THE POSSIBLE ROLE OF SMALL GTP BINDING PROTEIN RHO IN NUERO-GLIAL CELL DIFFENTIATION

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Rho is a smal GTP-binding protein to regulate actin cytoskeleton. Rho is known to be present abundantly in the brain, but its role in neural differentiation has been poorly understood. Activation of Rho induces neurite retraction and cell rounding in neuroblastoma cells cultured *in vitro*. In contrast, inactivation of Rho by a bacterial ADP-ribosyltransferase (C3) induces neurite extension and increase of differentiation marker enzyme activity such as acetylcholine esterase (AChE) in PC12 cells, an adrenergic cell line.

To clarify the involvement of Rho in other neuronal cell line and glial cell line, in this study, I first examined the effects of C3 on a cholinergic cell line (NG108-15) and a glioma (C6) for their differentiation. Treatment of NG108-15 cells and C6 glioma cells with C3 for 3 days induced neurite or process formation. Treatment with C3 also increased the activities of marker enzyme for differentiation, AChE and choline acetyltransferase (ChAT) in NG108-15 cells, and 2', 3'-cyclic nucleotide 3'-phosphohydrolase (CNP) in C6 cells. Prior treatment with H-89, an inhibitor of protein kinase A, abolished C3-induced increases of marker enzyme activities in NG108-15 cells. These results suggest that inactivation of Rho induces neuronal and glial differentiation, as well as adrenergic cell, and that protein kinase A is related to the C3-induced enzyme induction.

The above mentioned *in vitro* studies using cultured neural cells suggest a pivotal role of Rho in the regulation of neuro-glial cell differentiation. To evaluate possible role of Rho in postnatal development of the brain, changes in the Rho Protein and Rho-related proteins levels in the cytosol and membrane fractions of mice brain in 0-2 weeks after birth were examined. The activities of the marker enzymes for neuronal and glial cells were also measured to confirm neuro-glial development of the brain. Both AChE and ChAT activities of whole brain homogenate were progressively increased during the postnatal period. CNP activity increased markedly between one and two weeks after birth. In contrast, the amounts of RhoA and RhoB in the membrane fraction, which are considered to be in an active state, were highest in new born mouse brain and decreased during the postnatal period. The amount of Rho GDP dissociation inhibitor, a regulatory protein for Rho, was unchanged, while those of Rho target proteins, Rock-2 and citron, were gradually increased. All these results suggest a possible involvement of Rho protein in neuro-glial cell differentiation during postnatal development of the brain *in vivo*.

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