Clinical Implications

- In vitiligo, all the skin is affected.
- Modification of pigment distributions in the skin may be associated with other alterations, such as defects in the epidermal barrier.
- Stabilization of the disease, by targeting nonlesional skin, is an additional challenge for improving the benefits of treatment.
- Full-body phototherapy may stabilize the disease by inducing the differentiation of melanocytes, with consequent improvements in cell–cell adhesion.
- Counteracting extracellular and intracellular oxidative stress may contribute to reducing melanocyte detachment and disease progression.

Recently, immunohistochemical examination of E-cadherin in tissue samples collected from patients with vitiligo, after punch grafting, revealed that melanocytes from normally pigmented donor sites may migrate toward lesional skin and repopulate the depigmented areas because of decreased E-cadherin expression (Kovacs *et al.*, 2015). The mechanisms that underly the activation of melanocytes after punch grafting have not been fully explained. Nevertheless, the process might be regulated by epidermal and dermal cells that should be able to manage cell adhesion actively.

Therapeutic options for vitiligo are still limited. Although some mechanisms underlying the interplay between oxidative stress and immunity have been postulated (Richmond *et al.*, 2013), understanding mechanisms that cause oxidative stress could provide valuable information to identify new therapeutic targets. The challenge will be to maintain cells metabolically active as requirement for sustaining the energy demand and coping with oxidative stress. Moreover, the analysis has to be extended to pigmented skin to both identify early events in "silent" vitiligo melanocytes and prevent the spread of the disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Ardigo M, Malizewsky I, Dell'Anna ML *et al.* (2007) Preliminary evaluation of vitiligo using in vivo reflectance confocal microscopy. *J Eur Acad Dermatol Venereol* 21:1344–50

Bellei B, Pitisci A, Ottaviani M *et al.* (2013) Vitiligo: a possible model of degenerative diseases. *PLoS One* 8:e59782

Bertolotti A, Boniface K, Vergier B *et al.* (2014) Type I interferon signature in the initiation of the immune response in vitiligo. *Pigment Cell Melanoma Res* 27:398–407

Cario-André M, Pain C, Gauthier Y *et al.* (2007) The melanocytorrhagic hypothesis of vitiligo tested on pigmented, stressed, reconstructed epidermis. *Pigment Cell Res* 20:385–93

Dell'Anna ML, Picardo M (2006) A review and a new hypothesis for non-immunological pathogenic mechanisms in vitiligo. *Pigment Cell Res* 19:406–11

Denat L, Kadekaro AL, Marrot L *et al.* (2014) Melanocytes as instigators and victims of oxidative stress. *J Invest Dermatol* 134:1512–8

Eleftheriadou V, Thomas K, van Geel N *et al.* (2015) Developing core outcome set for vitiligo clinical trials: international e-Delphi consensus. *Pigment Cell Melanoma Res*

Ezzedine K, Eleftheriadou V, Whitton M *et al.* (2015) Vitiligo. *Lancet*

Ezzedine K, Lim HW, Suzuki T *et al.* (2012) Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res* 25:E1–13

Gauthier Y, Cario-Andre M, Lepreux S *et al.* (2003) Melanocyte detachment after skin friction in non lesional skin of patients with generalized vitiligo. *Br J Dermatol* 148:95–101

Haass NK, Herlyn M (2005) Normal human melanocyte homeostasis as a paradigm for understanding melanoma. *J Invest Dermatol Symp Proc* 10:153–63

Harris JE, Harris TH, Weninger W *et al.* (2012) A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive CD8+T-cell accumulation in the skin. *J Invest Dermatol* 132:1869–76

Jin Y, Birlea SA, Fain PR *et al.* (2012) Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. *Nat Genet* 44:676–80

Kovacs D, Abdel-Raouf H, Al-Khayyat M *et al.* (2015) Vitiligo: characterization of melanocytes in repigmented skin after punch grafting. *J Eur Acad Dermatol Venereol* 29:581–90

Richmond JM, Frisoli ML, Harris JE (2013) Innate immune mechanisms in vitiligo: danger from within. *Curr Opin Immunol* 25:676–82

Schallreuter KU, Salem MA, Gibbons NC *et al.* (2012) Blunted epidermal L-tryptophan metabolism in vitiligo affects immune response and ROS scavenging by Fenton chemistry, part 2: Epidermal H2O2/ONOO(−)–mediated stress in vitiligo hampers indoleamine 2,3-dioxygenase and aryl hydrocarbon receptor-mediated immune response signaling. *FASEB J* 26:2471–85

