Co-localization of CHL1 and ROBO1 During Embryonic Development in Mouse Brains

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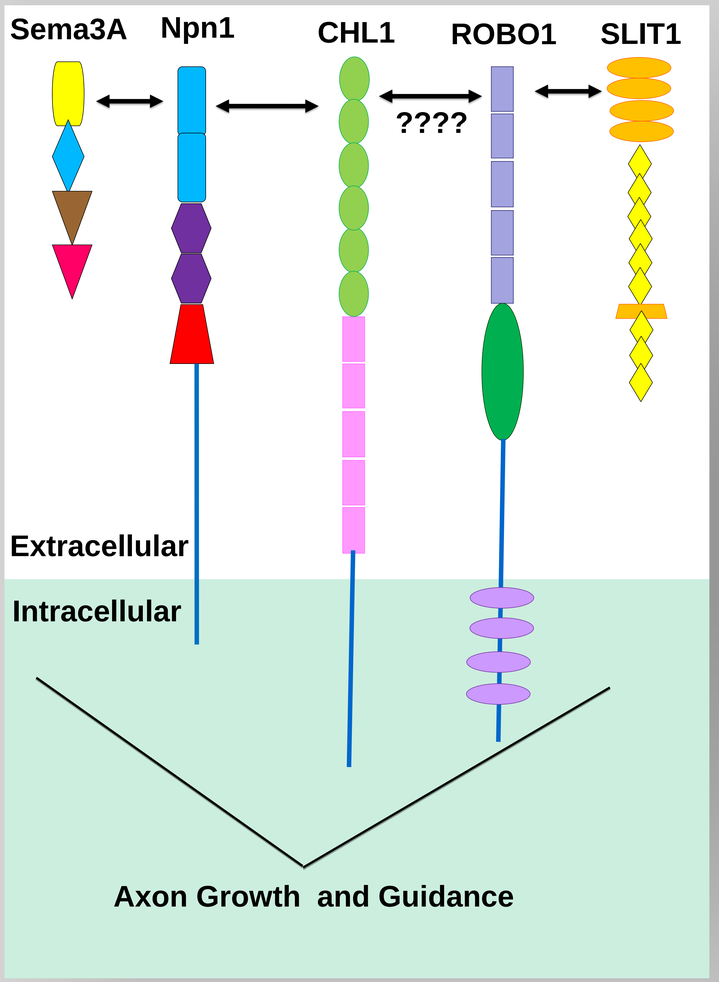
Abstract

Axons are an integral part of a neuron responsible for transmitting electrical signals within the brain. It is important to understand the mechanics of axon growth, including: how growth cones function and which proteins are involved in axon migration and how they co-exist with other proteins. This hierarchy of information is essential to combat certain types of cancers and neurological disorders such as Alzheimer's disease, a common form of dementia which involves the progressive deterioration of brain cells leading to loss in cognitive abilities. We currently know of several families of proteins that play major roles in regulating axon guidance. Two such families are neural cell adhesion molecules (CAMs) of the immunoglobulin superfamily (Maness and Schachner, 2007) and the family of SLIT proteins (Andrews, *et al.,* 2007). Close Homolog of L1 (CHL1), a CAM, is part of the mammalian L1 family, which is known to play a role in axon growth and migration of developing neurons (Wright, *et al.,* 2007). Roundabout1 (ROBO1), also a member of the immunoglobulin super family, plays a role in axon guidance in the developing forebrain by serving as a receptor for the guidance cue, SLIT1 (Andrews, *et al.,* 2007). Our objective in this study is to investigate a potential interaction between CHL1 and ROBO1. We hypothesize that these two proteins function together to regulate SLIT-mediated axon growth in developing neurons. The first step in our investigation involved the use of co-immunofluorescence staining to visualize CHL1 and ROBO1 colocalization. We demonstrate here that CHL1 and ROBO1 do colocalize in the intermediate zone of the cerebral cortex during axonal outgrowth (E15) and axonal targeting (E16). This colocalization suggests an interaction between these two proteins that may help promote axon growth and guidance.



