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BRAIN RESEARCH

Research Report

ADAM12 is expressed by astrocytes during experimental demyelination

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ABSTRACT

A disintegrin and metalloproteinase (ADAM) 12 represents a member of a large family of similarly structured multi-domain proteins. In the central nervous system (CNS), ADAM12 has been suggested to play a role in brain development, glioblastoma cell proliferation, and in experimental autoimmune encephalomyelitis. Furthermore, ADAM12 was reported to be almost exclusively expressed by oligodendrocytes and could, therefore, be considered as suitable marker for this cell type. In the present study, we investigated ADAM12 expression in the healthy and pathologically altered murine CNS. As pathological paradigm, we used the cuprizone demyelination model in which myelin loss during multiple sclerosis is imitated. Besides APC$^+$ oligodendrocytes, SMI311$^+$ neurons and GFAP$^+$ astrocytes express ADAM12 in the adult mouse brain. ADAM12 expression was further analyzed *in vitro*. After the induction of demyelination, we observed that activated astrocytes are the main source of ADAM12 in brain regions affected by oligodendrocyte loss. Exposure of astrocytes *in vitro* to either lipopolysaccharides (LPS), tumor necrosis factor α (TNFα), glutamate, or hydrogen peroxide revealed a highly stimulus-specific regulation of ADAM12 expression which was not seen in microglial BV2 cells. It appears that LPS- and TNFα-induced ADAM12 expression is mediated via the classic NFκB pathway. In summary, we demonstrated that ADAM12 is not a suitable marker for oligodendrocytes. Our results further suggest that ADAM12 might be implicated in the course of distinct CNS diseases such as demyelinating disorders.

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Abbreviations: ADAM, a disintegrin and metalloproteinase; APC, adenomatous polyposis coli; AT, annealing temperature; bFGF, basic fibroblast growth factor; BP, base pairs; BSA, bovine serum albumine; CC, corpus callosum; CNS, central nervous system; Cx, cortex; ctrl, control; cup, cuprizone; DMEM, Dulbecco's modified Eagle's medium; EAE, experimental autoimmune encephalomyelitis; FCS, fetal calf serum; GFAP, glial fibrillary acidic protein; Glut, glutamate; H_2O_2, hydrogen peroxide; HBEGF, heparin-binding epidermal growth factor; HPRT, hypoxanthine guanine phosphoribosyltransferase; IL1ß, interleukin 1ß; IHC, immunohistochemistry; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; LPS, lipopolysaccharides; MS, multiple sclerosis; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PBS, phosphate-buffered saline; PLP, proteolipoprotein; rt, real-time; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; SEM, standard error of the mean; sq, semi-quantitative; TNFα, tumor necrosis factor α

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1. Introduction

The "a disintegrin and metalloproteinase" (ADAM) family consists of numerous similarly structured multi-domain proteins involved in many physiological and pathological processes. Altered expression and functions of specific ADAMs are implicated in the pathophysiology of several diseases including rheumatoid arthritis (Valdes et al., 2004), Alzheimer's disease (Malinin et al., 2005; Tanabe et al., 2007), cardiac hypertrophy (Asakura et al., 2002), asthma (Chiba et al., 2009), and several types of cancer (Mochizuki and Okada, 2007). They are found in various species and are expressed in many organs and tissues (Wolfsberg et al., 1995) such as the skeletal muscle (Yagami-Hiromasa et al., 1995), the placenta (Gilpin et al., 1998), and the central nervous system (CNS) (Bernstein et al., 2004). The prototypical ADAM protein contains an extracellular pro-metalloproteinase, a disintegrin-like, a cysteine-rich and an epidermal growth factor-like domain, followed by a transmembrane- and a cytoplasmic tail domain (Kveiborg et al., 2008). Functions of different ADAMs include cell adhesion (Najy et al., 2008), cell fusion (Yagami-Hiromasa et al., 1995), proteolysis (Moss et al., 2007), and facilitating of cell proliferation (Kodama et al., 2004) and migration (Estrella et al., 2009) allowing them to take part in different biological processes. These include fertilization (Pasten-Hidalgo et al., 2008), angiogenesis (Mahoney et al., 2009) and, as so far most elaborately investigated, the development and progression of cancer (Mochizuki and Okada, 2007). In the CNS, ADAMs have been suggested to play a role during brain development (Lin et al., 2008) and to contribute to axon extension (Fambrough et al., 1996) and neurogenesis (Pan and Rubin, 1997).

