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Wound Healing in Development

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Abstract

Wound healing is the inherent ability of an organism to protect itself against injuries. Cumulative evidence indicates that the healing process patterns embryonic morphogenesis and may result in either organ regeneration or scarring – phenomena that are developmental stage-or age-dependent. Skin is the largest organ. Its morphogenesis and repair mechanisms have been studied extensively due not only to its anatomical location, which allows easy access and observation, but also its captivating structure and vital function. Thus, this review will focus on using skin as a model organ to illustrate new insights into the mechanisms of wound healing that are developmentally regulated in mammals, with special emphasis on the roles of Wnt signaling pathways and their crosstalk with TGF- β signaling. Relevant information from studies of other organs is discussed where it applies, and the clinical impact from such knowledge and emerging concepts on regenerative medicine are also discussed in perspective.

Keywords

Wound healing; Development; Morphogenesis; Scarless; Scarring; Myofibroblasts; Wnt

INTRODUCTION

In the biological world, every species has the ability to restore the disruption of the normal continuity of body structure after physical injury. Formation of scar as a replacement of previously functional components is hallmark of the postpartum injury repair of mammals. Scars and associated abnormalities not only hinder the normal function of the organ, but also pose great challenges in clinical management. Skin is the largest organ. Its morphogenesis and injury repair have been studied extensively due not only to its anatomical location, which allows easy access and observation, but also its captivating structure and vital function.

In skin, the process of wound repair can be generalized into three cascading phases each with characteristic cellular events: 1) inflammation; 2) fibroplasia or proliferative phase; and 3) scar formation. Inflammation is triggered by the formation of a fibrin clot and the degranulation of aggregated platelets that release chemotactic factors essential for the recruitment of leukocytes, principally neutrophils, as well as bone-marrow derived stem cells or fibrocytes which exit from the blood stream into the wound. Residential

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macrophages or mast cells also degranulate and release additional soluble mediators to activate and recruit endothelial cells for angiogenesis and fibroblasts for fibrillogenesis. The latter occurs in 2 to 3 days after inflammation has decreased and marks the onset of the proliferative phase. Tissue debris and invading microorganisms are cleared by the inflammatory responses, and granulation tissue is being formed in the proliferative phase (3 to 7 days) to replace the fibrin clot via re-epithelialization, angiogenesis, and fibroplasia. Fibroblasts and myofibroblasts are responsible for extracellular matrix (ECM) synthesis, principally fibronectin (Fn) and collagen, and ECM deposition (Gurtner et al., 2008; Singer and Clark, 1999). Their migration and subsequent reorganization of the granulation tissue matrix bring about a reduction in the size of the wound (wound contraction). Other cell types such as hair follicle stem cells are also activated and participate in re-epithelialization (Ito et al., 2005). In addition, epithelial cells undergo epithelial to mesenchymal transition (EMT) and participate in dermal repair (Kalluri and Weinberg, 2009; Kong et al., 2006). The proliferative phase is marked by dynamic forward and feedback interactions between cells and their surrounding ECM components in a spatially and temporally controlled fashion by growth factors and cytokines. The newly formed granulation tissue is subsequently remodeled into scar tissue (scar forming and scar remodeling phase). The scar remodeling phase is characterized by the maturation of the ECM that transitions from mostly Type III to Type I collagen via additional ECM synthesis or removal (mediated by the actions of proteases and protease inhibitors) and collagen crosslinking. Depending on the nature of injury and the size and location of the wound, scar remodeling and maturation can take place from weeks to months.

