Wound Healing in Mammals and Amphibians: Toward Limb Regeneration in Mammals

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Abstract Mammalian fetal skin regenerates perfectly, but adult skin repairs by the formation of scar tissue. The cause of this imperfect repair by adult skin is not understood. In contrast, wounded adult amphibian (urodeles and anurans) skin is like mammalian fetal skin in that it repairs by regeneration, not scarring. Scar-free wound repair in adult *Xenopus* is associated with expression of the paired homeobox transcription factor *Prx1* by mesenchymal cells of the wound, a feature shared by mesenchymal cells of the regeneration blastema of the axolotl limb. Furthermore, mesenchymal cells of *Xenopus* skin wounds that harbor the mouse *Prx1*-limb-enhancer as a transgene exhibit activation of the enhancer despite the fact that they are *Xenopus* cells, suggesting that the mouse *Prx1* enhancer possesses all elements required for its activation in skin wound healing, even though activation of the same enhancer in the mouse is not seen in the wounded skin of an adult mouse. Elucidation of the role of the *Prx1* gene in amphibian skin wound healing will help to clarify the molecular mechanisms of scarless wound healing. Shifting the molecular mechanism of wound repair in mammals to that of amphibians, including reactivation of the *Prx1*-limb-enhancer, will be an important clue to stimulate scarless wound repair in mammalian adult skin. Finding or creating *Prx1*-positive stem cells in adult mammal skin by activating the *Prx1*-limb-enhancer may be a fast and reliable way to provide for scarless skin wound repair, and even directly lead to limb regeneration in mammals.

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Abbreviations

AEC	Apical epithelial cap
D	Dermis
E	Epidermis
ECM	Extracellular matrix
EPC	Endothelial progenitor cell
H	Hypodermis
MIF	Migration inhibitory factor
MMP	Matrix metalloproteinase
PDGF	Platelet-derived growth factor
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor

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1 Structure of Mammalian Adult Skin

The skin in mammals is the largest organ of the body and is composed of two layers, the epidermis and dermis. In addition to these two layers, the hypodermis, which is sometimes considered a layer of the skin, lies beneath the dermis and is composed mainly of adipose tissue. It is noteworthy that there are significant differences in the anatomy and physiology of each skin layer between species such as humans and mice (Wong et al. 2011), and principle structures based on human skin are overviewed here (Fig. 1).