ADAM12 possesses extracellular metalloproteinase and cell-binding properties as well as intracellular signaling capacities (Kveiborg et al., 2008). A variety of functions have been suggested for ADAM12, including support of releasing growth factors such as heparin-binding epidermal growth factor (HBEGF) (Asakura et al., 2002; Kodama et al., 2004) and insulin-like growth factor (IGF) 1 (Loechel et al., 2000) as well as interaction with cell surface integrins. ADAM12 is, therefore, likely to mediate cell adhesion, differentiation, proliferation, and migration (Kveiborg et al., 2008). Furthermore, it was suggested that ADAM12 takes part in myogenesis (Gilpin et al., 1998), bone growth (Kveiborg et al., 2006), and fetal development (Cowans and Spencer, 2007). Besides such physiological functions, ADAM12 seems to be involved in pathological processes such as cardiac hypertrophy (Asakura et al., 2002), osteoarthritis (Valdes et al., 2004), and cancers of various tissue origins (Kveiborg et al., 2008).

So far, little is known about the function of ADAM12 within the CNS. It has been suggested that ADAM12 is necessary for proper brain development and regionalization, since it is expressed in highly restricted regions of the neuroepithelium (Lin et al., 2008). An increase of ADAM12 expression was found in human glioblastomas where it correlates with the proliferative activity of tumor cells (Kodama et al., 2004). Among brain resident cells, ADAM12 is supposed to be almost exclusively expressed by oligodendroglia under physiological conditions and is, therefore, considered to be a suitable oligodendrocyte marker (Bernstein et al., 2004). During experimental autoimmune encephalomyelitis (EAE), an animal model widely used to investigate pathological mechanisms of human multiple sclerosis (MS), ADAM12 expression is up-regulated in the mouse spinal cord due to infiltrating T-cells (Toft-Hansen et al., 2004).

In order to further investigate the role of ADAM12 within the intact and pathologically altered rodent brain, we analyzed the expression of ADAM12 in C57BL6 mice. The cuprizone demyelination model was used as a model for MS, since feeding of cuprizone induces highly reproducible demyelination of different brain areas (Kipp et al., 2009). The cellular source of ADAM12 in the intact and demyelinated brain was investigated by consecutive slice immunohistochemistry (IHC) and immunofluorescence double labeling. Primary cell culture experiments were additionally performed to gain insight into stimulus-specific regulation of ADAM12 expression.

2. Results

2.1. *ADAM12 is expressed by oligodendrocytes and cortical neurons*

It was recently reported that ADAM12 is expressed in the rodent brain. With the exception of very few immunopositive pyramidal neurons in the developing rat brain, ADAM12 was exclusively localized to oligodendrocytes (Bernstein et al., 2004). In order to confirm these findings in the murine CNS, we performed immunofluorescence double labeling with an anti-ADAM12 antibody and antibodies for respective cell markers in young male C57BL6 mice. SMI311 was selected to provide a specific marker for neurons. In contrast to markers for individual non-phosphorylated neurofilaments that identify different subsets of neurons and are, therefore, especially suitable for defining anatomic and functional differences in normal and pathologically altered neurons, SMI311 is a general marker for adult neurons and differentiating neuronal precursors (Ulfig et al., 1998). An anti-adenomatous polyposis coli (APC) antibody was selected to visualize late stage oligodendrocyte cells (Groebe et al., 2009; Norkute et al., 2009; Pott et al., 2009). Colocalization of ADAM12 and 95% of APC^+ oligodendrocytes was confirmed within the corpus callosum (CC) (Fig. 1) and the cerebral cortex (not shown). ADAM12 staining in oligodendrocytes was restricted to the cell membrane (arrow in Fig. 1I) and the cytoplasm sparing the cell nucleus. Unexpectedly, immunofluorescence double labeling for ADAM12 and SMI311 revealed that ADAM12 is also expressed by numerous $SMI311^+$ neurons in the telencephalic cortex of the adult mouse brain (Fig. 2). ADAM12 staining in neurons was mainly confined to the neuronal cell bodies sparing dendritic branches (arrows in Fig. 2I). Staining intensity was similar to that observed in APC^+ cells.

Since only few $ADAM12^+$ neurons were noted in the study conducted by Bernstein et al., we performed Western Blot analysis to confirm the specificity of the anti-ADAM12 antibody. Proteins were isolated from the CC and the cortex and applied in different concentrations (20 μg, 10 μg and 5 μg) for sodium dodecylsulfate polyacrylamide gel electrophoresis