

Adult and fetal wound healing

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1. ABSTRACT

Mammalian wound healing is an intricate process involving the coordinated interaction of a variety of tissue types and cellular lineages. This is governed by a complex array of physical, biological and chemical signals. In adult tissue, the successful completion of wound healing inevitably results in the formation of scar. However, animal studies of embryonic tissue have shown that early gestation fetal wounds heal with complete restoration of tissue structure and function. The exact mechanisms underlying scarless healing in the fetus remain to be elucidated.

2. INTRODUCTION

Unlike in the salamander, tissue injury in adult mammalian vertebrates leads to incomplete regeneration of the damaged organ. Under most circumstances, the defect is repaired via an inflammatory response culminating in scar formation. This is a result of fibroblast collagen secretion and myofibroblast wound contraction. Though an efficient mechanism in the short-term, the scar response never restores injured tissue to its original state and may compromise function.

Approximately 1.25 million people suffer from burn injuries annually in the United States (1). An

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additional 6.5 million are plagued with chronic skin ulcers due to underlying conditions such as venous stasis or diabetes mellitus (2). Unfortunately, no reliable therapeutic option currently exists on the market and the outcome of scar formation remains unpreventable under these circumstances. Thus, chronic wounds represent a major health problem costing the healthcare system some \$ 20 billion in 2002 (<http://www.ahrq.gov/clinic/epcsums/woundsum.htm>). Researchers, however, remain hopeful citing that full-thickness fetal skin wounds heal without scar formation. Future therapeutic progress potentially may rest on the ability of scientists to apply what is learned from scarless fetal wound healing to models of adult injury.

3. ADULT CUTANEOUS WOUND HEALING

3.1. Process of wound healing

Normal adult wound repair is a highly regulated process generally recognized to follow three temporally overlapping phases: inflammation, tissue formation, and tissue remodeling.

3.1.1. Coagulation and inflammation

Cutaneous wounds often result in the disruption of the vascular network. In response, platelet aggregation leads to the formation of a fibrin clot preventing further extravasation of blood or plasma (3). Platelets also secrete a multitude of growth factors and cytokines that modulate fibroblast activity such as transforming growth factor β 1 (TGF- β 1) and platelet derived growth factor (PDGF) (4). In the absence of hemorrhage, chemotactic factors and vasoactive mediators are released by injured epithelial cells, or generated by the clotting and activated complement pathways (3).

Neutrophils respond to many of the same chemotactic agents and begin to infiltrate the site of injury prior to the activation and/or arrival of fibroblasts and monocytes. There, neutrophils release degradative enzymes and phagocytose foreign material while also secreting pro-inflammatory cytokines (5). Neutrophil infiltration abates within a few days in the absence of infection. Soon after, circulating monocytes diapedese the endothelium and become tissue macrophages that target neutrophils for phagocytosis. In the presence of foreign body or infection, a persistent neutrophil-rich inflammatory response instead results, leading to poor wound healing and excess fibrosis (6).

Monocytes are attracted to the wound site in response to a variety of chemoattractants derived from intravascular and extravascular compartments. Upon conversion into activated macrophages at the wound site, a host of cytokines is secreted. Although inflammatory by nature, these mediators remain critical for normal wound healing as evidenced in previous studies of macrophage-depleted guinea pigs which exhibited defective wound repair (7). Monocytes and activated macrophages can bind to the extracellular matrix (ECM) through cell surface integrin receptors. Adherence to the ECM induces ECM phagocytosis, promoting wound debridement. Attachment

to the ECM also induces selective mRNA expression by macrophages leading to increased expression and subsequent secretion of colony stimulating factor 1 (CSF-1, necessary for macrophage survival), tumor necrosis factor (TNF- α , inflammatory cytokine), PDGF (chemotactic agent for fibroblasts) as well as c-fos and c-jun (transactivating factors necessary for many activation signals) (8, 9). Similar to neutrophils, a persistent macrophage response may lead to excess scar formation, an unwanted outcome (10).

3.1.2. Reepithelialization

The process of reepithelialization begins within hours after injury. In the presence of basement membrane damage, epidermal cells migrate over a provisional matrix derived from fibroblast. Keratinocytes originating from the interfollicular epidermis or from neighboring hair follicles are the first to migrate into the wound margins (11). Transiently amplifying epidermal stem cells in the basal layer surrounding the wound indeed supply a portion of the migrating cells (12). In hair bearing skin, stem cells found in the infundibular hair bulge have also been shown to contribute to reepithelialization of denuded skin (13). More recent studies have further clarified a lack of a role of bulge stem cells in epidermal homeostasis while confirming their important contribution to wound repair (14).