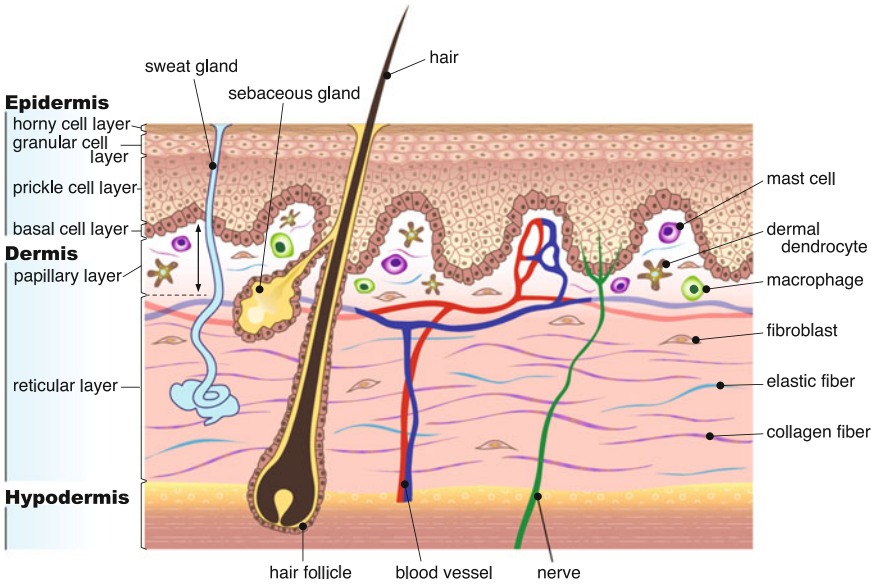

Fig. 1 Adult human skin is a layered organ consisting of an epidermis and a dermis. The epidermis is composed of four distinct layers: horny, granular, prickle, and basal cell layers (from top to bottom). The dermis is a highly elastic, tough, and flexible tissue made up of a meshwork of collagenous, reticular, and elastic fibers. It is divided into two functional layers, the papillary dermis and reticular dermis. These two layers are separated by a vascular plexus, which is fed by another vascular plexus located at the base of the reticular dermis (not shown). The papillary dermis contains a higher density and a greater variety of cells than does the reticular dermis. Fibroblasts, the main cells in the dermis, are essentially located in the papillary layer and are found only in very small numbers in the reticular dermis. They play an important part in production of the extracellular matrix. The skin also contains hair follicles, glands (A sweat gland and a sebaceous gland are shown here), and nerve endings responsible for the sense of touch and pain.

The epidermis, which is a terminally differentiated and stratified squamous epithelium, is the most superficial layer of the skin and is classified into several layers, including the basal layer at the bottom. The basal cell layer, the innermost cell layer of the epidermis, is a single layer consisting of basal cells including the epidermal stem cell subpopulation. The stem cell population in the basal layer gives rise to distinguishable layers, such as prickle, granular, and horny cell layers, as the cells from the basal layer move outwards and progressively differentiate into them. The prickle cell layer, the suprabasal cell layer, is composed of five to ten layers that appear connected to each other by prickle-like structures. The granular cell layer is composed of two or three layers of cells containing flattened nuclei and many granules. The main component of the granules is released as corneum lipid into the intercellular space of the horny cell layer. The horny cell layer is the outermost cell layer where keratinocytes are enucleated and become corneocytes as a terminal differentiation. Corneocytes consist of a stabilized array of keratin filaments contained within a covalently cross-linked protein envelope and serve as

a protective surface (Matoltsy et al. 1968). Additionally, there are several special cells in the epidermis, including melanocytes, which provide pigment to the keratinocytes, Langerhans, and dendritic cells, which have immunological functions, and Merkel cells (putative mechanosensory cells).

The dermis is composed of two layers, the papillary and reticular layers. The upper layer, the papillary layer, edges into the epidermis across the basement membrane and nourishes it. This layer contains (1) cellular components, including fibroblasts, mast cells, macrophages, and dermal dendrocytes, and (2) extracellular matrix (ECM) components, namely stromal components (e.g., collagen and elastic fibers) and matrix components (glycoproteins and proteoglycans, etc.). The lower layer, the reticular layer, is thicker than the papillary layer and is characterized by an ECM containing a network of coarse collagen and elastic fibers. This layer also contains cellular components such as fibroblasts. The nerves and blood vessels come into the dermis and display intricate patterns of branching. The nerves often run along larger blood vessels (Mukouyama et al. 2002).

The epidermis of the skin and its appendages (e.g., hair follicles, sebaceous glands, sweat glands, and nails) are derived embryonically from the prospective epidermal ectoderm and the neural crest cells, which are also ectodermal in origin. The skin appendages have their roots in the dermis or even in the hypodermis, both of which are derived from embryonic mesoderm such as somites and lateral plate.

2 Phases of Repair After Skin Injury

The skin is an intricate structure as described above, and it functions as armor for protection of the body from external environments and as an anatomical barrier from pathogens and physical damage, water resistance, UV protection, and so on. The armor of the vertebrate body is always exposed to irritants and assailants and is therefore sometimes injured. When the skin is injured, the skin initiates a complex process of events, namely wound healing that involves inflammation as well as formation and remodeling of new tissue, which require orchestrated regulation of different cell types. The wound healing process results in reconstitution of injured skin, although the process in adult mammals is imperfect and less restorative, and the wounds heal with a scar (Fig. 2). In fact, skin wounds deep through the basement membrane trigger scar formation and, therefore, the wounded dermis is repaired with scar formation.