DEVELOPMENTAL STAGE-SPECIFIC WOUND HEALING

Healing of early gestational fetal wounds

Intriguingly, skin wounds of early gestational stage heal in a regenerative fashion without scar formation. It occurs in an hyaluronic acid-rich ECM environment and is independent of the amniotic environment (Armstrong and Ferguson, 1995) (Adzick et al., 1985). Figure 1 illustrates the major wound healing events that occur in early fetal, late fetal, and postnatal stages (Fig. 1). Accordingly, skin repair at the early fetal stage is characterized by rapid re-epithelialization in which actin “purse string” filaments are formed at the edge of the wound providing coordinated cell movement to close the defect (McCluskey and Martin, 1995). Since blood vessels are yet to form, there is no “bleeding” or clot formation to initiate inflammation, and wound healing is further accomplished by the interactions between the two cell-layered primordial epidermis and the underlying mesenchymal tissue (Gallicano et al., 2001). Transition from scarless to scar forming repair coincides with the development and maturation of blood vessels, the inflammatory system, and skin structure, most evidently the hair follicle (from hair germ/dermal condensation to the formation of mature hair follicle) (Fig. 1). As a part of the haemostatic response, a fibrin clot is formed at the site of injury. At this time a variety of cells, i.e., existing fibroblasts and mast cells, as well as leukocytes and fibrocytes (Bucala et al., 1994) which exit from blood vessels via extravasation, are recruited into the wound site. Additionally, stem cells from the basal layer of the epidermis (now a stratified multi-cellular layer), and from hair follicles (bulge) are activated and migrate into the wound for re-epithelialization (Ito et al., 2005) (Fig. 1). Collectively, repair of early fetal skin injury follows or mimics the process of organ morphogenesis by cells or tissues that are less differentiated and with greater phenotypic plasticity, and results in organ regeneration without scar formation. In contrast, repair of late fetal and postnatal skin injury, although participated by both local and distant stem or progenitor cells, occurs in a matured organ environment and results in scar formation.

Organ morphogenesis vs. wound healing

Based on the available information, the switch from scarless to scar forming repair during fetal development seems unique to skin. For instance, the diaphragmatic injury of fetuses results in scar formation regardless of the age of the fetus (Longaker et al., 1991). The pattern of injury repair of other organs during fetal development is less well known owing to their internal location and/or earlier timing in morphogenesis, which are challenging for access experimentally and by observation. For the purpose of comparison, we provide in Table 1 the gestational period and timing of organ morphogenesis for skin and various other organs from rodents (mouse and rat), sheep, and human (Table 1). It is noteworthy that the transition from scarless to scar forming repair coincides rather consistently with the timing of hair follicle formation among these organisms. Therefore, it is reasonable to assume that mechanisms which control embryonic organ morphogenesis, i.e., hair follicle formation in the present case, might have functioned to control wound healing.

In the human embryo, a two-layered primitive epidermis (periderm and basal layer) develops around 4 to 8 weeks (~30 to 60 days; comparable to about 10 days of a mouse embryo) (Gallicano et al., 2001; Lane, 1986). Stratification and keratinization of the epidermis as well as hair follicle formation takes place from 9 to 14 weeks (~60 to 100 days), and the epidermis fully develops by the 16th week (112 days) (Lane, 1986) (Table 1). The dermal layer, in contrast, is composed of loose connective tissue with mesenchymal cells distributed in an ECM rich in hyaluronic acid, which later on becomes more structurally condensed and contains Type III collagen, Fn, and proteoglycans (Coolen et al., 2009). Interestingly, myofibroblasts can be induced in the dermal mesenchyme, regardless of the nature and size of the skin wound which was made during the mid gestational stage of fetal sheep (60 to 90 days; term 147 days and hair follicle formed between 57 to 89 days; Table 1) (Cass et al., 1997). Of significance, these myofibroblasts later disappeared in the incisional or small (2 mm) punch excisional wounds that healed scarlessly. They, however, persisted and were abundant in large punch wounds that scar (Cass et al., 1997). It was clear from this study that myofibroblast induction in the mid gestational skin wounds of sheep was caused by a wounding and, although not proven at the time, their persistence might lead to scarring.

The role of myofibroblasts in organ fibrosis during injury repair has since been confirmed and elucidated (Darby and Hewitson, 2007). From the perspective of organ morphogenesis, however, we speculate that healing of the small wounds in the fetal sheep model might have occurred relatively quickly in a scarless fashion before hair follicle formation. On the other hand, healing of the large wounds, which would require a longer period of time, might have proceeded in parallel with skin morphogenesis, i.e., hair follicle formation. It is therefore conceivable that the mechanism which took place in governing hair follicle morphogenesis might have interacted with the process of wound healing to sustain myofibroblast differentiation. Recent studies on the mechanism of hair follicle formation in development and of myofibroblast differentiation during wound healing attest to the fact that such interaction may indeed occur; and the Wnt signaling pathway (discussed in the later section) which plays a critical role in the development of multicellular organisms has been proven to not only control hair follicle morphogenesis in development (DasGupta and Fuchs, 1999), but also is essential in myofibroblast differentiation (Carthy et al., 2011). Additional evidence of overlap between organ morphogenesis and wound healing comes from observations that the process of postnatal wound repair, in part, patterns embryonic morphogenesis. For instance, the fetal form of ECM, such as hyaluronic acid, tenascin, the fetal form of Fn, and type III collagen, is synthesized and deposited by fibroblasts during the early proliferative stage of postnatal wound healing, and is later replaced by scar tissue consisting of principally Type I collagen (Cass et al., 1998; Merkel et al., 1988; Whitby et al., 1991) (Arai et al., 1997; Coolen et al., 2009; French-Constant and Hynes, 1989; Pagni

