Chapter 6

The Role of Actin Remodelling Proteins in Wound Healing and Tissue Regeneration

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64673

Abstract

The actin cytoskeleton is an essential network of filaments that is found in all cells and has an important role in regulating cellular activities. The dynamic regulation of cytoskeletal synthesis, remodelling and function is critical for many physiological processes and is integral for the successful repair of wounds. Wound healing relies on the fine balance between cellular proliferation, adhesion and migration, resulting in tightly controlled equilibrium between tissue regeneration and fibrosis. The actin cytoskeleton regulates all these processes and is therefore an important factor contributing to the re-establishment of the skin barrier function, restoration of the skin anatomical structure and wound repair; however, it also inevitably results in scar formation. Regulation of the actin cytoskeleton is tightly controlled by several large protein families, which are discussed in this chapter. Members of the FERM superfamily of proteins, the filamin and tropomyosin families of actin-associated proteins as well as the gelsolin family of actin remodelling proteins are all important regulators of the actin cytoskeleton, which can affect different stages of wound healing. Targeted therapies against different proteins involved in cytoskeletal regulation may lead to novel therapeutic interventions aimed at improving wound healing and reducing scar formation.

Keywords: actin cytoskeleton, wound healing, skin regeneration, fibrosis

1. Introduction

The actin cytoskeleton is made up of a complex network of microtubules, actin filaments, intermediate filaments and stress fibres, providing a cellular engine that drives motility, adhesion and contraction downstream of complex signalling pathways. The actin cytoskeleton

is also involved in modulating cell signalling, growth, differentiation and gene expression, while components of the actin cytoskeleton further work in synergy to provide stronger cell stability during stress [1, 2].

Cutaneous wound repair is a dynamic process triggered in response to tissue injury, which aims to restore the skin barrier function, and involves a sequence of events including acute inflammation, reepithelialisation, collagen deposition and contraction and remodelling [3]. Common to all tissue repair processes is the migration of cells into the wound space including fibroblasts, epithelial cells and endothelial cells. It is the active assembly and disassembly of the filamentous actin and reorganisation of its networks that underpins the important cell processes, which occur during wound healing.

Changes in the distribution of actin-associated proteins during epidermal wound healing in vivo were first reported in 1992 [4]. Filamentous actin was found in all the living epidermal layers before, after and during wound healing while different actin-associated proteins, namely talin, filamin and gelsolin, showed a reduced expression at the leading edge of migrating epidermis, which returned to normal levels once the epidermis has reformed [4].

The precise orchestration of actin polymers into filaments and their interactions with various proteins regulating actin remodelling, stability, branching and bundling is what underpins cellular migration and outcomes of wound healing. Central to the ability of fibroblasts and keratinocytes to move into the wounded area is a dynamic and responsive actin cytoskeleton and the molecules that regulate actin filament dynamics and change the rate of cell migration can also alter the rate of wound healing [5]. Understanding the role of the actin cytoskeleton in cellular functions vital for tissue repair and regeneration and how different regulators of the actin cytoskeleton control this intricate balance between actin polymerisation and disassembly will be critical for the development of novel therapeutic approaches. New therapies that can regulate the actin cytoskeleton could lead to improved wound healing outcomes. Here, we will focus on describing the role of different actin cytoskeleton regulators and how they are able to modulate the cytoskeleton and influence different stages of wound healing.

2. Actin dynamics during wound healing

Actin-based cell motility relies on the balanced activity of specific actin-binding proteins that drive the dynamics of the actin system and govern its special organisation [6]. A number of different structural and dynamic aspects of cell behaviour are dependent on the actin cytoskeleton, including cell morphology, polarity, adhesion complex formation, vesicle trafficking and phagocytosis, cytokinesis and movement [7]. Actin microfilaments are the smallest components of the actin cytoskeletal network and play a role in cellular motility, structure and division [6]. Two types of actin microfilaments have been categorised; individual non-polymerised globular actin subunits termed G-actin and long filamentous polymerised fibres termed F-actin assembled from individual G-actin subunits. Microfilament actin (F-actin) exists in equilibrium with a soluble monomeric actin (G-actin) and this balance is

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often shifted in response to changes in cellular environment, cellular migration, adhesion and wound repair [8]. During wound healing, activation of neutrophils during the inflammatory phase of wound repair induces changes in cell shape, migration, degranulation and phagocytic responses, all of which require cytoskeletal restructuring. In addition, the reestablishment of the skin barrier function as well as endothelial vessel integrity in wounds is dependent on actin cytoskeleton integrity [9]. Microtubules and intermediate filaments are larger structures of the cytoskeleton composed of α and β tubulin dimers which function in both cellular movement and division [6]. Intermediate filaments are involved in the formation of adhesion complexes namely hemidesmosomes, desmosomes and focal adhesions and directly interact with proteins of the extracellular matrix [10]. Key roles of the intermediate filaments include signal transduction, cytoskeletal crosstalk between the organelles in the cytoplasm and organisation of the cytoplasm [11].

Stress fibres are also a component of the actin cytoskeleton network allowing a cell to modulate its responses to tissue injury. Mammalian cells contain three types of stress fibres: ventral stress fibres attached to focal adhesions at both ends, dorsal stress fibres attached to focal adhesions at one end, and transverse arcs which are the acto-myosin bundles that do not attach to focal adhesions directly [12]. The major role of stress fibres is to maintain a balance between contraction and adhesion. This balance results in stable actin bundles, which maintain a constant length under tension, especially in ventral stress fibres attached to the extracellular matrix on both sides [13].

