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Wound repair: a showcase for cell plasticity and migration

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Abstract

A skin wound requires several cell lineages to exhibit considerable plasticity as they migrate towards and over the site of damage to contribute to repair. The keratinocytes that re-epithelialize the tissue, the dermal fibroblasts and potentially other mesenchymal stem cell populations that repopulate damaged connective tissue, the immune cells that counter infections, and endothelial cells that re-establish blood supply and facilitate the immune response - all of these cells are "dynamic" in that they are activated by immediate wound cues, they reprogram to adopt cell behaviours essential for repair including migration, and finally they must resolve. In adult tissues, repair is unique in its requirement for dramatic cell changes and movements otherwise associated only with development and disease.

Introduction

During embryonic development, individual cells and groups of cells are continually migrating and epithelial sheets are folding and fusing in order to sculpt tissues and organs and ensure that the right cell lineages are where they need to be in the developing organism. Beyond foetal stages however, cell movements are most often associated with pathologies such as metastatic spread of cancer cells. But there is one healthy scenario, the repair response at any site of tissue damage, where several cell lineages exhibit considerable plasticity as they are marshalled to migrate towards and over the site of damage in order to heal the wound. In several ways wound healing appears to recapitulate many of the cell and tissue migrations of embryonic morphogenesis.

Wound repair begins with the temporary plugging of both damaged vessels and the breach in the barrier layer with a fibrin clot or scab. In subsequent hours and days, and even weeks, many cell lineages work in a concerted fashion to repair the defect as best they can. Innate immune cells, largely neutrophils and macrophages, are recruited from local tissues and from the blood circulation. The epidermis repairs from the wound margins and potentially from epithelial appendages if any remain, and the dermis is reconstituted by fibroblasts migrating in from various sources to form a temporary wound granulation tissue which is heavily vascularised by local angiogenesis. Each of the lineages involved in these cell and tissue migrations is dramatically altered by local wound signals; their transcriptomes change significantly and in many ways they can be considered as transiently reprogrammed during the healing period. Considering each of these dynamic cell populations in turn, we discuss how each contributes to the repair process and what goes wrong if the reprogramming events fail.

The wound inflammatory response

After tissue damage, an innate immune response is immediately triggered, with neutrophils spilling out passively from damaged blood vessels or actively recruited by diapedesis from local wound capillaries (Figure 1). Macrophages tend to follow in the wake of neutrophils with their numbers peaking at later stages; these cells derive from two sources: tissue residents already in the vicinity of the wound, and recruited monocytes drawn from the local wound vasculature.

Inflammation, *per se*, is not absolutely critical for healing, because embryos can repair tissue damage before the first innate immune cells are established [1], and neonatal mice null for the leukocyte lineage switching ets-family transcription factor PU.1, and lacking innate immune cell lineages, can repair wounds very effectively [2]. However, adult tissue repair appears much more dependent, at least on macrophages, with classic anti-macrophage serum knockdown experiments in rabbits exhibiting poor healing [3], and more recent, temporally-regulated diptheria toxin-mediated killing of macrophages in mice revealing differing healing defects depending on what phase of healing is targeted [4]. Certainly the inevitable inflammatory response to tissue damage has potent and important paracrine influence on the repair process.

Neutrophils are considered to be primarily bactericidal at wounds, killing by means of reactive oxygen species (ROS) and neutrophil extracellular traps [5]. In recent years we have learned more about their diapedesis and extravascular migration to sites of tissue damage by intravital investigations in mice; for example, neutrophils have been observed responding to adenosine triphosphate and formyl-peptide signals from necrotic cells [6]. Studies in the translucent zebrafish have perhaps provided the clearest opportunities to visualise neutrophil migration towards and away from sites of tissue damage and wound infection. This model has contributed to the identification of evolutionarily-conserved immediate damage signals following tissue damage, including calcium [7] and hydrogen peroxide [8,9], and subsequent studies in *Drosophila* show that these are the same attractants that draw hemocytes (fly innate immune cells) to wounds [10,11]. Recent zebrafish studies of calcium dynamics in responding neutrophils indicate enrichment at the leading edge, at a distance and then as cells get closer to the wound they exhibit long, whole cell calcium pulses, and disruption of these calcium dynamics severely compromises homing of neutrophils to the wound [12]. *In vivo* imaging of the resolution of the innate immune response has begun to offer opportunities to identify the molecular mechanisms regulating reverse migration and agents that might modulate this process [13,14]. Neutrophil behaviour changes upon wound activation to enable their important microbiocidal and phagocytic functions, but interestingly, this change in phenotype appears not to disturb the cell's capacity to respond to subsequent wound signals [15]. A role for pro-resolving factors in inflammation, and their potential use in driving therapeutic resolution of leukocytes from wounds comes from a

study in mouse where treatment with Chemerin 15 dampened the inflammatory response and improved healing [16].

Macrophages fulfil a portfolio of roles that change over the duration of healing. Initially they are bactericidal, and voraciously phagocytose cell and matrix debris, particularly clearing red blood cells and any spent neutrophils at the wound site. The long-term influence of these early macrophages became evident when Lucas et al [4] depleted them specifically during early time-points after injury – their absence retarded re-epithelialisation as well as reduced wound granulation tissue and eventual scar size. Later in the repair process, macrophages develop pro-repair capacity, for example promoting wound angiogenesis by release of vascular endothelial growth factor (VEGF) and other angiogenic factors [4].