2.1 Hemostasis

Wound healing needs hemostasis for the first step. At the onset of skin injury, capillary blood enters the wound bed, and this is followed by a requirement to reduce continuous blood loss. Humoral and cellular components, such as fibrinogen and

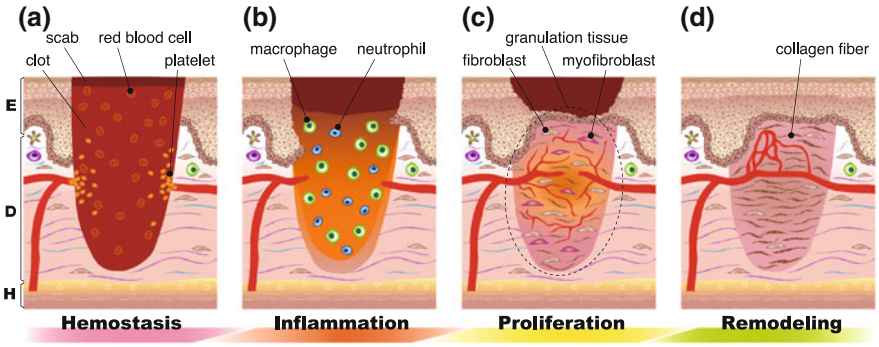

Fig. 2 Wound healing is a complex process encompassing a number of overlapping phases, including hemostasis, inflammation, proliferation, and remodeling. **a** Platelets adhere to damaged blood vessel walls. At this point, the platelets act to initiate blood clotting by activating coagulation factors. The resulting clot consists of platelets, red blood cells, and extracellular matrix molecules. **b** The inflammatory phase begins immediately and lasts for a few hours to a few days in acute wounds. Inflammatory cells such as phagocytic neutrophils and macrophages invade the clot, phagocytizing and triggering inflammatory response. Macrophages play an important role in subsequent angiogenesis, matrix deposition, reepithelialization, and fibroblast migration by secreting chemokines. **c** Migration and proliferation of fibroblasts and endothelial cells, which accompany reepithelialization, result in the formation of granulation tissue. Fibroblasts gradually replace the provisional matrix with a collagen-rich matrix and transform into myofibroblasts. **d** The transition from granulation to scar tissue occurs, leaving a collagen-rich scar tissue that is slowly remodeled in the following months under the wound surface that has been completely covered with a neoepidermis

platelets, stem blood loss and additionally provide signals that contribute to the earliest phases of wound healing. For arrest of bleeding, fibrinogen is activated in response to injured epithelium to form fibrin meshes that trap platelets, which adhere to the ruptures of blood vessels, preventing further blood loss. In addition, as platelets come into contact with damaged ECM components, they release coagulation factors, leading to formation of blood clots on the injured tissue. Various growth factors and chemokines such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor beta (TGF-beta), all of which have pivotal roles in the following phases, are rapidly released (Abe et al. 2001). This earliest phase is called hemostasis, and it occurs over a period of minutes to hours under normal circumstances.

2.2 Inflammation

The subsequent inflammatory phase is characterized by invasion of phagocytic neutrophils, the most abundant type of white blood cells in mammals, in the wound area (Enoch et al. 2006), usually within the first 24 h after injury. Neutrophils release chemotactic factors that attract monocytes to egress from blood vessels and enter injured tissue, in addition to phagocytosing foreign particles, bacteria and necrotic

cell components. Macrophages as well as monocytes are essential for further wound healing due to their contribution to angiogenesis, matrix deposition, and epithelialization (Bellingan et al. 1996). A distinct "cocktail" of chemokines secreted by macrophages attracts fibroblasts to migrate from the surrounding undamaged tissues into the wound site. The inflammatory phase typically lasts for several days.

2.3 Proliferation

Attracted fibroblasts migrating into the damaged site, which are dominant cells in the wound area, start growing. Fibroblast proliferation is oxygen-dependent, and thus a sufficient peripheral oxygen partial pressure is necessary for adequate wound healing (Gordillo and Sen 2003). Extension of blood vessels into the injured tissue allows oxygen to be transported further into the wound region, and the extension is achieved as follows.

