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Review Article

Animal models of skin regeneration

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ABSTRACT

Cutaneous injury in the majority of vertebrate animals results in the formation of a scar in the post-injured area. Scar tissues, although beneficial for maintaining integrity of the post-wounded region often interferes with full recovery of injured tissues. The goal of wound-healing studies is to identify mechanisms to redirect reparative pathways from debilitating scar formation to regenerative pathways that lead to normal functionality. To perform such studies models of regeneration, which are rare in mammals, are required. In this review we discussed skin regenerative capabilities present in lower vertebrates and in models of skin scar-free healing in mammals, e.g. mammalian fetuses. However, we especially focused on the attributes of two unusual models of skin scar-free healing capabilities that occur in adult mammals, that is, those associated with nude, FOXN1-deficient mice and in wild-type African spiny mice.

1. Introduction

Vertebrates can respond to injury by healing with regeneration or repair. Whereas regeneration restores damaged organs with normal architecture and the functionality of uninjured tissues, repair heals wounds with a fibrotic scar that often impairs functionality. Teleost fish and urodele amphibians have remarkable capabilities for regeneration when their limb or fin has been amputated [1,2]. In contrast, mammals respond to similar injuries with a repair process that heals the post-amputated region through a scar-forming process [2–4].

Among vertebrates two types of regeneration have been recognized: epimorphic (so-called true regeneration) is a process that is based on the formation of a blastema structure, considered as a hallmark of regeneration [5–7]. The blastema is composed of progenitor/stem cells that are formed underneath the epithelial apical cap covering the post-amputated region. During the regeneration process blastema cells differentiate into cells that give rise to regenerative tissues that restore amputated parts of the body [6]. The blastema structure has been observed and analyzed during fin regeneration in zebrafish [8], limb regeneration in axolotls [9,10], and even in mammals during regrowth of holes punched into the

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ears of rabbits and some strains of rodents from *Muridae* family: MRL [11], nude [12] and spiny [13] mice. Regeneration of digit tips occurs in mouse [7]. Compensatory regrowth is the process in which already differentiated/mature cells proliferate to replace damaged tissues. The best example of compensatory regrowth is liver in which partial hepatoctomy/chemical injury/viral hepatitis triggers liver-cell proliferation to restore damaged tissue [14].

Although regeneration or repair healing processes take place in all parts of the body, it is the skin, the front line protective barrier that participates most extensively in these processes. While the skin wound healing with concomitant scar tissues formation has been comprehensively investigated, skin regeneration has had less attention primarily because of the lack of suitable models for higher organisms. Since studies over the last few years have identified organisms with post-wounded skin regenerative abilities among both lower vertebrates [15–18] and mammals [13,19–23], in this review we have focused on studies of mammalian models of skin repair vs. skin regeneration.

2.   Classical stages of skin wound repair

Skin, as the largest organ of the animal's body, separates internal organs from the external environment. As a front line of defense from external insults skin is the most frequently subjected to injuries. Mammalian skin is composed of two layers, epidermis and dermis, that are separated by a basement membrane [3,4]. Epidermis, the most superficial part of the skin, comprises several layers of keratinocytes. A single cell basal layer that includes the epidermal stem cell population gives rise to spinous, granular and the outermost stratum corneum layers. Dermis is composed by heterogeneous population of cells and extracellular components. Cellular components of the dermal part of the skin mostly contains: dermal fibroblasts, macrophages and mast cells. The extracellular matrix, produced mainly by dermal fibroblasts, contains collagens, glycoproteins, proteoglycans and elastin.

The classical skin wound healing process in adult mammals is comprised of three overlapping phases: (i) inflammation, (ii) tissue proliferation/formation and (iii) remodeling [3,4,24].

The first stage, inflammation, is preceded by homeostasis and coagulation achieved through formation of a provisional wound matrix that occurs immediately after injury. Initially, a platelet plug, followed by a fibrin matrix, forms a structure that attracts invading cells, mostly inflammatory response cells. Subsequently, neutrophils, followed by monocytes, migrate into injured tissues to phagocytize foreign particles (bacteria and necrotic tissues). Some monocytes differentiate into macrophages; both participate in angiogenesis and matrix deposition.

The second stage, proliferation, starts as a re-epithelialization of a wound within hours after injury. Epidermal cells migrate to cover the wound area and one to two days post injury they proliferate at the wound margin [3]. Attracted to the wound site, fibroblasts proliferate to form granulation tissue together with a network of blood vessels formed through angiogenesis. Fibroblasts produce an extracellular matrix that is composed predominantly of collagen III and I [24].

The third stage – remodeling – is stimulated by matrix metalloproteinases (MMPs). MMPs are a group of endopeptidases, released by fibroblasts and macrophages that modulate components of the extracellular matrix (ECM). Collagen III is replaced by collagen I, myofibroblasts contract to decrease scar surface. Then, endothelial cells, macrophages and myofibroblasts undergo apoptosis. Scar tissue, as the final product of repair healing process, is formed [3,4]. However, of vital importance is that the post-injured area never regains the properties and appearance of uninjured skin. Furthermore, hair follicles, sebaceous gland, sweat glands do not recover after serious skin injuries. Fortunately, although scar-forming skin wound healing is the most common process occurring during skin injuries, there are examples of a perfect scar-free healing resolutions to skin injuries.

3.   Skin healing in lower vertebrate model organisms

For a long time zebrafish and urodele amphibians have been recognized as model organisms to study regeneration phenomena [5,8,25]. Amputated fins, jaws, the tip of heart in zebrafish and limbs in salamander undergo a regeneration process that achieves full functional reconstruction of lost/damaged tissues [5,8]. As expected, studies on reparative vs. regenerative skin wound healing processes in these model organisms have recently gained increasing attention. Studies by Richardson et al. [15] on laser performed full-thickness skin wounds in zebrafish revealed scar-free skin healing. Post-wounded skin underwent a fast, re-epithelialization process (within 24 h). Transgenic lines in zebrafish showed a massive inflammatory response, rich in neutrophils and macrophages that peaked 24 h post-wounding. The inflammatory response was followed by formation of granulation tissue that resolved to form a post-wounded skin with normal epidermis and dermis indistinguishable from the unwounded skin [15].

Most of what we know about regenerative abilities in amphibians comes from studying the post-amputated limb. During this process it was observed that tissue which covers amputated limbs never formed a scar. Studies on skin healing showed that skin injuries in axolotls [16,17] and *Xenopus laevis* [18,26] heal in a scar-free, regenerative manner. Healing of the excisional wounds made in the skin of the axolotls tail was accompanied by fast re-epithelialization (8 h after injury), a very low inflammatory response and low fibrosis as indicated by expression of α-smooth muscle actin, [16]. Since bleomycin, an antibiotic that induces pulmonary and skin fibrosis, caused scar formation in axolotl injured skin, the authors concluded that regenerative skin healing is not the result of a lack of fibrotic molecular machinery [16]. Seifert et al. extended previous studies by examining the post-injured skin healing process in adult axolotls with full excisional wounds made outside of the regenerative structures, that is, in tails or limbs, in the dorsal flank muscle [17]. Their study showed regenerative skin wound healing that was characterized by a short homeostasis period, lack of scab formation and a relatively long phase of new extracellular matrix production/remodeling. Scar-free healing was observed in