Strategic depletion experiments illustrate that macrophages can orchestrate key behaviours in several host cell lineages within the healing wound. How they might instruct various aspects of the repair process is not yet entirely clear but they are known to express numerous growth factors and cytokines at various phases of the repair process; one of these, for example, TGFβ1 is known to have profound effects at the wound site, particularly in its influence on fibroblast deposition of scar collagen [17]. Further insights have been gained through microarray comparisons of wild type versus PU.1 knockout mouse wounds, which revealed several inflammation-dependent wound induced genes [18]. This dataset reflects both the leukocyte transcriptional response to injury, as well as the indirect influence on the surrounding tissue, and mining for transcripts induced in wound fibroblasts only after macrophage recruitment has been informative about the pro-fibrotic influence of inflammation. For example, knocking down spp1/osteopontin, which is induced in scar-associated fibroblasts by macrophage-derived, platelet-derived growth factor (PDGF), significantly reduced the extent of wound granulation tissue formation and scarring [19].

The changeable cellular phenotype of macrophages, and the range of differentiation and activation states [20] helps to explain the pleiotropic nature of these cells and their complex functions in wound repair. To simplify their descriptions, the extreme ends of the most distinct polarization options (resting, M0; bactericidal and pro-inflammatory, M1; anti-inflammatory/pro-repair M2) are often described. The spectrum of phenotypes has been revealed through sampling wound macrophages harvested from polyvinyl alcohol (PVA) sponges in mice [21,22]. That prior experience can influence the fates of these cells is a

feature anticipated to be of relevance to the wound context. For example, in response to environmental changes such as bacteria exposure in an early wound, cells will have been epigeneticially programmed or primed [23,24], which can in turn provide a level of restriction for future characteristics [25]. New insights into how macrophages might be primed prior to wound exposure comes from studies in *Drosophila* where there is clear evidence that macrophages are incapable of even sensing a wound until first primed by engulfment of apoptotic debris and a calcium flash mediated signalling cascade [26].

Changes in immune cell phenotype/plasticity during the wound inflammatory response may be pivotal in influencing how they interact with the wound cells sharing their environment. There have long been hints that tissue scarring is evolutionarily linked to the type-2-cell mediated immune response to parasitic infections that lead to fibrous encapsulation of helminths as a host protection response [27]. Just as macrophage phenotype-switching via IL4R activation drives parasitic encapsulation, it can lead to tissue scarring; a recent study shows that this might be mediated via Relm-α signalling which, in turn, drives expression of persistent collagen cross-linking enzymes leading to the bundled unresolvable collagen of a dermal scar [28].

Addressing the extent to which the changing immune environment during the time-course of wound repair reflects reprogramming of individual macrophages, or successive incoming waves of cells with different characteristics will require technically challenging live imaging investigations. A recent study in healing wounds in the scalp of living mice goes some way towards characterising macrophage influx into wounds and reveals a surprisingly early, transient population that leave wound vessels via micro-hemorrhages, rather than by diapedesis [29].

Epidermal repair

One of the key tissue movements of any skin wound healing episode is re-epithelialisation. In a skin wound context, the triggers for rapid keratinocyte activation are numerous, and include damage signals such as H_2O_2 and calcium, changes in mechanical tension, pathogen sensing, loss of electrical gradient, and serum exposure [30-33]. Features of activation include induction of stress signalling cascades leading to immediate early gene activation (e.g. Fos and early growth response genes, EGRs [34,35]), which in turn mediate vast transcriptional changes. The dramatic epithelial response to injury is not only protective

through promoting the immune response and limiting DNA damage [36,37], but also it actively drives repair by initiating a transient reprogramming of the edge cells [38,39], a phenotypic change that has been equated to a partial epithelial-to-mesenchymal transition (EMT)[40,41]. This renders the wound-edge cells migratory and invasive [42], immunogenic (and thus self-limiting)[43], and proliferative [44]. These migrating cells are clearly very vulnerable *en route* because of the loss of protective cornified and pigmented stratified layers of normal skin and their exposure to increased stressful stimuli including inflammation-triggered ROS at the wound site. To cope with these stresses, the epidermal cells activate a series of interacting glutathione-NRF2-thioredoxin pathways that together enhance keratinocyte viability during healing [37].

It is the basal cells of a stratified epidermis that attract most of the attention in cell migration studies; arguably more plastic than the differentiated apical layers, the basal cells at the immediate wound margin are considered to take the leadership role, guiding collective migration of the tissue layer. However, recent *in vitro* work and *in vivo* studies in model organisms particularly amenable to live imaging, indicate that suprabasal cells and also many rows of "follower" cells are activated, and in turn contribute in non-passive ways to forward migration of the epithelial sheet (Figure 1). Immunostaining of repairing blister wounds showed changes in integrin expression in suprabasal cells [45,46]. Electron microscopy (EM) of full-thickness mouse wounds shows that both basal and immediately suprabasal layers extending 70 or more rows back from the leading edge exhibit considerable loosening of adhesions between neighbours. It appears that up-regulation of EphrinB1 in the basal and suprabasal cells may lead to down regulation of components of both tight and adherens junction leaving epithelial cells only loosely linked to one another by modified desmosomal junctions. This loosening of junctions between cells releases tension and provides space for shuffling forward of follower cells [44]. A study of wounds made in *Drosophila* embryos similarly revealed changes in "follower" cells; specifically, ratcheting, and myosin-mediated cell:cell junction shrinkage episodes leading to cell intercalations were observed, just as occur in several embryonic morphogenetic processes [47]. In the embryo these intercalations are believed to drive tissue extension, but in wound healing it may be less about actively extending the tissue, but rather, a need for release of epithelial tension to enable forward movement [48]. It is not yet known if similar molecular mechanisms are at play in local epidermal stem cell populations (e.g. interfollicular epidermis [49], hair follicle

bulge [50], sweat glands and ducts [51,52]), allowing for their release and recruitment to a healing wound. Although the functional importance of the transient contribution of these cells to re-epithelialisation is debated [53], understanding how their migrations are regulated would be valuable to many contexts.

