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Cells in focus

Connective tissues: signalling by tenascins

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Abstract

Different connective tissue cells secrete different types of tenascins. These glycoproteins contribute to extracellular matrix (ECM) structure and influence the physiology of the cells in contact with the tenascin containing environment. Tenascin-C expression is regulated by mechanical stress. It shows highest expression in connective tissue surrounding tumors, in wounds and in inflamed tissues where it may regulate cell morphology, growth, and migration by activating diverse intracellular signalling pathways. Thus, integrin and syndecan signalling is influenced by tenascin-C and the levels and/or activies of several proteins involved in intracellular signalling pathways are regulated by its presence. Tenascin-X is important for the proper deposition of collagen fibers in dermis and patients with a tenascin-X deficiency suffer from Ehlers Danlos syndrome. Tenascin-R (and -C) is prominent in the nervous system and has an impact on neurite outgrowth and synaptic functions, and tenascin-W is found in the extracellular matrix of bone, muscle, and kidney.

Keywords: Cell adhesion; Signalling; Adhesion modulation; Cell shape

Cell facts

- bone: osteoblasts produce tenascin-C, -W
- cartilage: perichondrial cells produce tenascin-C
- tendon: fibroblasts produce tenascin-C
- smooth muscle cells produce tenascin-W, -C
- skeletal muscle: endo-, peri-, and epimysial fibroblasts produce tenascin-X
- dermal fibroblasts produce tenascin-X
- tumors: stromal fibroblasts produce tenascin-C
- wounds: fibroblasts produce tenascin-C
- nervous system: glial cells produce tenascin-R, -C, -X.

1. Introduction

The name connective tissue already exemplifies its primary function, namely to bind together and support various body structures and to fill the spaces between them. Connective tissue is, therefore, mostly

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considered as supportive for the functioning of other more important organs, and often no specific autonomous function is attributed to it. However, during organ development it is well known that the mesenchyme (connective tissue) is required for epithelial differentiation and it is the mesenchyme which determines the fate of the epithelium through mesenchymal epithelial interactions. In the adult, organ homeostasis depends on undisturbed mesenchymal–epithelial interactions as well, and changes in the stromal connective tissue can influence pathological reactions of epithelia including cancer development (Ingber, 2002). Finally, in the adult, specialized connective tissue includes tendon, bone, and cartilage. Clearly these structures are of eminent importance and anyone having suffered from a bone fracture, from the rupture of a tendon or from osteoarthritis will appreciate the importance of intact connective tissue.

A common feature of all connective tissues is that it mainly consists of extracellular matrix (ECM) within which the connective tissue cells are sparsely distributed. The ECM is primarily made of fibrous proteins like type I collagen, the major protein that accounts for 25% of the total protein mass of our bodies. However, besides many different types of collagens the ECM contains a multitude of other glycoproteins, proteoglycans and the carbohydrate polymer hyaluronic acid (for a review see Bosman & Stamenkovic, 2003). The relative proportion of these constituents is responsible for the physical properties of the respective extracellular matrices which can be as different in structure and function as the basement membrane in the kidney serving as a filtration barrier, the resilient tendon ECM, the cushioning cartilage ECM in our joints, or the tough mineralized matrix of bones and teeth.

Tenascins are a family of four ECM glycoproteins in vertebrates (cf. this issue). They are typically present in many different connective tissues. Tenascins contribute to matrix structure and they influence the behavior of the cells in contact with the ECM. The focus of this article will, therefore, be an overview over the effects of the tenascins on cellular functions.

2. Cell origin and plasticity

Most connective tissues are generated from mesoderm, a primary germ layer of the embryo lying between ectoderm and endoderm. Mesoderm-derived mesenchyme give rise to muscular, circulatory, lymphatic, urogenital, and skeletal systems as well as the linings of the body cavities. The continued presence of certain mesenchymal cells in the adult holds promise for molecular medicine through the use of stem cells in tissue engineering (Caplan & Bruder, 2001).

One tenascin producing cell type which does not belong to connective tissue, namely glial cells, is also

