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Comparison of Wild-Type versus Mutant L1CAM Expression in Cultured Neurons

David R. Cannata
Sacred Heart University

Eva Murphy
Sacred Heart University

Cassandra Lew
Sacred Heart University

Samantha Pignatelli
Sacred Heart University

Comparison of Wild-Type versus Mutant L1CAM Expression in Cultured Neurons

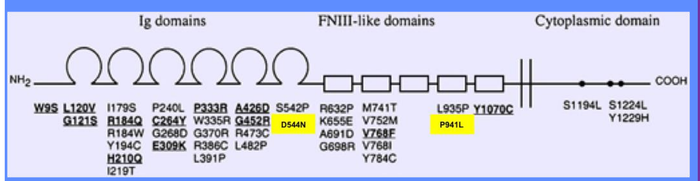
D. Ryan Cannata, Eva Murphy, Cassandra Lew, Samantha K. Pignatelli,
and Mark Jareb; Department of Biology, Sacred Heart University

Abstract

The correct targeting of proteins to axons and dendrites of neurons is essential for the proper development of the nervous system. L1CAM is an axonally-targeted protein responsible for multiple aspects of neuronal development. L1CAM mutations are known to result in a developmental syndrome characterized by cognitive and motor disabilities. We investigated the cellular distribution of known L1CAM mutant proteins, P941L and D544N, in cultured embryonic chick forebrain neurons to test the hypothesis that aberrant protein targeting of these mutants plays a role in the developmental abnormalities associated with the syndrome. Preliminary data suggests that the P941L L1CAM mutant is targeted normally to the axon suggesting that downstream signaling events are abnormal. In contrast, the D544N L1CAM mutant does not appear to reach the cell surface of the neuron.

Introduction

L1 syndrome is caused by mutations in the L1CAM protein. The syndrome affects 1 in every 30,000 newborn human males, characterized by hydrocephalus and seizures in severe cases, as well as lower-limb muscle spasticity and adducted thumbs. L1CAM is an axonal protein involved in axon-pathfinding during neural development, myelination of axons, cell migration, and transmembrane signaling at tyrosine kinase receptors. The ectodomain of L1CAM is composed of six immunoglobulin-like and five fibronectin(III)-like repeats, with a single transmembrane helical segment and short intracellular C-terminus (Haspel and Grumet, 2003). The severity of L1 syndrome varies with different mutations. The D544N mutation is localized in the sixth immunoglobulin domain and has been shown to affect homophilic binding when expressed in non-neural cells (De Angelis, et al. 2002). The P941L mutation is localized in the fourth fibronectin domain and when expressed in PC12 cells affects cell migration and neurite outgrowth (Tagliavacca et al., 2012). The current study investigates the cellular trafficking and subsequent axonal targeting of mutant human L1CAM constructs of D544N and P941L.



Schafer, H; and Muerkoster, S.S. (2008).

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Materials And Methods

Culture: Chick forebrain neurons were cultured from incubated fertilized eggs between 8-9 days of development. Forebrains were dissected, treated with trypsin, and dissociated into single cells by trituration in HBSS. The cells were plated on acid-washed coverslips treated with poly-L-lysine (1 mg/ml) in Neurobasal media with B27.

Transfection: P941L or D544N human L1CAM construct (Addgene) were co-transfected with mRuby via Nucleofection (Amaxa). 2 µg of each DNA construct were mixed with 100 µl of Nucleofector solution containing $4x10^6$ forebrain cells. The solution was electroporated using the Nucleofector II and subsequently plated on poly-lysine treated coverslips.

Immunocytochemistry: Transfected cultures were incubated with anti-L1 antibodies (5G3; Novus; 1:200) live for 2-5 minutes at 37°C and then fixed. To visualize internal staining some cultures were incubated for 1 hour at 37°C after fixation. Endogenous cell-surface NgCAM was labeled using 8D9 (DSHB) antibodies (1:2) live for 2-5 minutes at 37°C and then fixed. After primary antibody labeling all cultures were then incubated sequentially with FITC-donkey-anti-mouse secondary antibodies (1:400; Jackson), mouse anti-FITC (1:200; Sigma) and a Dylight488-donkey-anti-mouse secondary (1:400; Jackson) and visualized.

Results

Figure 1. Micrographs of example neuron cultured among glial cells illustrating live antibody labeling of endogenous NgCAM. **(A)** Phase contrast. **(B)** Live antibody labeling of NgCAM. Neurons exhibit polarized expression of NgCAM to their axons. Faint labeling of dendrites at background fluorescence levels are observed. (bar=25 µm)

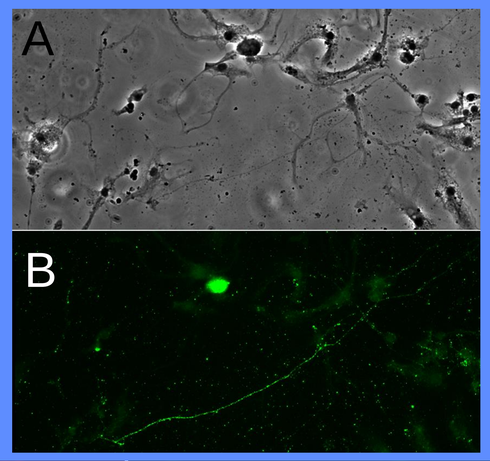


Figure 2. Micrographs of neuron co-transfected with mRuby and P941L L1CAM mutant **(A)** Phase contrast. **(B)** mRuby. **(C)** P941L mutant. Live L1CAM antibody labeling is observed in the axon, branches of which loop around the cell body and some presumptive dendrites. Arrows indicate a presumptive dendrite visualized with mRuby that is not labeled by the L1CAM antibody.

