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Research Report

Expression of netrin-1 and its receptors DCC and neogenin in rat brain after ischemia

*Atsushi Tsuchiya, Takeshi Hayashi, Kentaro Deguchi, Yoshihide Sehara, Toru Yamashita, HanZhe Zhang, Violeta Lukic, Makiko Nagai, Tatsushi Kamiya, Koji Abe**

Department of Neurology, Graduate School of Medicine, Dentistry and Pharmacy, Okayama University, 2-5-1 Shikata-cho, Okayama, 700-8558, Japan

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ABSTRACT

It is very important to investigate the mechanism of axonal growth in the ischemic brain in order to consider a novel mean of therapy for stroke. Netrins are chemotropic factors for axon with chemoattractant or chemorepellant guidance activities, and deleted in colorectal cancer (DCC) and neogenin are receptors for netrins. In this study, we examined expressions of netrin-1, DCC, and neogenin in the brain after 90 min of transient middle cerebral artery occlusion (tMCAO). Netrin-1 was expressed in neurons at the peri-ischemic area with a peak at 14 days. DCC was expressed both in neurons and astrocytic feet with a peak at 14 days, though neogenin was expressed in endothelial cells at MCA territory with a peak at the same time point. These results suggest that netrin-1 is involved in the promotion of axonal growth. The expression of netrin-1 and DCC was overlapped both in the spatial and temporal patterns, indicating that DCC plays a role in netrin-1's axonal growth promoting effects. The location of neogenin positive cells differed from that of netrin-1 positive cells, thus its angiogenic activity may not have relevance with netrin-1.

1. Introduction

Once the central nervous system (CNS) was severely damaged, neurological deficit would permanently continue because tissue regeneration is quite limited. However, even if new neurons were not provided, a functional recovery should take place if axons grew after injury. It is therefore very important to investigate the mechanism of axonal growth of regeneration in the ischemic brain in order to consider a novel mean of therapy for stroke.

Chemotropic factors are important for axon pathfinding, and a growing number of chemotropic factors have been identified in the past several years. Some of these molecules are bifunctional; they exert either chemoattractive or chemorepulsive activity depending on which receptor to bind (Mueller, 1999; Wong et al., 1999; David and McKerracher, 2001). Netrins are chemotropic factors for axon with chemoattractant or chemorepellant guidance activities both in vitro and in vivo (Mueller, 1999). Netrins continue to be expressed in the adult vertebrate CNS (Kennedy et al., 1994; Livesey and

* *Corresponding author*. Fax: +81 86 235 7368.
E-mail address: neurotakeshi@hotmail.com (K. Abe).

Abbreviations: CBF, cerebral blood flow; CNS, central nervous system; DAB, diaminobenzidine tetrahydrochloride; DAPI, 4,6-diamidino-2-phenylinodoleHCL; DCC, deleted in colorectal cancer; GFAP, glial fibrillary acidic protein; MCA, middle cerebral artery; NAGO, N-acetyl glucosamine oligomer; PB, phosphate buffer; PBS, phosphate-buffered saline; tMCAO, transient middle cerebral artery occlusion

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Hunt, 1997; Meyerhardt et al., 1999; Wong et al., 1999; Madison et al., 2000), but their role in injured brain is unknown. Netrin receptors that mediate netrins' attractive or repulsive responses have also been identified, deleted in colorectal cancer (DCC), and neogenin are chemoattractive receptors for netrins (Keino-Masu et al., 1996; Park et al., 2004). DCC is the high affinity cell surface receptor for netrin-1 and is widely expressed in the developing brain but is down-regulated prior to maturation. In adult rodents, on the other hand, DCC mRNA has been located in the substantia nigra, striatum, and cerebellum (Livesey and Hunt, 1997; Volenec et al., 1997). Neogenin has been cloned from chicken, mouse and human (Vielmetter et al., 1994; Keeling et al., 1997; Meyerhardt et al., 1997), and has been reported to bind with netrin-1 (Keino-Masu et al., 1996). However, a role for neogenin in axon guidance has not yet been demonstrated.

In the present study, we examined the expression of netrin-1, DCC, and neogenin in the ischemic brain following transient middle cerebral artery occlusion (tMCAO).