Keratinocyte migration is achieved through several phenotypic alterations. These include retraction of intracellular tonofilaments, dissolution of intercellular desmosomes, and formation of peripheral cytoplasmic actin filaments (15). Hemidesmosomal linkages between the basement membrane and epidermal cells are also lost (16). The culmination of these changes allows epidermal cells to migrate over the wound defect in order to restore epidermal continuity.

The molecular events underlying these phenotypic changes have been the subject of much investigation. Migrating keratinocytes have been shown to express characteristics of basal and suprabasal lineages. For example, these cells contain transglutaminase, components of differentiated keratinocytes in the stratum granulosum. Activation of the transglutaminase A gene is essential for facilitated repair of skin injury (17). In addition, wound keratinocytes exhibit receptors for fibronectin, a protein not present under normal circumstances. Similar to activated macrophages and fibroblasts, these keratinocytes have been found to secrete fibronectin while migrating across the wound surface (18). The exact signals initiating the epidermal migration process remain unclear, although experimental manipulations such as reducing extracellular calcium concentration have been shown to impart cultured keratinocytes with a similar phenotype, while normal concentrations drive terminal differentiation (19). Interestingly, it appears that migrating epidermal cells also exhibit the ability to digest collagen and dissect out dessicated eschar and other non-viable tissue by secretion of collagenase, also known as matrix metalloproteinase 1 (MMP-1), and plasminogen activator, respectively (20, 21).

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The factors inducing keratinocyte proliferation have not been fully elucidated. One hypothesis put forward by Heilmann and Swartz is known as the “free-edge” effect whereby the absence of neighboring cells at the wound margin acts as a proliferation and migration signal (22). Local growth factors such as epidermal growth factor (EGF), TGF- α (23), heparin binding EGF, keratinocyte growth factor (KGF) (24), and epiregulin (25) stimulate reepithelialization via autocrine or paracrine effects on the EGF receptor. Clearly, this process is complex and likely involves a multitude of indispensable orchestrated signals.

3.1.3. Formation of granulation tissue

Granulation tissue begins to form approximately 4 days after injury. It is a vascularity rich neostroma comprised of macrophages, fibroblasts, and loose connective tissue. The initial fibrin clot functions as a chemokine to stimulate macrophages and fibroblasts to migrate into the wound space. These macrophages provide a continuing source of growth factors necessary for neoangiogenesis (26). Fibroblast movement across the fibrin clot is contingent upon the presence of serum factors and proteolytic enzymes, such as MMP-1, gelatinase (MMP-2) and stromelysin (MMP-3) (27). The rate of granulation tissue formation appears to be dependent on interaction of the fibroblast integrin receptor with fibronectin (28).

After migrating into the wound space, fibroblasts commence laying down a provisional matrix mainly composed of collagen and proteoglycans. Expression of both TGF- β 1 and TGF- β 2 is increased in adult wounds and exogenous administration leads to increased collagen, protein, and inflammatory cell accumulation (29). Collagen rebuilds the damaged tissue in the early stage of wound healing and is critical for healthy scar formation. When collagen density reaches a critical level, fibroblast proliferation and collagen synthesis are suppressed (30). Dysregulation of this negative feedback loop results in pathologic scar formation with densely packed collagen bundles and occasionally fibrotic disorders such as keloid formation, morphea, and scleroderma (31).

3.1.4. Neovascularization

Neoangiogenesis is a critical component of wound healing and depends on local and circulating endothelial cells and a variety of factors such as the ECM. A number of cytokines have also been implicated in neoangiogenesis including TGF- α , TGF- β , TNF- α , PDGF, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), interleukin-8 (IL-8), angiogenin, angiotropin, angiopoietin 1, and thrombospondin (32). In addition, low oxygen tension, lactic acid, plasminogen activator, and collagenases all play a role in the neovascularization process (31). After granulation tissue has reached a mature state, neoangiogenesis is halted by activation of apoptotic pathways (33).

