Wnt Signaling During Cutaneous Wound Healing

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11.1 Introduction

The skin is an intricate structure composed of the epidermis, dermis, and a dermal adipocyte layer [1]. It is the largest organ in the human body acting as a barrier against external microorganisms and dehydration [2]. The skin further contributes to homeostasis by participating in thermal regulation by sensing and responding to disturbances.

Regeneration is a process of restoration, renewal, and growth crucial to the ability of cells and organs to be resilient to damage [3]. It is important to distinguish between repair, healing via formation of scar tissue, and regeneration, which is restoration to the pre-injury state [4]. Full-thickness skin loss in adult mammals typically results in a reparative rather than regenerative response, leading to the formation of scar tissue [3]. Deposition of a collagen-rich matrix in the neo-dermis makes it prone to contracture, decreased elasticity, and tensile strength, and promotes hypertrophic scar formation [5]. Epithelialization without epidermal appendage development over a large surface area leads to alopecia and thermal imbalance [5]. This repair depends on the differentiation and proliferation of involved cells, including epidermal stem cells (ESCs), keratinocytes, and fibroblasts, together with the assistance of various biological signals [6].

Healing of skin wounds parallels embryonic skin development in many ways. Both processes involve the differentiation, migration, proliferation,

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and apoptosis of various cell types to create the multilayered tissue that constitutes the skin [3]. While skin wounds in early mammalian embryos regenerate without scar tissue formation and complete restitution of the normal skin architecture [7], this is not the case with adult wounds [8]. However, many of the same key signaling pathways that are activated during embryonic skin development are also activated during postnatal wound healing, e.g., Wnt/β-catenin, Notch, and Hedgehog pathways [9], creating interest in better understanding the role of these pathways.

Maintenance of epidermal homeostasis is achieved by separate populations of stem cells in the skin: stem cells that come from the bulb region of the hair follicles, interfollicular epidermis, as well as sebaceous gland [10]. While both epidermal and bulb stem cells have demonstrated the potential to regenerate epidermis, an effective cell-based approach utilizing these populations to promote "scarless" wound healing remains elusive [3]. Interestingly, recent data demonstrate that the epidermis of wounded adult mice can regenerate hair follicles under the influence of Wnt-responsive interfollicular stem cells [11]. Here, we present a summation of data, which provide strong evidence for an alternative approach for enhancing cutaneous regeneration after injury: augmenting the endogenous Wnt pathway to activate tissue-resident stem cells.

11.2 Three Wnt Signaling Pathways

The Wnt signaling pathway is an evolutionarily conserved pathway that regulates crucial aspects of cell fate determination, cell polarity, cell migration, neural patterning, and organogenesis during embryonic development [12]. The name Wnt is resultant from a fusion of the name of the Drosophila segment polarity gene wingless and the name of the vertebrate homolog, integrated or int-1 [13]. Wnt proteins regulate a dizzying array of cellular processes including cell fate determination, motility, polarity, primary axis formation, organogenesis, and stem cell renewal [13]. As the signaling pathways that play crucial role during embryogenesis are tightly regulated, the expressions of the Wnt proteins and Wnt antagonists are exquisitely restricted both temporally and spatially during development [14].

Intracellular Wnt signaling diversifies into three main branches: (1) the β-catenin pathway (canonical Wnt pathway), which activates target genes in the nucleus; (2) the planar cell polarity (PCP) pathway, which involves jun N-terminal kinase (JNK) and cytoskeletal rearrangements; and (3) the Wnt/Ca^{2+} pathway [3]. In humans, there are currently 19 different known Wnt proteins and ten different frizzled (Fzd) receptors [15]. Frizzled genes encode integral membrane proteins that function in multiple signal transduction pathways. They have been identified in diverse animals, from sponges to humans. The family is defined by conserved structural features, including seven hydrophobic domains and a cysteine-rich ligand-binding domain. Frizzled proteins are receptors for secreted Wnt proteins, as well as other ligands, and also play a critical role in the regulation of cell polarity. Frizzled genes are essential for embryonic development, tissue and cell polarity, formation of neural synapses, and regulation of proliferation, and many other processes in developing and adult organisms. Here we focus on canonical/ß-catenin-dependent Wnt signaling, which has been implicated in tissue regeneration and repair.

