Elastic Fibre Proteins in Wound Healing

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As essential components of people's connective tissues, elastic fibres give tissues such as major blood vessels, skin and the lungs their elasticity. In humans, the fibrillin family is composed of three highly conserved proteins, fibrillin-1, -2 and -3, all of which are engaged in the formation of microfibrils. Fibrillin-2 and -3 are mainly expressed in fetal tissues, while fibrillin-1 is continuously expressed throughout adulthood in tissues such as the heart, aorta, lung, nervous system and skin. Mutations in the FBN1 gene, which encodes fibrillin-1, are associated with MFS, isolated autosomal dominant ectopia lentis 1, mitral valve-aorta-skeleton-skin (MASS) syndrome, Weill–Marchesani syndrome (WMS), stiff skin syndrome, acromicric and geleophysic dysplasias and Marfanoid-progeroid-lipodystrophy syndrome.

elastic fibre proteins wound healing fibrillin-1 LTBP4 tropoelastin TGFβ signalling integrin

1. Introduction

Elastic fibres endow tissues and organs with elasticity and extendibility in response to mechanical forces. Aberrant formation and destruction of elastic fibres leads to many diseases, such as Marfan syndrome (MFS) [1], cutis laxa and aneurysms [2]. Elastic fibres are formed predominantly from elastin and fibrillin microfibrils [3]. Elastic fibre proteins guide and facilitate elastogenesis, where tropoelastin globules are deposited on a fibrillin microfibril scaffold, a process which is facilitated by fibulin-4 and -5 and latent TGFβ-binding protein (LTBP)-4. In addition to elastogenesis, elastic fibre proteins have been implicated in wound healing: for instance, in keloid disease and hypertrophic scarring, disorganised and reduced elastin and fibrillin has been observed [4][5]. Furthermore, elastic fibre proteins are important players in regulating TGFβ signalling [6] and integrin-mediated cell attachment and spreading, which can further contribute to wound healing.

2. The Role of Elastic Fibre Proteins in Wound Repair

In addition to their role in elastogenesis, there is increasing evidence demonstrating the importance of these elastic fibre proteins in wound repair. In a periodontal disease model, fibrillin-1 expression was strongly elevated at the beginning of the destruction of periodontal tissue, but decreased with wound healing [7]. This decrease in fibrillin-1 expression during wound healing has been associated with the differentiation of fibroblasts to myofibroblasts in dental pulp healing [8]. Overexpression of fibulin-5 in a dermal ulcer model showed that fibulin-5 expression facilitates wound healing in vivo [9]. Numerous reports have demonstrated the role of tropoelastin in the

inflammation and proliferation stages of wound healing; for example, tropoelastin induced transient expression of chemokines, which are necessary for tissue recovery [10]. Elastic fibre proteins are also important for the extracellular regulation of TGFβ, an important mediator of wound healing [11]. Thus, in the following section, researchers review the role of elastic fibre proteins in TGFβ sequestration and activation had reviewed.

2.1. Elastic Fibre Proteins and TGFβ Signalling

TGFβ is secreted as a large latent complex (LLC) covalently linked to members of the LTBP family. A disulphide bond is formed between LTBP1, 3 and 4 and the TGFβ pro-domain (latency-associated peptide (LAP)), and the LLC then deposits into the extracellular matrix via the interactions between LTBPs and fibrillin and fibronectin [12]. LTBPs influence TGFβ signalling by at least two mechanisms: promoting effective secretion of latent TGFβ from cells [13][14], and the localisation of latent TGFβ in the matrix [12]. LTBP4 interacts with different isoforms of TGFβ (TGFβ1, β2, β3), and two different LTBP4 SNPs enhance and reduce TGFβ signalling, respectively [15]. Co-immunoprecipitation showed an interaction between LTBP4 and the TGFβ receptor 2, and knock-down of LTBP4 reduced the expression of TGFβ receptor 2 and signalling [16]. Lu et al. found that knock-down of LTBP4 in systemic scleroderma skin fibroblasts reduced the extracellular level of TGFβ and the downstream targets of TGFβ signalling [17].