Changes in cell shape, adhesion and migration properties are all regulated by the continuous remodelling of the actin cytoskeleton. Cell motility is powered by controlled assembly and disassembly of the actin cytoskeleton, and the migration speed is dependent on the membrane tension created by the coalescence of the actin filaments growing against the tense membrane [14]. In order to migrate in response to extracellular signals, cells first assemble actin at the cell front driving the extension of membrane protrusions called lamellipodia and filopodia [15]. At the leading edge of the cell, adhesions are formed with the extracellular matrix, hence anchoring the protrusions to move the cell body. The combination of the acto-myosin contractibility and disassembly of the adhesion structures at the rear of the cell allows the cell body to move forward [8]. Lamellipodia, filopodia and membrane ruffles are components of the actin cytoskeleton involved in both cell motility and cell-matrix adhesions [16]. Lamellipodia consist of a network of branched actin filaments that produce the force for cell protrusions at the leading edge. The assembly of actin-based projections is regulated by GTPases of the Rho family, which link the surface receptors to the organisation of the actin cytoskeleton. While Rho GTPase is instrumental in formation of stress fibre and focal adhesion formation, Rac1 and Cdc42 signal the formation of lamellipodia and filopodia, respectively.

Filapodia are thin cellular processes consisting of long parallel actin filaments arranged into tight bundles. Membrane ruffling is characterised by the dynamic movement of the membrane protrusions, consisting of lamellipodia and filopodia, in response to the extracellular signals. Away from the leading edge of the cell at the site of slow actin turnover, lamellas are formed and are characterised by proteins involved in the movement of stress fibres, namely tropomyosin and myosin II [8]. Initial integrin mediated cell-matrix adhesions, termed focal

complexes, develop underneath lamellipodia and are driven by actin polymerisation. These are highly dynamic structures that exist for a limited time. A proportion of the stable focal complexes develop into elongated focal adhesions, which are associated with contractile stress fibres [17]. A vital function of focal adhesions is the anchoring of polymerised actin filament stress fibres into bundles, which provide contractile force required for effective translocation of a cell body during cellular migration [18]. Different components of the actin cytoskeleton of the moving cell and adhesion sites formed in response to GTPase signalling in fibroblasts are shown in **Figure 1**. The main changes in the actin cytoskeleton during wound healing include lamellipodia remodelling during keratinocyte migration and wound reepithelialisation, infiltration of inflammatory cells and migration of fibroblasts required for the deposition and remodelling of the extracellular matrix and dermal wound contraction [19, 20].

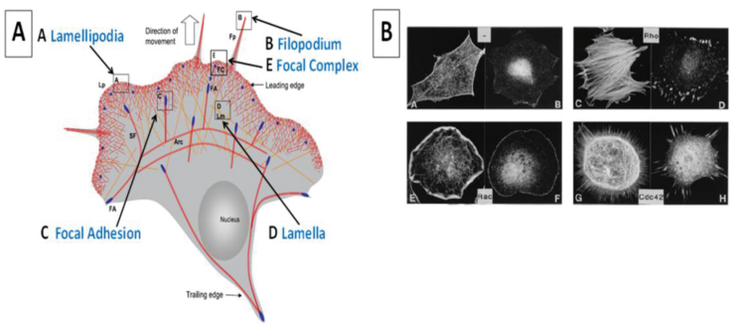

Figure 1. Actin cytoskeleton of the moving cell. (A) Arrangement of the actin cytoskeleton in a moving cell A lamellipodia, B filopodium, C focal adhesion, D lamella, E focal complex. (B) Formation of different actin cytoskeleton components in response to GTPase signalling in fibroblasts. Actin filaments visualised with phalloidin staining in A, C, E and G and adhesion complexes visualised with an anti-vinculin antibody in B, D, F and H. Quiescent fibroblasts in A and B show few organised actin filaments or adhesion complexes. In response to Rho stress fibre formation C and adhesion complex formation D is evident. Microinjection of Rac induces lamellipodia E and associated focal adhesion complexes, while microinjection of Cdc42 induces filopodia formation G and associated adhesion complexes H. Figure adapted from [8, 25].

In resting cells, there is little actin turnover, and fast-growing actin ends are blocked, with large pools of actin monomers in a complex with polymerising-inhibiting or sequestering proteins. In response to wounding, a local increase in actin polymerisation is initiated by uncapping the actin ends and by severing existing filaments leading to *de novo* polymerisation. The barbed ends of the actin filament are the hotspots for the majority of biochemical reactions that control filament assembly and a number of actin remodelling, capping, severing and sequestering proteins modulate their affinity for barbed ends in a spatial and temporal manner [21]. Some actin remodelling proteins also affect the actin filament barbed ends by indirect activity and control of the flux of actin monomers available at the barded end [22]. Signal transduction networks that translate environmental signals into intracellular changes govern actin dynamics and interplay between extracellular environment and cell motility. Many actin-binding proteins accumulate at sites of actin-rich lamella and have been shown to regulate actin dynamics in motile keratinocytes [23]. Focal adhesion formation in fibroblasts is a complex process initiated by the ligation and clustering of the integrin subunits and signalling via

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RhoGTPases, which influence both actomyosin contractibility and actin stress fibre formation [24].