11.3 Canonical Wnt Signaling

The hallmark of canonical Wnt signaling is the accumulation and translocation of the adherens junction-associated protein, β-catenin, into the nucleus [16]. β-Catenin has been shown to perform two apparently unrelated functions: cell-cell adhesion in addition to a signaling role as a component of the Wnt/wg pathway. Wnt/wg signaling results in β-catenin accumulation and transcriptional activation of specific target genes during development. Dysregulation of β-catenin signaling plays a role in the genesis of a number

of malignancies, suggesting an important role in the control of cellular proliferation or cell death. Without Wnt signaling, cytoplasmic β-catenin is degraded by a β-catenin destruction complex [17]. Phosphorylation of β-catenin within this complex by casein kinase and GSK3 targets it for ubiquitination and subsequent proteolytic destruction by the proteosomal complex [18]. Binding of Wnt to its receptor complex composed of the Fz (frizzled) and the LRP5/6 triggers a series of events that disrupt the APC/Axin/GSK3 complex that is required for the targeted destruction of β-catenin [19, 20], allowing consequent stabilization and accumulation in the cytoplasm [3]. Stabilized β-catenin translocates into the nucleus, exerting its effect on gene transcription by functioning as a transcriptional coactivator [13]. A large number of binding partners for β-catenin in the nucleus have been uncovered and perhaps the best characterized are the members of the LEF/TCF DNA-binding transcription factors (Fig. 11.1) [21].

11.4 Wnt Signaling in Tissue Regeneration and Repair

The importance of Wnt in tissue regeneration has been highlighted by studies demonstrating impaired regenerative capacity in animals when Wnt signaling is reduced [22, 23]. Additionally, the Wnt pathway regulates cell proliferation in the adult epidermis, which directly impacts the rate and extent of skin wound healing [24]. Wnt proteins also serve as niche signals for at least two types of skin stem cells that contribute to skin wound healing: those in the bulge region of the hair follicle, and those in the basal layer of the interfollicular epidermis [25].

