Regulation of fibroblast migration by tenascin-C

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Abstract

Synthesis of new tissue by fibroblasts is required for tissue rebuilding in response to injury. Fibroblast migration from surrounding healthy tissue into the fibrin–fibronectin provisional matrix deposited upon injury is a key rate-limiting step of this stage of tissue repair. These events must be tightly regulated. Excessive deposition of scar tissue is the major hallmark of fibrotic disease. Tenascin-C is an extracellular matrix glycoprotein that is transiently expressed upon tissue injury, where it is specifically localized to the wound edge, and persistently up-regulated in fibrotic disease. We have shown that full-length tenascin-C promotes fibroblast migration within fibrin–fibronectin matrices and we have mapped the domains within the molecule critical for enhancing migration. We also demonstrated that specific fragments of tenascin-C inhibit fibroblast migration. These results suggest that transient expression of tenascin-C at the wound boundary is key to tissue repair: its induction recruits fibroblasts into the wound and fragments resulting from its breakdown prevent excessive fibroblast infiltration. Our results demonstrate how fibroblast migration in three-dimensional provisional matrices may be differentially regulated by proteolysis of matrix molecules and could explain how persistent expression of tenascin-C contributes to the progression of fibrotic disease.

Fibroblasts mediate tissue rebuilding after injury

An effective response to tissue injury, for example following trauma, degenerative conditions or age-related disease, is essential to restore structural integrity and to maintain functionality. Immediately upon injury, a provisional ECM (extracellular matrix), consisting predominantly of fibrin and plasma fibronectin, is synthesized. Deposition of this matrix temporarily fills the wound bed and prevents excessive blood loss by acting as a haemostatic plug. At approx. 4 days following injury, fibroblasts in healthy tissue adjacent to the wound proliferate and migrate into the provisional matrix. Fibroblasts adhere to the three-dimensional fibrin scaffold via interactions with $\alpha v\beta 3$ integrin cell surface receptors [1]. Cell binding to distinct domains of fibronectin via $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrins, syndecan-4 and CD44, and $\alpha 4\beta 1$ integrins are essential for fibroblast migration into the provisional matrix [2]. Once in the wound bed, fibroblasts synthesize new matrix to replace that which has been lost or damaged. They initially assemble fibronectin-rich granulation tissue upon the framework of the provisional matrix. This in turn forms a scaffold for the subsequent deposition of collagen types I and III. The resultant scar tissue is remodelled and collagen cross-linked over the following years, although it fails to exactly recapitulate the cellularity, vascularity and tensile strength of the original tissue. Fibroblast infiltration into the provisional matrix-filled wound bed is the key rate-limiting step of tissue rebuilding after injury: too little results in reduced granulation tissue formation characteristic of chronic non-healing wounds; too much causes excessive deposition of scar tissue, which is the major hallmark of fibrotic disease [3]. Thus it is vital that fibroblast migration on fibrin–fibronectin matrices is tightly regulated.

Key words: extracellular matrix, fibroblast migration, fibrosis, provisional matrix, tenascin-C, tissue repair.

Abbreviations used: ECM, extracellular matrix; EGF-L, epidermal growth factor-like repeats; TNIII, fibronectin type III-like repeats.

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Tenascin-C is transiently expressed upon tissue injury

Tenascin-C is a large hexameric ECM glycoprotein that is specifically expressed upon tissue damage, where it is up-regulated within 24 h of injury. Expression of tenascin-C is down-regulated and protein cleared from the tissue by the time repair is complete. Persistent expression of tenascin-C is associated with fibrotic diseases such as pulmonary fibrosis, scleroderma and liver cirrhosis, and with chronic non-healing wounds (for a detailed review of tenascin-C expression see [4]).

At sites of tissue injury tenascin-C is specifically localized to the wound edge under migrating keratinocytes and fibroblasts [5]. Tenascin-C-null mice exhibit reduced fibronectin deposition in corneal suture wounds. These wounds are also devoid of migrating keratinocytes [6]. Furthermore, mice that lack tenascin-C exhibit reduced inflammatory cell and myofibroblast infiltration in concanavalin A-induced hepatic fibrosis [7]. These results indicate that tenascin-C promotes cell migration upon injury. However, tenascin-C appears to act in a tissue- and injury-specific manner. Tenascin-C-null mice exhibit prolonged infiltration of polymorphonuclear cells in dermatitis induced by 2,4-dinitrofluorobenzene compared with wild-type, but not in dermatitis induced by PMA [8].