Integrins are activators of TGFβ by binding to and unfolding LAP to release mature TGFβ from the latent complex to enable TGFβ receptor binding [18]. Binding of LAP to LTBP is required to provide resistance to the pulling force [19]. Recently, Campbell et al. also showed by cryo-EM that αvβ8 could activate latent TGFβ without releasing mature TGFβ from the latent complex [20]. Fibrillin-1 has been linked to the regulation and bioavailability of TGFβ in the extracellular matrix via direct interaction with LTBP1 and LTBP4 and via stabilising the LLC [21][22]. Although the mechanisms are not fully elucidated, many studies support a role for fibrillin-1 in TGFβ sequestration. For example, fibrillin-1 mutations are associated with MFS, which is linked to an increase in TGFβ activation in connective tissues [22], and osteoblasts from $Fbn1^{-/-}$ mice have more activated TGFβ [23]. In addition, fibrillin-1 was found to influence pSmad2-dependent TGFβ signalling via regulating the expression of miR-503 in fibroblasts [24].

In fibulin-4-deficient aortic smooth muscle cells, elevated TGFβ signalling was observed due to increased levels of TGFβ2 [25]. Interestingly, Burger et al. found that in vascular smooth muscle cells, reduced fibulin-4 expression enhanced the activation of TGFβ, but there was no change in TGFβ signalling when fibulin-4 was absent [26]. Fibulin-5 expression is reported to be regulated by TGFβ in fibroblasts and mammary epithelial cells [27][28][29][30], and fibulin-5 overexpression in 3T3-L1 cells elevated the TGFβ-stimulated activation of ERK1/ERK2 and p38 MAPK [30], indicating that fibulin-5 is also involved in TGFβ signalling.

2.2. Role of Elastic Fibre Proteins Supporting Integrin-Mediated Cell Adhesion

In addition to their role in supporting TGFβ secretion and activation, elastic fibre proteins support integrin-mediated cell adhesion. Integrins αvβ3, α5β1, αvβ6, α8β1, αvβ6, αvβ1 and αvβ5 can bind to the TB4 domain of fibrillin-1 via an RGD sequence in cell-based assays or protein–protein interaction analyses [31][32][33][34][35]. In addition, fibrillin-1

was found to influence integrin-mediated focal adhesion formation by regulating the expressions of miR-612 and miR-3185 in fibroblasts [24]. Bax et al. found that the C-terminal GRKRK sequence in tropoelastin supports cell adhesion via interaction with αvβ3 [36]. The same group also found that αvβ5 can interact with the central region of tropoelastin to mediate cell adhesion [37], and Bochicchio et al. found that domains 12 to 16 of tropoelastin can interact with integrins αv and α5β1, thus promoting cell spreading and attachment [38]. Modelling data linked these findings to show that different regions on tropoelastin bind to multiple sites on integrin αvβ3 to co-operatively support signalling [39]. Fibulin-5 binds human smooth muscle cells (SMC) via integrins α5β1 and α4β1, and influences SMC proliferation and migration, but does not support the activation of receptor tyrosine kinases [40]. In addition, Furie et al. found that fibulin-5 binds to keloid-derived fibroblast-like cells (FLC) and regulates FLC adhesion and proliferation through integrin β1 [41].

Collectively, elastic fibre proteins play an important role in wound healing via regulating the deposition and activation of TGFβ and supporting integrin-mediated cell adhesion, as shown in **Figure 1**.