For cellular migration, dynamic rearrangements of the actin cytoskeleton occur to form protrusive structures and generate intracellular forces required for cell movement. The actin-based motility is best described in four steps: polarisation, protrusion of lamellipodia, formation of attachment sites and retraction of cell rear end [6]. Fibroblast locomotion during wound healing is the result of series of coordinated cellular events and main motor protein involved in mediating formation of lamellipodia of migrating cells is Myosin I. However, during wound healing, Myosin II, motor protein, is involved in the contraction of transverse actin fibres during lamellar contractile phase of wound healing [26]. In addition, release of Myosin II contractibility accelerates the healing of large wounds in long term by mobilisation of large cell sheet or rows of cells behind the leading edge [27].

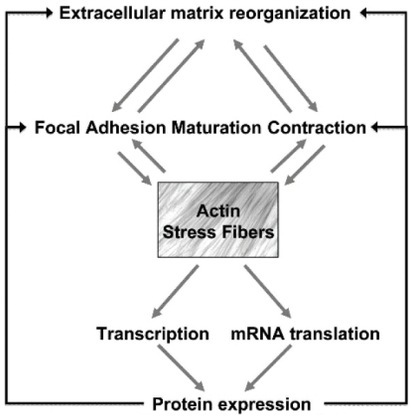

Figure 2. The role of actin cytoskeleton in regulating myofibroblast function. Actin cytoskeleton involvement in bidirectional signalling augmenting extracellular matrix organisation, focal adhesion turnover and contraction as well as transcriptional regulation of proteins instrumental in these processes vital for outcomes of wound repair. Figure adapted from [24].

Myofibroblasts are modified fibroblasts characterised by the presence of the contractile apparatus and formation of robust stress fibres. These cells are involved in the contraction and remodelling of the extracellular matrix but are also found in aberrant tissue remodelling in fibrotic disorders. The actin cytoskeleton regulates several mechanical functions during myofibroblast differentiation including focal adhesion formation, contraction and matrix remodelling and simultaneously regulates transcription of genes involved in the same mechanical functions and therefore plays an important role in amplifying the signal leading to myofibroblast differentiation. The bidirectional signalling between matrix stiffness, focal adhesion augmentation and stress fibre formation during actin cytoskeletal regulation of myofibroblast function is illustrated in **Figure 2** [28].

2.1. Scar-free foetal and adult wound healing

Whereas adult wound keratinocytes crawl forwards over the exposed substratum closing the deficit, a wound in embryonic epidermis is closed by contraction of an actin purse

string. Blocking the assembly of this actin cable in chick and mouse embryos by drugs or by inactivation of small GTPase Rho severely hinders the reepithelialisation process [29]. Foetal wounds reepithelialise quickly via contraction of actin-myosin fibres in a "purse-string" like manner drawing the edges of the wound together. This is facilitated by the rapid polymerisation of the F-actin some five to six cells back from wound edge and is anchored by the E-cadherin at the leading edge to facilitate coordinated movement [30]. Foetal wound fibroblasts do not express alpha smooth muscle actin, suggesting that they do not change their phenotype into contractile myofibroblasts observed in adult wounds and these differences may account for differences in repair outcomes in foetal vs adults wound tissue [30]. Changes in the expression profile of proteins associated with actin cytoskeleton are indicative of the switch between scar-free regeneration and scar forming repair. Wounding has a differential effect on cytoskeletal proteins including gelsolin and paxillin associated with actin dynamics both in foetal and adult skin wounds [19, 31]. Interestingly, wounding also has an effect on the expression of filamentous F-actin. While "scar-free" foetal wounds have predominantly epidermal expression of F-actin, the "scar forming" adult wounds have predominantly dermal F-actin expression and this developmental switch in actin expression might be important in foetal wound contraction and "scar-free" wound healing [32]. The importance of the actin cytoskeleton in healing of foetal wounds was demonstrated at embryonic day E17 by the addition of cytochalasin-B, an inhibitor of actin polymerisation, which completely prevented epithelial wound closure with no actin cable structures evident while at embryonic day E19, dermal actin filaments formed spherical structures around the wound margin but did not affect already limited wound repair response (**Figure 3**) [19].

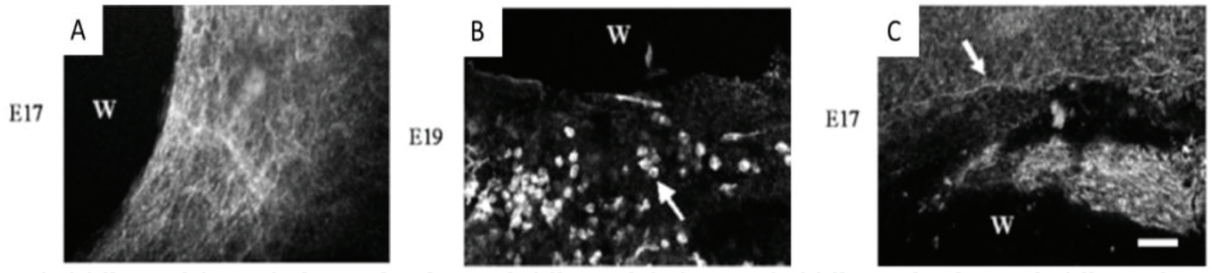

Figure 3. Effect of inhibiting actin polymerisation and protein proliferation on actin cable formation in E17 foetal wounds. Phalloidin-FITC binding to actin in E17 and E19 skins foetal skin treated with 10 μg cytochalasin-B per ml (A and B, respectively). Phalloidin-FITC binding to actin in E17 foetal skin treated with 2 mM hydroxyurea (C). Scale bar = 50 μm in (C) and applies to all images. Figure adapted from [19] and modified.