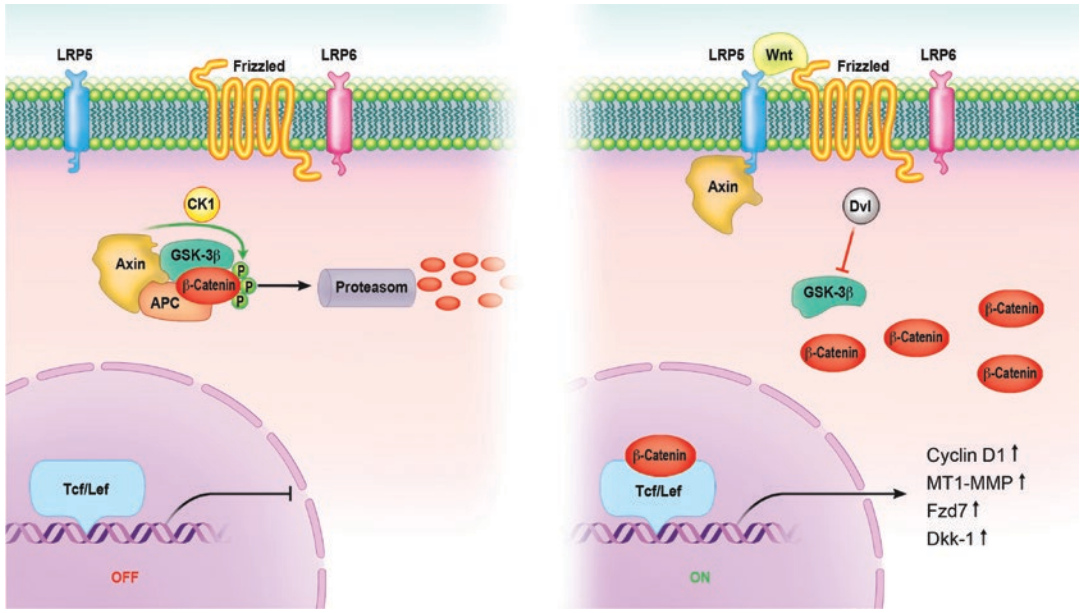

Fig. 11.1 Canonical Wnt signaling pathway. In the absence of signal, action of the destruction complex (CKIα, GSK-3β, APC, Axin) creates a hyperphosphorylated β-catenin, which is a target for ubiquitination and degradation by the proteosome. Binding of Wnt ligand to a frizzled/LRP-5/6 receptor complex leads to stabilization of hypophosphorylated β-catenin, which interacts with TCF/LEF proteins in the nucleus to activate transcription. In a canonical pathway, CKIα, GSK-3β, APC, and Axin act as negative regulators and all other components act positively. *APC* adenomatous polyposis coli, *CK* casein kinase, *GSK* glycogen synthase kinase, *Fzd* frizzled receptor, *LRP* low-density lipoprotein receptor-related protein, *Tcf/Lef* T-cell-specific transcription factor/lymphoid enhancer-binding factor

Recent studies have shown that fibroblast growth factor (FGF)-9 modulates hair follicle regeneration after skin injury in adult mice and that FGF-9 triggers Wnt activation in wound fibroblasts [26]. Through a unique feedback mechanism, activated fibroblasts then express FGF-9, thus amplifying Wnt activity throughout the wound dermis during a crucial phase of skin regeneration (Fig. 11.2). Skin wounds express various Wnt proteins during the early phases of healing, with transcripts of Wnts 1, 3, 4, 5a, and 10b being present in murine full-thickness cutaneous wounds up to 7 days after injury [9]. In the epithelium, Wnt 10b protein can be detected in migrating epithelial cells up to 3 days after wounding, while Wnt 4, 5a, and 10b localize to hair follicles [9]. Wnt 2a and 4 are expressed in the dermis, although reports vary with respect to the time course of their expression (range: 30 h to 7 days after wounding) [3]. It appears that Wnt signaling, through its ability to activate stem cells with induction of their self-renewal and proliferation, serves as a positive stimulus for wound repair [27]. Collectively, these data demonstrate that endogenous Wnt signaling is a prerequisite for tissue repair, but there are obvious caveats [22]. Most experimental methods used to study Wnt signaling in tissue healing rely on techniques that, in general, produce unrestrained Wnt pathway activation [22].