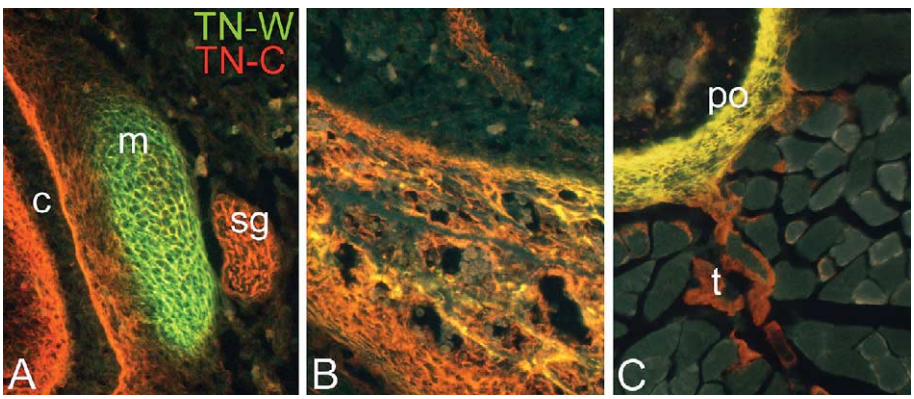

Fig. 1. Tenascin-C and tenascin-W in connective tissues. (A) Anti-tenascin-C (red) stains cartilage in the neurocranium (c) and a salivary gland (sg) in a coronal section through an embryonic mouse head (Day 16.5). Anti-tenascin-W (green) stains the connective tissue of a developing muscle of mastication (m). (B) Both tenascin-C (red) and tenascin-W (green, with co-localization appearing yellow) are found in the bony matrix of the embryonic mandible (Day 16.5). (C) In the adult mouse tenascin-C (red) and tenascin-W (co-localization with tenascin-C is yellow) are found in the periosteum (po) of the ribs. Only tenascin-C is detectable in intercostal tendon (t).

included in this review. Glial cells, however, act in many ways like supportive cells for the nervous system. Interestingly, glial cells secrete ECM proteins common to connective tissue cells, including tenascins.

Each of the connective tissues expresses a different member or set of tenascins (see "Cell Facts"). In most cases cells produce only one type of tenascin, but on the immunohistochemical level tenascin-C sometimes coincides with tenascin-W staining in developing bones and in certain muscles (Scherberich et al., 2004); (Fig. 1). In general, however, the regulation of tenascin expression is distinct for each gene. Tenascin-C expression exhibits an interesting mechanism of activation, not only by certain growth factors but also through the application of mechanical stress to a tissue or even to cells in culture (Chiquet, Renedo, Huber, & Flück, 2003). This feature of tenascin-C fits with its expression pattern at locations known to experience mechanical loading such as the skeleton and tendons. Since tenascin-C favors osteoblast differentiation (Mackie & Ramsey, 1996) its presence may be important in bone remodeling (Webb et al., 1997). A special feature of tenascin-C is its appearance under pathological conditions including cancer, wounds, and inflammatory conditions (for reviews see Jones & Jones, 2000; Chiquet-Ehrismann & Chiquet, 2003).

Tenascin-R is exclusively expressed in the central nervous system where it is mainly secreted by oligodendrocytes. Tenascin-X is located in dermis and skeletal muscle while tenascin-W is most prominent in the skeleton, intestinal smooth muscle and in the kidney. Interestingly, tenascin-W is also expressed in the limbus, the source of corneal stem cells (Scherberich et al., 2004).

3. Functions

The most studied function of tenascins is their modulation of cell adhesion and cell spreading. It was found early on that tenascin-C inhibited cell adhesion to fibronectin and promoted growth of tumor cells (Chiquet-Ehrismann, Mackie, Pearson, & Sakakura, 1986). Recently, a mechanism of action was described. It was found that cell spreading was inhibited through the binding of tenascin-C to the XIIIth fibronectin-type III repeat of fibronectin. This repeat is required for fibronectin's interaction with syndecan-4 to promote full cell spreading. This inhibition of syndecan-4 binding to fibronectin results in increased growth of tumor cells (Huang, Chiquet-Ehrismann, Moyano, Garcia-Pardo, & Orend, 2001) but suppresses proliferation of normal cells (Orend, Huang, Olayioye, Hynes, & Chiquet-Ehrismann, 2003).

Several intracellular signalling systems have been proposed to be affected by the interaction of cells with tenascin-C (Fig. 2). If cells are plated on tenascin-C coated substrata they either remain rounded or extend actin-rich, fascin-containing protrusions but do not spread and form stress fibers as on fibronectin (Fischer, Tucker, Chiquet-Ehrismann, & Adams, 1997). In the context of a three-dimensional matrix consisting of fibrinogen and fibronectin it was shown that tenascin-C suppresses focal adhesion kinase and RhoA activity (Midwood & Schwarzbauer, 2002). These are clearly key molecules regulating cell shape through their influence on the actin cytoskeleton and may thus be important transducers of the adhesion-modulating activities of tenascins. Recently it was shown that cGMP-dependent protein kinase phosphorylates the actin-binding protein LASP thereby influencing cytoskeletal organization and cell motility (Butt et al., 2003). Since cGMP-dependent protein kinase activity was shown to be required for tenascin-C mediated focal adhesion disassembly (Murphy-Ullrich et al., 1996) LASP phosphorylation