et al., 1991). Although, the mechanism which controls the transition of ECM is currently unknown, the observation nonetheless, suggests that the process governing embryonic organ morphogenesis may be activated upon wounding in postnatal organisms. The recent discovery of a correlation/dependency between wound size- and Wnt-dependent de novo hair follicle regeneration in wounds of adult mouse skin presents a remarkable example of such an overlap in mechanisms between organ morphogenesis and wound healing (Ito et al., 2007). Since Wnt signaling is also activated by wounding (Okuse et al., 2005), these findings collectively indicate that the healing process may be governed by crosstalk between wound-induced factors to Wnt signaling.

MOLECULAR MECHANISMS UNDERLYING DEVELOPMENTALLY REGULATED WOUND HEALING

Ample studies have provided evidence of differences at both cellular and molecular levels between scarless and scar forming wound repair. We have assembled a list of major signaling pathways, transcriptional factors, and growth factors and cytokines that are involved in both organ morphogenesis and wound healing, contrasting scarless vs. scar forming repair (Table 2). In the following sections, instead of providing a review for the individual components, the focus is on Wnt signaling. It is because Wnt signaling is central to developmental organ morphogenesis, that the newly discovered crosstalk of Wnt with factors such as TGF- β gives insights and rationale to the link between developmentally regulated organ morphogenesis and wound healing.

Wound healing and organ morphogenesis: the Wnt connection

Signaling through Wnt morphogen is fundamental for embryonic development of multicellular organisms across the animal kingdom. Based on the mode of action, Wnt proteins activate two distinct pathways, namely canonical and non-canonical (Fig. 2). The key player of the canonical Wnt pathway is β -catenin, which is stabilized in the cytoplasm in response to ligand-receptor binding and then translocated into nucleus where it binds to the TCF-LEF transcription factors to regulate gene expression (Logan and Nusse, 2004). In contrast, activation of the non-canonical Wnt pathway leads to an increase in intracellular calcium followed by stimulating the activities of protein kinase C (PKC) and phospholipase C (PLC), or the activation of jun N-terminal kinase (JNK) that regulates planar cell polarity (Montcouquiol et al., 2006). Recent studies imply that both branches of Wnt signaling may differentially participate in scarless versus scar-forming wound healing processes (outlined in Fig. 2).

The canonical Wnt pathway and scar wound healing

As implicated in previous sections, a notable event in skin development that coincides with the transition from scarless to scar forming wound repair is the formation of hair follicles (DasGupta and Fuchs, 1999; Stelnicki et al., 1997)(Figure 1 and Table 1). The involvement of canonical Wnt/ β -catenin signaling in the initiation and morphogenesis of hair follicle has been well established (Fuchs, 2007). The connection between Wnt signaling and wound healing phenotype was established in a recent study using the reporter BAT-gal mouse model to monitor the activity of canonical Wnt signaling in wound healing. Accordingly, the activity of Wnt signaling was increased in postnatal wounds but not fetal wounds with a scarless healing phenotype. The differential activity of Wnt signaling between scar forming and scarless wound healing was strengthened by in vitro studies where Wnt (Wnt3a) had an greater effect on fibroblasts from postnatal mouse skin compared to fetal fibroblasts engaged in cell proliferation, hyaluronic acid and type 1 collagen synthesis, and differential TGF- β isoform ($-\beta 1$ vs. $-\beta 3$) expression (Carre et al., 2010). This and the previously mentioned discovery of a Wnt-dependent de novo hair follicle regeneration in wounds of adult mouse

that is wound-size dependent (Ito et al., 2007) open a whole new perspective and link between developmentally regulated skin morphogenesis and wound healing.