Wagner RY, Luciani F, Cario-Andre M *et al.* (2015) Altered E-cadherin levels and distribution in melanocytes precedes manifestations of vitiligo. *J Invest Dermatol* 135:1810–20

Wu J, Zhou M, Wan Y *et al.* (2013) CD8+T cells from vitiligo perilesional margins induce autologous melanocyte apoptosis. *Mol Med Rep* 7:237–41

See related letter to the editor on pg 1921

EDA Fibronectin in Keloids Create a Vicious Cycle of Fibrotic Tumor Formation

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During the early phase of wound healing, first plasma fibronectin (FN) and then *in situ* FN are deposited at the site of injury. *In situ* FN—FN made by tissue cells at the injury site—often contains an extra domain A (EDA) insert. Multiple wound-related signal transduction pathways control the deposition of EDA FN, and the EDA insert can in turn trigger pathways that induce inflammation, increased extracellular matrix molecule deposition including FN and collagen, and activation of fibroblasts. Together these pathways can create a vicious cycle that leads to fibrosis or keloid formation.

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FN is an extracellular matrix (ECM) glycoprotein critical for embryogenesis, morphogenesis, and wound repair (Clark, 2014). Although plasma fibronectin (FN), which is synthesized by the liver, circulates in blood as non-complexed molecules in a 1:10 ratio with fibrinogen (Greiling and Clark, 1997), little FN ECM exists in fully developed tissue (Yamada and Clark, 1996). However, tissue injury or inflammation leads to rapid plasma FN deposition in complex with fibrin followed by so-called cell FN deposition by tissue cells a few days later (Yamada and Clark, 1996). Plasma FN exists as 250 kDa glycoprotein consisting of two chains, one with and one without a variable domain (v). Although cell FN comes from the same gene as plasma FN, it contains additional spliced variants with extra domains A and/or B (EDA and/or EDB; Figure 1). Both plasma and cell FN are polymerized into fibrils and deposited into the ECM of most tissues. Most of the secondary structure of FN is organized into individually folded domains based on amino acid homology (Petersen *et al.*, 1983). These domains, termed Type I, II, and III, also represent biologically sites that participate in the formation of matrix fibrils and provide binding sites for cells, other matrix molecules, and growth factors (Schwarzbauer and DeSimone, 2011; Zhu and Clark, 2014). As FN is extremely sensitive to proteolysis, FN must be deposited continuously from blood or from *in situ* tissue cell production to sustain its presence (Deno *et al.*, 1983). With healing of a wound or resolution of inflammation, both plasma FN and cell FN disappear rapidly from the tissue (Welch *et al.*, 1990). Here we focus on FN containing EDA (EDA FN) that has been found to persist in sites of skin fibrosis (Bhattacharyya *et al.*, 2014) and in keloids, as reported in this issue (Andrews *et al.*, 2015).

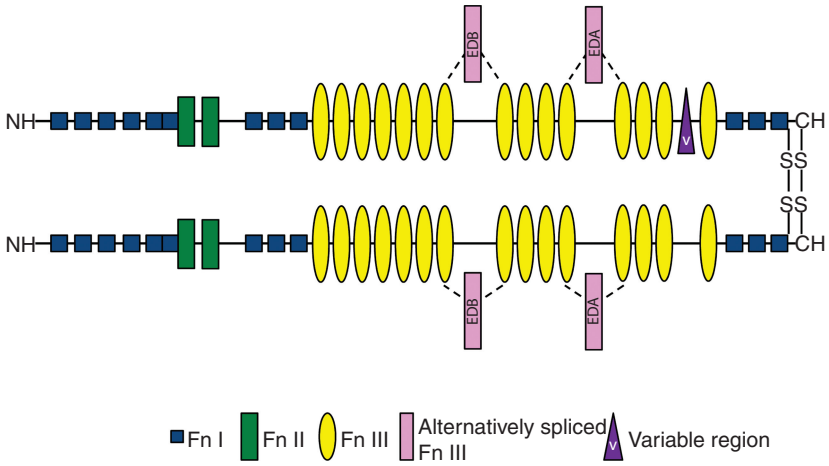

Figure 1. Depiction of the modular structure of fibronectin. Plasma fibronectin consists of two chains, made up of repeating, modular structures: type I (blue), type II (green), and type III (yellow). One chain contains the variable region (purple) and the other does not. Alternative splicing of the gene results in the synthesis of fibronectin that contains the extra domains A (EDA) and B (EDB; pink).

