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Analysis of combinatorial variability reveals selective accumulation of the fibronectin type III domains B and D of tenascin-C in injured brain

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ABSTRACT

Tenascin-C (Tnc) is a multimodular extracellular matrix glycoprotein that is markedly upregulated in CNS injuries where it is primarily secreted by reactive astrocytes. Different Tnc isoforms can be generated by the insertion of variable combinations of one to seven (in rats) alternatively spliced distinct fibronectin type III (FnIII) domains to the smallest variant. Each spliced FnIII repeat mediates specific actions on neurite outgrowth, neuron migration or adhesion. Hence, different Tnc isoforms might differentially influence CNS repair. We explored the expression pattern of Tnc variants after cortical lesions and after treatment of astrocytes with various cytokines. Using RT-PCR, we observed a strong upregulation of Tnc transcripts containing the spliced FnIII domains B or D in injured tissue at 2–4 days post-lesion (dpl). Looking at specific combinations, we showed a dramatic increase of Tnc isoforms harboring the neurite outgrowth-promoting BD repeat with both the B and D domains being adjacent to each other. Isoforms containing only the axon growth-stimulating spliced domain D were also dramatically enhanced after injury. Injury-induced increase of Tnc proteins comprising the domain D was confirmed by Western Blotting and immunostaining of cortical lesions. In contrast, the FnIII modules C and AD1 were weakly modulated after injury. The growth cone repulsive A1A2A4 domains were poorly expressed in normal and injured tissue but the smallest isoform, which is also repellant, was highly expressed after injury. Expression of the shortest Tnc isoform and of variants containing B, D or BD, was strongly upregulated in cultured astrocytes after TGFβ1 treatment, suggesting that TGFβ1 could mediate, at least in part, the injury-induced upregulation of these isoforms. We identified complex injury-induced differential regulations of Tnc isoforms that may well influence axonal regeneration and repair processes in the damaged CNS.

Introduction

Tenascin-C (Tnc) is a glycoprotein present in the extracellular matrix (ECM) of a variety of tissues where it plays multiple roles in cell growth, migration and adhesion during development but also under conditions of tissue remodeling in the adult organism such as wound healing and tumor growth (Bartsch, 1996; Faissner, 1997; Jones and Jones, 2000; Joester and Faissner, 2001; Chiquet-Ehrismann and Chiquet, 2003). In the central nervous system (CNS), Tnc is widely expressed at early stages of development, principally by astrocytes and radial glia, where it has a variety of functions such as influencing neurite outgrowth and guidance and in the development of glia (Faissner, 1997; Meiners et al., 1999; Joester and Faissner, 2001; Garcion et al., 2001; Rigato et al., 2002). Tnc expression is down-regulated in most regions of the adult CNS but it is strongly upregulated around lesion sites of CNS stab wound injuries, associated with a subset of GFAP positive astrocytes (McKeon et al., 1991; Laywell et al., 1992; Brodkey et al., 1995; Zhang et al., 1997; Tang et al., 2003). Tnc upregulation is also seen following other forms of CNS damage, such as in the dentate gyrus after unilateral entorhinal cortex lesion (Deller et al., 1997) or the hippocampus after injection of kainic acid (Niquet et al., 1995; Nakic et al., 1996).

Tnc is encoded by a single gene giving rise to a number of alternatively spliced variants that differ in their number of fibronectin type III (FnIII) domains (see Fig. 1 for structural details). The smallest Tnc variant contains a series of 8 constant FnIII repeats that are present in all rodent Tnc molecules. Numerous larger isoforms of Tnc are generated by the insertion of up to six (in mice) and seven (in rats) additional alternatively spliced FnIII domains between the 5th and 6th constant FnIII domains of the basic structure. In mice, 28 Tnc isoforms