3. FERM superfamily of proteins

The FERM domain (F for 4.1protein, E for ezrin, R for radixin and M for moesin) is a widespread domain found in many cytoskeletal associated proteins at the interface between the plasma membrane and the actin cytoskeleton. The function of FERM domain is to localise the proteins

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to the plasma membrane, and therefore, members of the FERM superfamily of proteins mediate the linkage between the actin cytoskeleton and cell membrane and are characterised by the presence of the conserved FERM domain at the N-terminus and often an actin binding domain at the C-terminus. FERM proteins are involved in cellular motility and membrane-cytoskeletal interactions and play roles in promotion of cancer and wound healing [33]. The main members of this family include protein 4.1R, ezrin, radixin and moesin, often referred to as ERM proteins. Ezrin is a component of the microvilli of the plasma membrane, meosin is involved in binding major cytoskeletal structures to the plasma membrane, while radixin is involved in the binding of the barbed end of actin filaments to the plasma membrane [21, 34]. Earlier studies have shown that ezrin, radixin and meosin colocalise with F-actin in the endothelial cells in vitro and in vivo and play a role in formation of focal F-actin branching points, while their interaction with phosphorylated protein kinase C (PKC) has been shown to be important during wound repair [35]. Inhibition of PKC activity results in delayed wound repair, reduced association with ERM proteins and reduced F-actin branching points. In addition, phosphorylation of ERM proteins by PCK improved in vitro wound healing of cancer cells [36]. Furthermore, in vivo studies examining the healing of hepatic injury in meosin knockdown mice showed reduced inflammatory infiltrate, fibrosis and collagen deposition at the wound margins of these mice compared with control animals [37].

The role of the FERM protein superfamily during wound healing has also been demonstrated in 4.1R knockout mice ($4.1R^{2013/2013}$) and their cultured keratinocytes. Protein 4.1R is present in the cytoplasm and the leading edge of the moving cell [34]. Absence of 4.1R protein leads to reduced adhesion, spreading and migration of keratinocytes. In addition, diminished focal adhesion complexes and reduced integrin Beta-1 expression were directly linked to absence of 4.1R protein in vitro. Using its FERM domain, 4.1R protein was shown to interact with the Ras GTPase-activating-like protein 1, a scaffolding protein that binds and cross-links actin filaments, allowing migration to take place [34]. In addition, ezrin/radixin/moesin proteins have been shown to be involved in the development of diabetes including the secretion and utilisation of insulin and may contribute to the pathogenesis and progression of diabetic angiopathy, nephropathy and cardiomyopathy [38]; all of which have been implicated in the development of diabetic ulcers. These proteins may be novel targets for therapeutic interventions aimed at preventing diabetic complications; however, further research is required to elucidate their exact mechanisms before they can be developed for specific treatments.

The FERM superfamily of proteins consists of over 30 proteins including the Kindlin family of focal adhesion proteins. Kindlin-1 and Kindlin-2 have been implicated in integrin signalling and focal adhesion turnover, while deletion of the Kindlin-1 is associated with a congenital skin disease—Kindler Syndrome, where patients experience skin atrophy, blister formation and impaired wound healing [39–42]. Kindlin-2 is an important regulator of focal adhesion stabilisation and maturation of focal adhesions and stress fibres in myofibroblasts. In addition, the upregulation of Kindlin-2 observed in myofibroblasts during wound healing suggests a role for Kindlin-2 in skin fibroblasts and tissue regeneration [41]. More recently, talin and Ehm2 have been added to the FERM superfamily both of which have roles in wound repair [21, 34].

3.1. Talin

Talin, a member of the FERM family of proteins, is concentrated in regions of cell-substrate and cell-cell contacts. Using its FERM domain, talin acts as a "hyper-activator" of integrin receptors linking the cytoplasmic tail of integrin receptors to the actin cytoskeleton and increasing the affinity of the integrin extracellular domain to the extracellular matrix, hence regulating cell adhesion-dependent processes including tissue remodelling [43]. Talin knock-out results in abnormal cellular migration and early embryonic lethality [8]. Integrin adhesion receptors connect the extracellular matrix to the actin cytoskeleton and serve as bidirectional mechanotransducers during wound healing mediating actin cytoskeletal remodelling in response to stiffening of the extracellular matrix [44]. The inside out-signalling of integrin receptors regulates the ligand binding affinity of the cell surface receptors in response to changes in environmental factors important for cell survival, including tissue injury [45]. Cytoplasmic talin is activated in response to phosphatidylinositol 4,5-biphosphate (PIP2) binding which also terminates the auto-inhibition of talin through the talin head-rod binding. Once activated, the talin subdomain interacts with the β integrin tails, forms the talin specific