EDA FN inductive pathways

During embryonic development, the EDA domain of FN is highly expressed. However, its expression is transient as it disappears with the increasing age of an organism (Vartio *et al.*, 1987; Pagani *et al.*, 1991). In fact, the EDA domain of FN is largely absent from normal, adult tissue (Oyama *et al.*, 1989). It becomes upregulated, however, under specific circumstances of tissue remodeling, such as during wound repair, tissue injury, inflammation, and fibrosis (ffrench-Constant *et al.*, 1989; Peters *et al.*, 1989; Satoi *et al.*, 2000; Singh *et al.*, 2004; Muro *et al.*, 2008). The mechanisms that trigger upregulation of the EDA FN isoform are both cell type specific and context dependent, making it critical to understand the regulation of this process in response to extracellular signals. The molecular mechanism controlling alternative mRNA splicing of the EDA exon depends on spliceosome assembly and RNA secondary structure, as well as the serine/arginine (SR)-rich family of proteins (Lavigueur *et al.*, 1993; Mermoud *et al.*, 1994 Cramer *et al.*, 1997; Buratti and Baralle, 2004). Growth factors, stress signals, ECM proteins, cytokines, and stiffness have all been connected upstream of these SR proteins, ultimately leading to increased EDA FN expression (Naro and Sette, 2013).

Over two decades ago, transforming growth factor-β (TGF-β) was first identified as a modulator of the EDA isoform levels in fibroblasts, resulting in an increased ratio of EDA to total FN (Balza *et al.*, 1988; Borsi *et al.*, 1990; reviewed in Leask and Abraham (2004)). Differential cell type–specific signaling axes downstream of TGF-β have also been identified in the regulation of EDA FN splicing. In mouse embryonic fibroblasts, it was shown that activation of the PI3K/AKT/mTOR signaling pathway facilitates phosphorylation and activation of the splicing factor SF2/ASF (also known as SRSF1), resulting in the increased expression of EDA FN. It was proposed that the modulation of AKT activity was a direct result of TGF-β-induced downregulation of PTEN (White *et al.*, 2010). These studies suggest that TGF-β family members can control EDA levels by altering activity and/or expression levels of splicing factors.

In addition to TGF-β, growth factors, such as HGF and EGF, as well as stress signals, have been implicated in regulating increased levels of EDA FN (Inoue *et al.*, 1999). Interestingly, it is known that HGF works through the PI3K cascade to increase levels of EDA FN (Magnuson *et al.*, 1991; Seebacher *et al.*, 1988; Blaustein *et al.*, 2004), further implicating this signaling pathway in regulating alternative splicing of FN. Recent evidence suggests that cell–ECM interactions will alter levels of EDA FN. Srebrow *et al.* (2002) demonstrated that a laminin-rich basement membrane can modulate the alternative splicing of EDA. Treatment of fibroblasts with type III and V collagens promoted the splicing and increased assembly of EDA FN (Zoppi *et al.*, 2012). Furthermore, the EDA domain itself has recently been implicated in promoting FN synthesis and fibrillogenesis by dermal fibroblasts (Shinde *et al.*, 2015). Further studies designed to understand the molecular

