The affects on septin7 expression in multiple cell culture techniques

J. E. Engel and Bloodgood BL
Division of Biological Sciences, University of California, San Diego

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 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 septin7 within the spine neck we over-expressed GFP-tagged septin7 in cultured hippocampal neuronal slices. I hypothesize 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 projection 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 head and dendritic branch.

Results

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.

**Figure 2:** Cell membrane protein copy of Gels

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 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 gel transferred a horizontal slant, however, not a significant difference so you can conclude that the two GFP bands are the same size.

**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.

**Figure 4:** Why Septin?

**Conclusion**

What's next?

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

ACKNOWLEDGMENTS

We would like to thank Kelly Martynuik for her assistance during the conduction of this study. We would also like to thank Beatriz Cruz-Moreno for her imaging help.