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Research Report

Expression and function of myelin-associated proteins and their common receptor NgR on oligodendrocyte progenitor cells

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ABSTRACT

Nogo-A, oligodendrocyte myelin glycoprotein (OMgp) and myelin-associated glycoprotein (MAG) are known as myelin-associated proteins that inhibit axon growth by binding a common receptor, the Nogo66 receptor (NgR). In the CNS, Nogo-A, OMgp and MAG are predominantly expressed by oligodendrocytes. As our previous study revealed that oligodendrocyte progenitor cells (OPCs) did not inhibit neurite outgrowth, it is not clear whether these myelin-associated proteins are expressed in OPCs, and what functions they perform if they are expressed in OPCs. In the present study, with OPCs induced from neural precursor cells (NPCs) derived from rat embryonic spinal cord, and oligodendrocytes differentiated from OPCs, we have observed the expression patterns of Nogo-A, OMgp, MAG and NgR in NPCs, OPCs and oligodendrocytes by immunostaining and western blot assay. We found that Nogo-A could be detected in all tested cells; OMgp could be detected in OPCs and oligodendrocytes, but not in NPCs; MAG was only detected in oligodendrocytes; while NgR could be detected in NPCs and OPCs, but not in oligodendrocytes. These results indicated that the expression pattern of MAG and NgR in OPCs was totally different from that of oligodendrocytes, which might be one of the factors that led to the discrepancy between the two cells in promoting neurite outgrowth. By respectively blocking Nogo-A, OMgp and NgR expressed on OPCs with their corresponding antibodies, we further investigated their roles in the proliferation and differentiation of OPCs, as well as the possible signal pathways involved in. Our results showed that when OPCs were cultured under proliferation condition, blocking Nogo-A, OMgp or NgR did not affect the proliferation of OPCs, but could all significantly prolong their processes. And this effect on OPC processes might involve the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. When OPCs were cultured under differentiation condition (containing triiodothyronine, T3), blocking Nogo-A, OMgp or NgR could all inhibit the differentiation of OPCs, and this effect might involve the extracellular signal-regulated kinases1/2 (Erk1/2) signaling pathway. These results suggested that under proliferation environment, the functions of Nogo-A, OMgp and NgR expressed in OPCs might be to control the length of processes, thus maintaining the morphology of OPCs. While in differentiation environment, the functions of Nogo-A, OMgp and NgR expressed in OPCs turned to promote the

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differentiation of OPCs, thus facilitating the maturation of oligodendrocytes. And NgR, as the common receptor for Nogo-A and OMgp, might be the main molecule that mediated these functions in OPCs.

1. Introduction

The inability of axonal regeneration after central nervous system (CNS) injury is caused by many factors. One of the main obstacles is the myelin-associated inhibitors, including Nogo-A (Chen et al., 2000; GrandPre et al., 2000; Prinjha et al., 2000), myelin-associated glycoprotein (MAG) (McKerracher et al., 1994; Mukhopadhyay et al., 1994) and oligodendrocyte myelin glycoprotein (OMgp) (Kottis et al., 2002; Wang et al., 2002). These inhibitors could highly restrict the neurite outgrowth in vitro (Schwab and Caroni, 1988) and axon regeneration after injury in vivo (Schnell and Schwab, 1990). Among them, Nogo-A has been more thoroughly studied. It has two functional domains contributing to the inhibition of neurite growth: one is Amino-Nogo (Prinjha et al., 2000) and another is a short 66-amino acid (aa) residue called Nogo-66 (GrandPre et al., 2000). Amino-Nogo is located on the cytoplasmic side of cell membrane, and may work as an inhibitor when there is disruption of myelin, while Nogo-66 is located on cell surface, between two hydrophobic domains. Nogo-66 can inhibit neurite growth through binding its receptor NgR, a GPI protein predominantly expressed by neurons (Fournier et al., 2001). And a leucine-rich repeat domain of NgR is the key for the binding of Nogo-66. Interestingly, another two inhibitors, OMgp and MAG, share the same receptor of Nogo-A (Domeniconi et al., 2002; Fournier et al., 2001; Liu et al., 2002). Besides the inhibitory effect on neurite outgrowth, some of these myelin-associated proteins may also involve in the development of CNS. It was reported that during cortical development, Nogo-A was expressed on migrating and postmigratory neurons and radial glial cells, which regulated the radial migration of cortical neuronal precursor cells (Mathis et al., 2010). In addition, OMgp expressed in oligodendrocytes was found to play roles in regulating nodal formation and myelination (Apostolski et al., 1994; Huang et al., 2005; Nie et al., 2006). Similar situation also existed in their common receptor NgR. It has been reported that NgR in embryonic cells participated in the regulation of homeoprotein Nanog expression. Activation of NgR increased the expression levels of Nanog mRNA and protein, which inhibited the differentiation of embryoid bodies (Gao et al., 2010).