The canonical Wnt pathway and developmentally regulated TGF- β and PAI-1, a marker of organ fibrosis

The differential expression and precise role of TGF- β isoforms during skin development and in the scarless healing of mammalian fetal skin wounds have generated much interest in the field of wound healing. Although their exact role remains to be elucidated, it has been suggested that high TGF- β 3 activity may be anti-scarring and/or correlate with a scarless healing phenotype (Shah et al., 1995). Nonetheless, it is known that the effect of TGF- β on ECM production during injury repair is in part mediated through the modulation of matrix degrading proteases and their inhibitors, principally PAI-1 (plasminogen activator inhibitor-1) (Li et al., 2003). We have previously shown that PAI-1 and its counterpart urokinase-type plasminogen activator (uPA), a serine protease, are differentially expressed in early and late gestational mouse skin and skin wounds, i.e., in normal fetal skin uPA level is higher in E14.5 than in E18 mice, while PAI-1 is lower in E14.5 than in E18 mice (Huang et al., 2002). After wounding, E14.5 wounds show a moderate increase in uPA and a minimal increase in PAI-1, while E18 wounds show a transient increase in uPA but a significant, sustained increase in PAI-1. In addition, the likely role of PA-1 in scarring in fetal skin injury repair was demonstrated by the addition of aprotinin (a serine protease inhibitor) to E14.5 wounds, which caused an increase in collagen deposition (Huang et al., 2002). The relationship between TGF- β 3 and PAI-1 was subsequently investigated using embryonic day 14.5 TGF- β 3 knockout mice and an ex vivo limb wounding study (Li et al., 2006). The results showed not only that extracts from fetuses and fibroblasts of the knockout mouse had a higher PAI-1 expression, but also that PAI-1 expression was increased in both epidermis and dermis of knockout mouse limb wounds. Therefore, the developmentally regulated TGF- β isotype expression and their differential effect on scar formation during wound healing may be partly caused by their capacity to regulate PAI-1 expression (Laiho et al., 1987).

The role of Wnt signaling on PAI-1 expression and organ fibrosis was validated by recent studies using a mouse model of obstructive kidney fibrosis where the ectopic expression of Wnt1 activated PAI-1 expression in vivo and its promoter activity in vitro. In contrast, the blockade of Wnt/beta-catenin signaling by Dickkopf-1 gene inhibited PAI-1 induction indicating that PAI-1 is a direct downstream target of Wnt/beta-catenin signaling (He et al., 2010). Collectively, the evidence so far indicates that activation of canonical Wnt signaling may be pivotal to both developmentally regulated organ morphogenesis and wound healing via its interaction with not only the TGF- β signaling pathway (further discussed in the section on “myofibroblast differentiation”), but also PAI-1 induction.

The canonical Wnt pathway and HIF-1 α

Given the facts that HIF-1 α is associated with mid-gestation sheep skin wounds (Cass et al., 1997) (Table 1) and TGF- β 3 induction, there is potentially a transcriptional crosstalk between β -catenin, TCF, and HIF-1 α that controls the switch between scarless versus scarring repair (Schaffer et al., 2003; Scheid et al., 2002). Such crosstalk was demonstrated in human colon cancer cells where, under hypoxia condition, HIF-1 α competes with TCF4 for binding to β -catenin, leading to suppression of transcriptional activity mediated by the β -catenin/TCF4 complex (Kaidi et al., 2007). Thus, further investigation on the roles of β -catenin/TCF and β -catenin/HIF-1 α in the wound healing response could be beneficial for not only delineating the mechanism underlying scarless versus scar forming repair, but also providing rationale for therapeutic design to control excessive scarring during wound healing.

