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Research report

Localization of nectin-2δ at perivascular astrocytic endfoot processes and degeneration of astrocytes and neurons in nectin-2 knockout mouse brain

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ABSTRACT

Nectins are Ca^{2+}-independent immunoglobulin-like cell–cell adhesion molecules. In the nervous system, among four members (nectin-1, -2, -3, and -4), nectin-1 and -3 are asymmetrically localized at puncta adherentia junctions formed between the mossy fiber terminals and the dendrites of CA3 pyramidal neurons in the mouse hippocampus and heterophilic *trans*-interactions between nectin-1 and nectin-3 are involved in the selective interaction of axons and dendrites of cultured neurons. By contrast, nectin-2, which has two splicing variants, nectin-2α and -2δ, has not been well characterized in the brain. We showed here that nectin-2α was expressed in both cultured mouse neurons and astrocytes whereas nectin-2δ was selectively expressed in the astrocytes. Nectin-2δ was localized at the adhesion sites between adjacent cultured astrocytes, but in the brain it was localized on the plasma membranes of astrocytic perivascular endfoot processes facing the basement membrane of blood vessels. Genetic ablation of *nectin-2* caused degeneration of astrocytic perivascular endfoot processes and neurons in the cerebral cortex. These results uncovered for the first time the localization and critical functions of nectin-2 in the brain.

1. Introduction

Nectins are Ca^{2+}-independent immunoglobulin-like cell–cell adhesion molecules and are composed of four members (nectin-1, -2, -3, and -4) that mediate cell–cell adhesion (Takai et al., 2008a, 2008b). Nectins have three extracellular immunoglobulin domains, a single transmembrane region, and a cytoplasmic region. The extracellular region of nectin on one cell interacts *intrans* with the same or different nectin family member on the adjacent cell. The cytoplasmic tail of nectins binds the actin filament (F-actin)-binding protein afadin, which mediates an interaction with the actin cytoskeleton. In addition to cell–cell adhesion, nectins regulate a variety of cell functions, including cell migration polarization, differentiation, proliferation, and survival.

At the mossy fiber–CA3 pyramidal cell synapses in the mouse hippocampus, nectin-1 and -3 are asymmetrically distributed, being localized exclusively at the pre- and post-synaptic sides of puncta adherentia junctions, respectively (Mizoguchi et al., 2002). Heterophilic *trans*-interactions of nectins are stronger than their homophilic *trans*-interactions. Of the various *trans*-interactions among the nectin family members, the interaction between nectin-1 and -3 is the strongest (Harrison et al., 2012; Satoh-Horikawa et al., 2000). The asymmetric localization of nectin-1 and -3 and the strong heterophilic *trans*-interaction between them are involved in the selective interaction of axons and dendrites (Togashi et al., 2006).

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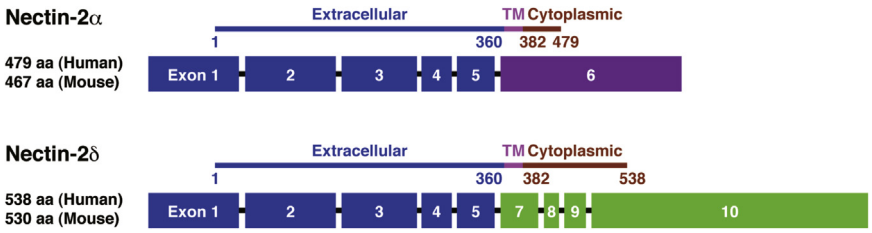

Fig. 1. Structures of two splice variants of nectin-2 and their exons. Both variants consist of an extracellular domain, a transmembrane (TM) domain, and a cytoplasmic domain. Nectin-2α is encoded by exons 1–6, whereas nectin-2δ is encoded by exons 1–5 and exons 7–10.

In contrast to nectin-1 and -3, nectin-2 has not been well characterized in the brain. Nectin-2, also termed as poliovirus receptor (PVR) related 2 (PVRL2/PRR2) and CD112, has two splice variants, nectin-2α and -2δ (Fig. 1), and both transcripts are ubiquitously expressed in human tissues (Eberle et al., 1995). The *nectin-2* gene was originally cloned as a murine homolog of the *PVR* gene (Morrison and Racaniello, 1992), but its gene product was later shown to serve as an entry receptor for herpes simplex viruses (Warner et al., 1998). Nectin-2 is localized at intercellular borders in various tissues and organs, including blood vessels and the heart, suggesting a role for homophilic interactions between hematopoietic and endothelial cells and between cardiac myocytes (Lopez et al., 1998; Satomi-Kobayashi et al., 2009). In the testis, nectin-2 and -3 are localized on Sertoli cells and spermatids, respectively, at Sertoli-spermatid junctions, and genetic deletion of *nectin-2* causes the male-specific infertility with abnormal differentiation of spermatocytes in the mouse testis (Bouchard et al., 2000; Mueller et al., 2003; Ozaki-Kuroda et al., 2002). In addition, *nectin-2*-knockout (*nectin-2*$^{-/-}$) mice show a disruption of intercalated discs between cardiac myocytes following aortic banding, resulting in severe heart failure (Satomi-Kobayashi et al., 2009). This finding suggests the role of nectin-2 in the maintenance of cardiac structure and function under stressed conditions. Genetic association between *nectin-2* and various human disorders, such as Alzheimer's disease (Harold et al., 2009; Logue et al., 2011; Takei et al., 2009), coronary heart disease (Freitas et al., 2002), multiple sclerosis (Schmidt et al., 2006), and cleft lip/palate (Jagomagi et al., 2010), has been reported. Moreover, a high expression level of nectin-2 is associated with a better prognosis of acute myeloid leukemia (Graf et al., 2005) and translocation of *nectin-2* gene is identified in peripheral T-cell lymphoma (Almire et al., 2007). However, to date, the localization and functions of each isoform of nectin-2 in the brain remain unknown. We therefore studied the localization and functions of nectin-2 in the brain.