In their effort to repair a breach in the epidermal barrier, migrating wound-edge keratinocytes and follower cells lay down new basement membrane components ahead of themselves [54], and a well-organised substratum looks to be essential to successful re-epithelialisation [55]. However, these cells also encounter and sense, via integrins, the new and unusual provisional wound extracellular matrix (ECM). Sensing this alternative substrate is an important trigger of the wound response; contact with wound- or dermis-associated matrix proteins (*e.g.* collagen I, fragmented ECM components) causes many cellular changes including induction of protease expression [56]. Adopting this degradative phenotype is considered necessary for the epidermal tongue to cut its pathway between scab and healthy wound granulation tissue until it meets its opposing partner to fuse to and seal the wound gap (Figure 1). Migrating keratinocytes appear to navigate this route between tissue layers guided by ECM-integrin "outside-in" signalling, as their integrin profile makes them selective about their substrates including an avoidance of fibrinogen/fibrin [57].

Several years ago we showed that many of the transient gene changes that occur in the advancing epidermal front such as upregulation of the epidermal growth factor (EGF) receptor, may be downstream not just of immediate early gene activation [34,35], but also dependent on epigenetic unsilencing mechanisms mediated by clearance of particular polycomb-deposited histone marks [38,39], which need to be reinstated after the normal epidermal architecture is restored. Changes in epithelial cell behaviour exhibited in a repairing wound are not dissimilar to a number of pathologies involving migrating epithelial cells including metastatic spread of some cancers, and so *transient* epigenetic regulation may be a key strategy that limits cancer-associated cell behaviours at the wound site.

Wound-associated fibroblast dynamics

Wound-associated fibroblasts are important for repopulating lost tissue in the wound defect, depositing new collagen matrix, and also contributing to closure through their contractility (Figure 1). As with the keratinocytes, fibroblasts sense and then "activate" in response to many aspects of tissue damage, including the immediate cues such as serum

exposure, changes in mechanical tension, and pathogens [58-60]. These cells then adapt to the changing wound environment as healing progresses, fine-tuning their behaviours in response to changing mechanical properties, inflammatory and fibrogenic signals, and oxygen levels [60-62], producing first temporary, and then more permanent matrix with varying composition.

Alpha-smooth muscle actin induction and increased collagen I expression have become hallmarks of dermal fibroblast activation [63], although the wound-induced phenotypic changes occurring in these cells extends much deeper. Re-enacting wound-associated stimuli on *in vitro* fibroblasts cultures has demonstrated the plasticity of this cell population – with epigenetic reprogramming [64,65] that is certain to facilitate their dramatic changes in gene expression and behaviours.

It has always been presumed that a skin wound activates dermal fibroblasts at the margin of the damage, and it is these edge-cells that are triggered to migrate into the wound bed where they can make their contribution. The most definitive recent lineage studies in mice have refined what we know about the origins and eventual fates of locally-derived wound fibroblasts; labelling sub populations of skin fibroblasts at developmental stages and then wounding adult mice demonstrated that the initial wound infilling comes from the lower (*i.e.* reticular) dermis of adjacent unwounded skin, with a subsequent later wave, behind the advancing wound epidermis, deriving from upper (*i.e.* papillary) dermal cells of the adjacent skin [66]. The nature and timing of these migrations may, in part, contribute to the aberrant architecture of wound scar tissue and explain why it is generally impotent at regenerating new appendages (*e.g.* hair and sweat glands) since the lower dermal cells do not respond to the inductive signals [67].

Although these data suggest that reticular wound-margin dermal fibroblasts make the major contribution to regenerating the dermis, there is evidence that alternative stem cell populations can infiltrate the wound also. Although their ultimate contribution to repairing tissue remains controversial and under investigation, even the smallest number of infiltrating cells could be potent in their paracrine effects [68,69], or provide a subordinate cell source for repair, or contribute to wound-associated pathologies. For example, multipotent bone marrow-derived mesenchymal stem cells (MSCs)[70,71] appear to be recruited to sites of tissue injury. These, or even other multipotent cell populations [72], could be already residing in the skin (*e.g.* peri-vascularly or in the adipose tissue [73,74]), or

circulating as with "fibrocytes" [75,76] and recruited to the site of injury in a manner similar to leukocytes.

Live-imaging to dissect the mechanisms of fibroblast or MSC migration at a cellular level has been difficult because of the tissue depth and lack of specific cell markers, but with technical advances in microscopy and improved transcriptional delineation of cell types [66], visualising the movements of different cell populations into the wound may soon be possible. Nevertheless, *in vitro* studies and histology of *in vivo* wounds have shown that a range of chemoattractants such as insulin [77], platelet derived growth factor [78] and CCL21 [79], can help draw fibroblasts and MSC into wounds through directional cell migration. Growth factors also appear to mediate the ultimate fate of wound fibroblasts, with options including apoptosis [80,81] or dedifferentiation and a return to relative quiescence [82].