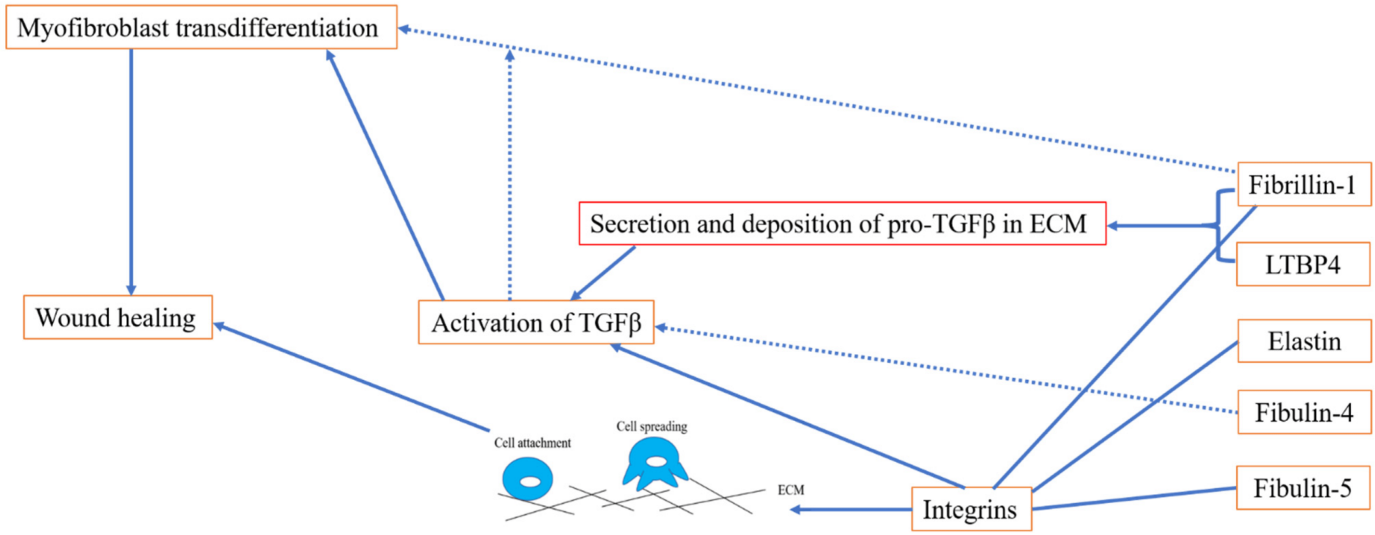

Figure 1. Diagram of elastic fibre proteins in wound healing. Deposition and sequestration of pro-TGFβ in the ECM is crucial for the proper regulation of TGFβ via fibrillin-1 and LTBP4. In addition, fibrillin-1 may be involved in myofibroblast transdifferentiation in a TGFβ-dependent way. Fibrillin-1, tropoelastin and fibulin-5 are also involved in the process of wound repair by regulating cell adhesion via integrins.

3. Perspectives

Although the roles of fibrillin-1, tropoelastin, LTBP4, fibulin-4 and -5 in elastogenesis have been widely studied, many scientific questions remain to be elucidated. Deciphering whether interactions between LTBP4 and tropoelastin support either elastogenesis or LTBP4-mediated TGFβ signalling in wound healing, and the role fibrillin plays in these processes, are of great significance in tissue regeneration and elastic fibre diseases. Additionally, deciphering the order and hierarchy of interactions between all the elastic fibre proteins is important to understand the sequence of events and molecular requirements for elastogenesis. Considering the importance of

myofibroblasts in wound healing, exploring the detailed molecular mechanisms of how elastic fibre proteins influence myofibroblast differentiation may provide opportunities for novel therapeutics for wound repair. For instance, elucidating whether changes in the expression of elastic fibre proteins or dysfunction of elastic fibres in scar tissue alters their biomechanical properties, such as contractility, to negatively influence myofibroblast differentiation would be an important future research direction.

