Role of the actin cytoskeleton in wound healing and scar formation

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Abstract

The actin cytoskeleton is an essential network of filaments found in all cells and is important in the process of migration, adhesion and proliferation. All these processes are fundamental to wound repair. Members of the gelsolin family of actin-severing proteins are important regulators of cytoskeletal organisation. Manipulation of these proteins has revealed important roles in wound repair, suggesting that they may be potential new targets for therapeutic intervention to help improve wound healing and reduce scar formation.

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Adult wound repair and the actin cytoskeleton

Cutaneous wound repair is characterised by acute inflammation, contraction, collagen deposition and re-epithelialisation, a sequence of events involving the interaction of various cell types [1]. Chief among these are inflammatory cells, fibroblasts and keratinocytes, the latter two being responsible for rebuilding and repairing the wound.

After wounding, an influx of inflammatory cells release cytokines to stimulate migration of fibroblasts into the wound. The fibroblasts synthesise extracellular matrix (ECM) proteins such as collagen to replace the damaged dermis and, as the dermis is reconstituted, epidermal keratinocytes migrate across the new tissue to effect wound closure by the process of re-epithelialisation. Changes in cell adhesion, shape and motility are therefore essential processes for wound repair and, to accomplish these, keratinocytes and fibroblasts must be able to translate a multitude of incoming signals into appropriate responses.

The actin cytoskeleton consists of a network of filaments found in all cells. It is dynamic and remodelled in response to a variety of stimuli to generate the mechanical forces necessary for changes in cell contraction, adhesion and motility that underpin tissue repair. These changes include the lammellipodial crawling of keratinocytes during wound re-epithelialisation, infiltration of inflammatory cells and migration of fibroblasts required for the deposition and remodelling of the ECM and dermal contraction at the wound site [2-4].

Actin-remodelling proteins of the gelsolin family [2, 5] are instrumental in reorganising the actin cytoskeleton. Members of this family usually contain either three or six copies of a repeat unit of about 125 amino acids originally found in gelsolin and referred to as 'gelsolin domains'. The family now includes gelsolin, Flightless I (FliI), adseverin, CapG, villin, advillin, protovillin and supervillin. These proteins regulate actin filaments by severing pre-existing filaments and/or capping the filament ends, a process involving the gelsolin domains [6, 7]. After severing, they remain attached to the 'barbed' ends of the broken filament, thereby preventing annealing or addition of actin monomers. Actin filaments are subsequently uncapped by interaction with phosphoinositides, leading to rapid actin assembly [2]. This is the first step in enabling cells to reorientate their cytoskeleton

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to drive changes in motility, adhesion and contraction.

An additional function of gelsolin is its ability to scavenge any actin that is exposed to extracellular spaces or released into the circulation after tissue injury [8]. Spillage of large amounts of actin can overwhelm the capacity of circulating gelsolin and result in its depletion.

The persistence of actin within the microvasculature can contribute to the pathogenesis of organ injury at sites removed from the primary insult [9-11]. The principal evidence for this hypothesis is a precipitous drop in circulating levels of gelsolin after severe injury or illness [9-11]; the demonstration of actin and actin-gelsolin complexes in the plasma of critically ill patients [10, 12]; and a relationship between reduced plasma levels of gelsolin and poor clinical outcome in a wide spectrum of diseases [9-11].

Role of gelsolin family in wound repair and burn injury

There is limited information concerning the involvement of the actin-remodelling proteins of the gelsolin family in the wound repair process. In adult skin, gelsolin is expressed primarily in suprabasal keratinocytes and dermal fibroblasts and appears to be reduced in keratinocytes at the leading edge of migrating epidermis in suction blister wounds [13]. Studies with gelsolin knockout mice indicate that, in skin fibroblasts, absence of gelsolin causes a variety of motility and actin-related defects, including pronounced stress fibres and an inability to sever and remodel actin filaments [14].

Plasma gelsolin levels decrease to 10% of their normal levels after burn injury [15]. Treatment of burned animals with intravenous infusion of gelsolin attenuates burn induced pulmonary vascular dysfunction. Using microarray technology CapG, Villin and Supervillin, all members of the gelsolin family are significantly reduced in response to burn injury; this down-regulation persists even 14 days post-wounding [16]. Clearly there is a direct effect of burn injury on members of the gelsolin family which may be critical in the process of tissue repair and scar formation.