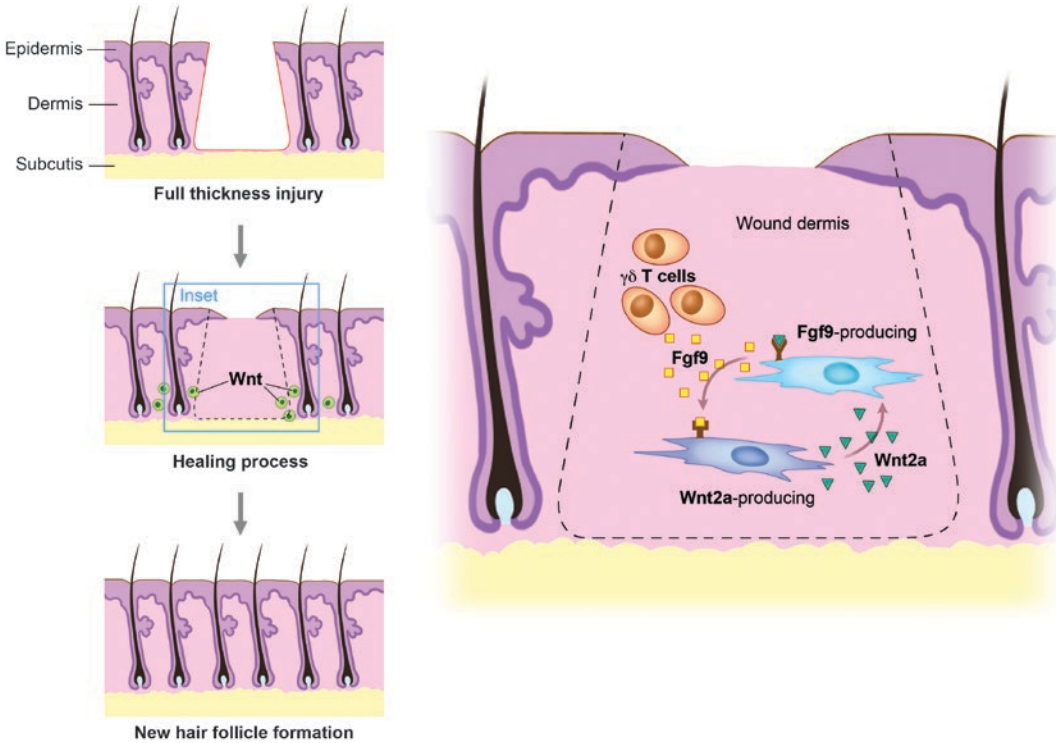

Fig. 11.2 Wnt signaling maintains the hair-inducing activity in skin repair. Fibroblast growth factor (Fgf) 9 is a secreted signaling molecule that is expressed in epithelium. Mesenchymal Fgf signaling interacts with β-catenin-mediated Wnt signaling in a feed-forward loop that functions to sustain mesenchymal Fgf responsiveness and mesenchymal Wnt/β-catenin signaling. Wnt2a is a canonical Wnt ligand that activates mesenchymal Wnt/β-catenin signaling, whereas Fgf9 is the only known ligand that signals to mesenchymal Fgf receptors (FGFRs). Mesothelial Fgf9 and mesenchymal Wnt2a are principally responsible for maintaining mesenchymal Fgf-Wnt/β-catenin signaling, whereas epithelial Fgf9 primarily affects epithelial branching. In summary, Fgf signaling is primarily responsible for regulating mesenchymal proliferation, whereas β-catenin signaling is a required permissive factor for mesenchymal Fgf signaling. *Fgf* fibroblast growth factor

11.5 Wnt Signaling and Stages of Wound Healing

11.5.1 Hemostasis and Inflammation

Wound healing is classically described as a process involving three overlapping phases. The first stage of physiological or acute wound healing is dedicated to hemostasis and formation of a provisional wound matrix, which occurs immediately after injury and is completed after some hours [28]. This matrix is comprised of activated platelets and fibrin molecules, fibronectin, vitronectin, and thrombospondins, forming a scaffold structure for the migration of leukocytes, keratinocytes, fibroblasts, and endothelial cells, while functioning as a reservoir of growth factors [29]. Recent data suggests that Wnt signaling is essential for development of megakaryocytes and for stimulating proplatelet function in vitro [30]. Interestingly, canonical Wnt has been shown to inhibit platelet aggregation whereas noncanonical Wnt-5A stimulates platelet aggregation [3].

Hemostasis triggers the inflammatory phase, which is characterized by the presence of erythema (rubor), warmth (calor), edema (tumor), and pain (dolor) [3]. At a cellular level, inflammation involves blood vessel dilation, increased vascular permeability, and leukocyte recruitment to the site of injury. Two leukocyte populations sequentially dominate the inflammatory events of wound healing: neutrophils and macrophages [31]. Both provide the critical function of wound debridement, whereas the latter population is critical in orchestrating the subsequent steps in wound healing. Wnt signaling has been shown to be involved in the regulation of inflammatory processes: Wnt5a is induced in human macrophages in response to mycobacteria and conserved bacterial structures, contributing to the regulation of pro-inflammatory cytokines via its receptor frizzled (Fzd) 5 [32]. Wnt5a is also induced in other infectious and inflammatory diseases such as tuberculosis, sepsis, psoriasis, rheumatoid arthritis, and atherosclerosis [33]. β-Catenin-dependent Wnt signaling enhances the inflammatory response [34].

11.5.2 Proliferation

During the proliferative phase of healing, approx. 3–10 days after injury, the body seeks to cover the wound surface through the formation of granulation tissue to restore the vascular network [35] and re-epithelialization. Under the control of regulating cytokines like IFN-γ and TGF-β, the synthesis of collagen, fibronectin, and other proteins by fibroblasts forms the basis for the new matrix of connective tissue and the restoration of mechanical strength to injured tissue [36]. Subsequently, the synthesis of collagen increases throughout the wound, while the proliferation of fibroblasts declines successively [33].