References

1. Ramirez, F.; Caescu, C.; Wondimu, E.; Galatioto, J. Marfan syndrome; A connective tissue disease at the crossroads of mechanotransduction, TGFbeta signaling and cell stemness. Matrix. Biol. 2018, 71–72, 82–89.
2. Halabi, C.M.; Kozel, B.A. Vascular elastic fiber heterogeneity in health and disease. Curr. Opin. Hematol. 2020, 27, 190–196.
3. Thomson, J.; Singh, M.; Eckersley, A.; Cain, S.A.; Sherratt, M.J.; Baldock, C. Fibrillin microfibrils and elastic fibre proteins: Functional interactions and extracellular regulation of growth factors. Semin. Cell Dev. Biol. 2019, 89, 109–117.
4. Ghazawi, F.M.; Zargham, R.; Gilardino, M.S.; Sasseville, D.; Jafarian, F. Insights into the pathophysiology of hypertrophic scars and keloids: How do they differ? Adv. Skin Wound Care 2018, 31, 582–595.
5. Jumper, N.; Paus, R.; Bayat, A. Functional histopathology of keloid disease. Histol. Histopathol. 2015, 30, 11033–11057.
6. Godwin, A.; Singh, M.; Lockhart-Cairns, M.P.; Alanazi, Y.F.; Cain, S.A.; Baldock, C. The role of fibrillin and microfibril binding proteins in elastin and elastic fibre assembly. Matrix. Biol. 2019, 84, 17–30.
7. Handa, K.; Abe, S.; Suresh, V.V.; Fujieda, Y.; Ishikawa, M.; Orimoto, A.; Kobayashi, Y.; Yamada, S.; Yamaba, S.; Murakami, S.; et al. Fibrillin-1 insufficiency alters periodontal wound healing failure in a mouse model of Marfan syndrome. Arch. Oral. Biol. 2018, 90, 53–60.
8. Yoshiba, N.; Yoshiba, K.; Ohkura, N.; Takei, E.; Edanami, N.; Oda, Y.; Hosoya, A.; Nakamura, H.; Okiji, T. Correlation between Fibrillin-1 Degradation and mRNA Downregulation and Myofibroblast Differentiation in Cultured Human Dental Pulp Tissue. J. Histochem. Cytochem. 2015, 63, 438–448.
9. Lee, M.J.; Roy, N.K.; Mogford, J.E.; Schiemann, W.P.; Mustoe, T.A. Fibulin-5 promotes wound healing in vivo. J. Am. Coll. Surg. 2004, 199, 403–410.

10. Almine, J.F.; Wise, S.G.; Hiob, M.; Singh, N.K.; Tiwari, K.K.; Vali, S.; Abbasi, T.; Weiss, A.S. Elastin sequences trigger transient proinflammatory responses by human dermal fibroblasts. FASEB J. 2013, 27, 3455–3465.
11. Margadant, C.; Sonnenberg, A. Integrin-TGF-beta crosstalk in fibrosis, cancer and wound healing. EMBO Rep. 2010, 11, 97–105.
12. Zilberberg, L.; Todorovic, V.; Dabovic, B.; Horiguchi, M.; Couroussé, T.; Sakai, L.Y.; Rifkin, D.B. Specificity of latent TGF-beta binding protein (LTBP) incorporation into matrix: Role of fibrillins and fibronectin. J. Cell. Physiol. 2012, 227, 3828–3836.
13. Miyazono, K.; Olofsson, A.; Colosetti, P.; Heldin, C.H. A role of the latent TGF-beta 1-binding protein in the assembly and secretion of TGF-beta 1. EMBO J. 1991, 10, 1091–1101.
14. Annes, J.P.; Chen, Y.; Munger, J.S.; Rifkin, D.B. Integrin alphaVbeta6-mediated activation of latent TGF-beta requires the latent TGF-beta binding protein-1. J. Cell. Biol. 2004, 165, 723–734.
15. Lamar, K.M.; Miller, T.; Dellefave-Castillo, L.; McNally, E.M. Genotype-Specific Interaction of Latent TGFbeta Binding Protein 4 with TGFbeta. PLoS ONE 2016, 11, e0150358.
16. Su, C.T.; Huang, J.W.; Chiang, C.K.; Lawrence, E.C.; Levine, K.L.; Dabovic, B.; Jung, C.; Davis, E.C.; Madan-Khetarpal, S.; Urban, Z. Latent transforming growth factor binding protein 4 regulates transforming growth factor beta receptor stability. Hum. Mol. Genet. 2015, 24, 4024–4036.
17. Lu, J.; Liu, Q.; Wang, L.; Tu, W.; Chu, H.; Ding, W.; Jiang, S.; Ma, Y.; Shi, X.; Pu, W.; et al. Increased expression of latent TGF-beta-binding protein 4 affects the fibrotic process in scleroderma by TGF-beta/SMAD signaling. Lab. Investig. 2017, 97, 591–601.
18. Shi, M.; Zhu, J.; Wang, R.; Chen, X.; Mi, L.; Walz, T.; Springer, T.A. Latent TGF-beta structure and activation. Nature 2011, 474, 343–349.
19. Buscemi, L.; Ramonet, D.; Klingberg, F.; Formey, A.; Smith-Clerc, J.; Meister, J.J.; Hinz, B. The single-molecule mechanics of the latent TGF-beta1 complex. Curr. Biol. 2011, 21, 2046–2054.
20. Campbell, M.G.; Cormier, A.; Ito, S.; Seed, R.I.; Bondesson, A.J.; Lou, J.; Marks, J.D.; Baron, J.L.; Cheng, Y.; Nishimura, S.L. Cryo-EM Reveals Integrin-Mediated TGF-beta Activation without Release from Latent TGF-beta. Cell 2020, 180, 490–501.e16.
21. Isogai, Z.; Ono, R.N.; Ushiro, S.; Keene, D.R.; Chen, Y.; Mazzieri, R.; Charbonneau, N.L.; Reinhardt, D.P.; Rifkin, D.B.; Sakai, L.Y. Latent transforming growth factor beta-binding protein 1 interacts with fibrillin and is a microfibril-associated protein. J. Biol. Chem. 2003, 278, 2750–2757.
22. Neptune, E.R.; Frischmeyer, P.A.; Arking, D.E.; Myers, L.; Bunton, T.E.; Gayraud, B.; Ramirez, F.; Sakai, L.Y.; Dietz, H.C. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat. Genet. 2003, 33, 407–411.