3.1.5. Wound contraction and remodeling

Wound contraction and remodeling are the last phases of wound healing and occur during the second week post-injury when fibroblasts undergo a phenotypic switch to cells with increased smooth muscle actin expression known

as myofibroblasts (34). Wound contraction is under the regulation of growth factors such as TGF- β 1, TGF- β 2, and PDGF (35). Remodeling is responsible for removing excess connective tissue and is made possible by MMPs (36). Additionally, the number of fibroblasts is decreased and there is a progressive decline in the vascularity of the wound. During this phase scar tissue becomes paler and the wound gains strength when the collagen matures. The fibroblasts secrete mature type I collagen which forms along the lines of tension in the wound instead of immature randomly deposited type III collagen. This remodeling of collagen may be disturbed by a number of factors, leading to a raised scar. The persistence of a raised scar can lead to hypertrophic scars and keloid formation (37).

3.2. Cytokines

The major growth factors directly involved in promoting wound healing are PDGF, TGF- β , TGF- α , FGF, VEGF, EGF and HGF. The role of IL-1 α , IL-1 β and TNF- α will also be discussed.

3.2.1. TGF- β

TGF- β is a pleiotropic growth factor with important effects on wound healing. Three isoforms of TGF- β are expressed in mammalian skin, with > 85% being TGF- β 1 in adult wounds. Most of the major cell types involved in wound healing secrete TGF- β including epithelial cells, macrophages, lymphocytes, endothelial cells, and smooth muscle cells (29). TGF- β is chemotactic for fibroblasts, keratinocytes, and inflammatory cells and stimulates collagen type I production by fibroblasts. In addition, TGF- β increases production of other matrix proteins such as fibronectin and glycosaminoglycans. TGF- β also downregulates expression of matrix degrading proteases and integrin receptors, while increasing expression of their inhibitors (38).

3.2.2. PDGF

PDGF is a family of isoforms consisting of disulfide-bonded homo- or hetero-dimer of PDGF A-chain and B-chain gene products. Both homo- and hetero-dimers are present in platelets, but PDGF-AB is the most common form (39). PDGF is able to play an indirect role in many aspects of wound healing through stimulating the release of other growth factors by macrophages. PDGF also modulates the synthesis of fibronectin and hyaluronic acid (40). Furthermore, *in vitro* studies have linked PDGF to wound contraction due to effects on myofibroblasts (41). Finally, PDGF influences wound remodeling by regulating fibroblast secretion of collagenase (42).

3.2.3. EGF

EGF and EGF-like homologues comprise an ever expanding family of polypeptide molecules including EGF, TGF- α , coagulation factors (factors IX and X), angiotensin II and ECM molecules like fibrillin (23). The EGF-R is a subfamily of receptor kinases that operate mainly through G-protein, mitogen-activated protein kinase (MAPK) and JAK-STAT transduction pathways (43). In normal adult skin, EGF receptors are primarily located within the basal epidermal population (44). EGF plays a role in the induction of these cells to migrate, divide, differentiate, and

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reestablish cellular adhesion via desmosomes and hemidesmosomes (23). EGF was the first growth factor shown to accelerate reepithelialization of normal partial thickness surgical wounds on donor skin (45). Very recently, EGF has also been shown to promote wound healing by increasing myofibroblast proliferation and collagen synthesis in a rat full thickness wound model (46).

3.2.4. FGF

In mammals, the FGF family is composed of at least 22 structurally homologous polypeptides (47). FGFs transduce their signals through 5 related FGF receptors (FGFR 1-5), high-affinity transmembrane tyrosine kinases (48). Many FGFs have an affinity for binding to heparin-like glycosaminoglycans and can therefore be stored and released by digesting the ECM (49). FGFs play key roles in regulating cell proliferation, migration, and differentiation. For example, FGF-1 and FGF-2 stimulate fibroblast migration and proliferation while FGF-1, FGF-2, FGF-4 and FGF-5 have been shown to stimulate angiogenesis (50). The expression of FGF-7 is strongly upregulated after skin injury and is restricted to fibroblast and T cells at the wound site (51). Impaired wound reepithelialization was observed in dominant-negative FGF-7 experiments indicating a role in stimulating keratinocyte migration and proliferation (24). As potent mitogenic and chemotactic factors, FGFs have been clear candidates for improving wound healing.