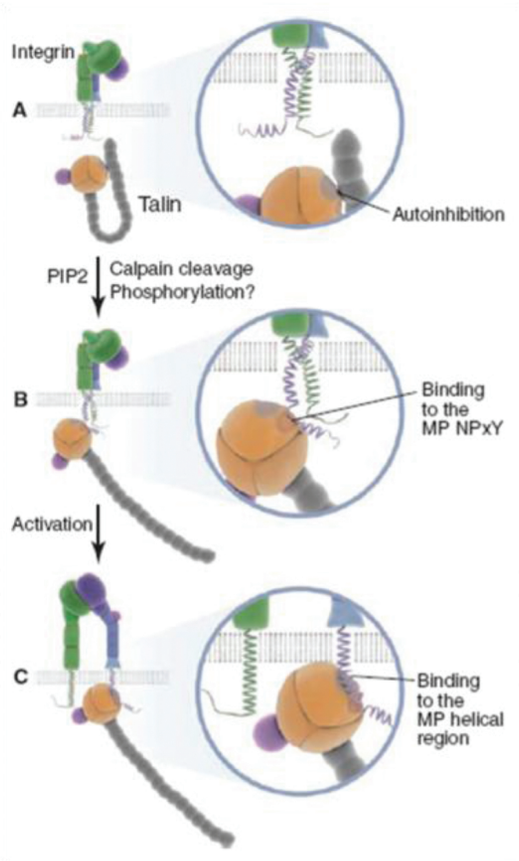

Figure 4. Talin activation of integrin receptor subunits. (A) PIP2 binding to the cytoplasmic talin activates the talin protein by ending the auto-inhibitory interaction with the rod domain. (B) Talin subdomain engages with the membrane proximal NPxY motif in the β integrin cytoplasmic tail. (C) Talin-specific loop structure forms with binding to the MP helical region of the integrin cytoplasmic chain, hence disrupting the connection between α/β subunit integrin cytoplasmic tails. Pulling forces at the β integrin tail reorient the transmembrane domains, hence disrupting the packing of the α/β transmembrane domains. Figure adapted from [46].

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loop structure and disrupts the connection between the cytoplasmic tails linking the integrin receptors and the actin cytoskeleton [46]. This model of integrin activation by talin is an example of how cytoplasmic proteins can regulate activation state of integrin receptors and can transduce the biochemical signals into an array of cellular signalling transduction pathways, a crucial function for cellular adhesion, migration, angiogenesis, extracellular matrix assembly and wound remodelling [47]. **Figure 4** illustrates a schematic model of talin activation of integrin subunits; however, this process is likely to be more complex and involves spatial activation and interaction of different proteins involved in actin dynamics. Recent findings have shown that talin associates with the actin remodelling protein Flightless I (Flii) in wounded keratinocytes and this interaction may contribute to Flii regulation of adhesion-dependent signalling pathways during wound repair [48].

3.2. Ehm2

Ehm2 is a member of the FERM family that has been identified as a positive regulator of keratinocyte adhesion and motility. Ehm2 is upregulated in response to tissue injury and its levels are up to three times higher in acute wounds compared with chronic wound samples [34]. Ehm2 expression is highest in wounds undergoing active healing, where high expression was observed at the wound edge suggesting a functional role for Ehm2 during wound repair. In vitro knock-down of Ehm2 reduces NWasp protein expression and cellular adhesion, migration and motility without affecting cell growth, cell cycle or apoptosis, suggesting that Ehm2 is an important actin modulator of cell migration during healing of acute wounds [34]. Therefore, in common with other FERM family members, these findings suggest that Ehm2 promotes wound healing via the process of reepithelialisation.

4. Filamin family of proteins

The filamin (FLN) family of proteins consists of three proteins, namely Filamin-a (FLNa), Filamin-b (FLNb) and Filamin-c (FLNc). While FLNa and FLNb are enriched at the cell periphery and focal adhesions, FLNc is mainly localised in muscle Z-disc. These proteins function as actin filament cross-linking proteins and serve as scaffolds to over 90 different binding partners including channels, receptors, intracellular signalling molecules and transcription factors [49]. FLN proteins are required for the recycling, trafficking and stabilisation of membrane proteins and facilitate the signal transduction at specific locations within the cell. In addition, the FLN family of proteins act as cohesive proteins to stiffen the F-actin networks, cross-linking the filament structures and reconstituting many aspects of cell mechanics [49]. In humans, mutation in the FLNa gene results in disrupted neuronal cell migration, while FLNa overexpression also prevents migration [50]. However, genetic knockdown of FLNa in embryonic fibroblasts results in no defect in migration, suggesting a compensatory mechanism by FLNb. The main family member, FLNa, has been the predominant focus of research and has been shown to play a role in wound healing.

4.1. Filamin-a

FLNa acts as a negative regulator of integrin activation by blocking talin binding to the β integrin tail, and subsequent proteolysis and depletion of FLN. Phosphorylation of the β integrin tail dissociates FLN from integrins, hence allowing activation of integrins via talin and other members. FLNa binding with different partners leads to different outcomes in cell adhesion, spreading and migration: association with F-actin leads to formation of orthogonal F-actin networks with unique mechanical and physiological properties; interaction with Migfilin and R-Ras induces and enhances integrin activation respectively; interaction with RalA induces filopodia formation, while interaction with ROCK and Rho GTPases leads to increased actin cytoskeleton remodelling required for cell migration [49].

Human wounds heal through a combination of granulation tissue formation (via production of extracellular matrix and neovascularisation) and wound contraction (via fibroblast-mediated contraction). FLNa has been shown to protect fibroblasts against force-induced apoptosis by stabilising cell-matrix contacts [51]. Moreover, fibroblast spreading and adhesion are dependent on FLNa, consistent with its known role in cytoskeletal dynamics [52]. Studies in mice show that FLNa stabilises actin filaments in fibroblasts and mediates wound closure by promoting elastic deformation and maintenance of tension in the collagen matrix [53]. FLNa accumulates at membrane ruffles where it interacts with different binding proteins and regulates fibroblast interactions with their mechanical environment [54]. When FLNa was blocked using short hairpin RNA, fibroblasts were unable to maintain tension in collagen matrices, and they had reduced migration in vitro. In addition, FLNa-deficient fibroblasts were less able to realign collagen matrix fibres in response to tension, and they demonstrated impaired ability to form cell extensions, a deficit reversed with pharmacologic stabilisation of the actin cytoskeleton. When FLNa was deleted conditionally in dermal fibroblasts in a mouse model, full-thickness wounds healed significantly more slowly and was associated with decreased matrix deposition. No side effects or contradictions were observed in these mouse models suggesting that targeted therapies against FLNa may be worth pursuing. As researchers continue to unlock the molecular mechanisms of fibroblast mechanotransduction, novel therapies may be developed to target and manipulate fibroblast behaviour for a wide range of cutaneous diseases [55].