The non-canonical Wnt pathway and scarless fetal wound healing

A prominent feature of fetal scarless wound healing is the rapid re-epithelialization by a collective cell movement mediated by the actin cable via a purse-string like contraction mechanism (Buck, 1979; Martin and Lewis, 1992; Odland and Ross, 1968) (Martin and Lewis, 1992; McCluskey and Martin, 1995) (Fig. 1). The molecular mechanism which modulates the event was uncovered in the study where PCP (the Wnt/planar cell polarity) mutant mice, including *Celsr1*^{-/-}, *PTK7*^{-/-}, *Scrb1*^{-/-}, *Grhl3*^{-/-}, and *Vangl2*^{+/-}/*Grhl3*^{+/-}, which display a neural tube defect or disorientation of cochlea stereocilia, were defective in wound closure at E12 (Caddy et al., 2010). The evidence not only demarcates the differential role of non-canonical and canonical Wnt signaling pathways in scarless vs. scar forming wound healing, but also strengthens the notion that wound healing is developmentally regulated by mechanisms controlling organ morphogenesis. With the availability of mouse mutants of Wnt5a present in fetal skin and regulating cell movement during limb development and of *Ror2* which mediates Wnt5a signaling, the mechanism of Wnt-signaling in skin morphogenesis and wound healing may be elucidated in the near future (Gros et al., 2010; Oishi et al., 2003; Yamaguchi et al., 1999).

Myofibroblast differentiation: a convergence of the canonical Wnt and TGF- β signaling pathways

The hallmark of myofibroblasts is alpha smooth muscle actin expression which is induced by TGF- β treatment (Carthy et al., 2011). Myofibroblast differentiation propelled by the increased level of TGF- β in wounds during injury repair, promotes scarring and is a paradigm in organ fibrosis (Desmouliere et al., 2005). A recent study shows that treating fibroblasts with Wnt3a promotes the formation of a myofibroblast-like phenotype, in part, by upregulating TGF- β signaling through SMAD2 in a canonical Wnt/ β -catenin-dependent manner (Carthy et al., 2011). Since Wnt3a can also be induced by other inflammatory cytokines such as TNF α (Al-Aly et al., 2007), the above observation greatly strengthens a canonical Wnt signaling pathway-mediated mechanism in developmentally regulated wound healing that parallels organ morphogenesis (see Fig. 1 and previous section on “Organ morphogenesis and wound healing”).

Conclusion of the Wnt signaling-mediated organ morphogenesis and wound healing

Scar formation is the central issue in the field of wound healing. Emerging evidence strongly supports that wound healing parallels organ morphogenesis. Depending on the age of the organism and the nature of the injury wounds can heal under the control of Wnt signaling either in a regenerative fashion without scar or in scarring. Specific examples are presented in this review to establish the concept. Furthermore, the knowledge from recent studies on the crosstalk between Wnt and factors such as TGF- β provides not only insights regarding observations on scarless vs. scar forming repair during fetal development, but also a rational framework to further test key issues in wound healing, such as inflammation, angiogenesis, and myofibroblast differentiation, in organ regeneration vs. scarring.

Hypertrophic scars and keloids are two types of skin fibrosis occurring in humans with challenging consequences in health and in clinical management (Tuan and Nichter, 1998). Due to the difficulties in sample procurement and the lack of appropriate animal models which closely resemble the pathophysiology of that found in humans, not much is known concerning Wnt signaling pathways in either hypertrophic scarring or keloid formation. Nonetheless, evidence so far has shown that there is an accumulation of nuclear and cytoplasmic β -catenin in both hypertrophic and keloid tissues (Russell et al., 2010; Sato, 2006) indicating a possible involvement of Wnt signaling in the disease mechanism. The prolonged duration of elevated β -catenin and activation of the tcf-reporter in hyperplastic wound and primary dermal cells (Cheon et al., 2005) plus the stabilization of β -catenin in

mesenchymal cells of transgenic mice which leads to hyperplastic cutaneous wounds (Cheon et al., 2002) afford further support for a role between Wnt signaling and scarring in humans. Further characterization of the downstream events at the transcriptional level in response to Wnt/ β -catenin activation would be insightful for elucidating the cellular and molecular mechanisms underlying hypertrophic scar and keloid formation during postnatal injury repair and identifying targets for development of therapeutics.