The blood vessel network in the wound region is formed through angiogenesis and vasculogenesis of endothelial cells. For the first step of angiogenesis, in which new blood vessels originate from preexisting ones, growth factors such as VEGF and TGF-beta stimulate endothelial cells in the nearby healthy tissue to release proteases such as matrix metalloproteinases (MMPs) into the wound area (Urlich et al. 2005). MMPs digest the basement membrane, allowing endothelial cells to escape from their parent vessel, elongate, and form a new capillary sprout extending away from the original vessel. Newly formed blood vessels enter the collagenous network, which is a major component of the ECM produced by fibroblasts and fibrocytes (circulating cells that rapidly enter sites of tissue injury and exhibit potent immune-stimulatory activities) at the wounded site, and form highly vascularized "granulation tissue".

Vasculogenesis, in contrast, is defined as the formation of new blood vessels by differentiation of endothelial progenitor cells (EPCs) usually circulating in the blood and also contributes to enhanced blood vessel growth during the wound healing process (Tepper et al. 2005; Velazquez 2007). Some distinct factors have been proposed to play a major role in mobilization and recruitment of EPCs to hypoxic and injured sites. These factors include cognate and non-cognate ligands to CXCR4 (a chemokine receptor), that is, CXCL12 and macrophage migration inhibitory factor (MIF) (Ceradini et al. 2004; Grieb et al. 2010).

Nutrients and oxygen provided through blood vessels extending into the injured tissue promote fibroblast proliferation. Fibroblasts generally differentiate further into myofibroblasts that generate a tensile strength across the wound, leading to contraction and further closure of the wound. Fibroblasts continue to secrete growth factors such as TGF-beta, PDGF and VEGF which activate the migration and proliferation of keratinocytes in the epidermis, creating the epithelial layer that covers the top of the wound. This process is accompanied by a constant transition from the proliferative phase into the next phase of remodeling in the wound-healing process.

2.4 Remodeling

The granulation tissue is remodeled into a relatively acellular fibrous scar tissue. Eventually, granulation tissue is converted into a scar. The scar differs from normal dermis: (1) the number of elastic fibers is reduced in scar tissue and (2) type I collagen fibers in the scar are broken by MMPs, and growth factors influence the amount of collagen available for cross-linking but do not affect the cross-linking process itself (Mast 1992). As the scar matures, the density of blood vessels returns to normal and the number of fibroblasts is reduced by apoptosis (Mast 1992). The remodeling phase can last for several months or even years. During this period, type I collagen fibers in the scar are crosslinked by the enzyme lysyl oxidase into thick bundles oriented parallel to the surface of the wound and the ECM is remodeled into a more mature structure with greater integrity. Due to myofibroblast contraction, complete wound closure occurs and the wound strength is increased from 20 % of normal tensile strength at 3 weeks after injury to about 80 % within 2 years in human skin (Levenson et al. 1965).

3 Wound Healing in the Mammalian Fetus

It is well known that skin wounds in early mammalian embryos can heal perfectly with no sign of scar tissue formation and complete restitution of the normal skin architecture (Whitby and Ferguson 1991). There are many differences between the healing process of embryonic and adult wounds. However, most of those differences do not appear to be the reason for embryonic scarless healing. Most differences merely stem from the differences in development itself. However, some differences, as overviewed below, have been shown to be involved in fetal scarless wound healing.

3.1 Inflammation

One of the most important differences between embryonic and adult wound-healing processes is the inflammatory response. The embryonic response in the number of inflammatory cells recruited to the wound is different from that of the adult response, and the embryo skin has less activated cells at the wound site and longer duration of inflammatory cells at the wound site (Hopkinson-Woolley et al. 1994; Cowin et al. 1998; Wulff et al. 2012). Therefore, the possibility that recruitment of inflammatory cells to a wound site affects scar formation prompted researchers to manipulate the recruitment of these cells. For example, sheep embryos that were artificially stimulated to produce an inflammatory response showed an adult-like response with scar formation (Ozturk et al. 2001). On the

other hand, PU.1 null mice, which lack macrophages and functioning neutrophils, took more time to repair skin wounds than did wild-type siblings and could not repair the skin scarlessly (Martin et al. 2003). These studies suggest that an appropriate balance of inflammatory response is required for the wound healing.