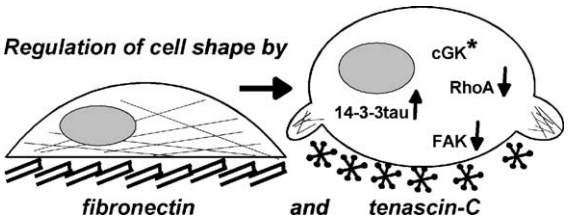

Fig. 2. Tenascin-C signaling and cell shape. This diagram summarizes the effect of a fibronectin substratum (disulfide-linked dimeric molecules assembled into fibrils underneath cell on the left) and a tenascin-C substratum (six-armed molecules underneath cell on the right) on cell shape, the actin cytoskeleton (fine lines within cells) and intracellular signaling molecules induced or suppressed by tenascin-C. Whereas, cells on fibronectin are flat and assemble a network of actin stress fibers, cells on tenascin-C are rounded and extend actin-rich processes. The expression of 14-3-3 tau is elevated on a tenascin-C substratum, the activities of focal adhesion kinase (FAK) and RhoA are repressed and cGMP-dependent protein kinase activity (cGK*) is required.

may represent an intracellular mechanism mediating this effect. Finally, the upregulation of phosphoserine/threonine binding adapter protein 14-3-3 tau by cells plated on tenascin-C could represent a further intracellular signal with the potential of interaction with many signalling pathways (Martin, Brown-Luedi, & Chiquet-Ehrismann, 2003). Each of these signalling molecules could in addition take part in the propagation of the reported effects of tenascin-C on DNA replication. Another direct effect of tenascin-C on proliferation has been proposed through direct binding and activation of the EGF-receptor by tenascin-C (Swindle et al., 2001). From all these reports it becomes clear that the cell type used for the studies as well as the experimental context will influence the cellular responses observed as summarized in Orend and Chiquet-Ehrismann (2000).

In the nervous system tenascin-C and tenascin-R have been shown to interact with neuronal membrane proteins such as the Ig-CAM F3/F11/contactin (Zacharias, Norenberg, & Rathjen, 1999) and the beta2 subunit of the voltage-gated sodium channel and may therefore affect channel localization and function (Srinivasan, Schachner, & Catterall, 1998). The phenotypes of tenascin knockout mice are surprisingly mild; however, mice deficient in tenascin-C (Evers et al., 2002) or tenascin-R indeed do show neuronal deficits (Montag-Sallaz & Montag, 2003). Furthermore, tenascin-C seems to be required for correct reinnervation of skeletal muscles (Cifuentes-Diaz et al., 2002). Mice deficient in tenascin-X have a hyperstretchable skin accompanied by reduced amounts of collagen fibers (Mao et al., 2002). This mimics the Ehlers Danlos phenotype observed in humans deficient in tenascin-X (see below).

4. Associated pathologies

A recessive form of Ehlers Danlos syndrome is caused by a deficiency in tenascin-X (Schalkwijk et al., 2001). These patients suffer from hypermobile joints, hyperelastic skin, and easy bruising. Ehlers Danlos syndrome is a heritable connective tissue disorder generally characterized by defects in fibrillar collagen structure and/or metabolism. Frequently it is a dominant disease caused by mutations in the type V collagen gene. Other forms include mutations in type III collagen, deficient processing of type I collagen or reduced crosslinking of collagens due to deficiencies in the enzyme lysyl hydroxylase. All of these mutations result in reduced amounts of collagen fibers in the skin, in tendons and other connective tissues leading to their reduced stability and hyperstretchability. The mechanism of how a deficiency in tenascin-X affects collagen deposition in these connective tissues is to date unknown.

In the adult, tenascin-C expression is associated with pathological conditions such as cancer, wound healing and inflammatory diseases as reviewed in Chiquet-Ehrismann and Chiquet (2003). In all these situations tenascin-C could have similar effects on the cells. Probably tenascin-C promotes the migration of the wound epithelial cells to close the injury and increases infiltration of carcinoma cells from a tumor into the surrounding tissue. Furthermore, the immune system is also affected by tenascin-C which was shown to inhibit T-cell proliferation and to interfere with leukotriene B4 induced chemotaxis of PMNs and monocytes (for references see Chiquet-Ehrismann & Chiquet, 2003). These effects of tenascin-C could be beneficial in the case of injuries but detrimental in the process of carcinogenesis.

The presence of tenascin-C in many cancer tissues including difficult to operate brain tumors has made this molecule a promising target for in vivo diagnosis as well as for therapy through antibody-mediated localization of radionuclides (for references see Chiquet-Ehrismann & Chiquet, 2003). Furthermore, strategies are being developed to use peptides from tenascin-C and other ECM proteins to function as potent therapeutic reagents to increase neuronal regeneration following central nervous system injury (Meiners & Mercado, 2003).

In summary, we have seen that tenascins add to connective tissue function in many different ways. Though we have started to have a glimpse at the cell physiological effects mediated by tenascins the details and exact mechanisms of action remain to be clarified.

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