Irrespective of origin and state of differentiation, the heterogeneous collection of mesenchymal cells within the granulation tissue are all thought to be "dynamic", altering their cellular phenotype and migratory behaviour when faced with the complex wound environment. Single cell analysis of dissociated wound beds has the potential to inform about cellular origins, and their varying capacities for reprogramming in response to a wound, and their potentially unique contributions to repair. As discussed in the inflammation section above, fibroblasts in the wound granulation tissue go on to lay down a collagenous scar matrix and this is heavily influenced by signals from invading innate immune cells. It will be important in future to begin to observe (ideally intravitally) how the migratory behaviour of fibroblasts impacts on matrix deposition at the wound site and whether therapeutic modulation of this can be utilised to block wound scarring.

Wound angiogenesis

Healing wounds are pink because of a considerable angiogenic response at sites of tissue damage (Figure 1), which is presumed to be in response to the increasing metabolic demands of the repairing wound. Just as with wound fibroblast activities, the sprouting of vessels in wound granulation tissue is not easily amenable to live imaging (although translucent zebrafish models might soon change this), but there are lessons to be learned from developmental angiogenesis. A study in mouse and zebrafish embryos indicates that endothelial tip cell sprouting requires VEGF and that macrophages (which might also be the

primary source of the VEGF) act on these sprouts to nurture vessel anastomosis [83]. Consistent with this, deletion of VEGF expression in macrophages severely reduced angiogenesis in open murine excisional wounds [84]; however, more recent studies indicate that macrophages can also have a "dampening" role on wound angiogenesis via non-canonical Wnt signalling and activation of Flt1 (a non-signalling VEGF receptor), which acts to suppress VEGF-mediated angiogenesis [85]. Also, a study in RhoB null mice showed that this non-constitutively expressed small GTPase, acting via VEZF1, is necessary for wound blood vessel sprouting, and that coincident with reduced wound angiogenesis in KO mouse wounds, there was enhanced lymphangiogenesis, which is normally much delayed by comparison to blood vessel sprouting [86]. These studies suggest a complex interplay between these two vessel types at the wound site, and of course both will need to remodel during the resolution phase of wound repair, to re-establish normal cutaneous vessel architecture. This field of research is likely to rapidly expand in the coming years (presumably benefitting from angiogenesis studies in developmental biology and cancer), as we know that poor wound angiogenesis is fundamental to chronic healing.

Conclusions

The wound is a complex *in vivo* masterclass in cell migration, with many cell lineages performing together to heal the wound. Whist immune cells are designed for recruitment to distant sites, several of the other lineages involved would presumably, in the absence of a nearby wound cue, live out their lives as fairly dormant, largely immobile cells. For several of them, it seems clear that they undergo major reprogramming in order to commence their migrations and perform other new functions in the wound. We clearly need to understand these reprogramming events better in order to learn how we might enhance healing when it goes wrong, and, to that end, we need to develop better imaging approaches that allow us to visualise these migration as they occur *in vivo* in mammalian skin wounds, and to extrapolate from the genetically tractable models such as flies and fish. Normal, healthy wound repair is a showcase for the adaptability and migratory potential of the cells in our skin as they recapitulate processes that they last undertook when they were cells undergoing morphogenetic episodes in the embryo.

References and recommended reading

of special interest (*)
of outstanding interest (**)

1. Hopkinson-Woolley J, Hughes D, Gordon S, Martin P: **Macrophage recruitment during limb development and wound healing in the embryonic and foetal mouse**. *J Cell Sci* 1994, **107 (Pt 5)**:1159-1167.

2. Martin P, D'Souza D, Martin J, Grose R, Cooper L, Maki R, McKercher SR: **Wound healing in the PU.1 null mouse--tissue repair is not dependent on inflammatory cells**. *Curr Biol* 2003, **13**:1122-1128.

3. Leibovich SJ, Ross R: **The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum**. *Am J Pathol* 1975, **78**:71-100.

4. Lucas T, Waisman A, Ranjan R, Roes J, Krieg T, Muller W, Roers A, Eming SA: **Differential roles of macrophages in diverse phases of skin repair**. *J Immunol* 2010, **184**:3964-3977.

5. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A: **Neutrophil extracellular traps kill bacteria**. *Science* 2004, **303**:1532-1535.

6. McDonald B, Pittman K, Menezes GB, Hirota SA, Slaba I, Waterhouse CC, Beck PL, Muruve DA, Kubes P: **Intravascular danger signals guide neutrophils to sites of sterile inflammation**. *Science* 2010, **330**:362-366.

7. Sieger D, Moritz C, Ziegenhals T, Prykhozhij S, Peri F: **Long-range Ca2+ waves transmit brain-damage signals to microglia**. *Dev Cell* 2012, **22**:1138-1148.

8. Niethammer P, Grabher C, Look AT, Mitchison TJ: **A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish**. *Nature* 2009, **459**:996-999.

9. Yoo SK, Starnes TW, Deng Q, Huttenlocher A: **Lyn is a redox sensor that mediates leukocyte wound attraction in vivo**. *Nature* 2011, **480**:109-112.

10. Moreira S, Stramer B, Evans I, Wood W, Martin P: **Prioritization of competing damage and developmental signals by migrating macrophages in the Drosophila embryo**. *Curr Biol* 2010, **20**:464-470.

11. Razzell W, Evans IR, Martin P, Wood W: **Calcium flashes orchestrate the wound inflammatory response through DUOX activation and hydrogen peroxide release**. *Curr Biol* 2013, **23**:424-429.