Here we found that nectin-2α was expressed in both cultured mouse neurons and astrocytes whereas nectin-2δ was selectively expressed in astrocytes. Astrocytes are critical for providing structural, trophic, and metabolic support to neurons and regulating synaptic functions (Marco and Pierre, 1996). Anatomically, astrocytes locate between neurons and blood vessels in the brain. Astrocytes extend processes to synapses (perisynaptic processes), which ensheath or touch synapses, and regulate synaptic formation by releasing soluble factors that can increase synapse number (Ullian et al., 2004), synapse stabilization via Eph/ephrin signaling (Nishida and Okabe, 2007), synaptic transmission by uptaking glutamate released from synapses (Allen, 2014), by releasing gliotransmitters (Newman, 2003), and by limiting spillover of neurotransmitters into neighboring synapses (Oliet et al., 2004), and synaptic elimination (Stevens et al., 2007). They also extend processes to the basement membrane of blood vessels (perivascular endfoot processes), which enwrap vascular walls, regulate the blood brain barrier (Alvarez et al., 2013) and blood flow (Petzold and Murthy, 2011), and transport water, ions, and nutrients from blood (Barros, 2013). We showed using immunofluorescence and immunoelectron microscopies that nectin-2δ was localized at the adhesion sites between adjacent astrocytes in culture, but that in the brain it was localized on the plasma membranes of astrocytic perivascular endfoot processes facing the basement membrane of blood vessels. Genetic ablation of *nectin-2* caused degeneration of astrocytic perivascular endfoot processes and neurons in the cerebral cortex.

2. Results

2.1. Expression of nectin-2 in cultured astrocytes

We investigated which nectins are expressed in cultured neurons and astrocytes. Hippocampal neuron cultures were established from embryonic day 18.5 mouse embryos, whereas astrocyte cultures were obtained by differentiation of neurospheres prepared from mouse pups. Whole cell lysates were prepared from the neurons and the astrocytes at 14 and 7 days *in vitro* (DIV), respectively, and subjected to Western blotting with the indicated antibodies (Abs). The lysates of mouse forebrain were used as a control. Nectin-1 and −2 were expressed in both astrocytes and neurons. Nectin-3 was expressed in neurons, but not in astrocytes (Fig. 2**A**). Notably, as regard to nectin-2, nectin-2α (lower band) was detected in the forebrain, astrocytes, and neurons, whereas nectin-2δ (upper band) was detected in astrocytes by the anti-nectin-2 monoclonal antibody (mAb). This result was consistent with immunoblottings by using the anti-nectin-2α polyclonal Ab (pAb) and the anti-nectin-2δ pAb. In *nectin-2*$^{-/-}$ astrocytes, nectin-2α/δ, −2α, or −2δ was not detected, indicating that each Ab specifically recognized each variant of nectin-2 (Supplemental Fig. 1). When endogenous nectin-2 was silenced by the nectin-2 siRNA, nectin-2α and nectin-2δ were reduced by 82% and 79%, respectively, in the lysate of the astrocytes transfected with the nectin-2 siRNA (Fig. 2**B**). In the astrocytes transfected with the nectin-2 siRNA, the amount of nectin-1 was not changed as compared with that in the astrocytes transfected with the control siRNA (Fig. 2**B**). Nectin-3 was not detected in the astrocytes transfected with the control siRNA and the nectin-2 siRNA (Fig. 2**B**). The anti-nectin-2α pAb detected two extra bands (Fig. 2**A and B, asterisk**), but these bands were also detected in the *nectin-2*$^{-/-}$ astrocytes, indicating that these two bands were non-specific bands (Supplemental Fig. 1). These results indicate that nectin-2α is commonly expressed in both astrocytes and neurons, while nectin-2δ is highly expressed in astrocytes.

2.2. Localization of nectin-2 at adhesion sites between adjacent cultured astrocytes

We examined the localization of nectin-2 in cultured astrocytes by immunofluorescence microscopy. In a high cell density culture, the intense signals for nectin-1, -2α/δ, -2α, -2δ, afadin, and N-cadherin were observed at adhesion sites between cultured astrocytes (Fig. 3**A**). When HA-tagged nectin-2α or -2δ was exogenously