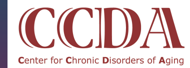
IMP2 Expression In The Mouse Nervous System



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Abstract

Insulin-like growth factor-II (IGF-II) mRNA-binding protein-2 (IMP2) is one of the three homologs (IMP1-3) that play critical roles in the posttranscriptional regulation of gene expression in several tissues. Recent studies point out important roles of ZBP1 (zipcode binding protein; a.k.a. IMP 1) in axon guidance and regeneration by regulating the localization and local translation of specific mRNAs. However, the function of IMP2 is least understood, largely because an isoform-specific antibody is not available, which makes the conventional techniques to locate protein expression unfeasible. We custom made an IMP2-specific antibody. We used Western blot and immunocytochemistry to test its specificity on the cultured cells following overexpression of IMP 1-3 isoforms, respectively. Using this IMP2-specific antibody, we examined IMP2 expression in the mouse nervous system. We found that IMP2 expresses extensively in the neuronal soma and processes on both the cortical and dorsal root ganglion neuron cell culture. Its expression in the nervous system is sustained postnatally, unlike that of IMP1 and IMP3. Ongoing experiments are aimed at further understanding IMP2 expression patterns during injury and assessment of its role to facilitate mRNA localization during axon regeneration in the adult nervous system.

Introduction

Spinal cord and nerve injures often result in axon degeneration, which leads to many functional deficits. So far the approaches to enhance axon regeneration are limited. Recent i*n vitro* studies demonstrate that mRNA localization and local protein synthesis are crucial to axon growth (Harris et al., 1987; Campbell and Holt, 2001; Yao et al., 2006), and injury to the peripheral nerves induces robust localized mRNAs and proteins locally synthesized in axons (Twiss et al., 2000). The process of local protein synthesis consists of transport of mRNAs in associated with their binding proteins from the cell body to axon terminals. IMP family proteins include IMP1-3 and their alternative splice variants. ZBP1 localizing ß actin mRNA and regulating its local translation requires the molecular mechanism of sequence-specific recognition and binding to the "zipcode" in the 3' untranslated region of ß actin mRNA (Zhang et al., 2001; Kindler et al., 2005; Kiebler and Bassell, 2006). More recent *in vivo* studies further indicate involvement of ZBP1 in facilitating axon regeneration by transport multiple mRNA cargos into the axons (Donnelly et al., 2011; Willis et al., 2011). IMP family proteins have conserved two RNA-recognition motifs (RRMs) and four K-homology (KH) domains, which suggest different isoforms might target similar mRNAs (Nielsen et al., 2001; Yaniv and Yisraeli, 2002). Furthermore, our pilot study implicates that IMP2 could also enhance axon regeneration.

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Results

Materials & Methods

Dissociated Dorsal Root Ganglion (DRG) Neuronal Culture

The mice were euthanized and the DRG were removed. They were then incubated in collagenase for 20 minutes before they were dissociated. They were plated on the pre-coated coverslips in DMEM/F12 with glutamine Media (Invitrogen).

N2A cell transfection

The N2A cells were grown to 90% confluence in 12-well plates before they were transiently tranfected with plasmids: GFP or GFP-IMP1, -IMP2, -IMP3, -HCC, respectively. Lipofectamin® 2000 transfection reagent was used according to manufacture's instruction (Invitrogen) . Twenty-four hours post-transfection, the cells were harvested for Western blot analysis.

Crush Model

The sciatic nerve in the posterior of the leg was clamped to crush for 30 seconds. Five days after the surgery, the mouse was euthanized and perfused with 4% paraformaldehyde. The sciatic nerve was dissected and frozen in tissue-tek. They were sectioned using a cryostat.

Western Blot

Equal amount of protein samples were resolved on 10% SDS-Polyarcylamide gels and transferred onto a PVDF membrane. The membrane was probed with antibodies against IMP1 and IMP3 (1:500, from Drs. Yukio Sasaki and Gary Bassell at Emory University School of Medicine), IMP2 (1:500), GFP (1:1000; Clontech) and GAPDH (1:2000; Ambion) followed by horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies (1:3000; Amersham). SuperSignal West Pico Chemiluminescent substrate was used for detection of immunoreactivity on the membrane, which was then exposed to X-ray films (Kodak).

Immunostaining

Immunostaining was carried out on both the tissue sections and the cultured neurons. They were rinsed in phosphate buffered saline (PBS) and blocked in 2% bovine serum albumin in PBS with 0.1% Triton-X100 before they were incubated in a primary antibody. After incubation in fluorochrome-tagged secondary antibody, they were rinsed stringently and mounted. The following antibodies were used: rabbit anti-IMP2 (1:100 for tissue sections; 1:400 for cells), polyclonal rabbit MAP2 (1:100; Chemicon), monoclonal mouse anti-MAP2 (1:500; Sigma), monoclonal mouse anti-tau (1:100; Sigma), goat anti-rabbit Alexa Fluor® 546 (1:200 for tissue sections; 1:1000 for cells; Invitrogen), donkey anti-mouse Alexa Fluor® 488 (1:1000; Jackson ImmunoResearch).

Conclusion

We have custom made IMP2-specific antibody. This antibody specifically recognizes IMP2 and its variant, but not IMP1 or IMP3. Using this antibody, we found extensive IMP2 expression in the nervous systems, which is, sustained postnatlly. This study has laid groundwork for us to investigate the functional role of IMP2 in axon regeneration in the future. We expect that research along this direction could open up new therapeutic avenues to improve neurological recovery after spinal cord and nerve injuries.