3.2.5. HGF

Hepatocyte growth factor (HGF) was originally identified as a potent mitogen for mature hepatocytes (52). Since that time HGF has been shown to play an important physiological role in regeneration and protection of many tissues following injury (53). In the skin, HGF stimulates proliferation and migration of epidermal keratinocytes and melanocytes (54). Keratinocyte expression of HGF and its receptor (c-Met/HGF receptor) is increased in response to cutaneous wounding through autocrine signals (55). Transgenic overexpression of HGF resulted in increased vascularization and granulation tissue expansion (56). In the presence of neutralizing anti-HGF antibody, reepithelialization and neovascularization responses to wounding were significantly delayed (57). Very recently, double gene transfer of HGF and prostacyclin synthase in rats was shown to significantly enhance wound healing (58).

3.2.6. VEGF

VEGF is a heparin-binding homo-dimeric glycoprotein that binds targets a VEGF receptor specific to endothelial cells. VEGF expression is enhanced by molecular factors such as TGF- α , TGF- β , EGF, TNF- α , KGF, and low oxygen tension (59). VEGF has been shown to promote angiogenesis *in vivo* (60) and *in vitro* (61). Transgenic mouse studies demonstrated a pro-angiogenic paracrine effect of VEGF (62) with blockade leading to delayed wound healing (63). Recently increased VEGF expression has been linked to the formation of hypertrophic scars and keloids (64).

4. FIBROPROLIFERATIVE SCARRING

Fibroproliferative scarring refers to the end result of an amplified wound healing process leading to excessive

connective tissue production. The two main types of excess scarring that are observed in cutaneous wounds are referred to as hypertrophic and keloid scars.

4.1. Hypertrophic scars and keloid scars

Hypertrophic scars are defined as raised, erythematous, pruritic lesions that remain within the boundaries of the original wound (65). Hypertrophic scar formation usually begins six to eight weeks post-injury, reaching a plateau by six months. If located over joints or along extremities, debilitating contractures may result. Regression may be observed without intervention and the extent of scarring is directly related to the depth and location of injury (66). Keloid scars only form in humans and no animal model exists.

Keloid scars are also raised, erythematous and pruritic, but extend beyond the original wound boundaries (65). Keloids almost never regress spontaneously, do not lead to contractures, and are likely to recur if treated with surgical resection (67).

4.2. Pathogenesis of fibroproliferative scarring

Fibroproliferative scarring occurs due to an imbalance between the synthesis of the ECM and its degradation during tissue remodeling. The imbalance begins as early as the inflammatory phase of wound healing with increased levels of IL-8 (68). This leads to secretion of pro-fibrotic growth factors like TGF- β , insulin-like growth factor 1 and PDGF. Of these, the TGF- β family plays a major role in fibrosis (69). Interestingly, TGF- β 1 and TGF- β 2 have been reported to increase fibrosis while TGF- β 3 decreases fibrosis (39), with the latter recently entering Phase II clinical trials in humans for scar prevention and/or reduction. In addition, TGF- β 1 and - β 2 have been found to potently stimulate fibroblast contraction (70).

Unlike normal ECM which is rich in hyaluronic acid, the ECM in fibroproliferative wound healing is predominately chondroitin sulfate, a substance that may interfere with collagen degradation (71). Collagenolysis does not proceed normally in fibroproliferative states leading to impaired tissue remodeling (72). Furthermore, despite an excess of the cross linking enzyme lysyl oxidase, collagen in proliferative scars is often randomly oriented and inadequately cross-linked (71).

4.3. Histology of fibroproliferative scars

In contrast to the random collagen orientation of normal dermis, adult wounds are characterized by dense collagen bundles arranged parallel to the epidermis. They are connected to each other via random fine fibrillar strands of collagen or elastin. The collagen fibers in hypertrophic scars are flatter, less distinct and arranged in a wavy pattern but still run parallel to the epidermis. In contrast, keloid scars exhibit large and very coarse collagen fibers arranged more haphazardly in the dermis (73). Curiously, α -smooth muscle actin positive myofibroblasts are only found in hypertrophic scars and not keloids (74).