** 12. Beerman RW, Matty MA, Au GG, Looger LL, Choudhury KR, Keller PJ, Tobin DM: **Direct In Vivo Manipulation and Imaging of Calcium Transients in Neutrophils Identify a Critical Role for Leading-Edge Calcium Flux**. *Cell Rep* 2015, **13**:2107-2117.

This is a lovely light sheet imaging study showing calcium pulses in zebrafish neutrophils as they migrate towards a wound. Technically clever, they expressed human TRPV1 calcium channel in zebrafish neutrophils, and stimulated with capsaicin (the zebrafish orthologue is not sensitive to capsaicin) to show how disrupting neutrophil calcium dynamics blocks their capacity for wound homing.

13. Burgon J, Robertson AL, Sadiku P, Wang X, Hooper-Greenhill E, Prince LR, Walker P, Hoggett EE, Ward JR, Farrow SN, et al.: **Serum and glucocorticoid-regulated kinase 1 regulates neutrophil clearance during inflammation resolution**. *J Immunol* 2014, **192**:1796-1805.

14. Robertson AL, Holmes GR, Bojarczuk AN, Burgon J, Loynes CA, Chimen M, Sawtell AK, Hamza B, Willson J, Walmsley SR, et al.: **A zebrafish compound screen reveals modulation of neutrophil reverse migration as an anti-inflammatory mechanism**. *Sci Transl Med* 2014, **6**:225ra229.

15. Ellett F, Elks PM, Robertson AL, Ogryzko NV, Renshaw SA: **Defining the phenotype of neutrophils following reverse migration in zebrafish**. *J Leukoc Biol* 2015, **98**:975-981.

16. Cash JL, Bass MD, Campbell J, Barnes M, Kubes P, Martin P: **Resolution mediator chemerin15 reprograms the wound microenvironment to promote repair and reduce scarring**. *Curr Biol* 2014, **24**:1406-1414.

17. Ferguson MW, O'Kane S: **Scar-free healing: from embryonic mechanisms to adult therapeutic intervention**. *Philos Trans R Soc Lond B Biol Sci* 2004, **359**:839-850.

18. Cooper L, Johnson C, Burslem F, Martin P: **Wound healing and inflammation genes revealed by array analysis of 'macrophageless' PU.1 null mice**. *Genome Biol* 2005, **6**:R5.

19. Mori R, Shaw TJ, Martin P: **Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring**. *J Exp Med* 2008, **205**:43-51.

20. Lawrence T, Natoli G: **Transcriptional regulation of macrophage polarization: enabling diversity with identity**. *Nat Rev Immunol* 2011, **11**:750-761.

21. Crane MJ, Daley JM, van Houtte O, Brancato SK, Henry WL, Jr., Albina JE: **The monocyte to macrophage transition in the murine sterile wound**. *PLoS One* 2014, **9**:e86660.

22. Daley JM, Brancato SK, Thomay AA, Reichner JS, Albina JE: **The phenotype of murine wound macrophages**. *J Leukoc Biol* 2010, **87**:59-67.

23. Glass CK, Natoli G: **Molecular control of activation and priming in macrophages**. *Nat Immunol* 2015, **17**:26-33.

24. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, Stender JD, Chun HB, Garner H, Geissmann F, et al.: **Environment drives selection and function of enhancers controlling tissue-specific macrophage identities**. *Cell* 2014, **159**:1327-1340.

25. Okabe Y, Medzhitov R: **Tissue-specific signals control reversible program of localization and functional polarization of macrophages**. *Cell* 2014, **157**:832-844.

26. Weavers H, Evans I, Martin P, Wood W: **Corpse engulfment generates a molecular memory that primes the macrophage inflammatory response in Drosophila**. *Cell* 2016, In Press.

27. Allen JE, Sutherland TE: **Host protective roles of type 2 immunity: parasite killing and tissue repair, flip sides of the same coin**. *Semin Immunol* 2014, **26**:329-340.

** 28. Knipper JA, Willenborg S, Brinckmann J, Bloch W, Maass T, Wagener R, Krieg T, Sutherland T, Munitz A, Rothenberg ME, et al.: **Interleukin-4 Receptor alpha Signaling in Myeloid Cells Controls Collagen Fibril Assembly in Skin Repair**. *Immunity* 2015, **43**:803-816.

Here we learn of the novel insight that unresolvable wound scarring in skin might be a consequence of macrophage delivered signals to wound fibroblasts that lead to expression of specific collagen linking proteins.

* 29. Rodero MP, Licata F, Poupel L, Hamon P, Khosrotehrani K, Combadiere C, Boissonnas A: **In vivo imaging reveals a pioneer wave of monocyte recruitment into mouse skin wounds**. *PLoS One* 2014, **9**:e108212.

This is the first paper to live image early macrophage recruitment to skin wounds using intravital imaging approaches. The result is nice movies of macrophages transiently entering the wound, earlier than expected, and scouting around the margins.

30. Lisse TS, King BL, Rieger S: **Comparative transcriptomic profiling of hydrogen peroxide signaling networks in zebrafish and human keratinocytes: Implications toward conservation, migration and wound healing**. *Sci Rep* 2016, **6**:20328.

31. Freedberg IM, Tomic-Canic M, Komine M, Blumenberg M: **Keratins and the keratinocyte activation cycle**. *J Invest Dermatol* 2001, **116**:633-640.

32. Riding A, Pullar CE: **ATP Release and P2 Y Receptor Signaling are Essential for Keratinocyte Galvanotaxis**. *J Cell Physiol* 2016, **231**:181-191.