β-Catenin is an important regulator of fibroblast behavior during the proliferative phase of dermal wound repair [4]. β-Catenin protein levels and transcriptional activity are elevated in dermal fibroblasts during the proliferative phase of healing in murine cutaneous wounds and return to baseline during the remodeling phase [37]. Human wounds similarly show increased expression of β-catenin and its target genes, such as fibronectin and MMP7, during the proliferative phase [7]. While increased β-catenin activity during the proliferative phase is crucial for successful wound repair, prolonged or aberrant β-catenin activity beyond the normal parameters of healing contributes to excessive fibrosis and scar formation [38]. Indeed, human hypertrophic scars and keloids exhibit elevated β-catenin levels [39].

While Wnt ligands may participate in stimulating dermal β-catenin during wound repair, Wnt signaling is not crucial for maintaining elevated β-catenin levels during the proliferative phase of cutaneous healing [5]. This has been demonstrated in mice treated with an adenovirus expressing the Wnt signaling inhibitor Dickkopf (DKK1, which binds LRP6/Arrow), without a significant decline in β-catenin protein levels during the proliferative phase of skin wound healing, in contrast to the situation in bone repair [40]. This suggests that other factors play a role in regulating β-catenin levels during the proliferative phase of healing. Indeed, β-catenin levels in fibroblasts can be stimulated by growth

factors, such as TGF-β1, which are released during the early stages of wound repair [41]. Furthermore, β-catenin activity in dermal fibroblasts is regulated by extracellular matrix (ECM) components, such as fibronectin, which activates β-catenin through a GSK3ß-dependent, β1 integrin-mediated pathway [42]. Hypertrophic scars and keloids represent a dysregulated response to cutaneous wounding, resulting in an excessive deposition of ECM, especially collagen [36]. TGF-β is believed to be responsible for excessive ECM deposition in hypertrophic scars, keloids, and other fibrotic conditions [39]. Since β-catenin is known to accumulate during fibroproliferation, it is speculated that it could play a role in the mechanisms that lead to hypertrophic/keloid scarring [43]. β-Catenin and Wnt signaling are intrinsically involved in the formation of the dermis and of epidermal structures, both during wound repair and during skin development [5]. It will be interesting to elucidate whether non-Wnt activators of β-catenin, such as ECM proteins and growth factors, modulate β-catenin during skin development as they do during the response to injury [22].

11.5.3 Remodeling

Remodeling is the final phase of wound healing and occurs from day 21 to up to 1 year after injury [44]. In the skin, remodeling consists of deposition of matrix and subsequent changes in its organization and composition over time [45]. During the maturation of the wound the components of the ECM undergo certain changes. Fibrin clot formed in the early inflammatory phase is replaced by granulation tissue that is rich in type III collagen and blood vessels during the proliferative phase and subsequently replaced by a collagenous scar predominantly of type I collagen [36]. This type of collagen is oriented in small parallel bundles and is, therefore, different from the basket-weave collagen in healthy dermis. Wnt is responsible for the differentiation of myofibroblasts causing wound contracture, decreasing the surface of the developing scar [46]. Wnt has also been shown to be critical in the process of angiogenesis and endogenous enhancement of Wnt can correct vascular defects [47]. As angiogenic processes diminish, wound blood flow declines, and acute wound metabolic activity slows, eventually stopping.