3.2 Reepithelialization

After fetal skin is injured, surrounding epidermal cells rapidly move to fill the wound and to reepithelialize it. In this process, these cells are pulled forward by the contraction of actin fibers that draw the wound edges together as the opening of a purse is closed by a purse string (Martin and Lewis 1992). This process is unique to embryonic skin and different from that of adult wounds in which crawling of periwound epidermal cells resurface wounds, and the cells before crawling undergo retraction of intracellular tonofilaments and dissolution of most of the intercellular desmosomes that provide physical connections between the cells.

Epidermal cells are adherent to the extracellular matrix, which can change the speed of keratinocyte migration. Cell adhesion molecules such as fibronectin and tenascin and cell surface receptors such as integrins emerge earlier in healing fetal wounds than in adult wounds (Whitby and Ferguson 1991; Cass et al. 1998; Whitby et al. 1991). This can alter the phenotype of epidermal cells moving to fill in the wound, including composition of the underlying extracellular matrix can modify the speed of keratinocyte migration in the presence of growth factors (Nickoloff et al. 1988; Putnins et al. 1991).

3.3 Fibroblast Migration and Reorganization of the ECM

Fetal fibroblasts migrate to the wound site by chemoattractants derived from macrophages and neutrophils as adult fibroblasts do (see Sect. 2.2). Interestingly, fetal fibroblasts show a greater ability than adult fibroblasts to migrate, and it has been suggested that this is because fetal fibroblasts have more surface receptors for hyaluronic acid, which serves to enhance fibroblast migration (Chen et al. 1989). Their increased migration velocity during wound repair likely affects collagen deposition and distribution patterns. Fetal fibroblasts synthesize more type III and IV collagen than do their adult counterparts in vitro (Lorenz and Adzick 1993). Collagen type III fibers may allow a more reticular pattern of fiber deposition because they are smaller and finer than type I fibers, which predominate and are the principal component of both adult and fetal ECM. Collagen synthesis is slower in adult wounds than in fetal wounds and, therefore, adult fibroblasts probably have difficulty in synthesizing collagen in parallel with proliferation, whereas fetal fibroblasts simultaneously proliferate and synthesize collagen (Clark 1996). This difference in reorganization of the ECM gives rise to different tissue organization

at the wound site that is involved in contraction and degree of scarring. In the adult wound site, granulation tissue, which plays a considerable role in wound contraction, is mainly composed of myofibroblasts that is characterized by smooth muscle features acquired in fibroblasts in granulation tissue. Myofibroblasts can also be detected temporarily in fetal wounds at earlier time points than in scarring wounds that have progressively more active myofibroblasts (Cass et al. 1997; Ellis and Schor 1996).

3.4 Growth Factors for Embryonic Wound Healing without Scar Formation

Growth factors and their receptors play a pivotal role in wound healing and sometimes lead to a number of aberrations associated with abnormal wound healing such as pathological scarring. Embryonic skin contains much higher levels of morphogenetic growth factors than does adult skin because an embryo is rapidly developing and growing with a considerable expansion of skin volume. Differences in inflammatory responses between an embryo and an adult also give rise to differences in levels of the growth factors derived from these cells. Consequently, the growth factor profile at a fetal wound site is very different from that at an adult wound site in quality, quantity and duration. For example, the TGF-beta family, which contains at least three isoforms known as TGF-beta-1, TGF-beta-2 and TGF-beta-3, is multifunctional and is believed important in both tissue repair and scarring. In an embryonic wound site, the levels of TGF-beta-1 and TGF-beta-2 were lower and TGF-beta-3 was higher than in an adult wound site (Martin et al. 1993; Cowin et al. 2001; Soo et al. 2003). It was shown that blocking TGF-beta-1 and TGF-beta-2 or the addition of exogenous TGF-beta-3 in an adult wound site can reduce scar formation (Shah et al. 1994 and 1995). However, in other studies using a different animal model, TGF-beta-3 had no effect in reducing scar formation (Wu et al. 1997). Wound healing, particularly that of the epidermis has long been studied as a part of limb regeneration because it is recognized as an indispensable process for limb regeneration (Werner and Richard 2003).