33. Zhou J, Wang J, Zhang N, Zhang Y, Li Q: **Identification of biomechanical force as a novel inducer of epithelial-mesenchymal transition features in mechanical stretched skin**. *Am J Transl Res* 2015, **7**:2187-2198.

34. Martin P, Nobes CD: **An early molecular component of the wound healing response in rat embryos--induction of c-fos protein in cells at the epidermal wound margin**. *Mech Dev* 1992, **38**:209-215.

35. Grose R, Harris BS, Cooper L, Topilko P, Martin P: **Immediate early genes krox-24 and krox-20 are rapidly up-regulated after wounding in the embryonic and adult mouse**. *Dev Dyn* 2002, **223**:371-378.

36. Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R, Havran WL: **A role for skin gammadelta T cells in wound repair**. *Science* 2002, **296**:747-749.

** 37. Telorack M, Meyer M, Ingold I, Conrad M, Bloch W, Werner S: **A Glutathione-Nrf2-Thioredoxin Cross-Talk Ensures Keratinocyte Survival and Efficient Wound Repair**. *PLoS Genet* 2016, **12**:e1005800.

Key to survival of epidermal keratinocytes as they migrate forward to heal the wound is for them to upregulate protection/tolerance machinery to counter wound stresses such as ROS from the wound inflammatory response; this paper dissects out the complex and redundant signalling pathways that regulate this machinery.

38. Shaw T, Martin P: **Epigenetic reprogramming during wound healing: loss of polycomb-mediated silencing may enable upregulation of repair genes**. *EMBO Rep* 2009, **10**:881-886.

39. Na J, Lee K, Na W, Shin JY, Lee MJ, Yune TY, Lee HK, Jung HS, Kim WS, Ju BG: **Histone H3K27 Demethylase JMJD3 in Cooperation with NF-kappaB Regulates Keratinocyte Wound Healing**. *J Invest Dermatol* 2016.

40. Savagner P, Kusewitt DF, Carver EA, Magnino F, Choi C, Gridley T, Hudson LG: **Developmental transcription factor slug is required for effective re-epithelialization by adult keratinocytes**. *J Cell Physiol* 2005, **202**:858-866.

41. Nieto MA: **Epithelial plasticity: a common theme in embryonic and cancer cells**. *Science* 2013, **342**:1234850.

42. Bleaken BM, Menko AS, Walker JL: **Cells activated for wound repair have the potential to direct collective invasion of an epithelium**. *Mol Biol Cell* 2016, **27**:451-465.

43. Strid J, Sobolev O, Zafirova B, Polic B, Hayday A: **The intraepithelial T cell response to NKG2D-ligands links lymphoid stress surveillance to atopy**. *Science* 2011, **334**:1293-1297.

** 44. Nunan R, Campbell J, Mori R, Pitulescu ME, Jiang WG, Harding KG, Adams RH, Nobes CD, Martin P: **Ephrin-Bs Drive Junctional Downregulation and Actin Stress Fiber Disassembly to Enable Wound Re-epithelialization**. *Cell Rep* 2015, **13**:1380-1395.

Eph/EphrinB1 signalling in the wound edge epithelium leads to dissolution of adherens and tight, but not desmosomal junctions extending up to 70+ cells back from the wound margin, leading to "partial EMT" and facilitating migration of the "follower" cells.

45. Hertle MD, Jones PH, Groves RW, Hudson DL, Watt FM: **Integrin expression by human epidermal keratinocytes can be modulated by interferon-gamma, transforming growth factor-beta, tumor necrosis factor-alpha, and culture on a dermal equivalent**. *J Invest Dermatol* 1995, **104**:260-265.

46. Hertle MD, Kubler MD, Leigh IM, Watt FM: **Aberrant integrin expression during epidermal wound healing and in psoriatic epidermis**. *J Clin Invest* 1992, **89**:1892-1901.

47. Munjal A, Lecuit T: **Actomyosin networks and tissue morphogenesis**. *Development* 2014, **141**:1789-1793.

* 48. Razzell W, Wood W, Martin P: **Recapitulation of morphogenetic cell shape changes enables wound re-epithelialisation**. *Development* 2014, **141**:1814-1820.

A study in *Drosophila* embryonic wounds shows how stochastic myosin flashes trigger cell:cell junctional shrinking and intercalation events in the rows back from a wound edge to enable release of epidermal tension and forward sheet migration.

49. Lim X, Tan SH, Koh WL, Chau RM, Yan KS, Kuo CJ, van Amerongen R, Klein AM, Nusse R: **Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling**. *Science* 2013, **342**:1226-1230.

50. Brownell I, Guevara E, Bai CB, Loomis CA, Joyner AL: **Nerve-derived sonic hedgehog defines a niche for hair follicle stem cells capable of becoming epidermal stem cells**. *Cell Stem Cell* 2011, **8**:552-565.

51. Lu CP, Polak L, Rocha AS, Pasolli HA, Chen SC, Sharma N, Blanpain C, Fuchs E: **Identification of stem cell populations in sweat glands and ducts reveals roles in homeostasis and wound repair**. *Cell* 2012, **150**:136-150.

52. Plikus MV, Gay DL, Treffeisen E, Wang A, Supapannachart RJ, Cotsarelis G: **Epithelial stem cells and implications for wound repair**. *Semin Cell Dev Biol* 2012, **23**:946-953.

53. Garcin CL, Ansell DM, Headon DJ, Paus R, Hardman MJ: **Hair follicle bulge stem cells appear dispensable for the acute phase of wound re-epithelialisation**. *Stem Cells* 2016.