2. Results

2.1. *Change in netrin-1 expression*

In the sham control brains, no immunoreactivity for netrin-1 was found in every part of the brain (Fig. 1). At 1 day after the tMCAO, however, the cerebral cortex and caudate at the inner boundary area of the middle cerebral artery (MCA) territory became positively stained. It was demonstrated that neuropil was diffusely stained, indicating that neurite and axon expressed immunoreactive netrin-1. There was no immunoreactivity in the ischemic core or contralateral hemisphere. At 7 days after the tMCAO, immunoreactive netrin-1 was expressed in the cell body in the cerebral cortex and caudate at the ischemic boundary area. The immunoreactivity at the neuropil became less dense at this time point. At 14 days, the immunoreactivity became stronger and the number of positively stained cells was increased. At 28 days, the number of netrin-1 positive cells decreased and the degree of staining became weak.

Double immunofluorescent studies for netrin-1 and NeuN or glial fibrillary acidic protein (GFAP) were carried out in order to confirm the cell phenotype which expressed netrin-1 (Fig. 2). Immunoreactivity for GFAP became strong after the tMCAO in the outer ischemic boundary area, but was not overlapped with netrin-1 at any time points (Fig. 2). The immunoreactivity for NeuN became weak after the tMCAO in the ischemic area, but netrin-1 expressing cells were stained for NeuN (Fig. 2). In order to confirm the result, same procedures were repeated, which showed identical results.

2.2. *Change in DCC and neogenin expression*

In the sham control brain, no immunoreactivity for DCC was found in every part of the brain (Fig. 3, left column). At 1 day after the tMCAO, however, the cerebral cortex and caudate at the inner boundary area of the MCA territory became positively stained. The immunoreactive DCC was expressed in the cell body in the cerebral cortex and caudate at

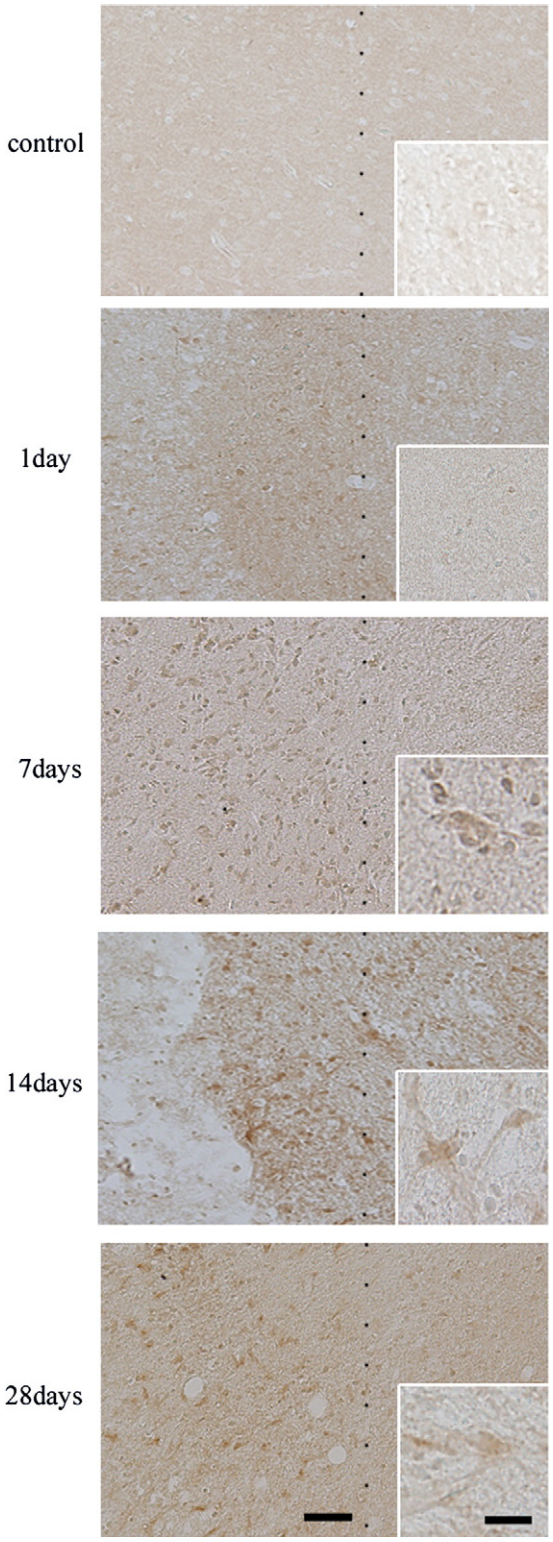

Fig. 1 – Representative photomicrographs of immnunohistochemistry for netrin-1. Left to the dotted line indicates the MCA perfused area. In spite of no immunoreactivity in the control brain, neuropil at the boundary area became positively stained at 1 day. At 7 and 14 days, cell bodies became more densely stained, which decayed at 28 days. Scale bar is 50 μm and 25 μm for inlets.