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The role of myofibroblasts in wound healing

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ABSTRACT

The importance of proper skin wound healing becomes evident when our body's repair mechanisms fail, leading to either non-healing (chronic) wounds or excessive repair (fibrosis). Chronic wounds are a tremendous burden for patients and global healthcare systems and are on the rise due to their increasing incidence with age and diabetes. Curiously, these same risk factors also sign responsible for the development of hypertrophic scarring and organ fibrosis. Activated repair cells – myofibroblasts – are the main producers and organizers of extracellular matrix which is needed to restore tissue integrity after injury. Too many myofibroblasts working for too long cause tissue contractures that ultimately obstruct organ function. Insufficient myofibroblast activation and activities, in turn, prevents normal wound healing. This short review puts a spotlight on the myofibroblast for those who seek therapeutic targets in the context of dysregulated tissue repair. "Keep your myofibroblasts in balance" is the message.

1. Introduction

1.1. Dysregulated wound healing – a debilitating condition

Wound healing is the physiological response to injury and ensures rapid repair of damaged tissues. The life quality and survival of patients are severely impacted when normal wound healing and tissue repair processes become deregulated. Poor or insufficient healing is characteristic for chronic wounds that cause mortality and morbidity in about 2% of affected patients in Western countries [1]. Conversely, excessive healing leads to the accumulation of collagenous scar extracellular matrix (ECM) – a condition called fibrosis [2]. Fibrosis is a key driver of progressive organ malfunction in many acute and chronic inflammatory and metabolic diseases and affects all vital organs, such as heart, liver, kidney and lung, as well as disfiguring scarring of the skin. Fibrosis is estimated to contribute to ~33% of deaths globally and ~45% in industrialized nations [3]. The healthcare costs associated with impaired wound healing and scarring has reached the tens of billions of dollars per year in the US, and these costs are expected to further increase. Addressing these and many other debilitating conditions afflicting patients in our aging population, major funding agencies have decided to give high priority to wound repair and regenerative medicine research [4]. Most recently, the NIH published new Funding opportunity announcements (FOA), spawned by the NIA U13-ASP/AAIM Workshop on Wound repair and healing in older adults (February 20-21, 2014): Non-healing ulcerative wounds in aging (R01, R21 and launched in July 2015 the FOA collaborative projects to accelerate research in organ fibrosis to "...develop novel therapeutic strategies aimed to lessen organ fibrosis; or develop novel technologies to study fibrosis".

Chronic and/or fibrotic healing occurs when body-inherent repair capacities are either impaired or overwhelmed. One regenerative medicine approach to support endogenous repair is to replace injured, diseased or aged tissues with fully functional counterparts to extend the healthy life expectancy of our aging population [1,5]. However, the field of regenerative medicine faces a number of challenges because of adverse host reactions that trigger body-own repair mechanisms [6]:

- implanted biomaterials/cells trigger immune and inflammatory responses;
- tissue/material grafts cause rejections, implant fibrosis and contractures;
- following implantation, regenerative stem cells often not only fail to acquire the desired functionality but become part of the dysregulated repair process and develop tumorigenic or fibrotic features.

Another approach in regenerative medicine is to support the adult's body's dormant regenerative capacities [7]. To understand

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how fundamental repair process can be activated in a controlled manner to restore/preserve the function of injured human adult organs, there is a critical need for innovative, transformational, and most importantly interdisciplinary research.

1.2. Wound healing phases

Wound healing is a highly regulated process of consecutive and overlapping phases: immediately after tissue injury, the blood coagulation cascade induces inflammatory cell accumulation and cytokine production [8,9]. Inflammatory cells such as mast cells, neutrophils and macrophages create the conditions that stimulate fibroblast migration, proliferation activation, and collagen production. Together with ongoing neovascularization in the wound bed granulation tissue is formed in this proliferation phase [6,10–16]. The combination of granulation tissue contraction by activated fibroblasts and re-epithelialization by keratinocytes then restores tissue integrity and reduces the wound size to a permanent scar [17]. In the final maturation phase, the overall numbers of fibroblastic cells and vessels are reduced by programmed cell death [18]. It is evident that disturbance of the fine equilibrium between cells, growth factors, and ECM in any of these phases will impact on the normal course of healing [8]. Given the complexity of the healing process and its versatility in handling a vast variety of different wound scenarios, it is actually amazing that failure does not occur more frequently. Only if all redundancies fail to compensate disturbances or if the tissue defect is too large, cell-cell and cell-ECM miscommunications will result in either chronic or excessive healing. In this review, I will focus on the role of fibroblasts and myofibroblasts that dominate the proliferation and maturation phase of wound healing and are the main culprits in causing pathologic tissue contractures. This review contains excerpts of a recently published more concise book chapter on the topic [19].