54. Margadant C, Raymond K, Kreft M, Sachs N, Janssen H, Sonnenberg A: **Integrin alpha3beta1 inhibits directional migration and wound re-epithelialization in the skin**. *J Cell Sci* 2009, **122**:278-288.

55. Nystrom A, Velati D, Mittapalli VR, Fritsch A, Kern JS, Bruckner-Tuderman L: **Collagen VII plays a dual role in wound healing**. *J Clin Invest* 2013, **123**:3498-3509.

56. Sudbeck BD, Pilcher BK, Welgus HG, Parks WC: **Induction and repression of collagenase-1 by keratinocytes is controlled by distinct components of different extracellular matrix compartments**. *J Biol Chem* 1997, **272**:22103-22110.

57. Kubo M, Van de Water L, Plantefaber LC, Mosesson MW, Simon M, Tonnesen MG, Taichman L, Clark RA: **Fibrinogen and fibrin are anti-adhesive for keratinocytes: a mechanism for fibrin eschar slough during wound repair**. *J Invest Dermatol* 2001, **117**:1369-1381.

58. Chang HY, Sneddon JB, Alizadeh AA, Sood R, West RB, Montgomery K, Chi JT, van de Rijn M, Botstein D, Brown PO: **Gene expression signature of fibroblast serum response**

predicts human cancer progression: similarities between tumors and wounds. *PLoS Biol* 2004, **2**:E7.

59. Wang J, Hori K, Ding J, Huang Y, Kwan P, Ladak A, Tredget EE: **Toll-like receptors expressed by dermal fibroblasts contribute to hypertrophic scarring**. *J Cell Physiol* 2011, **226**:1265-1273.

60. Achterberg VF, Buscemi L, Diekmann H, Smith-Clerc J, Schwengler H, Meister JJ, Wenck H, Gallinat S, Hinz B: **The nano-scale mechanical properties of the extracellular matrix regulate dermal fibroblast function**. *J Invest Dermatol* 2014, **134**:1862-1872.

61. Li W, Li Y, Guan S, Fan J, Cheng CF, Bright AM, Chinn C, Chen M, Woodley DT: **Extracellular heat shock protein-90alpha: linking hypoxia to skin cell motility and wound healing**. *EMBO J* 2007, **26**:1221-1233.

62. Grimstad O, Sandanger O, Ryan L, Otterdal K, Damaas JK, Pukstad B, Espevik T: **Cellular sources and inducers of cytokines present in acute wound fluid**. *Wound Repair Regen* 2011, **19**:337-347.

63. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C: **Alpha-smooth muscle actin expression upregulates fibroblast contractile activity**. *Mol Biol Cell* 2001, **12**:2730-2741.

64. Fitzgerald O'Connor EJ, Badshah, II, Addae LY, Kundasamy P, Thanabalasingam S, Abioye D, Soldin M, Shaw TJ: **Histone deacetylase 2 is upregulated in normal and keloid scars**. *J Invest Dermatol* 2012, **132**:1293-1296.

65. Glenisson W, Castronovo V, Waltregny D: **Histone deacetylase 4 is required for TGFbeta1-induced myofibroblastic differentiation**. *Biochim Biophys Acta* 2007, **1773**:1572-1582.

** 66. Driskell RR, Lichtenberger BM, Hoste E, Kretzschmar K, Simons BD, Charalambous M, Ferron SR, Herault Y, Pavlovic G, Ferguson-Smith AC, et al.: **Distinct fibroblast lineages determine dermal architecture in skin development and repair**. *Nature* 2013, **504**:277-281.

Here, elegant lineage tracking experiments show that the early pool of "healing" fibroblasts derive from the reticular dermis, which may explain the difficulty in regenerating epidermal appendages in wound scar tissue.

67. Lichtenberger BM, Mastrogiannaki M, Watt FM: **Epidermal beta-catenin activation remodels the dermis via paracrine signalling to distinct fibroblast lineages**. *Nat Commun* 2016, **7**:10537.

68. Smith AN, Willis E, Chan VT, Muffley LA, Isik FF, Gibran NS, Hocking AM: **Mesenchymal stem cells induce dermal fibroblast responses to injury**. *Exp Cell Res* 2010, **316**:48-54.

69. Wu Y, Chen L, Scott PG, Tredget EE: **Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis**. *Stem Cells* 2007, **25**:2648-2659.

70. Prockop DJ: **Marrow stromal cells as stem cells for nonhematopoietic tissues**. *Science* 1997, **276**:71-74.

71. Stappenbeck TS, Miyoshi H: **The role of stromal stem cells in tissue regeneration and wound repair**. *Science* 2009, **324**:1666-1669.

72. Johnston AP, Naska S, Jones K, Jinno H, Kaplan DR, Miller FD: **Sox2-mediated regulation of adult neural crest precursors and skin repair**. *Stem Cell Reports* 2013, **1**:38-45.

73. Feisst V, Brooks AE, Chen CJ, Dunbar PR: **Characterization of mesenchymal progenitor cell populations directly derived from human dermis**. *Stem Cells Dev* 2014, **23**:631-642.

74. Kramann R, Schneider RK, DiRocco DP, Machado F, Fleig S, Bondzie PA, Henderson JM, Ebert BL, Humphreys BD: **Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis**. *Cell Stem Cell* 2015, **16**:51-66.

75. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN: **Peripheral blood fibrocytes: differentiation pathway and migration to wound sites**. *J Immunol* 2001, **166**:7556-7562.

76. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A: **Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair**. *Mol Med* 1994, **1**:71-81.