5. Tropomyosin family of actin-associated proteins

Members of the tropomyosin family of actin-associated proteins display a tissue-specific and time-specific expression, while their association with actin filaments impairs a isoform-specific regulation of actin filament dynamics [56]. There are over 40 different isoforms of tropomyosin and many of them have functional relevance to actin filament dynamics: Tm5NM1 and Tm3 increase actin filament resistance to actin depolymerising drugs; TmBr3 increases actin filament sensitivity to actin depolymerising drugs; TmBr3 reduces actin stress fibre formation, while Tm5a and Tm2 inhibit Arp2/3-mediated filament branching in vitro [57]. Tropomyosin proteins assemble as the polymers in the major grove of the polymerised actin filaments and

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this association has been shown to regulate the molecules that control actin filament turnover [58]. Specific members of the tropomyosin family of actin-associated proteins have yet to be investigated in diabetic wound healing; however, tropomyosin receptor kinase A (TrkA) has been found to be increased in diabetic patients and linked to diabetic nephropathy [59].

High levels of Tm5NM1 expression have been shown to inhibit cell migration and invasion, while loss of Tm5NM1 leads to increased cell motility [57]. Elevated Tm5NM1 expression is associated with inhibition of Src activation [57]; stabilisation of mature focal adhesions [18]; increased myosin II recruitment and actin filament tension [58]; and increased paxillin phosphorylation [18]. These findings suggested that tropomyosins may be important regulators of actin function during physiological processes dependent on cell migration, including wound healing. The effect of wounding on Tm5NM1 expression has shown that Tm5NM1/2 expression is increased in response to wounding in mice skin and inversely correlates with paxillin phosphorylation and Rac activity regulating lamellapodial protrusions [60]. In addition, the effect of Tm5NM1 and Tm5NM2 isoform knock-down on wound healing using Tm5NM1/2$^{-/-}$ mice showed an accelerated wound healing response with smaller wound area and gape at day 7 post-wounding (**Figure 5**) suggesting a negative role for tropomyosins during wound repair [60]. Increased wound healing was not associated with increased cell proliferation or matrix remodelling but increased cell migration and activation of the paxillin/Rac signalling, suggesting that tropomyosin isoform expression has an important role in temporal regulation of wound repair [60]. Understanding how different actin remodelling proteins affect wound repair may hold clues for the development of novel therapies aimed at improved wound healing outcomes.

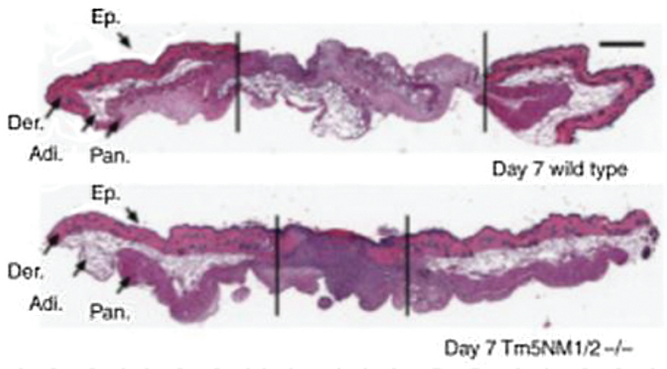

Figure 5. Cutaneous wound healing is accelerated at day 7 in Tm5NM1/2$^{-/-}$ mice. Representative H&E-stained transverse sections of full-thickness wounds after 7 days in Tm5NM1/2 and wild-type control mice (lines indicate the edges of the wound area). Adi., adipose tissue; Der., dermis; Ep., epidermis; Pan., panniculus. Bar = 500 μm. Figure adapted from [60].

6. Gelsolin family of actin remodelling proteins

The dynamic remodelling of the cytoskeleton is facilitated by the gelsolin family of remodelling proteins, which includes gelsolin, villin, adseverin, capG, advillin, supervillin and flightless I

[22]. These actin-binding proteins function in the cytoplasm of cells where they control actin organisation by severing pre-existing filaments, capping the fast-growing filament ends and nucleating or bundling actin filaments to enable filament reassembly into new cytoskeletal structures [61–64]. By remaining attached to the "barbed" ends of broken severed actin filament, these remodelling proteins prevent annealing of the broken filaments or addition of new actin monomers. Subsequently, the broken actin filaments are uncapped by interactions with phosphoinositides which results in rapid actin assembly and allows cells to reorientate the cytoskeleton and mediate the changes required for adhesion, motility and contraction [65]. The gelsolin family of actin remodelling proteins has three to six homologous gelsolin-like structural domains known as G1–G6 segmental domains, three actin binding regions and a number of calcium-independent monomer and filament binding domains. Villin, supervillin and Flii have evolved to contain additional domains allowing them to have multiple specific roles and interact with a variety of proteins. Villin contains an additional actin binding domain, termed villin head piece; supervillin contains an N-terminus domain capable of protein-protein interactions and nuclear localisation, while Flii contains a N-terminus leucine-rich repeat (LRR) domain also capable of multiple protein-protein interactions. In contrast, CapG only contains three gelsolin-like structural domains; however, it still retains full actin severing and capping ability and affects cell migration [8, 66]. There is a high homology in structure of different members of the gelsolin family; however, the differences in structure observed suggest evolutionary changes allowing specific and unique functional properties beyond actin remodelling [62, 67]. Indeed, specific functional roles have been demonstrated in cell motility, apoptosis and gene expression [68]. Several members including Flii, supervillin and gelsolin have roles in as nuclear receptor co-activators regulating gene expression [62, 69], and current studies have identified some of these proteins as new targets for improved healing and reduced scar formation [31].