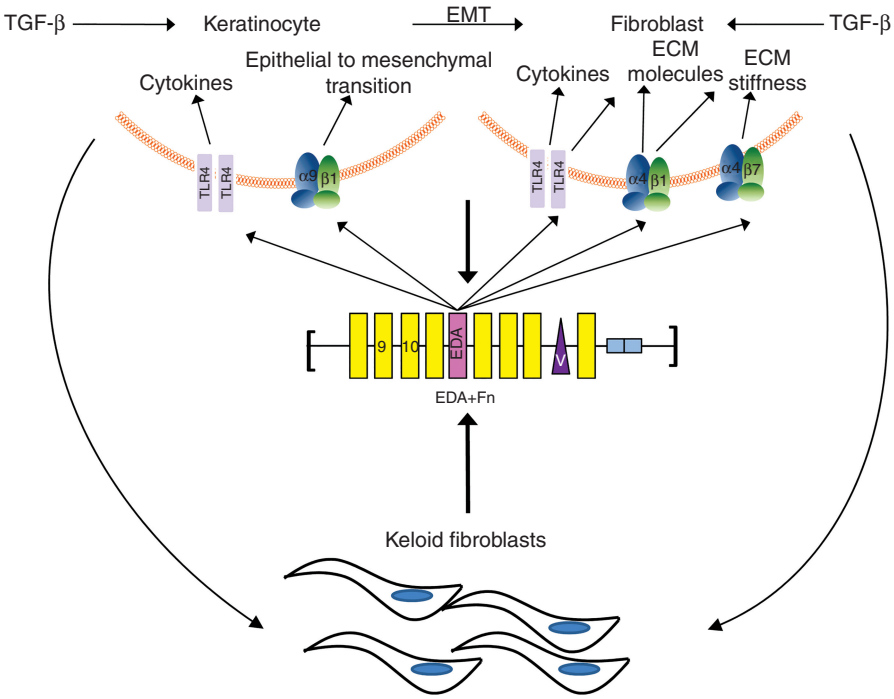

Figure 2. Feed-forward fibrotic loop contributes to Keloid formation. The influence of the pro-fibrotic cytokine, TGF-β, on both epithelial and fibroblast cell types can result in increased synthesis and deposition of EDA FN. Integrin receptors and TLR-4 on the surface of both cell types mediate intracellular signal transduction pathways in response to EDA FN resulting in cytokine production, epithelial–mesenchymal transition (EMT), fibroblast activation, and synthesis of matrix proteins. These cellular outcomes in response to EDA FN all contribute to the fibro-inflammatory environment driving keloid formation. EDA, extra domain A; FN, fibronectin; TGF-β, transforming growth factor-β; TLR-4, toll-like receptor 4.

mechanisms regulating EDA FN splicing should provide important insights into novel strategies for the treatment of fibrosis and excessive tissue remodeling seen in hypertrophy and keloids.

EDA FN triggered pathways

EDA FN and TGF-β, coupled with mechanical stress, are considered the leading influences in the differentiation of fibroblasts into myofibroblasts, a critical cell type in fibrotic disease (Desmouliere *et al.*, 1993; Vyalov *et al.*, 1993; Serini *et al.*, 1998). Animal models of scleroderma, atherosclerosis, cardiac allograft rejection, and adverse airway remodeling have demonstrated that EDA FN promotes the fibrotic phenotypes that drive pathological progression (Tan *et al.*, 2004; Arslan *et al.*, 2011; Kohan *et al.*, 2011; Booth *et al.*, 2012; Bhattacharyya *et al.*, 2014). In addition, EDA FN has been used clinically as an indicator of fibrotic disease and tissue remodeling in humans (van der Straaten *et al.*, 2004; Peters *et al.*, 2011). Recently, EDA FN was shown to increase in skin lesions and serum from patients with scleroderma (Bhattacharyya *et al.*, 2014).

EDA has also recently received attention due to its contribution to pathological inflammation. The major receptor believed to mediate EDA's role in inflammation is the innate immune receptor, toll-like receptor 4 (TLR-4). EDA FN has been shown to activate inflammatory pathways in dermal fibroblasts, mast cells, cytotoxic T Cells, and monocytes via TLR-4 (Okamura *et al.*, 2002; Gondokaryono *et al.*, 2007; Lasarte *et al.*, 2007; McFadden *et al.*, 2011; Kelsh *et al.*, 2014).

> Understanding the molecular pathways involving the expression of EDA fibronectin and its ability to enhance stimulation of those pathways—i.e., a positive feedback loop—it is hoped that new, more effective treatment of keloids can break this vicious cycle.

Other receptors, in addition to TLR-4, have been identified for the EDA domain of FN: integrins α4β1, α9β1, and α4β7 (Liao *et al.*, 2002; Shinde *et al.*, 2008; Kohan *et al.*, 2010). The role of these receptors in EDA-mediated fibrosis and inflammation is still somewhat uncertain. EDA FN was shown to work through α4β1 to promote a contractile phenotype in human dermal fibroblasts (Shinde *et al.*, 2015). EDA has also been shown to mediate TGF-β-dependent myofibroblast differentiation and collagen synthesis *via* the α4β7-dependent activation of the MAPK/ERK1/2 pathway. Integrin α9β1 has also been shown to regulate PI3K/AKT and ERK1/2 activation in response to EDA FN, ultimately leading to epithelial–mesenchymal transition in lung cancer cells (Sun *et al.*, 2014). The role of each of these receptors in the progression of fibrotic disease remains an important question.