Role of FliI in wound repair and scar formation

The actin remodelling protein FliI is a member of the gelsolin family of actin-severing proteins. It was originally identified in *Drosophila melanogaster* where mutations in the gene cause defects in the flight muscles which, consequently, are unable to support flight [17]. In the fly, *FliI* null mutations are embryonic lethal. The FliI protein is highly conserved between mouse and human and is the most evolutionarily conserved member of the gelsolin family [18], suggesting that it carries out important functions. FliI is present within migratory structures such as neurites, growth cones and sites of dynamic actin rearrangements such as filopodia, suggesting a role for FliI in actin reorganisation [19, 20].

In addition to its actin-binding, gelsolin-like domains, FliI has a leucine rich repeat (LRR) domain, a protein-protein interaction domain not found in other members of the gelsolin family. The LRR domain is thought to mediate membrane-protein and protein-protein interactions, either by binding directly as a ligand or as a regulator to enhance the affinity of binding [6, 17, 19]. The FliI LRR may endow FliI with the capacity to interact and respond to other specific protein molecules [17, 19] and a number of specific ligands have been identified [21, 22].

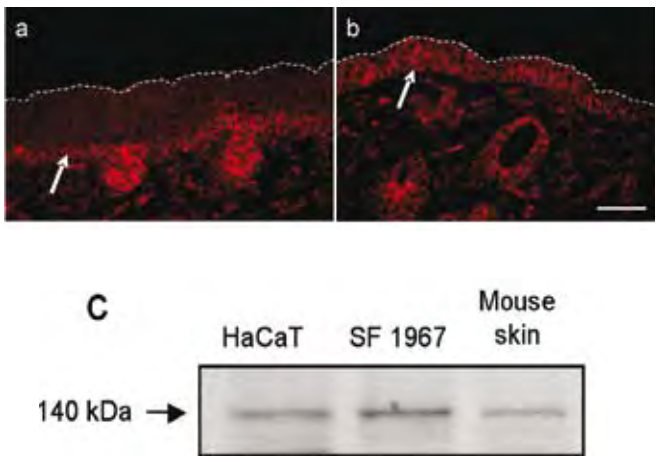
In serum-stimulated fibroblasts, FliI specifically colocalises with cytoskeletal structures connected with migration [19, 20]. It is associated with actin arcs, membrane ruffles and at the leading edge of cells, where it also colocalises with the GTP-binding proteins, ras, cdc42 and rhoA, that have central roles in regulating cytoskeletal reorganisation [23, 24]. The depolymerisation and repolymerisation of actin that occurs at membrane ruffles and which contributes to cell locomotion may be mediated by the actin-severing ability of FliI.

FliI and gelsolin are differentially expressed in the epidermis. FliI is present in human skin keratinocytes and dermal fibroblasts and in foetal and adult mouse skin (Figure 1). Their expression in skin appears to overlap but there is a key significant difference. FliI is abundant in the proliferative basal and differentiating suprabasal keratinocytes (Figure 1), whereas gelsolin is primarily expressed in the differentiating suprabasal cells [13]. As the proliferative basal cells are the cells that repopulate and repair the epidermis in response to injury, this points to a more significant role for FliI in wound repair. Using *FliI* transgenic and knockout mice and *in vitro* models of wound repair, we have found that FliI is a crucial mediator of wound healing and that it provides a mechanistic link between cytoskeletal remodelling in response to injury and induction of TGF-β1 expression.

Role of the actin cytoskeleton in scar-free foetal wound repair

Of major interest in the field of anti-scarring research is the fact that foetal skin has the capacity to heal a wound without scar formation [25]. This occurs by a process of regeneration

Figure 1. FliI is present in foetal and adult skin. Immunohistochemical staining of FliI in foetal (a) and adult (b) mouse skin. (c) Western blot detection of endogenous FliI (140kD) in extracts from human keratinocytes (HaCaT), skin fibroblasts and adult mouse skin with FliI antibody. Magnification bar in (b) refers to both images and = 50µm Arrows point to FliI positive cells.