Tenascin-C also has diverse effects on cell migration *in vitro* that reflect differences in cell type, species and the

Figure 1 | Tenascin-C stimulates cell migration within provisional matrices

Human adult dermal fibroblast-coated beads were cultured within three-dimensional matrices comprising fibrin, fibrin–fibronectin (fibrin-FN) or fibrin–fibronectin plus tenascin-C (fibrin-FN+TN-C). Cells exhibited little cell migration in the absence of fibronectin. The addition of tenascin-C further stimulated cell migration.

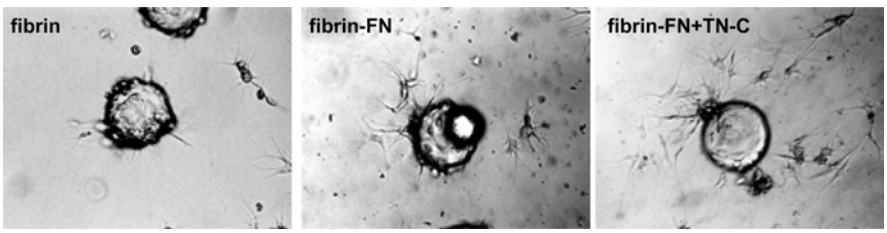
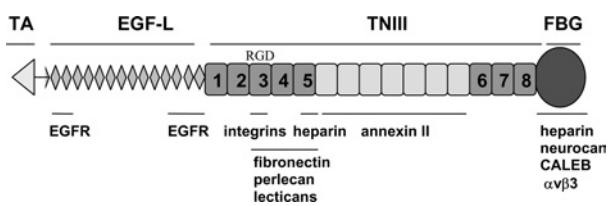


Figure 2 | The domain structure of tenascin-C

Each tenascin-C monomer comprises a tenascin-C assembly domain (TA), EGF-L, TNIII and the fibrinogen globe (FBG). Some of the known ligands for each domain, including cell surface receptors and other ECM molecules, are shown.



assay used in each study. Human corneal fibroblasts migrate on tenascin-C substrates, but soluble tenascin-C added to fibroblasts on collagen substrates inhibited migration [9]. Tenascin-C also inhibited chemotaxis of human monocytes and polymorphonuclear leucocytes through three-dimensional collagen or fibrin gels [10], but addition of soluble tenascin-C to glioma cells plated on fibronectin stimulated migration [11]. While these results indicate that tenascin-C has a role in regulating cell migration, its precise function at sites of tissue injury remains unclear.

Tenascin-C and tenascin-C fragments differentially regulate fibroblast migration

Our aim was to investigate the role of tenascin-C in regulating fibroblast infiltration in the provisional matrix deposited upon tissue injury. We cultured beads coated with human adult dermal fibroblasts within three-dimensional fibrin–fibronectin matrices in the presence or absence of tenascin-C. Little migration occurred in fibrin matrices alone. Cell migration was enhanced in fibrin–fibronectin matrices. The presence of full-length tenascin-C in the fibrin–fibronectin matrix further promoted fibroblast migration (Figure 1).

Tenascin-C is a large hexameric molecule, comprising multiple domains. Each domain binds to different cell surface receptors, including integrins, and ECM components, such as fibronectin (Figure 2). To identify which domain(s) of tenascin-C are critical for promoting fibroblast migration we synthesized recombinant proteins comprising each domain of the molecule. We found that one fragment within the TNIII (fibronectin type III-like repeats) promoted migration to the same extent as full-length tenascin-C, while another region of these repeats inhibited fibroblast migration almost completely.

Proteolytic regulation of tenascin-C function

These results suggest that inhibitory domains within TNIII are inaccessibly orientated in hexameric, full-length tenascin-C and/or that their inhibitory activity is attenuated by adjacent domains. Degradation of tenascin-C may create fragments with distinct activities from the full-length molecule by exposing such cryptic domains. Fragments of tenascin-C have been observed, for example in ruptured tendons [12] and in atherosclerotic plaques [13]. Furthermore, fragments comprising the EGF-L (epidermal growth factor-like repeats) domain of tenascin-C promote apoptosis of smooth-muscle cells *in vitro*, whereas full-length tenascin-C does not [13].