Wound repair of full-thickness skin injury ideally ends after a few months with a flat, non-tender scar that is ~70% as strong as normal skin (Gurtner *et al.*, 2011). Hypertrophic scars and keloids,

however, sometimes intervene. These two abnormal responses to wound repair have fundamentally different clinical and histological appearances and distinct clinical outcomes. Hypertrophic scars do not invade, but rather generate tensile stress, on the surrounding tissue, whereas keloids do the opposite (Bolognia *et al.*, 2012). Given these facts it is not surprising that hypertrophic scar fibroblasts, but not keloid fibroblasts, express α-smooth muscle actin, indicative of a myofibroblast phenotype that can generate substantial tensile forces on surrounding ECM (Ehrlich *et al.*, 1994). In contrast, keloid fibroblasts demonstrate a greater propensity to proliferate in serum-free medium (Russell *et al.*, 1988), resist cell apoptosis (Chodon *et al.*, 2000), and have increased sensitivity to TGF-β, which induces synthesis and deposition of ECM molecules including FN and collagen (Babu *et al.*, 1992; Bettinger *et al.*, 1996). In fact, TGF-β1 and β2, the TGF-β isoforms related to increased fibroblast ECM production and tissue fibrosis, are increased in keloid fibroblasts compared with normal fibroblasts and are thought to be largely responsible for fibroblasts' aggressive fibroproliferative nature in keloids (Xia *et al.*, 2004). Furthermore, TGF-β1 and β2 also increase the expression of EDA FN (Viedt *et al.*, 1995) and thus may be responsible for the increased EDA FN observed in keloids (Andrews *et al.*, 2015). Interestingly, EDA FN stimulates TLR-4 signaling (Bhattacharyya *et al.*, 2014) that in turn augments TGF-β responses (Bhattacharyya *et al.*, 2013). Thus, keloids may arise from a feed-forward mechanism in which EDA-FN stimulates both TLR-4 and integrins to augment TGF-β-driven fibroblast activation and ECM deposition including collagen and more EDA FN (Figure 2).

EDA FN and keloids

Although it has been known for some time that TGF-β1 and β2 act through connective tissue growth factor (Colwell *et al.*, 2005), more recently it has been found that β-catenin signaling is required for TGF-β-mediated fibrosis (Akhmetshina *et al.*, 2012), which helps explain why it was previously found that active TGF-β alone was insufficient to induce skin fibrosis (Campaner *et al.*, 2006). Moreover, in at least one scenario, TLR-4 was found to activate the beta-catenin signaling. Hence, TLR-4 stimulation by EDA FN might augment TGF-β responses both directly and indirectly creating a vicious cycle of keloidal fibrotic tumor formation. The activation of innate immune pathway signaling downstream of TLR--4, culminating in the production of cytokines and other pro-inflammatory mediators, suggests that EDA FN has the ability to act as a damage-associated molecular pattern (DAMP). Endogenous DAMPs that arise from the ECM, such as the EDA FN, are often generated during tissue remodeling and fibrosis. Subsequently, these DAMPs can trigger inflammation and further contribute to the fibrotic phenotype in a vicious, feed-forward cycle (Huebener and Schwabe, 2013). Targeting the pathways regulating the expression of EDA FN as well as its receptors may be key in controlling EDA FN–mediated inflammation and fibrosis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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REFERENCES

Akhmetshina A, Palumbo K, Dees C *et al.* (2012) Activation of canonical Wnt signalling is required for TGF-beta-mediated fibrosis. *Nat Commun* 3:735

Andrews JP, Marttala J, Macarak E *et al.* (2015) Keloid pathogenesis: potential role of cellular fibronectin with the EDA domain. *J Invest Dermatol* 135:1921–4

Arslan F, Smeets MB, Rien V, P W. *et al.* (2011) Lack of fibronectin-EDA promotes survival and prevents adverse remodeling and heart function deterioration after myocardial infarction. *Circ Res* 108:582–92

Babu M, Diegelmann R, Oliver N (1992) Keloid fibroblasts exhibit an altered response to TGF-beta. *J Invest Dermatol* 99:650–5