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Table 1. Differences between fetal and adult fibroblast

	Fetal	Adult
Collagen synthesis	+++ , immediately post-injury	+, at four days after injury
Prolyl hydroxylase activity	+++	+
HA Synthesis	cell density-independent	↓ with ↑ cell density
HA receptor	+++	+
Migration	rapid	slow
Proliferation	inhibition by TGF-β3	stimulation by TGF-β1 and -β2
Signal transduction	PKA pathway	MEK/ERK pathway

ERK: extracellular signal-regulated kinase, HA: hyaluronic acid, MEK: Map/Erk kinase, PKA: protein kinase A, TGF-β: transforming growth factor-beta

4.4. Treatment

Surgery is the oldest method of scar treatment; others include pressure therapy, corticosteroids, laser, topical silicone gel, radiation, and calcium channel blockers. These methods of treatment as well as other latest advances have been recently reviewed and will not be discussed further (75)

Classification of scars is the critical guiding force for determining therapy. Hypertrophic scars may regress spontaneously without treatment but often respond best to local measures such as topical corticosteroids. Keloids are much more difficult to treat and often require surgical excision in addition to adjunctive therapy (76). Growth factors and cytokines offer much promise as important vectors for treating fibroproliferative scars. In particular, interferons (IFN-α, IFN-β, IFN-γ) have been used often in combination with surgery (77-79).

5. FETAL CUTANEOUS WOUND HEALING

Scarless repair in fetal mammals, including humans, has been described for many years. Scarless fetal wound healing is determined by gestational age of the fetus and wound size (80). The transition from scarless to scarring wound repair occurs in the context of the developing fetal skin. In other words, fetal skin has been shown to heal scarlessly prior to a certain gestational age, after which typical scar formation prevails. The transition period is after 24-weeks of gestation in the human fetus (81), and between days 16.5 and 18.5 of gestation in the fetal rat (82). Moreover, larger size wounds only heal without scar formation at earlier gestational ages than smaller defects, suggesting a gradual reduction in scarless healing capability with advancing gestational age (80). Elucidation of the mechanisms underlying this scarless healing process may aid in the development of therapeutic interventions to abrogate scar formation in adults.

5.1. Fetal skin

The ECM plays an important role in wound healing. It is a dynamic layer of collagen, proteoglycans, and glycosaminoglycans that facilitates cellular migration and serves as a reservoir for growth factors. The unique matrix components of fetal ECM may partially be responsible for scarless wound healing. Fetal ECM differs from adult ECM in collagen composition, hyaluronic acid (HA) content, and proteoglycan ECM modulators.

Collagen type I is the principal component of both adult and fetal ECM. Fetal skin contains a higher ratio of type III to type I collagen than adult skin (30-60% vs. 10-20%, respectively) (46). With maturation, the relative amount of type III collagen in fetal skin diminishes, although the adult phenotype is not seen until the postnatal period (83). Since fetal skin contains more HA than adult skin, several investigators have proposed a role of HA in scarless healing (84, 85). Proteoglycan ECM modulators decorin, fibromodulin, lysyl oxidase, and MMPs serve a role in collagen synthesis, maturation, and degradation. Decorin production increases by 72% during the transition period in fetal rat skin and continues to increase into the postnatal period achieving a level 300-fold that of early gestational fibroblasts (86). Similarly, increased expression of lysyl oxidase and MMPs occurs with development (87). Fibromodulin has been shown to bind and inactivate TGF-β, and its expression was shown to diminish with skin maturation (88).

5.2. Fetal cells

5.2.1. Fetal platelets and inflammatory cells

Scarless wounds are characterized by a relative lack of inflammation (89), while induction of inflammation restores scar formation (90). The absence of an acute inflammatory infiltrate in scarless wounds may partly be explained by decreased fetal platelet degranulation and aggregation. Fetal platelets produce less PDGF, TGF-β1, and TGF-β2 than their adult counterparts (91). Although *in vitro* experiments have shown that fetal platelets degranulate upon exposure to collagen, aggregation has not been observed (92). Olutoye *et al.* showed an age-dependent aggregatory response to ADP exposure corresponding with the scarring transition period (93). The lack of aggregation may in part be explained by an HA-induced suppressive effect (94). In addition, unlike adult wound healing, fewer neutrophils are present in the fetal wound, and an age-dependent defect in the ability of fetal neutrophils to phagocytose pathogenic bacteria has been demonstrated in fetal sheep (95).