6.1. Gelsolin

Gelsolin, the most abundant member of this family, is involved in regulating the dynamics of the filamentous actin by binding, severing and capping actin filaments [65]. In resting cells, gelsolin is either inactive or associated with filaments as a capping protein, while stimulation of cells or increased Ca^{2+} levels lead to an increased gelsolin activity at the plasma membrane and severing and capping of filaments resulting in increased cytoskeletal rearrangements [6]. High gelsolin levels have been associated with stress fibre formation and gelsolin was found to play a role in promoting stress fibre formation and actin stabilisation [70]. Gelsolin is also a secreted protein where its role in plasma is to "clean up" actin filaments that have been released into circulation during burn injury and cell necrosis using its gelsolin domain [27–29]. Plasma gelsolin is able to inactivate pathogen-associated molecular pattern (PAMPs) molecules, like lipopolysaccharides (LPS) and LTA (lipoteichoic acid) resulting in decreased TLR-mediated NF-kB activity [30, 31] suggesting a potential protective role for plasma gelsolin against inflammation. Gelsolin has also been shown to play a role in inflammation with studies suggesting potential clinical applications for plasma gelsolin in diagnosis and disease activity evaluation as patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) who have significantly decreased plasma gelsolin levels compared with healthy controls [23,

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24]. A study examining the differential effect of wounding on actin and actin-associated protein in foetal skin explants showed that it is not the migration or proliferation of cells but rather formation of the actin cable that is important in early gestation foetal wound closure [19]. In foetal skin, gelsolin was observed surrounding the actin filaments at embryonic day 19 but not at embryonic day 17 which coincided with its upregulation in embryonic day 19 foetal skin but not embryonic day 17 foetal skin [19]. In adult skin, however, gelsolin is expressed predominantly in suprabasal keratinocytes at the leading edge of migrating epidermis [71] and studies examining the effect of gelsolin on wound repair indicate that increases in cellular gelsolin levels in mouse fibroblasts enhance cellular migration and results in increased rates of wound closure [72]. Moreover, absence of gelsolin in skin fibroblasts results in a variety of actin-related defects, including decreased motility and delayed wound closure potentially due to reduction in the reorganisation of cytoskeletal actin into contractile elements [66].

6.2. Flightless I

Flightless I (Flii) is a highly conserved multifunctional protein possessing an unique structure, containing six gelsolin domains and an additional 11 tandem repeats of a 23-amino acid leucine-rich repeat (LRR) motif not present in other family members [5]. The specificity of its structure allows Flii to regulate multiple intracellular and extracellular processes [5]. Flii uses its gelsolin domain to bind and remodel (via severing, capping and bundling) cytoplasmic actin monomers (G-actin) and actin filaments (F-actin) and it possesses F-actin severing ability [67]. Unlike other members of the gelsolin family, which enhance actin polymerisation, Flii inhibits actin polymerisation [73] and associates with focal adhesions inhibiting their turnover in a Rac1-dependant manner [74]. Unique specificity of its LRR domain allows Flii to interact with multiple signalling and structural proteins including paxillin, talin, vinculin, Ras, Cdc42 and LRR Flightless Interacting proteins 1 and 2 [48, 74]. The bipartite domain structure of Flii provides capacity for it to transduce cell signalling events into remodelling of the actin cytoskeleton and Flii has been proposed to be involved in a variety of signalling pathways, many of which are important in wound healing [74–77]. In addition, Flii binds to proteins other than actin, both in the cytoplasm and in the nucleus as well as outside the cell [76, 77]. It is sequestered in the cytosol by the active form of the calmodulin-dependent protein kinase type II (CaMK-II) protein [76]. Within the nucleus, it binds to a variety of coactivator complexes and to nuclear hormone receptor molecules, thereby mediating changes in transcription [76]. Flii may therefore provide a link between cell signalling pathways and actin-dependent morphogenetic processes including proliferation, migration and adhesion [31, 74].

Flii expression is increased in response to tissue injury in fibroblasts and LPS activation in macrophages [31, 77]. Flii is found in the nucleus, cytosol, lysosomes and like gelsolin is also a secreted protein by both fibroblasts and macrophages through a late endosome/lysosome pathway regulated by Rab7 and Stx11 [5, 77, 78]. Secreted Flii has been detected in human plasma [77], and acute and chronic human wound fluids [78, 79] and this secretion allows it to affect both intracellular and extracellular TLR-mediated signalling and subsequent production of pro-inflammatory cytokines important during wound repair [77]. Like gelsolin, secreted Flii has been shown to inactivate LPS, resulting in decreased TLR activation and downstream

inflammation-mediated signalling [77]. In addition, Flii has been shown to control inflammasome activation by way of direct blocking of caspase-1 and caspase-11 and by modulating their subcellular localisation [80]. These findings suggest that Flii upregulation in response to wounding may be directed towards regulating inflammation with unfortunate consequences on healing of wounded area.