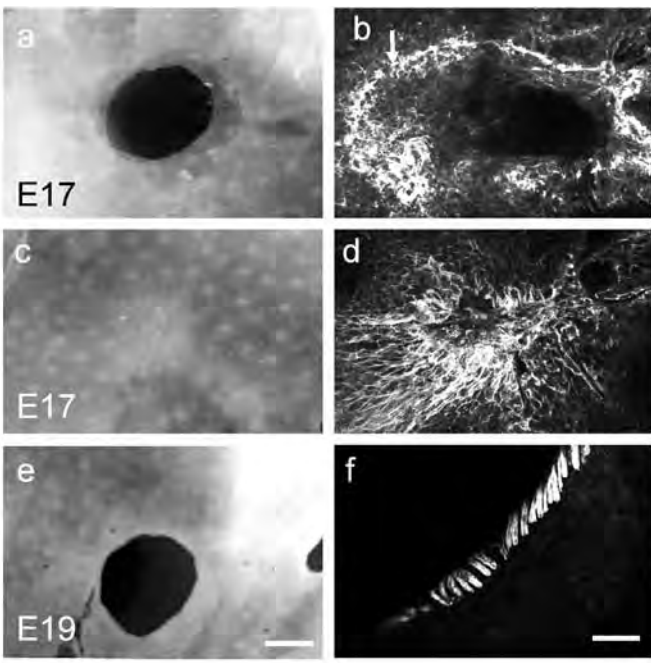


rather than repair, resulting in essentially perfect healing with the re-establishment of differentiated structures such as glands and hair follicles [1]. The capacity for scar-free healing remains until late in gestation in rodents and the third trimester in humans. After that period, and just before birth, a switch to 'adult' type wound healing occurs with concomitant scar formation.

Our studies have revealed that, in addition to a diminished inflammatory response and a different cytokine profile, the actin cytoskeleton is pivotal to foetal scar-free repair [4, 26, 27]. Skin from the embryonic Day 17 rat retains the ability to epithelialise an excisional wound when isolated in serum-supplemented suspension culture. This ability is lost by embryonic Day 19.

We have investigated this effect of gestational age on foetal epithelial wound closure by correlating the involvement of filamentous actin (F-actin) and its associated proteins, paxillin and gelsolin, in the wound margins of embryonic Day 17 and 19 rat skins, with the ability to close a full thickness excisional wound. Using fluorescent-phalloidin histochemistry and scanning confocal microscopy, actin polymerisation is observed some five to six cells back from the margin of wounds in the embryonic Day 17 skin as early as 3 hours post-wounding [4] (Figure 2). As the wounds close over the following 48-72 hours, the actin condenses around

Figure 2. Actin cables are formed in early but not late gestation foetal skin. Wounds created in E17 but not E19 foetal skin, when cultured in DMEM/10%FBS re-epithelialise and close over 72 hours. Wounded E17 foetal skin at (a) 3 hours post-wounding and (c) 72 hours post-wounding. (e) wounded E19 foetal skin 72 hours post-wounding. Phalloiding-FITC staining of F-actin reveals actin ring around E17 wound 48 (b) and 72 hours post wounding (d). No cables are formed in E19 foetal skin wound (f). Magnification bar in (e) = 600 µm and in (f) =50 µm. Arrow in (b) points to actin cable.



the epithelial margin before dispersing after wound closure.

In contrast, no organisation of actin is seen in the epithelial margin of wounds in skin from the embryonic Day 19 embryos. Chemical or mechanical disruption of the actin in wounded embryonic Day 17 skins prevents epithelial closure. In particular, incising the wound margin 24 hours after wounding results in the 'springing-open' of the E17 wound but not the E19 wound, reflecting the development of tension in the E17 wound margin. Expression of paxillin mRNA is upregulated following wounding at E17 but not at E19. Paxillin is also observed to colocalise with actin in E17 wounds, but not E19 wounds, indicating a potential role for paxillin in epithelial repair of the foetal wound. In contrast, gelsolin mRNA is upregulated in E19 foetal skin but not at E17 and gelsolin protein is observed surrounding actin filaments at E 19 but not E17.

The effect of wounding on the expression of contractile F-actin *in utero* in mice between embryonic Day 16 (E16) and embryonic Day 18 (E18) also reveals increased F-actin staining in the epidermis of E16 foetal wounds as early as 3 hours post-wounding, peaking in intensity after 24 hours [28]. In marked contrast, E18 foetal wounds show no increase in epidermal F-actin fluorescence, instead increased staining is observed in cells lying perpendicular to the wound margin within the dermis. These results demonstrate the importance of the cytoskeleton in wound epithelialisation in foetal wounds and may be important in foetal wound contraction and scar-free wound repair.

Conclusion

In conclusion, the actin cytoskeleton is an integral component of all cells and is crucial for cell motility, adhesion and proliferation. Manipulating specific members of the actin cytoskeleton could improve wound re-epithelialisation, matrix synthesis and wound contraction. Furthermore, it may be possible to manipulate levels of actin cytoskeletal proteins to promote healing via foetal wound repair mechanisms and thereby reduce scar formation.

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