Balza E, Borsi L, Allemanni G *et al.* (1988) Transforming growth factor beta regulates the levels of different fibronectin isoforms in normal human cultured fibroblasts. *FEBS Lett* 228: 42–4

Bettinger DA, Yager DR, Diegelmann RF *et al.* (1996) The effect of TGF-beta on keloid fibroblast proliferation and collagen synthesis. *Plast Reconstr Surg* 98:827–33

Bhattacharyya S, Kelley K, Melichian DS *et al.* (2013) Toll-like receptor 4 signaling augments transforming growth factor-beta responses: a novel mechanism for maintaining and amplifying fibrosis in scleroderma. *Am J Pathol* 182: 192–205

Bhattacharyya S, Tamaki Z, Wang W *et al.* (2014) FibronectinEDA promotes chronic cutaneous fibrosis through Toll-like receptor signaling. *Sci Transl Med* 6:232ra50

Blaustein M, Pelisch F, Coso OA *et al.* (2004) Mammary epithelial-mesenchymal interaction regulates fibronectin alternative splicing *via* phosphatidylinositol 3-kinase. *J Biol Chem* 279:21029–37

Bolognia JL, Jorizzo JL, Schaffer JV (2012) *Dermatology* 3rd (edn). Elsevier

Booth AJ, Wood SC, Cornett AM *et al.* (2012) Recipient-derived EDA fibronectin promotes cardiac allograft fibrosis. *J Pathol* 226: 609–18

Borsi L, Castellani P, Risso AM *et al.* (1990) Transforming growth factor-beta regulates the splicing pattern of fibronectin messenger RNA precursor. *FEBS Lett* 261:175–8

Buratti E, Baralle FE (2004) Influence of RNA secondary structure on the pre-mRNA splicing process. *Mol Cell Biol* 24:10505–4

Campaner AB, Ferreira LM, Gradnani A *et al.* (2006) Upregulation of TGF-beta1 expression may be necessary but is not sufficient for excessive scarring. *J Invest Dermatol* 126: 1168–76

Chodon T, Sugihara T, Igawa HH *et al.* (2000) Keloid-derived fibroblasts are refractory to Fas-mediated apoptosis and neutralization of autocrine transforming growth factor-beta1 can abrogate this resistance. *Am J Pathol* 157: 1661–9

Clark RAF (2014) Wound repair: basic biology to tissue engineering. In: Lanza RP, Langer RC, Vacanti JP (eds). *Principles of Tissue Engineering*, 4th edn. Elsevier: Amsterdam, Boston

Colwell AS, Phan TT, Kong W *et al.* (2005) Hypertrophic scar fibroblasts have increased connective tissue growth factor expression after transforming growth factor-beta stimulation. *Plast Reconstr Surg* 116:1387–90

Cramer P, Pesce CG, Baralle FE *et al.* (1997) Functional association between promoter structure and transcript alternative splicing. *Proc Natl Acad Sci USA* 94:11456–60

Deno DC, Saba TM, Lewis EP (1983) Kinetics of endogenously labeled plasma fibronectin: incorporation into tissues. *Am J Physiol* 245: R564–75

Desmouliere A, Geinoz A, Gabbiani F *et al.* (1993) Transforming growth factor-beta1 induces a-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 122: 103–1

Ehrlich HP, Desmouliere A, Diegelmann RF *et al.* (1994) Morphological and immunochemical differences between keloid and hypertrophic scar. *Am J Pathol* 145:105–3

ffrench-Constant C, Van De Water L, Dvorak HF *et al.* (1989) Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat. *J Cell Biol* 109: 903–14

Gondokaryono SP, Ushio H, Niyonsaba F *et al.* (2007) The extra domain A of fibronectin stimulates murine mast cells via toll-like receptor 4. *J Leukocyte Biol* 82:657–65

Greiling D, Clark RAF (1997) Fibronectin provides a conduit for fibroblast transmigration from a collagen gel into a fibrin gel. *J Cell Sci* 110: 861–70

Gurtner GC, Dauskardt RH, Wong VW *et al.* (2011) Improving cutaneous scar formation by controlling the mechanical environment: large animal and phase I studies. *Ann Surg* 254: 217–5

Huebener P, Schwabe RF (2013) Regulation of woun dhealing and organ fibrosis by toll-like receptors. *Biochim Biophys Acta* 1832:1005–7