Complete knockout of Flii leads to gastrulation failure and embryonic lethality [81], while Flii heterozygous and transgenic mice appear phenotypically normal [82] suggesting an important role in development. In foetal skin, Flii is transiently increased in E17 but not E19 mice skin; however, its expression is downregulated in the E17 keratinocytes immediately adjacent to the wound margin [83] suggesting that temporal regulation of Flii during healing may influence wound repair outcomes. In addition, Flii interaction with tight junction proteins Cld-4 and ZO-2 is instrumental in development of skin barrier function and recovery following injury [84]. Wound healing studies using Flii heterozygous and transgenic mice have demonstrated that reduced Flii expression results in improved rate of healing via effects on cellular migration, adhesion and proliferation [31, 48]. In contrast, Flii transgenic mice have thinner more fragile skin, reduced number of hemidesmosomes and impaired cellular migration and adhesion leading to delayed healing [31, 48]. In addition, studies using mice with an inducible fibroblast specific Flii overexpression have shown inhibited wound healing with larger wounds than non-induced controls, suggesting that fibroblast-derived Flii may have an important role during wound repair [85].

Flii impairs the turnover of focal adhesions via a Rac1-dependant mechanism and Flii interaction with Rac1-interacting proteins may be crucial to its effects on cell migration [74, 86]. In addition, Flii inhibits actin polymerisation [73] and this delicate balance of actin monomers and polymers can be altered using Flii neutralising antibodies (FnAb) raised against LRR domain of Flii [84] affecting collagen contraction, angiogenesis and wound healing outcomes [31, 87, 88].

Topical application of FnAb to wounds in preclinical models of wound repair results in a decreased wound area, a quicker rate of healing and decreased early scar formation [31, 88, 89] (**Figure 6**). Supporting these findings, both in vitro and in vivo studies have demonstrated that Flii plays a role in tissue scarring, collagen deposition and contraction [89, 90]. Using a preclinical model of porcine wound healing, studies have shown that Flii affects collagen I to collagen III ratio, impairs healing and contributes to the formation of early scars [89]. In addition, in vivo studies using human studies and animal models of bleomycin-induced hypertrophic scaring show that Flii-deficient mice exhibit reduced scarring in response to bleomycin as evident by decreased dermal thickness, smaller cross-sectional scar areas, fewer myofibroblast numbers and increased collagen I to collagen III ratios [91]. Use of FnAb in porcine models of wound healing is the first example of using antibodies in large animal in vivo to modify the regulators of actin cytoskeleton that lead to improved wound healing outcomes. No side effects, complications or contraindications were observed when FnAb was administered locally to mouse or pigs suggesting the potential for the development of this therapy for human use. Application of such approaches to regulate different modulators of

http://dx.doi.org/10.5772/64673

actin cytoskeleton may therefore lead to novel therapies aimed at optimal tissue regeneration and decreased scar formation following injury.

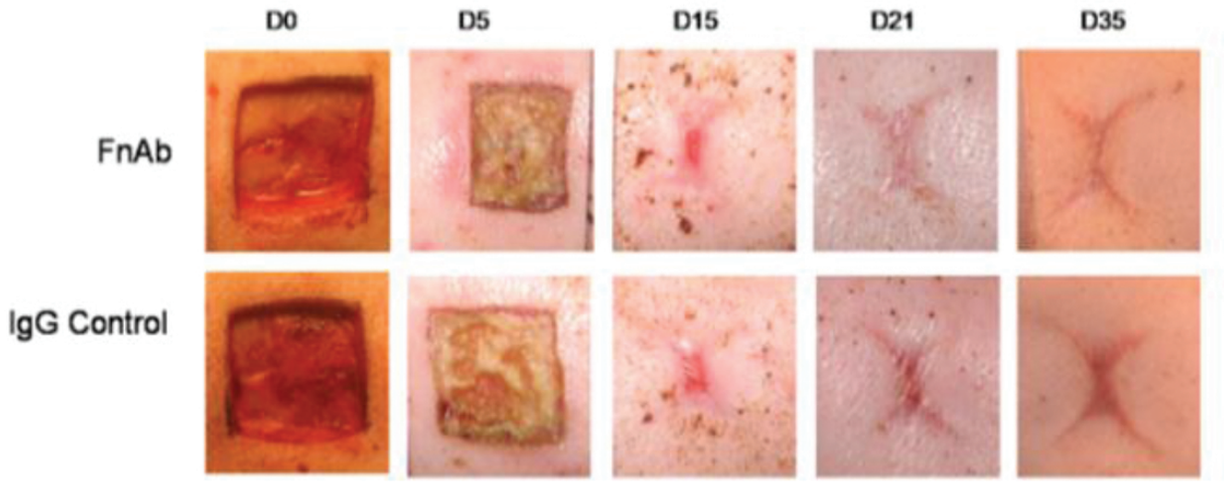

Figure 6. Treatment of excisional wounds with an FnAb improves wound healing and early scar appearance. Representative macroscopic images of wounds treated with either FnAb or a dose-matched IgG control on days 0, 5, 15, 21 and 35. These images were all taken from the same distance. The FnAb- and IgG control-treated wounds are from the same animal and the same position on either flank. Figure adapted from [89] and modified.

7. Conclusion

The actin cytoskeleton is an important regulator of numerous physiological processes that are important for efficient wound healing. Research has identified novel regulators of the actin cytoskeleton that can affect skin cell functions, tissue regeneration and repair. Identifying and understanding the role of the actin cytoskeleton and these regulating proteins will identify how they can affect outcomes of wound repair. The development of new approaches aimed at modulating actin remodelling proteins may therefore hold tremendous promise for therapeutic development and translation into clinical practice.

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