5.2.2. Fibroblasts

Because synthesis and remodeling of the ECM by fibroblasts is essential for wound healing, numerous studies have attempted to elucidate differences between fetal and adult fibroblasts (Table 1.). First, they display differences in synthetic function of collagen, HA, and other ECM components. Fetal fibroblasts produce higher protein and mRNA levels for most collagens (particularly type III, and type IV) than neonatal and adult cells (96, 97). The rate-limited enzyme of collagen synthesis (prolyl hydroxylase) has greater activity and different enzymatic regulation in human fetal fibroblasts as compared to adult fibroblasts (98, 99). Collagen synthesis by fibroblasts is delayed in the adult wound. In contrast, fetal fibroblasts synthesize collagen immediately post-injury. Furthermore, unlike in the adult, HA synthesis in fetal fibroblasts does not decrease with cell density (100). HA receptors are expressed at two- to four fold greater levels in fetal than adult fibroblasts (101).

Second, fetal fibroblasts display an elevated level of migratory activity compared to adult cells. Cultured fetal

fibroblasts migrate faster on type I collagen and HA gel than neonatal and adult fibroblasts. This may be explained partly by increased HA receptor levels in fetal fibroblasts and the secretion of an unidentified pro-migration paracrine factor (102, 103). Additionally, the migration of fetal and adult fibroblasts is differentially modulated by exogenous cytokines. The migration of subconfluent fetal cells is unaffected by TGF- β 1 and - β 2, but inhibited by TGF- β 3, while the migration of subconfluent adult cells was inhibited by all three isoforms. Similarly, the migration of confluent fetal cells was inhibited by all three TGF- β isoforms, although adult fibroblasts were unaffected by TGF- β 1 and - β 2, but stimulated by TGF- β 3 (104).

Third, fetal and adult fibroblasts also exhibit different proliferation responses to cytokine. TGF- β inhibits fetal human skin fibroblasts, while it is stimulatory for those of adults (105). In addition, curcumin, a natural product with wound healing activity, was found to totally abolish the inhibition of TGF- β 1 on fetal fibroblast proliferation without affecting the stimulatory effect on adult fibroblasts (105). Very recently, Giannouli *et al.* reported that TGF- β activated protein kinase A (PKA) induced the expression of cyclin-dependent kinase inhibitors (CKI) in fetal fibroblasts. Previous work showed that a specific PKA inhibitor blocked induction of CKIs and abrogated the TGF- β -mediated inhibitory effect, pointing to a central role of PKA. In contrast, proliferation of adult fibroblasts is enhanced by TGF- β via activation of the MEK-ERK pathway in a PKA-independent fashion (106).

These intrinsic differences between fetal and adult fibroblast demonstrate the complexity of scarless repair. Further unraveling the underlying mechanisms responsible for these differences depends heavily on our ability to more precisely define what exactly constitutes a fibroblast. No universally accepted fibroblast specific cell surface markers exist, further confounding the field of wound healing. Although fibroblast-specific protein 1 (FSP1) was previously thought to be specific for fibroblasts (107), our group has observed significant expression of FSP1 in bone marrow- and adipose-derived mesenchymal stem cells (unpublished data). Further work will be necessary to better define reliable and specific methods of identifying a fibroblast.

5.3. Cytokines

5.3.1. TGF- β

TGF- β isoforms are involved in all steps of wound repair and as mentioned above, have divergent effects on scar formation and wound healing. The expression of TGF- β 1 and - β 2 is increased in rabbit adult wounds, while it is unchanged in fetal wounds (108). Scarless wounds in fetal mice have less TGF- β 1 than neonatal or adult wounds (109). Insertion of PVA sponges containing TGF- β 1 into fetal wounds leads to scar formation (109). Similarly, treatment of adult rat wounds with neutralizing antibodies to TGF- β 1 and TGF- β 2 reduces scar formation (108, 110).

Furthermore, the relative proportion of TGF- β isoforms, and not the absolute amount of any one isoform, may determine the wound phenotype. In scarless fetal wounds,

TGF- β 3 expression is increased while TGF- β 1 expression is unchanged. Conversely, TGF- β 1 expression is increased and TGF- β 3 decreased in scarring fetal wounds (111). Treatment of adult wounds with exogenous TGF- β 3 reduces scar formation (112). This suggests the ratio of TGF- β 3 to TGF- β 1 may determine whether tissue regenerates with or without a scar.

5.3.2. Other growth factors

PDGF and FGF are additional pro-fibrotic cytokines. PDGF has prolonged expression during scar formation but disappears by 24 hours in fetal wounds (113). Treatment of fetal wounds with PDGF induces a marked increase in acute inflammation, fibroblast recruitment, and collagen deposition (114). The expression of FGF family of cytokines, including KGF1 and 2, increases with advancing gestational age (115). In contrast, VEGF increases two-fold in scarless wounds while its expression remains unchanged in scarring fetal wounds (116). Thus, an increased stimulus for angiogenesis and vascular permeability may assist in the rapid healing of fetal wounds.