Upon injury, induction of tenascin-C may promote fibroblast infiltration into the provisional matrix. This may explain the reduced fibronectin deposition in corneal suture wounds of tenascin-C-null mice [6]. Proteolysis of tenascin-C at the end of repair may create fragments that inhibit further fibroblast migration. In fibrotic diseases expression of tenascin-C is not down-regulated, nor does it appear to be degraded [14] resulting in high tissue levels of intact tenascin-C. Conversely, high levels of tenascin-C fragments are detected in wound exudates of chronic diabetic ulcers [15]. This differential proteolysis of tenascin-C may promote prolonged fibrosis or contribute to reduced granulation tissue formation by positively or negatively regulating fibroblast migration respectively.

Perspectives

Together, these results suggest that tenascin-C plays a key role in regulating fibroblast migration during the response to injury and indicate that its expression and degradation must be tightly controlled in order to ensure effective tissue rebuilding. Upon injury, fibroblasts must detach from the collagen-rich matrices of healthy tissue and adhere to and invade the fibrin–fibronectin provisional matrix. Potential

mechanisms of tenascin-C and tenascin-C fragment action may include modulation of cell adhesion, cytoskeleton organization and cell contractility, as well as regulation of protease activation and ECM degradation. Understanding more about these underlying mechanisms may have implications for the management of chronic wounds and treatment of fibrotic diseases.

References

1 Gailit, J., Clarke, C., Newman, D., Tonnesen, M.G., Mosesson, M.W. and Clark, R.A. (1997) Exp. Cell Res. **232**, 118–126
2 Ghosh, K., Ren, X.D., Shu, X.Z., Prestwich, G.D. and Clark, R.A. (2006) Tissue Eng. **12**, 601–613
3 Diegelmann, R.F. and Evans, M.C. (2004) Front. Biosci. **9**, 283–289
4 Chiquet-Ehrismann, R. and Chiquet, M. (2003) J. Pathol. **200**, 488–499
5 Betz, P., Nerlich, A., Tubel, J., Penning, R. and Eisenmenger, W. (1993) Int. J. Legal Med. **105**, 325–328
6 Matsuda, A., Yoshiki, A., Tagawa, Y., Matsuda, H. and Kusakabe, M. (1999) Invest. Ophthalmol. Visual Sci. **40**, 1071–1080
7 El-Karef, A., Yoshida, T., Gabazza, E.C., Nishioka, T., Inada, H., Sakakura, T. and Imanaka-Yoshida, K. (2007) J. Pathol. **211**, 86–94
8 Koyama, Y., Kusubata, M., Yoshiki, A., Hiraiwa, N., Ohashi, T., Irie, S. and Kusakabe, M. (1998) J. Invest. Dermatol. **111**, 930–935
9 Schmidinger, G., Hanselmayer, G., Pieh, S., Lackner, B., Kaminski, S., Ruhswurm, I. and Skorpik, C. (2003) J. Cataract Refract. Surg. **29**, 354–360
10 Loike, J.D., Cao, L., Budhu, S., Hoffman, S. and Silverstein, S.C. (2001) J. Immunol. **166**, 7534–7542
11 Deryugina, E.I. and Bourdon, M.A. (1996) J. Cell Sci. **109**, 643–652
12 Riley, G.P., Harrall, R.L., Cawston, T.E., Hazleman, B.L. and Mackie, E.J. (1996) Am. J. Pathol. **149**, 933–943
13 Wallner, K., Li, C., Shah, P.K., Wu, K.J., Schwartz, S.M. and Sharifi, B.G. (2004) Arterioscler. Thromb. Vasc. Biol. **24**, 1416–1421
14 Tourkina, E., Hoffman, S., Fenton, II, J.W., Lipsitz, S., Silver, R.M. and Ludwicka-Bradley, A. (2001) Arthritis Rheum. **44**, 1370–1381
15 Latijnhouwers, M.A., Bergers, M., Veenhuis, R.T., Beekman, B., Ankersmit-Ter Horst, M.F. and Schalkwijk, J. (1998) Arch. Dermatol. Res. **290**, 490–496

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