Perspectives in development of therapeutics for scar management

Management of scarring in the postnatal life poses a great clinical challenge in diverse medical areas. Early studies on wound healing have identified molecules and signaling pathways that play determinant roles in scar formation; however therapeutic approaches based on this knowledge was not successful and generated unreliable and unpredictable results (Occleston et al., 2010). Wound healing in adults takes place as a cascade of morphogenetic events acting in an attempt to re-store tissue compartmentation principally based on a wide range of cell types with limited plasticity found in a different matrix environment. Given the complexity of cellular components and dynamic changes in the microenvironment at the healing site, scar management may not be effective if implemented by a single treatment and thus development of treatment regimens synchronized with specific healing phases needs to be considered. Towards this direction, establishment of an integral view of the cell/signal transduction network in space and time that elucidates the progression of scarring is an essential prerequisite. Technically, this can be achieved by defining the spatiotemporal pattern of cell-cell/cell-ECM interaction and associated molecular expression followed by functional evaluation of molecules of interest in the organotypic culture or in vivo model. This information will be valuable in the search for targets among cell players and signaling pathways that participate in specific cascade of events during scar healing. For example, activation of Wnt/ β -catenin has been linked to the formation of normal versus pathological (such as hypertrophic scar, and keloid) scar. Although sharing the common outcome – formation of scar, they exhibit distinct phenotypes, such as rapid growth followed by slow regression in hypertrophic scarring, and persistent growth without spontaneous regression in keloids (Cheon et al., 2005; Gauglitz et al., 2010; Sato, 2006). Determination of cellular behavior in relation to the temporal and spatial organization of Wnt/ β -catenin activity in a distinct scarring context would provide insight into the development of a rational strategy to target scar formation which differ in pathogenesis.

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Figure 1. Wound healing in development

Wound repair in the early stage of embryonic development is characterized by rapid re-epithelialization in which actin “purse string” filaments are formed at the leading edge of the epithelial wound providing coordinated cell movement for wound closure. The process is closely intertwined with embryonic organ morphogenesis in a hyaluronic acid-rich ECM environment, and the wound heals in a regenerative fashion without scar formation. Transition from scarless to scar forming repair coincides with the development and maturation of blood vessels, inflammatory system, hair follicles (from hair germ/dermal condensation to mature hair follicles as indicated in the figure), and ECM in the dermis. At these stages and as a part of the haemostatic response, fibrin clot is formed at the site of injury. A variety of cellular components, i.e., existing fibroblasts and mast cells as well as leukocytes and fibrocytes via extravasation from blood vessels, are recruited into the wound site in response to injury for wound repair. Additionally, keratinocytes from the basal layer of epidermis and the activated stem cells from hair follicles (bulge) migrate into the wound for re-epithelialization. Recent evidence indicates that epidermal cells via epithelial-to-mesenchymal transition may also contribute to dermal repair.

Figure 2. Involvement of non-canonical and canonical Wnt pathways in cellular and morphogenetic events during development and wound healing

In the canonical Wnt/ β -catenin pathway (shaded grey), the signal is transduced through Dishevelled (Dvl) upon Wnt protein binding to its receptor Frizzled (Fz). The activation of Dvl leads to disassemble of the destruction complex consisting axin, adenomatous polyposis coli (APC), and GSK3 β , which results in the accumulation of β -catenin in the cytoplasm and nuclear translocation. After nuclear translocation, β -catenin interacts with transcription factors TCF/LEF to regulate target gene expression. Additional crosstalk between the Wnt/ β -catenin and TGF- β signaling pathways as well as Wnt/ β -catenin with other transcription factor, such as Hif-1 α controls cellular events essential in organ morphogenesis and wound repair during late fetal and postnatal stages. In contrast, the non-canonical Wnt/PCP signaling (shaded yellow) leads to the reorganization of actin cytoskeleton via the activation of Rho family of small GTPases (RhoA and Rac1) and their downstream protein kinases (JNK or Rho associated kinase (ROCK)). This signaling mechanism regulates polarized cell movement and establishes planar cell polarity (PCP) that are essential for epidermal wound repair, neural tube closure, and cochlea hair cell polarity during early phase of fetal development.

Table 1

A comparison on the gestational period and timing of morphogenesis between rodents, sheep, and human.