5.3.3. Interleukins

The interleukin family of cytokines plays an important role in chemotaxis and activation of inflammatory cell mediators. IL-6 stimulates monocyte chemotaxis and macrophage activation while IL-8 attracts neutrophils and stimulates neovascularization (117). Wounding stimulates a rapid increase in IL-6 and IL-8, which persists at 72 hours in the adult but disappears by twelve hours in the fetus (117, 118). Both IL-6 and IL-8 expression are significantly lower in early fetal fibroblasts at baseline and with PDGF stimulation compared to adult fibroblasts (117-119). Addition of IL-6 to fetal wounds produces scar in normally scarless wounds. Furthermore, IL-10 has an anti-inflammatory function by decreasing production of IL-6 and IL-8. When wounded fetal skin was harvested from early gestation IL-10 knock-out mice and grafted onto syngeneic adult mice, they healed with significant inflammation and scar formation (120). In an initial study in adult mice over-expressing IL-10, reduced inflammation and scarless healing was observed (121). This exciting work may have potential therapeutic implications for human adult wounds.

5.4. Molecular control of scarless repair

Several studies aimed at better defining the scarless fibroblast phenotype have examined cellular signaling pathways, specifically focusing on the role of receptor tyrosine kinases and adapter proteins such as Shc. The latter has been shown to couple receptor tyrosine kinases to MAPK (122). Shc serves as a key intermediate for discoid domain receptor (DDR) signaling and may contribute to hypoxia-induced factor stabilization and endothelial migration (123, 124). Although TGF- β signaling is mediated through intrinsic serine/threonine kinase receptors, tyrosine kinase receptor dependent signaling has been shown to modulate gene expression (125). Different receptor tyrosine kinase (RTK) phosphorylation patterns are observed between fetal and adult rat fibroblasts with increased amounts of EGF receptor, DDR, and Shc proteins in fetal fibroblasts

suggesting that RTK signaling may play a role in scarless repair (125).

Ultimately, the mechanistic differences between scarless and scarring repair may be regulated at the gene expression level. Homeobox genes are transcription factors that are implicated in the patterning and cell type specification events during development. These genes determine the direction taken by major developmental pathways involving activity of hundreds of genes. Their role in skin embryogenesis and wound healing is being investigated. Human homeobox genes MSX-1, MSX-2, and MOX-1 are differentially expressed during skin development (126). Additionally, human fetal scarless repair is associated with decreased expression of HOXB13 and increased PRX-2 expression (127). Given that scarless repair is inherent to developing skin, it seems likely that coordinated control of groups of genes by transcription factors, such as homeobox genes, has a crucial function during the repair process.

6. PERSPECTIVE

Scarring and scarless wound healing are complex events modulated by numerous growth factors, inflammatory cytokines, and other genes involved in the process of tissue repair. Further elucidation of the mechanisms underlying these differences will revolve around more detailed dissection of the regulatory factors controlling their unique signaling pathways. Moreover, scarless wound healing appears to be intrinsic to fetal skin, and even then age-dependent. ECM, inflammatory cells, fibroblasts, and cytokine expression patterns have all been implicated as potential mechanisms underlying scarless repair in the fetus. Tools such as microarray analysis may allow more rapid identification of the key candidate genes that lead to scarless repair, and such experiments are already underway and will hopefully shed light on this intricate fetal phenomenon.

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Abbreviations: CKI: cyclin-dependent kinase inhibitors, CSF-1: colony stimulating factor 1, DDR: discoid domain receptor, ECM: extracellular matrix, EGF: epidermal growth factor, FGF: fibroblast growth factor, FGFR: FGF receptors, FSP1: fibroblast-specific protein 1, HA: hyaluronic acid, IFN: interferons, IL: interleukin, KGF: keratinocyte growth factor, MAPK: mitogen-activated protein kinase, MMP: matrix metalloproteinase, PDGF: platelet derived growth factor, PKA: protein kinase A, RTK: receptor tyrosine kinase, TGF- β : transforming growth factor, TNF- α : tumor necrosis factor, VEGF: vascular endothelial growth factor.

Key Words: Wound Healing, Scarless, Fibroblast, Review

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