In the CNS, as myelin-associated inhibitors, Nogo-A, MAG and OMgp are predominantly expressed by oligodendrocytes (Chen et al., 2000; Trapp, 1990; Wang et al., 2002). During development, oligodendrocytes arose from OPCs (Vallstedt et al., 2005). According to previous reports, in the mouse spinal cord, OPCs emerged on embryonic day 12.5 (E12.5) from a ventral region, then migrated out and proliferated to spread into the entire spinal cord (Cai et al., 2001; Pringle and Richardson, 1993; Pringle et al., 1996). While the growth of corticospinal tract (CST) into the spinal cord occurred postnatally, and the leading CST axons reached the 8th cervical segment at postnatal day 2 (PD2) and the 5th lumbar segment at PD9 (Gianino et al., 1999). It indicated that the generation, migration and distribution of OPCs were earlier than the growth of CST; hence OPCs should have no inhibition on the axonal growth in vivo. With a dorsal root ganglion (DRG) and OPC/oligodendrocytes co-culture model, we found that OPCs were significantly more permissive to neurite outgrowth than mature oligodendrocytes (Ma et al., 2009). And with a spinal cord explant / OPCs co-culture model, we also observed that OPCs could contact neurites closely and did not show any inhibition on neurite outgrowth (Chen et al., 2010). These experiment evidences hinted that the function of OPCs was quite different from that of mature oligodendrocytes, especially on the inhibition of neurite growth. In this case, we wondered whether OPCs expressed myelin-associated inhibitors, and if these myelin proteins were expressed in OPCs, what functions they performed. In the present study, with OPCs induced from neural precursor cells (NPCs) derived from rat embryonic spinal cord, and oligodendrocytes differentiated from OPCs, we have systemically observed the expression patterns of Nogo-A, OMgp, MAG and NgR in NPCs, OPCs and oligodendrocytes. And by blocking Nogo-A, OMgp and NgR expressed on OPCs with their corresponding antibodies, we also investigated their roles in the proliferation and differentiation of OPCs, as well as the possible signal pathways involved in.

2. Results

2.1. *Induction and differentiation of OPCs*

Cells dissociated from E14.5 rat embryonic spinal cords were plated in serum-free N2/B27 medium supplemented with EGF and bFGF. Most cells died under such condition, and a subpopulation of cells proliferated and formed clusters which grew into floating neurospheres (Fig. 1A). Immunofluorescence staining showed that the cells in neurosphere were all nestin$^+$ NPCs (Figs. 1B–D).

To induce OPCs from NPCs, basal-NPC-medium was substituted by OPC medium gradually (as described in materials and methods). In OPC medium, neurospheres began to attach to the bottom of the flask, and single cells moved out of the spheres. After removing the necrotic spheres and fragmented cells, the remaining cells that adhered to the bottom of the flask displayed typical bipolar or tripolar morphology of OPCs (Fig. 1E). Immunostaining further confirmed that these cells expressed both PDGFR and A2B5, the specific markers of OPCs (Figs. 1F–H).

To identify the differentiation potential of OPCs, cells were seeded onto poly-L-lysine-coated coverslips in the medium containing T3 (30 μM) or 10% fetal bovine serum (FBS) without PDGF and bFGF, and cultured for 2–10 days respectively. In the presence of T3, after 2 days of differentiation