Species	Gestational Days (GD)*	Onset of scar forming repair (skin)	Hair follicle formation	Other organ morphogenesis
Mouse	20 GD	16 GD (Stelnicki et al., 1997)	14.5 GD (DasGupta and Fuchs, 1999)	Vascular development (8–10.5 GD)(Carmeliet et al., 1998); Lung (10 GD)(Warburton et al., 2006); Long bone (15 GD)(Patton and Kaufman, 1995); Liver (8.25 GD)(Lemaigre, 2009); Kidney (12.5 GD) (Bard et al., 2001); Heart (8 GD)(Brand, 2003); Intestine (14 GD)(Kim et al., 2007); Primary neurulation (10.5 GD)(Shum and Copp, 1996)
Rat	22 GD	18 GD (Ihara et al., 1990)	~15 GD (Ohsawa, 2001)	Lung (11 GD)(Yamada et al., 2002), Liver (11 GD) (Godlewski et al., 1997), Kidney (12.5 GD) (Bernhardt et al., 2006), Heart (9 GD)(Marcela et al., 2012)
Sheep	147 GD	100 –120 GD (Scheid et al., 2002)	57–89 GD (Lyne and Hollis, 1972)	Heart (15 GD)(Field, 1946), Long bone (~50 GD) (Richardson et al., 1976) Lung (<40 GD)(Pinkerton and Joad, 2000)
Human	266 GD	Late in the second trimester (~180 GD) (Lorenz et al., 1992)	65–84 GD (Akiyama et al., 2002)	Lung (30 to 50 GD) (Warburton et al., 2006); Liver (20 to 30 GD)(Haruna et al., 1996); Kidney (28 GD) (Faa et al., 2011); Heart (20 GD and on)(Brand, 2003); Intestine (20 to 30 GD)(Kim et al., 2007)

* GD = gestational days

Table 2

Signaling pathways and transcriptional factors involved in scarless vs. scar-forming wound healing

Scarless wound repair				Scar-forming wound repair			
Category	Evidence	Model organism	Ref	Category	Evidence	Model organism	Ref
Noncanonical Wnt/PCP pathway	Defect in wound closure in PCP (planar cell polarity) gene null mutants.	Mouse	(Caddy et al., 2010)	Canonical Wnt/ β -catenin pathway	Upregulation in wounded dermis; Wnt3a mimics the effect of TGF- β 1 on dermal fibroblasts; Wounding-induced de novo hair follicle regeneration.	Mouse	(Carre et al., 2010) (Ito et al., 2007)
TGF- β signaling	Anti-fibrotic effect of TGF- β 3. Increased ratio of TGF- β 3 to TGF- β 1. TGF- β 3 RNA increases in fetal skin.	Rat	(Shah et al., 1995); (Hsu et al., 2001) (Stelnicki et al., 1998b)	TGF- β signaling	Profibrotic Increased ratio of TGF- β 1 to - β 3. TGF- β 1 increases in postnatal skin; Smad3 null mutants accelerated wound healing and reduced infiltration of monocytes.	Rat	(Shah et al., 1994) (Hsu et al., 2001) (Ashcroft et al., 1999)
BMP2	Induced scar formation and skin maturation in 2 nd trimester fetus.	Sheep				Mouse	
Transcription factors Homeobox genes	HoxB13 decreased fetal skin wound; fast wound closure in null mutant. Prx-2 increase in wounded fetal skin; gene deletion alters fetal fibroblast phenotype.	Human Mouse	(Stelnicki et al., 1998a) (Mack et al., 2003) (Stelnicki et al., 1998a) (White et al., 2003) (Ting et al., 2005)	Transcription factors Homeobox genes	Msx-2 null mutants exhibit faster wound closure.	Mouse	(Yeh et al., 2009)
Grainy head like-3	Defect in closure of limb wound in null mutant.	Mouse					
HIF-1 α	Increased expression in transitional wounds with minimal scar formation.	Sheep	(Scheid et al., 2002)	VEGF	Exogenous VEGF converts the scarless to scar-forming repair; neutralization of VEGF decreases scar formation.	Mouse	(Wilgus et al., 2008)
Interleukins IL-10	Fetal skin of null mutant exhibited scar formation.	Mouse	(Liechty et al., 2000)	Interleukins IL-10 IL-6	Null mutant: accelerated wound healing and increased infiltration of macrophages. Null mutant: showed attenuated leukocyte infiltration, angiogenesis, and collagen accumulation.	Mouse Mouse	(Eming et al., 2007) (Lin et al., 2003)
Protease/protease inhibitor	Increased ratio of uPA to PAI-1 in the skin and skin wounds.	Mouse	(Huang et al., 2002)	Protease/protease inhibitor	Increased ratio of PAI-1 to uPA in the skin and skin wounds.	Mouse	(Huang et al., 2002)