23. Nistala, H.; Lee-Arteaga, S.; Smaldone, S.; Siciliano, G.; Carta, L.; Ono, R.N.; Sengle, G.; Arteaga-Solis, E.; Levasseur, R.; Ducy, P.; et al. Fibrillin-1 and -2 differentially modulate endogenous TGF-beta and BMP bioavailability during bone formation. J. Cell. Biol. 2010, 190, 1107–1121.
24. Zeyer, K.A.; Zhang, R.M.; Kumra, H.; Hassan, A.; Reinhardt, D.P. The Fibrillin-1 RGD Integrin Binding Site Regulates Gene Expression and Cell Function through microRNAs. J. Mol. Biol. 2019, 431, 401–421.
25. Ramnath, N.W.; Hawinkels, L.J.; van Heijningen, P.M.; te Riet, L.; Paauwe, M.; Vermeij, M.; Danser, A.H.; Kanaar, R.; ten Dijke, P.; Essers, J. Fibulin-4 deficiency increases TGF-beta signalling in aortic smooth muscle cells due to elevated TGF-beta2 levels. Sci. Rep. 2015, 5, 16872.
26. Burger, J.; van Vliet, N.; van Heijningen, P.; Kumra, H.; Kremers, G.J.; Alves, M.; van Cappellen, G.; Yanagisawa, H.; Reinhardt, D.P.; Kanaar, R.; et al. Fibulin-4 deficiency differentially affects cytoskeleton structure and dynamics as well as TGFbeta signaling. Cell Signal. 2019, 58, 65–78.
27. Kuang, P.P.; Joyce-Brady, M.; Zhang, X.H.; Jean, J.C.; Goldstein, R.H. Fibulin-5 gene expression in human lung fibroblasts is regulated by TGF-beta and phosphatidylinositol 3-kinase activity. Am. J. Physiol. Cell Physiol. 2006, 291, C1412–C1421.
28. Lee, Y.H.; Albig, A.R.; Regner, M.; Schiemann, B.J.; Schiemann, W.P. Fibulin-5 initiates epithelial-mesenchymal transition (EMT) and enhances EMT induced by TGF-beta in mammary epithelial cells via a MMP-dependent mechanism. Carcinogenesis 2008, 29, 2243–2251.
29. Topalovski, M.; Hagopian, M.; Wang, M.; Brekken, R.A. Hypoxia and Transforming Growth Factor beta Cooperate to Induce Fibulin-5 Expression in Pancreatic Cancer. J. Biol. Chem. 2016, 291, 22244–22252.
30. Schiemann, W.P.; Blobe, G.C.; Kalume, D.E.; Pandey, A.; Lodish, H.F. Context-specific effects of fibulin-5 (DANCE/EVEC) on cell proliferation, motility, and invasion. Fibulin-5 is induced by transforming growth factor-beta and affects protein kinase cascades. J. Biol. Chem. 2002, 277, 27367–27377.
31. Lee, S.S.; Knott, V.; Jovanović, J.; Harlos, K.; Grimes, J.M.; Choulier, L.; Mardon, H.J.; Stuart, D.I.; Handford, P.A. Structure of the integrin binding fragment from fibrillin-1 gives new insights into microfibril organization. Structure 2004, 12, 717–729.
32. Pfaff, M.; Reinhardt, D.P.; Sakai, L.Y.; Timpl, R. Cell adhesion and integrin binding to recombinant human fibrillin-1. FEBS Lett. 1996, 384, 247–250.
33. Bax, D.V.; Bernard, S.E.; Lomas, A.; Morgan, A.; Humphries, J.; Shuttleworth, C.A.; Humphries, M.J.; Kielty, C.M. Cell adhesion to fibrillin-1 molecules and microfibrils is mediated by alpha 5 beta 1 and alpha v beta 3 integrins. J. Biol. Chem. 2003, 278, 34605–34616.