11.6 Fetal and Adult Wound Healing

In the fetus, at least through the second trimester, skin and bone wounds heal in a regenerative manner [46]. Cutaneous wound healing in the early gestation fetus is remarkably different from that in the adult [48]. The most striking features of the fetal wound response are the speed and the absence of obvious scarring [49]. Investigators have now begun applying more comprehensive transcriptomic techniques to the study of scarless wound healing. In particular, there has been a focus on the time during fetal gestation when regenerative healing changes to adult wound healing with scar formation in order to understand the phenomena occurring immediately before and after this transition [8]. In rats, wounds made on the 16th day of gestation (gestation period: 21 days) histologically regenerate, but wounds made on the 18th day of gestation are associated with scarring [50]. The major objective of skin wounding research is restoration of the extracellular matrix architecture, and a subsequent return of strength and function to the injured skin, and therefore must overcome the fibrotic nature of postnatal wound healing. It is clear from studies conducted in mammals that normal skin development absolutely depends on a tight regulation of the activities of secreted signaling molecules that display potent organizing properties in the embryo [51]. These signaling molecules include members of the Hedgehog (Hh), transforming growth factor-beta (TGF-beta), and Wnt families of secreted factors (Table 11.1).

Table 11.1 Summary of developmental signaling pathways in mammalian skin development and repair

Signaling pathway	Skin section	Skin development	Skin repair
TGF-β	Epidermis	No significant role in hair follicle development	Inhibitory role in re-epithelialization
	Dermis	Role previously unknown Expressed in developing dermis	Reconstitution of the dermis: fibroblast proliferation and behavior, myofibroblast formation, matrix production, wound contraction
Wnt	Wnt	Development and morphogenesis of hair follicles	Regeneration of hair follicles in large wounds
	Dermis	Development of the dermis	Reconstitution of the dermis: fibroblast numbers and behavior, matrix production
Sonic hedgehog	Epidermis	Development and morphogenesis of hair follicles	Present in regenerated hair follicles
	Dermis	Role previously unknown	Involved in dermal reconstitution: effects on matrix, cellularity and vascularity
Notch	Epidermis	Epidermal differentiation	Role previously unknown
	Dermis	Role previously unknown	Involved in dermal reconstitution: effects on macrophage behavior, angiogenesis

11.7 Conclusions

Converting tissue repair to tissue regeneration remains a lofty goal, but exciting new techniques and methods of investigation, including high-throughput transcriptomic analysis, make it an increasingly realistic objective. Over the past decade, considerable insights into the molecular pathways driving the animal healing response and impairment have suggested new therapeutic targets and provided scientific rationale for future clinical trials. The wound epithelium in adult mammals is capable of responding to morphogenic signals from the dermis, as it does in the embryo during hair placode formation. Adult stem cells have tremendous therapeutic potential, and the skin epithelium represents an enormous source of accessible stem cells that might be a starting point for generating cells to replace diseased tissue. Skin stem cells have already been used to replace skin lost to burns; whether it will be possible to use skin stem cell plasticity to engineer treatments for other disorders remains to be determined.

Although increasing evidence supports a role for Wnt signaling in skin epithelial stem cell maintenance and/or determination, deregulated Wnt signaling activation has long been implicated in human cancers. Wnt signaling is essential at multiple steps during the complex organogenesis of the skin and its appendages. It is required to induce the formation of the dorsal dermis and regulate the size of the different skin appendage tracts. Later, Wnt signaling is required for the very early stages of skin appendage formation. Skin appendage distribution and pigmentation are regulated, in part by Wnt signaling. Disruption of the pathway can lead to the formation of skin appendage tumors. Any strategy that attempts to target the Wnt pathway to augment tissue regeneration will have to take into consideration the need to selectively and locally activate signaling in the tissue or area of interest while simultaneously restricting Wnt signaling in other parts of the body.

The intricate and dynamic nature of the wound environment suggests that successful therapies for treating wound healing disorders will not rely upon a single all-encompassing agent, but will likely require a multitude of factors for a finely tuned attenuation of endogenous Wnt signaling during the wound-healing process. Recognition of the complexity of the wound-healing process and its diseases as well as acceptance of the seriousness and mortality associated with repair pathologies will be critical steps in these future

efforts. Consequently, the combination of current knowledge in basic biology, identification of the limits of past clinical trials as well as translational research that includes development of improved animal models, harnessing of new technologies for more accurate imaging, and biomarker-based diagnostics will provide a strong basis to advance viable clinical approaches for treating patients with wound-healing pathologies. Identifying the relationships between developmental signaling pathways in adult wound repair and fetal skin development and/or regeneration will certainly propel the research community closer to this goal, and is a fruitful area of future investigation.

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