Methods

COS-7 cells were cotransfected with pcDNA3-CHL1 and pcDNA-ROBO1 plasmids. To cap CHL1, cells were incubated with anti-CHL1 goat polyclonal antibody (40 µg/ml). The cells were resuspended in DMEM containing 10 µg/ml of mouse anti-goat IgG to cluster CHL1 and incubated for 20 min on ice. Cells were incubated for 1 h at 37°C and then fixed with 4% PFA for 15 min. To label ROBO1, cells were incubated with anti-ROBO1 rabbit polyclonal antibody (20 µg/ml). ROBO1 and CHL1 were detected by incubating the cells with conjugated secondary antibodies.
Cryopreserved E16 wild-type mouse brains were permeabilized with 1% NP-40 in 1X PBS for 3 min (3X) , then washed in PBS. Sections were blocked overnight in 5% normal goat serum in 1X PBS. The next day, sections were incubated for 1 h at RT with goat polyclonal anti-CHL1 (R&D Systems Inc ; 75µl) and rabbit polyclonal anti-Robo1 (Abcam, 34µl). Sections were incubated with conjugated secondary antibodies for 45 min. Sections were mounted with Fluorashield containing DAPI. Fluorescent microscopy was performed using an Advanced Microscopy Group (EVOS) digital microscope.

Results

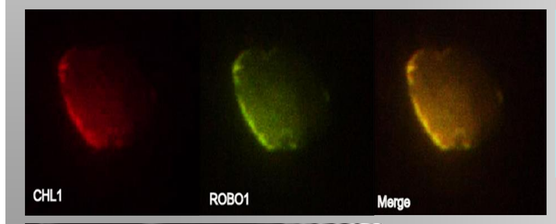


Figure 1: CHL1 capping (red) was induced by cross-linking with CHL1 antibody and detected with a TRITC-labeled secondary antibody. ROBO1protein (green) labeled with ROBO1 antibody and a FITC-labeled secondary antibody was recruited to CHL1 caps. Merged images show co-localization of CHL1 and ROBO1 (yellow).

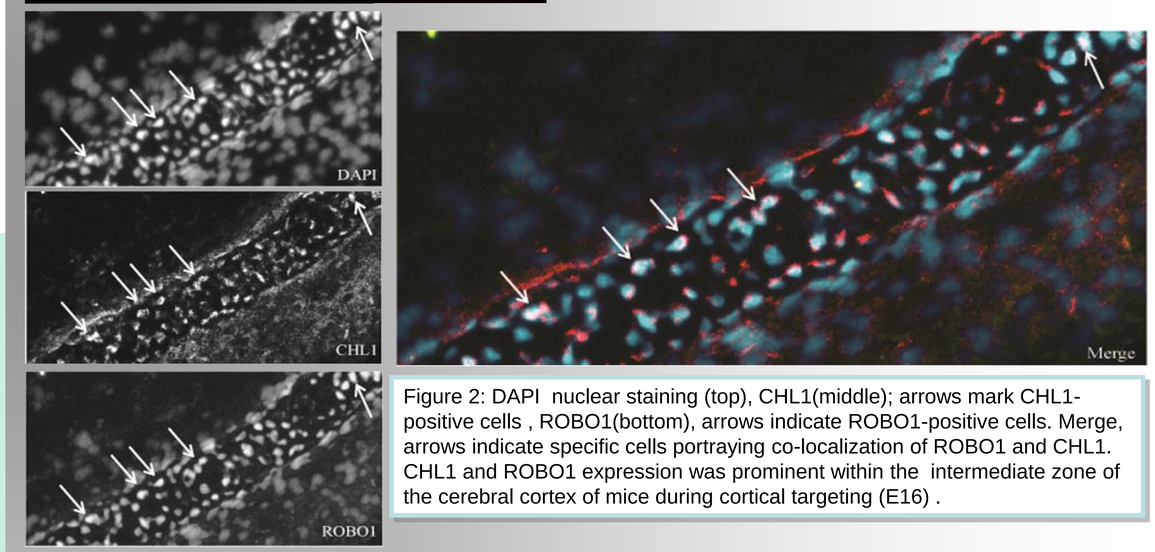


Figure 2: DAPI nuclear staining (top), CHL1(middle); arrows mark CHL1-positive cells , ROBO1(bottom), arrows indicate ROBO1-positive cells. Merge, arrows indicate specific cells portraying co-localization of ROBO1 and CHL1. CHL1 and ROBO1 expression was prominent within the intermediate zone of the cerebral cortex of mice during cortical targeting (E16) .

Conclusion

The results of this study demonstrate a potential cooperation between CHL1 and ROBO1 to control axon targeting. An interaction was demonstrated *in vitro* using co-capping experiments and co-localization was observed during the time of axonal cortical targeting (E16). Other studies (Andrews, et al, 2007 and Wright, et al, 2007) indicate a separate role for both ROBO1 and CHL1 in axon growth, thus a link between the two proteins is possible. To further investigate this interaction, the binding will be confirmed using co-immunoprecipitation.

References

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