4 Relationship Between Wound Healing and Limb Regeneration in Amphibians

Amphibians, in particular urodele amphibians such as newts and salamanders, exhibit perfect regeneration of many organs, including the skin, and this remarkable ability would save the animals from their traumas over the course of their life. A newt, for example, can completely regenerate its tail, limbs, jaws and ocular tissues such as the lens. Unlike urodele amphibians, anuran amphibians

such as frogs and toads have a limited regenerative ability that sometimes depends on their developmental stage. Regarding limb regeneration, *Xenopus laevis* can completely regenerate its developing limb buds before metamorphosis, but this regenerative capacity declines as metamorphosis proceeds (Dent 1962; Muneoka et al. 1986) The decline of regenerative capacity is due to intrinsic properties of limb cells rather than extrinsic properties such as neurotrophic factors or growth hormones (Sessions and Bryant 1988; reviewed by Suzuki et al. 2006). Despite the declined capacity of limb regeneration in the adult *Xenopus*, they can perfectly heal wounded skin, and the healing process from cutaneous traumas appears similar histologically to that in urodele amphibians (Suzuki et al. 2005; Yokoyama and Maruoka et al. 2011). We call the perfect wound healing in amphibians "skin regeneration" hereafter in this review.

The wound healing has long been studied as a part of limb regeneration because it can be recognized as a crucial and necessary event for limb regeneration.

4.1 Limb Regeneration in Amphibians

4.1.1 Immediate Reepithelialization and Subsequent Dedifferentiation

A hemostatic response occurs within a few seconds after amputation of a limb. Then the wound surface at the amputated site is covered with epidermal cells migrating from the circumference of the stump. Within 12 h after amputation, this wound-healing phase by the epidermis is thought to be achieved by cell movement with little cell division (Repesh and Oberpriller 1978; Carlson et al. 1998).

Over the next few days, this thin layer of epithelial cells thickens into a multilayered wound epithelium, called the apical epithelial cap (AEC). Underneath the AEC, mesenchymal cells around the wound site that have been saved from cell death enter the cell cycle and execute dedifferentiation. In this earliest process of limb regeneration, matrix metalloproteinases (MMPs) as well as AEC factors promote the conversion of mesenchymal cells to an undifferentiated state, so-called blastema cells (Yang and Bryant 1994). By the end of this process, a cone-shaped blastema, a cluster of blastema cells plus wound epithelium including the AEC, is formed, and this is the origin of tissue restoration, which induces and maintains limb regeneration (Muneoka and Sassoon 1992).

4.1.2 Redevelopment and Positional Memory for Repatterning

From 3 to 7 weeks after amputation, the basic pattern and main components of the limb are restored by the redevelopment phase, which includes re-differentiation and proliferation of the participating cell population and re-patterning by molecular mechanisms similar to those for developmental limb formation (Muneoka et al. 1992).

An important aspect of the re-development process is that each cell at the amputated site remembers its own position and what it should restore to regenerate a complete replica of the original limb structure. During limb development, for example, the transcriptional condition for a specific combination of gene expression in a cell successively progresses to form a skeletal pattern along the axes (reviewed by Tamura et al. 2010). The final state of the transcriptional condition in the genome is thought to be fixed and memorized but masked in a cell (probably by epigenetic regulation, reviewed by Yakushiji et al. 2009). Stimulation by limb amputation unveils the mask on the transcriptional condition and rewinds it into the final state that has been fixed in the cell. The program for patterning reprogresses from the middle (at the final state) to re-finish the development, restoring only the lost part of the pattern. To achieve this re-patterning from the amputated point, the genomic condition on position and pattern should not be erased after limb amputation, although the cell condition is initialized into an undifferentiated state. The molecular nature of this model remains unresolved, and it is essential to clarify the molecular mechanism of positional memory in amphibians for the purpose of successful organ regeneration in mammals.

