion that encompasses an excitatory synapse and is separated from the parent dendrite by a thin spine neck. The spine neck is essential for regulating the movement of molecules between the spine and dendrite and for creating a specialized electrical signaling domain within the spine head. Neuronal activity can regulate the physical dimensions of the spine, which can profoundly affect the way in which the spine contributes to signal processing within the neuron. However, the molecules that underlie this process remain elusive.

Figure 1: Why Septin?

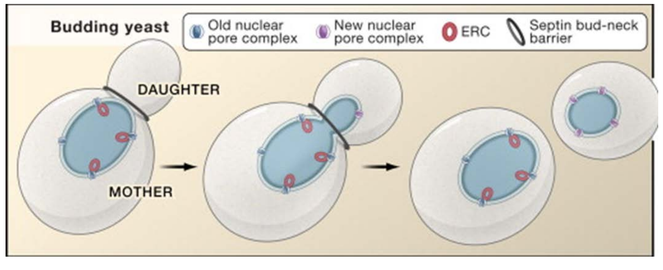

Figure 1. Septins have been shown to act as scaffolding molecules that recruit proteins and form the ring-like structure important for budding during cell division. Thus, it is possible for septins to act in a similar manner to regulate the cytoarchitecture of dendritic spine necks (Macara et. al., 2008).

Methods

The methods used during this study was plating HEK293A cells until the confluency was at 50% to continue with the transfection.

Proceeding with transfecting, after waiting 24 hours, HEK293A cells with eGFP-septin7 using the process of Calcium phosphate and PEI solution to find the most useful transfection in terms of toxicity. Cells were co-transfected with eGFP-septin7 and dsRED, dsRED allows overlap visualization of the cellular expression. The transfection efficiency is controlled by also transfecting cells with normal GFP.

Cell lysis occurs when GFP is visualized under the microscope, want to make sure cell confluency doesn't get too high. If the cells become overgrown they start to die. Lysis is the process of breaking down a cells wall and compromising its integrity. This is done to remove the protein of interest for further analysis.

Gel Electrophoresis was used to achieve the septin protein pattern within the HEK293A cells.

The Western Blot technique was used to achieve the replica protein pattern from the gel.

Hippocampal slice cultures were prepared from postnatal day 6-8 using mouse pups of both sexes.

Cultured neurons were biolistically transfected with dsRed and septin7 tagged with GFP or dsRed and pSUPER two days in vitro .

dsRed and GFP were imaged using a resonance scanner one week in vitro.

Results

Figure 2: Cell membrane protein copy of Gels

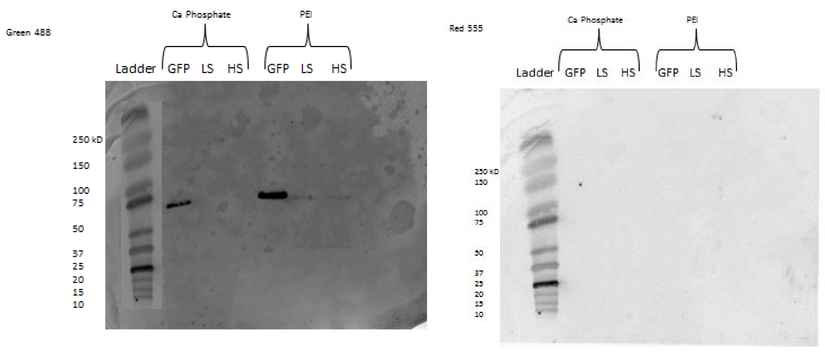

Figure 2. In the images above they are representing the copy of the gel that was ran with the protein pattern of the HEK293 cells co-transfected with dsRed and septin7.

Figure 3: Imaging dsRed and GFP

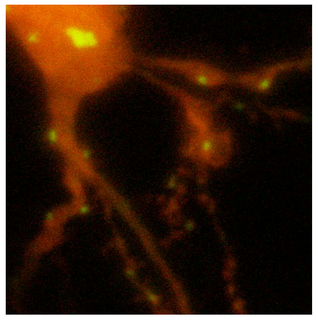

Figure 3. A pyramidal neuron was co-transfected with dsRed and septin7 tagged with GFP. The septin7 puncta are shown in green.

Conclusion

What we did:

Primary antibodies added:

Chicken anti-GFP

Rabbit anti-septin7

Secondary antibodies added:

Goat anti-chicken 488 (to recognize primary antibody for GFP)

Goat anti-rabbit 488 (to recognize septin7-GFP)

This should not have been added!

Donkey anti-rabbit 555 (to recognize septin7-GFP)

Western (488):

GFP worked, band for both transfections (CaP and PEI), bands are both relatively about 60-70 kDa which corresponds to GFP (28 kDa) and dsRED (28 kDa). Band for PEI is thicker meaning the transfection efficiency was greater. Band for PEI GFP looks slightly above CaP because the gel transferred a horizontal slant, however, not a significant difference so you can conclude that the two GFP bands are the same size.

Septin seems that it might have worked because there are two faint bands for low septin and high septin in the PEI transfection. However, the bands are not in the predicated spot. The band should be around 100 kDa because Septin-GFP fused protein is ~75kDa and dsRED is ~28 kDa and the cells were co-transfected with septin-GFP and dsRED. It is possible that the normal GFP antibody picked up the GFP tagged to septin which would explain the faint signal. In addition, 2 secondary antibodies were added to target septin7, one had an emission of 488 and the other was 555 but had the same species which could mean they likely were competing with each other which could also explain a faint signal. There is no signal for septin7-GFP in the CaP transfection.

Western (555)

As expected there is no signal for GFP in both transfections because the antibody for GFP has an emission of 488 not 555.

Septin7 antibody did not work for either transfection because if the primary septin7 antibody worked the secondary antibody should have picked it up and there is no signal. This could be a fault of the septin7 antibody that was purchased from Novas or it could be an issue of competing secondary antibodies.

What's next?

Only use two secondary antibodies with different emissions (488 and 555).

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