Inoue T, Nabeshima K, Shimao Y *et al.* (1999) Hepatocyte growth factor/scatter factor (HGF/SF) is a regulator of fibronectin splicing in MDCK cells: comparison between the effects of HGF/SF and TGF-β1 on fibronectin splicing at the EDA region. *Biochem Biophys Res Commun* 260:225–31

Kelsh R, You R, Horzempa C *et al.* (2014) Regulation of the innate immune response by fibronectin: synergism between the III-1 and EDA domains. *PLoS One* 9:e102974

Kohan M, Muro AF, Bader R *et al.* (2011) The extra domain A of fibronectin is essential for allergen-induced airway fibrosis and hyperresponsiveness in mice. *J Allergy Clin Immunol* 127:439–6

Kohan M, Muro AF, White ES *et al.* (2010) EDA-containing cellular fibronectin induces fibroblast differentiation through binding to α4β7 integrin receptor and MAPK/Erk 1/2-dependent signaling. *FASEB J* 24:4503–12

Lasarte J, Casares N, Gorraiz M *et al.* (2007) The extra domain A from fibronectin targets antigens to TLR4-expressing cells and induces cytotoxic T cell responses *in vivo*. *J Immunol* 178:748–56

Lavigueur A, La Branche H, Kornblihtt AR *et al.* (1993) A splicing enhancer in the human fibronectin alternatve ED1 exon interacts with SR proteins and stimulates U2 snRNP binding. *Genes Dev* 7:2405–17

Leask A, Abraham DJ (2004) TGF-beta signaling and the fibrotic response. *FASEB J* 18: 816–27

Liao YF, Gotwals PJ, Koteliansky VE *et al.* (2002) The EIIIA segment of fibronectin is a ligand for integrins alpha9beta1 and alpha4beta1 providing a novel mechanism for regulating cell adhesion by alternative splicing. *J Biol Chem* 277:14467–74

Magnuson VL, Young M, Schattenberg DG *et al.* (1991) The alternative splicing of fibronectin pre-mRNA is altered during aging and in response to growth factors. *J Biol Chem* 266: 14654–62

McFadden JP, Basketter DA, Dearman RJ *et al.* (2011) Extra domain A-positive fibronectin-positive feedback loops and their association with cutaneous inflammatory disease. *Clin Dermatol* 29:257–65

Mermoud JE, Cohen PT, Lamond AI (1994) Regulation of mammalian spliceosome assembly a protein phosphorylation mechanism. *EMBO J* 13:5679–88

Muro AF, Moretti FA, Moore BB *et al.* (2008) An essential role for fibronectin extra type III domain A in pulmonary fibrosis. *Am J Respir Crit Care Med* 177:638–45

Naro C, Sette C (2013) Phosphorylation-mediated regulation of alternative splicing in cancer. *Int J Cell Biol* 2013:Article ID 151839-http://dx.doi.org/10.1155/2013/151839

Okamura KY, Watari M, Jerud ES *et al.* (2002) The extra domain A of fibronectin activates Toll-like receptor 4. *J Biol Chem* 276:10229–33

Oyama F, Murata Y, Suganuma N *et al.* (1989) Patterns of alternative splicing of fibronectin pre-mRNA in human adult and fetal tissues. *Biochemistry* 28:1428–34

Pagani F, Zagato L, Vergani C *et al.* (1991) Tissue-specific splicing pattern of fibronectin messenger RNA precursor during development and aging in rat. *J Cell Biol* 113:1223–9

Peters JH, Grote MN, Lane NE *et al.* (2011) Changes in plasma fibronectin isoform levels predict distinct clinical outcomes in critically-ill patients. *Biomark Insights* 6:59–68

Peters JH, Maunder RJ, Woolf AD *et al.* (1989) Elevated plasma levels of ED1+ ("cellular") fibronectin in patients with vascular injury. *J Lab Clin Med* 113:586–97

Petersen TE, Thogersen HC, Skorstengaard K *et al.* (1983) Partial primary structure of bovine plasma fibronectin: three types of internal homology. *Proc Natl Acad Sci USA* 80: 137–41

Russell SB, Trupin KM, Rodriguez-Eaton S *et al.* (1988) Reduced growth-factor requirement of keloid-derived fibroblasts may account for tumor growth. *Proc Natl Acad Sci USA* 85: 587–91