77. Khamaisi M, Katagiri S, Keenan H, Park K, Maeda Y, Li Q, Qi W, Thomou T, Eschuk D, Tellechea A, et al.: **PKCdelta inhibition normalizes the wound-healing capacity of diabetic human fibroblasts**. *J Clin Invest* 2016.

78. Schneider L, Cammer M, Lehman J, Nielsen SK, Guerra CF, Veland IR, Stock C, Hoffmann EK, Yoder BK, Schwab A, et al.: **Directional cell migration and chemotaxis in wound healing response to PDGF-AA are coordinated by the primary cilium in fibroblasts**. *Cell Physiol Biochem* 2010, **25**:279-292.

79. Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H: **Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type**. *J Immunol* 2008, **180**:2581-2587.

80. Akasaka Y, Ono I, Kamiya T, Ishikawa Y, Kinoshita T, Ishiguro S, Yokoo T, Imaizumi R, Inomata N, Fujita K, et al.: **The mechanisms underlying fibroblast apoptosis regulated by growth factors during wound healing**. *J Pathol* 2010, **221**:285-299.

81. Desmouliere A, Redard M, Darby I, Gabbiani G: **Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar**. *Am J Pathol* 1995, **146**:56-66.

82. Darby I, Skalli O, Gabbiani G: **Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing**. *Lab Invest* 1990, **63**:21-29.

83. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C: **Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction**. *Blood* 2010, **116**:829-840.

84. Stockmann C, Kirmse S, Helfrich I, Weidemann A, Takeda N, Doedens A, Johnson RS: **A wound size-dependent effect of myeloid cell-derived vascular endothelial growth factor on wound healing**. *J Invest Dermatol* 2011, **131**:797-801.

85. Stefater JA, 3rd, Rao S, Bezold K, Aplin AC, Nicosia RF, Pollard JW, Ferrara N, Lang RA: **Macrophage Wnt-Calcineurin-Flt1 signaling regulates mouse wound angiogenesis and repair**. *Blood* 2013, **121**:2574-2578.

* 86. Gerald D, Adini I, Shechter S, Perruzzi C, Varnau J, Hopkins B, Kazerounian S, Kurschat P, Blachon S, Khedkar S, et al.: **RhoB controls coordination of adult angiogenesis and lymphangiogenesis following injury by regulating VEZF1-mediated transcription**. *Nat Commun* 2013, **4**:2824.

This paper is exceptional in that it begins to drill down to the molecular mechanisms underpinning wound angiogenic sprouting; in doing so it reveals a reciprocal interrelationship between wound angiogeneisis and lymphangiogenesis with the former blocked and the latter enhanced in RhoB knockout mice.

Figure 1. Histological overview of a wound, highlighting dynamic cell populations required for successful repair.

(Top image) Day 3 skin wound histology (H&E, excisional 4mm wound to the shaved back skin of an adult male mouse). Scale, 50 μm.

(i) Wound edge arteriole (Day 1) with wound-polarised adherence of leukocytes to the endothelial wall. Schematic illustrates diapedesis from the vessel and their subsequent migration towards the wound, and potent paracrine influence (green). Scale: 20 μm.

(ii) Wound edge dermis of a Day 7 wound. Schematic illustrates dermal fibroblasts acquiring a migratory (blue) and subsequently contractile (myofibroblast – with stress fibres) phenotype. Scale: 50 μm.

(iii) Extensive angiogenesis in a Day 5 wound bed (some vessels indicated with arrowheads). Schematic illustrates the branching and sprouting, led by tip cells (green) and resulting in a transient, dense vascular network in the wound granulation tissue. Scale: 50 μm.

(iv) Wound edge epidermis (Day 1 wound – pink cells) migrating across the wound bed, boring a path between the granulation tissue and overlying scab. Schematic illustrates the reduction in keratinocyte cell:cell contacts, and the involvement of suprabasal and follower cell, and production of new substratum as they progress. Scale: 50 μm.
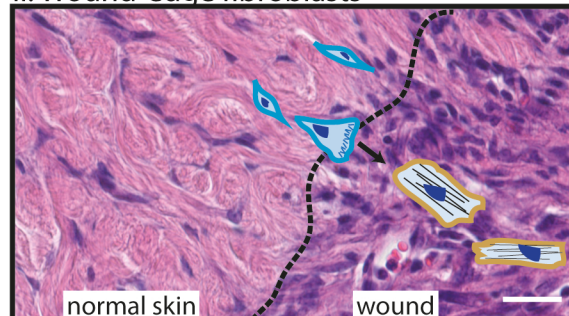
Overview of wound response (Day 3)

scab

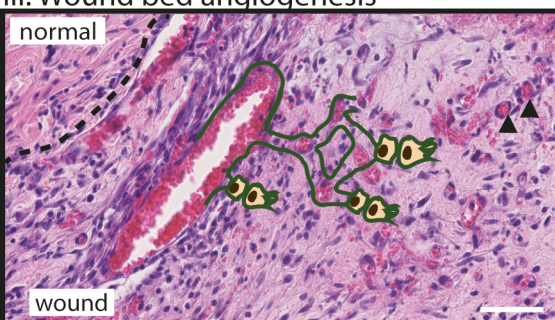advancing epidermis

iv

i

ii

normal skin

inflammatory response

wound bed granulation tissue

i. Inflammatory cell recruitment

normal skin

wound

ii. Wound-edge fibroblasts

normal skin

wound

iii. Wound bed angiogenesis

normal

wound

iv. Advancing epidermis

wound

normal skin