34. Jovanovic, J.; Takagi, J.; Choulier, L.; Abrescia, N.G.; Stuart, D.I.; van der Merwe, P.A.; Mardon, H.J.; Handford, P.A. alphaVbeta6 is a novel receptor for human fibrillin-1. Comparative studies of molecular determinants underlying integrin-rgd affinity and specificity. J. Biol. Chem. 2007, 282, 6743–6751.
35. Del, C.J.; Reed, N.I.; Molnar, K.; Liu, S.; Dang, B.; Jensen, S.A.; DeGrado, W.; Handford, P.A.; Sheppard, D.; Sundaram, A.B. A disease-associated mutation in fibrillin-1 differentially regulates integrin-mediated cell adhesion. J. Biol. Chem. 2019, 294, 18232–18243.
36. Bax, D.V.; Rodgers, U.R.; Bilek, M.M.; Weiss, A. Cell adhesion to tropoelastin is mediated via the C-terminal GRKRK motif and integrin alphaVbeta3. J. Biol. Chem. 2009, 284, 28616–28623.
37. Lee, P.; Bax, D.V.; Bilek, M.M.; Weiss, A.S. A novel cell adhesion region in tropoelastin mediates attachment to integrin alphaVbeta5. J. Biol. Chem. 2014, 289, 1467–1477.
38. Bochicchio, B.; Yeo, G.C.; Lee, P.; Emul, D.; Pepe, A.; Laezza, A.; Ciarfaglia, N.; Quaglino, D.; Weiss, A.S. Domains 12 to 16 of tropoelastin promote cell attachment and spreading through interactions with glycosaminoglycan and integrins alphaV and alpha5beta1. FEBS J. 2021, 288, 4024–4038.
39. Ozsvar, J.; Wang, R.; Tarakanova, A.; Buehler, M.J.; Weiss, A.S. Fuzzy binding model of molecular interactions between tropoelastin and integrin alphaVbeta3. Biophys. J. 2021, 120, 3138–3151.
40. Lomas, A.C.; Mellody, K.T.; Freeman, L.J.; Bax, D.V.; Shuttleworth, C.A.; Kielty, C.M. Fibulin-5 binds human smooth-muscle cells through alpha5beta1 and alpha4beta1 integrins, but does not support receptor activation. Biochem. J. 2007, 405, 417–428.
41. Furie, N.; Shteynberg, D.; Elkhatib, R.; Perry, L.; Ullmann, Y.; Feferman, Y.; Preis, M.; Flugelman, M.Y.; Tzchori, I. Fibulin-5 regulates keloid-derived fibroblast-like cells through integrin beta-1. Int. J. Cosmet. Sci. 2016, 38, 35–40.