Satoi S, Kitade H, Hiramatsu Y *et al.* (2000) Increased extra domain-A containing fibronectin and hepatic dysfunction during septic response: an *in vivo* and *in vitro* study. *Shock* 13:492–6

Schwarzbauer JE, DeSimone DW (2011) Fibronectins, their fibrillogenesis, and *in vivo* functions. *Cold Spring Harbor Perspect Biol* 3:A005041

Seebacher T, Manske M, Kornblihtt AR *et al.* (1988) Cellular fibronectin is induced by epidermal growth factor, but not by dexamethasone or cyclic AMP in rat liver epithelial cells. *FEBS Lett* 239:113–6

Serini G, Bochaton-Piallat ML, Ropraz P *et al.* (1998) The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. *J Cell Biol* 142:873–1

Shinde AV, Bystroff C, Wang C *et al.* (2008) Identification of the peptide sequences within the EIIIA (EDA) segment of fibronectin that mediate integrin a9b1-dependent cellular activities. *J Biol Chem* 283:2858–70

Shinde AV, Kelsh R, Peters JH *et al.* (2015) The alpha4beta1 integrin and the EDA domain of fibronectin regulate a profibrotic phenotype in dermal fibroblasts. *Matrix Biol* 41: 26–35

Singh P, Reimer CL, Peters JH *et al.* (2004) The spatial and temporal expression patterns of integrin alpha9beta1 and one of its ligands, the EIIIA segment of fibronectin, in cutaneous wound healing. *J Invest Dermatol* 123: 1176–81

Srebrow A, Blaustein M, Kornblihtt AR (2002) Regulation of fibronectin alternative splicing by a basement membrane-like extracellular matrix. *FEBS Lett* 514:285–9

Sun XJ, Fa PP, Cui ZW *et al.* (2014) The EDA-containing cellular fibronectin induces epithelial mesenchymal transition in lung cancer cells through integrin a9b1-mediated activation of PI3-K/AKT and Erk1/2. *Carcinogenesis* 35:184

Tan MH, Sun Z, Opitz SL *et al.* (2004) Deletion of the alternatively spliced fibronectin EIIIA domain in mice reduces atherosclerosis. *Blood* 104:11–8

van der Straaten HM, Canninga-van Dijk MR, Verdonck LF *et al.* (2004) Extra-domain-A fibronectin: a new marker of fibrosis in cutaneous graft-versus-host defense. *J Invest Dermatol* 123:1057–62

Vartio T, Laitinen L, Narvanen O *et al.* (1987) Differential expression of the ED sequence-containing form of cellular fibronectin in embryonic and adult human tissues. *J Cell Sci* 88:419–30

Viedt C, Burger A, Hansch GM (1995) Fibronectin synthesis in tubular epithelial cells: up-regulation of the EDA splice variant by transforming growth factor beta. *Kidney Int* 48:1810–7

Vyalov SL, Gabbiani G, Kapanci Y (1993) Rat alveolar myofibroblasts acquire alpha-smooth muscle actin expression during bleomycin-induced pulmonary fibrosis. *Am J Pathol* 143: 1754–65

Welch MP, Odland GF, Clark RAF (1990) Temporal relationships of F-actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction. *J Cell Biol* 110:133–45

White ES, Sagana RL, Booth AJ *et al.* (2010) Control of fibroblast fibronectin expression and alternative splicing *via* the PI3K/Akt/mTOR pathway. *Exp Cell Res* 316:2644–53

Xia W, Phan TT, Lim IJ *et al.* (2004) Complex epithelial-mesenchymal interactions modulate transforming growth factor-beta expression in keloid-derived cells. *Wound Repair Regen* 12: 546–6

Yamada KM, Clark RAF (1996) Provisional matrixIn: *Molecular and Cellular Biology of Wound Repair* Clark RAF ed 2nd (edn). Plenum: New York New York 51–93

Zhu J, Clark RA (2014) Fibronectin at select sites binds multiple growth factors and enhances their activity: expansion of the collaborative ECM-GF paradigm. *J Invest Dermatol* 134: 895–901

Zoppi N, Ritelli M, Colombi M (2012) Type III and V collagens modulate the expression and assembly of EDA+fibronectin in the extracellular matrix of defective Ehlers-Danlos syndrome fibroblasts. *Biochim et Biophys Acta* 1820:1576–87