4.1.3 Nerve Dependency of Blastema Formation

Nerve dependency is a characteristic of limb regeneration in amphibians. Nerve axons in the limb secrete neurotrophic factors into the blastema that are essential for its formation and growth, and surgical removal of axons in the limb therefore inhibits proper growth of the blastema, gives rise to no regenerate, and results in simple restoration of wounded skin (Stocum 2011).

Simple skin removal with little injury to nerve axons ends in wound healing, but if nerves are deviated into the site of the wound, outgrowth of a blastema-like structure can be induced (Bodemer 1959; Lheureux 1977; Reynolds et al. 1983; Maden and Holder 1984). Endo et al. (2004) clearly demonstrated that skin grafting from a different position of the limb with nerve deviation induces limb formation, suggesting a close relationship between wound healing and limb regeneration mediated by nerve signals in amphibians.

4.2 Perfect Wound Healing in Amphibian Skin

Amphibian skin is composed of two layers, a layered outer epidermis and a spongy inner dermis as in mammalian skin. The epidermis of an adult frog has a typical stratified squamous epithelium composed of germinative basal, spinous, granular, and cornified cells (Yoshizato et al. 2007). Many aspects of amphibian skin wound healing remain unclear, but some studies have suggested that the initial phase of wound healing in urodeles shares mechanisms with that in mammals.

Despite histological homologies of amphibian skin to mammalian skin and the common process of early healing, regenerative ability differs remarkably; mammalian wound healing results in a scar formation as described above (Ferguson and O'Kane 2004), while amphibian wound healing leads to a perfect restoration of tissue architecture and function (Levesque et al. 2010; Yokoyama and Maruoka et al. 2011; Seifert et al. 2012), although there is an exception (Poll 2009). The processes of wound healing in the metamorphosed *Xenopus* and axolotl are outlined here.

4.2.1 Immediate Hemostasis and Subsequent Reepithelialization

When the skin is injured, hemostasis begins within a few seconds as seen after limb amputation. Then the wound site is closed by epidermal tissue composed of two or three layers, referred to as wound epidermis, within several hours to one day by migration of epidermal cells using pseudopodial projections. Therefore, the epidermis is in direct contact with the subcutaneous muscle and connective tissue underneath (Levesque et al. 2010; Yokoyama and Maruoka et al. 2011). It should be noted that this reepithelialization in amphibians is very fast; a wound with a diameter of 1.5 mm in axolotl skin can be fully reepithelialized within 8 h (Levesque et al. 2010).

4.2.2 Dedifferentiation and Proliferation

Within 24 h after injury, in the case of metamorphosed *Xenopus*, the subcutaneous musculature underneath the wound epidermis is disrupted and a few mononuclear cells emerge there. Then, within 4 days after injury, the number of mononuclear cells greatly increase (Yokoyama and Maruoka et al. 2011). Similar mononuclear cells can also be seen in the surrounding dermal layer, and these appear to be the source of mesenchymal cells that repair the wound. These cells are eosin-negative, highly proliferative and express *Prx1* and *Tbx5*, reliable markers of blastema cells in limb regeneration (Suzuki et al. 2005; Satoh et al. 2007; Yokoyama and Maruoka et al. 2011). Furthermore, these mononuclear cells harboring the mouse *Prx1*-limb-enhancer as a transgene exhibit activation of the enhancer despite the fact that they are *Xenopus* cells, suggesting that the mouse *Prx1* enhancer possesses all elements required for its activation in skin wound healing. However, this activation of the same enhancer in the mouse is not seen at skin wound site of an adult mouse (Yokoyama and Maruoka et al. 2011).