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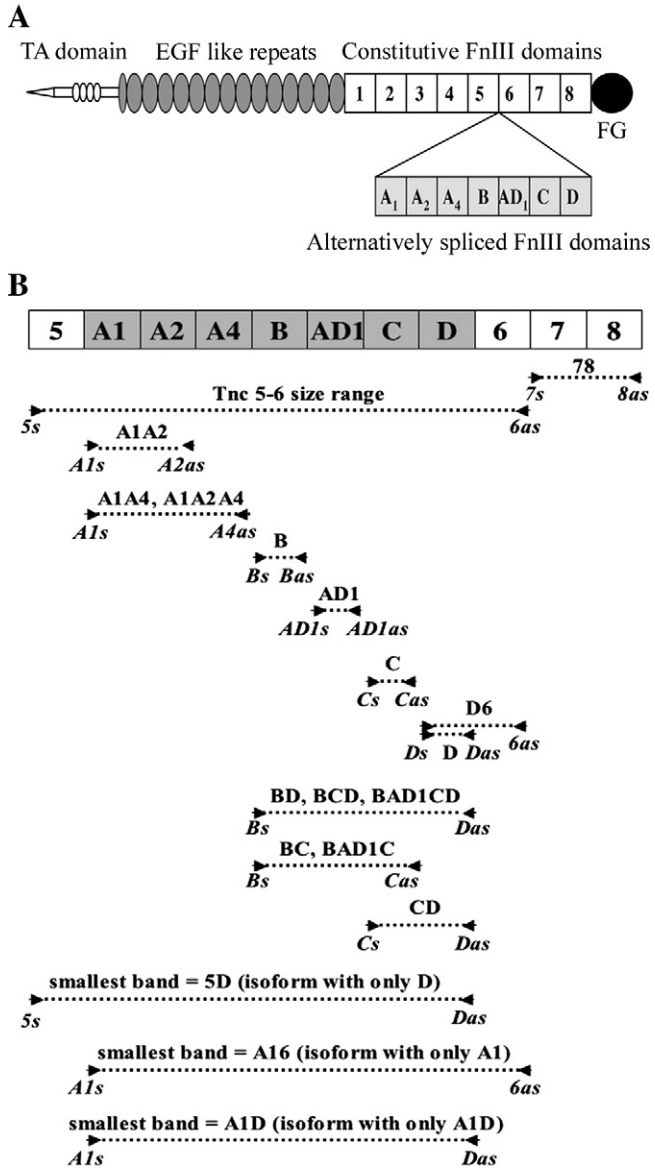

Fig. 1. (A) Schematic representation of the structural organization of the tenascin-C monomer. Tnc is produced as a hexamer and each monomer exhibits a multimodular composition with a serial arrangement of characteristic structural domains. The amino-terminal sequence or Tnc assembly domain (TA) contributes to the central knob, a cysteine-rich structure that assembles six monomers via disulfide bridges to form the native hexameric protein, the hexabrachion. This region is followed by 14.5 EGF-like repeats (31 aa each) and 8 fibronectin type III (FnIII) modules (90 aa each) in the smallest Tnc variant. The sequence is terminated by a domain (FG) homologous to fibrinogen-β and -γ (Joester and Faissner, 2001). Numerous larger isoforms of Tnc are generated by the insertion of up to seven additional alternatively spliced FnIII domains between the 5th and 6th constant FnIII domains of the basic structure in rats. These alternatively spliced domains have been named A1, A2, A4, B, C and D and, recently, we have discovered an additional spliced domain for the rat, called AD1. (B) The diagram illustrates the strategy used to measure expression of the various alternatively spliced domains and their combinations. Primer pairs were used that bridge single domains, two domains, three domains, four domains and the whole alternatively spliced region.