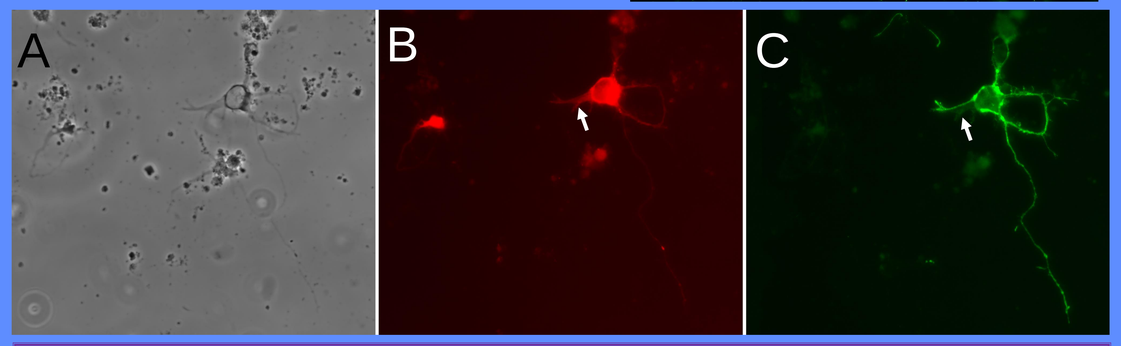
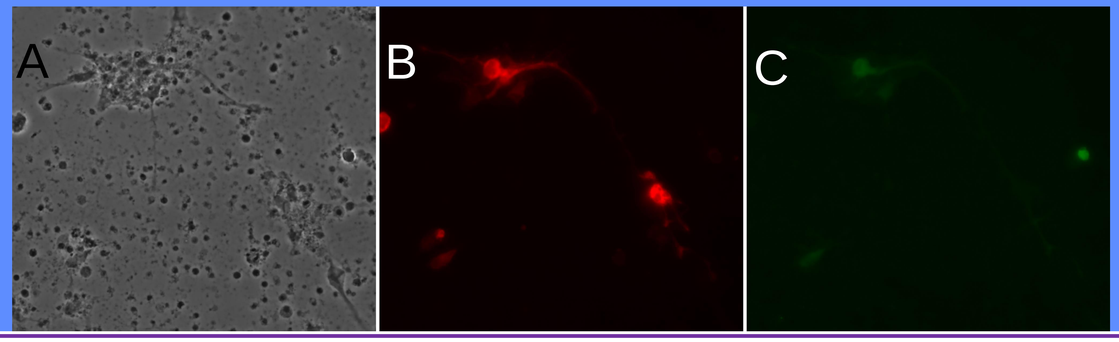


Figure 3: Micrographs of neuron co-transfected with mRuby and D544N L1CAM mutant **(A)** Phase contrast. **(B)** mRuby **(C)** D544N. L1CAM antibody labeling post-fixing is observed in the cell body. Live labeling of D544N neurons do not exhibit any appreciable staining suggesting that D544N never reaches the cell surface (not shown).



Discussion

L1CAM is an axonally-targeted protein responsible for multiple aspects of neuronal development. The current study examined the cellular distribution of two L1CAM mutants, D544N and P941L, in cultured chick embryonic forebrain neurons. We hypothesized that aberrant protein targeting of these mutants plays a role in the abnormal developmental manifestations of L1 syndrome, such as cognitive and motor disabilities.

Our data suggest that the P941L L1CAM mutant is targeted normally to the axon suggesting that downstream signaling events are abnormal. Taggliavacca et al. (2012) reports that P941L when transfected in PC12 cells reaches the cell surface but these cells exhibit delays in cell migration and decreased neurite amount and length compared to wild-type L1CAM. We hypothesize that the P941L mutant exhibits normal cellular trafficking and localization but the mutation prevents its interaction with some relevant protein thereby altering L1-mediated signaling.

D544 is a highly conserved surface site among L1 orthologues (De Angelis, 2002). Current literature suggests that the D544N mutant reduces homophilic L1:L1 binding. However, our data suggests that the D544N mutant does not reach the cell surface of the neuron. No immunofluorescence was observed in embryonic chick forebrain neurons transfected with the D544N L1CAM mutant when labeled live with antibody. In contrast, when primary antibody labeling was done on fixed neurons some faint labeling was observed in the cell body (Figure 3). We hypothesize that this mutation causes L1CAM to become trapped inside the endoplasmic reticulum, and therefore cannot be trafficked to the plasma membrane of the neuron.

Conclusions & Future Directions

The current study suggests differences in cell-surface expression among D544N and P941L L1CAM mutants. Live immunostaining of the L1CAM primary antibody showed presence of the P941L mutant at the cell surface as well as axonal localization, but D544N did not appear to reach the cell surface. Future research will investigate cell-signaling and protein-protein interactions in these mutants, as well as examine possible ER localization of D544N.

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