4.2.3 Skin Regeneration

Within 10 days after injury, an almost normal dermis and well-organized muscle begin reappearing (Levesque et al. 2010; Yokoyama and Maruoka et al. 2011). In the case of the *Xenopus*, immature exocrine glands appear within this dermis,

suggesting that skin derivatives are regenerated at this stage (Yokoyama and Maruoka et al. 2011). In the case of the axolotl, however, it takes more than 45 days for recovery of the basement membrane in the healing skin (Levesque et al. 2010). Eventually, within a couple of months, the wound is indistinguishable from the surrounding skin, and no scar forms, indicating that the skin wound healing is perfect in amphibians.

5 Perspectives on Perfect Wound Healing in Mammalian Adult Skin

As repeatedly discussed in this review, injured mammalian adult skin can be healed, but the wound healing is imperfect with scar/cicatrix formation and few skin derivatives. Although many researches have revealed cellular events and molecular mechanisms involved in the process of imperfect wound healing, the nature or cause of the imperfectness remains unclear. In contrast to the situation in the adult stage, embryonic skin can repair the wound without a scar. However, the repair appears not to be completely equivalent to that in the adult skin because the embryonic skin structure before wounding is immature, and embryonic wound healing therefore must include recovery of the immature skin and subsequent development into mature skin. Despite its nonequivalence, embryonic skin wound healing that does not form a scar is highly suggestive for successful perfect wound healing in the adult skin. Elucidating differences in molecular characteristics between embryonic and adult skin wound healing will help to ascertain the condition of scarless wound healing and regeneration of skin derivatives such as hairs and secretion glands.

On the other hand, in amphibians, wound healing of the skin is perfect, and the skin even in metamorphosed adults of both urodeles (Seifert et al. 2012) and anurans (Yokoyama and Maruoka et al. 2011) repairs the wound without scar. Wound healing must be a trigger of limb regeneration because an ectopic limb can be generated from a wound on the side of a urodele limb. Moreover, mesenchymal cells for skin wound healing and limb blastema mesenchymal cells share some molecular mechanisms, including *Prx1* activation, suggesting their close relationship. Although nerve signals are the key to connect wound healing to limb regeneration, *Prx1* activation is a nerve-independent event (though its maintenance is nerve-dependent) (Suzuki et al. 2007). Thus, regeneration of amphibian skin shares cellular and molecular features of the early events of limb regeneration but regenerates only skin in a nerve-independent manner.

The Prx1 molecule itself may contribute to scarless wound healing. In fact, Prx1 can directly activate tenascin-C expression in cultured fibroblasts (McKean et al. 2003). Since a high level of tenascin-C protein was observed in an axolotl skin wound, which can be healed in a scarless manner (Seifert et al. 2012), *Prx1* may regulate scarless skin wound healing through activation of tenascin-C

expression. Elucidation of the role of the *Prx1* gene itself in amphibian skin wound healing will help to clarify the molecular mechanisms of scarless wound healing. Mouse adult skin cannot reactivate *Prx1*-limb-enhancer after being wounded, although the enhancer contains all elements required for skin regeneration because the mouse sequence is sufficient for reactivation during amphibian wound healing (Yokoyama and Maruoka et al. 2011). It is possible that fibrosis, which fulfills the injured skin region including the dermal layer in adult mammals, uses a distinct molecular mechanism for the initial step, which is totally different from that in amphibians. However, Levesque et al. (2010) showed that a fibrosis-like response can be induced also in axolotl skin by bleomycin treatment as in mammal skin. This finding suggests that amphibian skin is a comparable model to skin in adult mammals for studying fibriosis or scar formation. Shift of the molecular mechanism to that in amphibians, including reactivation of *Prx1*-limb-enhancer, will be an important clue for successful perfect wound healing in mammalian adult skin. In this sense, it would be interesting to examine the *Prx1*-enhancer activity in embryonic skin wound healing in mammals. Finding or creating *Prx1*-positive stem cells for skin regeneration in the adult mammal skin that have activated *Prx1*-limb-enhancer may be a fast and secure way for perfect wound healing, and this will directly lead to limb regeneration in mammals.

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