have so far been identified in the CNS which differ in the combination of additional FnIII repeats (Joester and Faissner, 1999; von Holst et al., 2007). The alternatively spliced domains have been named A1, A2, A4, B, C and D and, recently, we have discovered an additional spliced domain in rat Tnc, called AD1 (unpublished results) as it corresponds to the AD1 domain, previously only described in humans and chicken (Derr et al., 1997). Diverse functions have been ascribed to several of the spliced FnIII domains through studies using fusion proteins and blocking antibodies. For example, a FnIII A1,A2,A4 peptide exhibits growth cone repulsive properties, whereas the recombinant proteins FnIII D, FnIII D6 and FnIII BD promote neurite outgrowth from various types of neurons (Götz et al., 1996; Meiners et al., 1999, 2001; Rigato et al., 2002). These results suggest large functional diversity of Tnc isoforms. *In vivo* and *in vitro* studies demonstrate that Tnc can provide permissive as well as inhibitory cues for axonal growth and guidance (Faissner and Kruse, 1990; Lochter et al., 1991; Husmann et al., 1992; Götz et al., 1997; Meiners and Geller, 1997; Meiners et al., 1999). Interestingly, expression of Tnc isoforms and spliced FnIII domains is differentially regulated during development, and periods of increased axonal growth in the developing CNS are closely correlated with expression of large isoforms (Prieto et al., 1990; Bartsch et al., 1992; Tucker, 1993; Joester and Faissner, 1999, 2001).

Given the potentially diverse actions of differentially spliced Tnc molecules, it is possible that the expression of different forms of Tnc might have functional significance in glial scarring and axonal regeneration. To date, it is not known which alternatively spliced Tnc forms and FnIII domains are preferentially expressed after injury. Here, we examined the expression of Tnc isoforms and spliced FnIII domains in a cortical and basal forebrain injury model. *In vitro*, astrocytic expression of Tnc is increased by TGFβ1 and bFGF, both of which are upregulated after CNS damage (Meiners et al., 1993; Mahler et al., 1997; Smith and Hale, 1997). However, the factors regulating the expression of specific isoforms in these cells are unknown. Thus, we have also examined the effects of injury-related cytokines on the production of Tnc isoforms by cultured astrocytes.

Materials and methods

Cytokines and primary antibodies

Recombinant cytokines: human Transforming Growth Factor β1 (TGFβ1), human basic Fibroblast Growth Factor (bFGF), human Epidermal Growth Factor (EGF), human Transforming Growth Factor α (TGFα), human Platelet Derived Growth Factor-AB (PDGF-AB) and murine Vascular Endothelial Growth Factor (VEGF) were from R&D Systems (Abingdon, Oxon, UK) and from PeproTech Inc. (Rocky Hill, New Jersey). The rabbit polyclonal antibody (pAb) KAF14 was raised against purified Tnc from postnatal mouse brains as described previously (Faissner and Kruse, 1990). The monoclonal antibody (mAb) 578 recognizes specifically the FnIII domain D and mAb 19H12 binds to an epitope located on the FnIII combination A1A2A4 (Götz et al., 1996). Mouse Tnc used in Western Blots was purified from P7–P14 mouse brains as described previously (Faissner and Kruse, 1990). Mouse anti-GFAP (Glial Fibrillary Acidic Protein) mAb was from Sigma and rabbit anti-GFAP pAb was from Dako.

Surgical procedures

Adult female Sprague Dawley rats (Charles River, Margate, UK) weighing approximately 200 g were anesthetized under halothane. 3 mm deep lesions were generated unilaterally by lowering a sterile scalpel into the right-hand side of the cerebral cortex with the other hemisphere serving as a control (Asher et al., 2000). Animals were allowed to survive for 2 to 28 days after the operation before they were terminally anesthetized and decapitated. Brains were either immediately dissected or stored at −70 °C. For immunohistochemistry we used adult male CD1 mice (20–30 g). For cortical or basal forebrain lesions, a Scouten wire knife (Kopf Instrument, Harvard Apparatus, UK) was used. Animals were perfused and sacrificed 7 days post-lesion. Brains were removed and stored first in 4% paraformaldehyde and then in 30% sucrose. All procedures were conducted in compliance with the UK Animals Scientific Procedures Act 1986.