2. Myofibroblasts tip the balance between normal and abnormal wound healing

2.1. The consequences of myofibroblast actions

The seminal works of Gabbiani et al. have established that granulation tissue contraction is actively promoted by specialized fibroblasts, which have been accordingly named "myofibroblasts" [20]. It is one of the main challenges for effective wound management to understand how the controlled formation, persistence, and disappearance of myofibroblasts gets out of balance in pathological wound repair. After restoring tissue integrity in physiological wound healing, myofibroblast activities cease and excessive cell numbers are reduced by apoptosis [21]; the signals triggering massive cell death and the timing are not entirely clear. It is however evident that persistence of myofibroblasts activity will lead to tissue deformation by contracture. In the skin, contractures manifest as hypertrophic scars [22] and "stiff skin" in the fibrotic lesions of scleroderma [23]. Contractures in palmar facia are the hallmark of Dupuytren disease [24–26]. Myofibroblast-produced tissue contractures can become life-threatening when fibrosis affects vital organs such as liver [7,27], heart [28–30], lung [31,32] and kidney [33,34]. In addition, myofibroblasts play a role in the stroma reaction against tumors which promotes cancer progression by creating a stimulating microenvironment for epithelial tumor cells [35]. Furthermore, the formation and contraction of fibrotic capsules by myofibroblasts is a common late stage of the foreign-body response against implants, leading to disfiguration (e.g., breast implants) and/or implant failure (e.g., glucose sensors) [36]. Finally, mesenchymal stem cell (MSC)-to-myofibroblast phenotypic conversion is a potential risk for cell therapies [37,38].

2.2. Myofibroblast identification

It is now well appreciated that myofibroblasts comprise a heterogeneous population of cells that exist in different activation states to produce and contract collagen ECM – not necessarily at the same time [39]. Fibroblast-to-myofibroblast activation is a multi-step event controlled by the continuously changing chemical and mechanical microenvironment in tissue under repair. Provisional fibrin clot ECM which is laid down during the hemostatic phase needs to be replaced by stronger collagen ECM. This ECM change requires in-migration of fibroblastic cells from adjacent tissues and the circulation. This first fibroblast activation step, sometimes referred to as "proto-myofibroblast" [17], is a response to changes in the ECM and the cytokine environment created by platelets and inflammatory cells [8,40]. In order to be able to migrate, proto-myofibroblast develop low contractile features, notably bundles of cytoplasmic actin and myosin and cell-ECM contacts. The analogous cytoskeletal structures formed in cell culture are called stress fibers and focal adhesions, respectively [17]. Protomyofibroblasts can then further mature into "differentiated myofibroblasts" by specializing on their contractile function which ultimately produces the mechano-resistant scar. In order to be able to produce significantly higher forces, focal adhesions with the ECM and contractile stress fibers are enlarged and incorporate α-smooth muscle actin (α-SMA). Expression of α-SMA is most often used in diagnosis and experimental conditions to define myofibroblast differentiation. It has been shown that the presence of this actin isoform not only enhances fibroblast contraction [41] but also guides the myofibroblast activation program in an intracellular mechanical feedback loop [38,42]. Together with the mechanical strain and stiffening of the ECM produced by myofibroblast activities, intracellular and extracellular mechanical stress promote the formation of new and persistence of already activated α-SMA-positive myofibroblasts [43,44].

Neo-expression of α-SMA in stress fibres is the most commonly used molecular marker for myofibroblasts [45] but it is not unique to myofibroblasts and functional myofibroblasts exists without expressing α-SMA as discussed above. In a tissue context, different molecular markers can be exploited to discriminate morphologically related cells and spatially close cells such as fibroblasts, myofibroblasts, vascular smooth muscle cells and pericytes [46]. Mature smooth muscle cells express α-SMA in addition to desmin, smooth muscle myosin heavy chain, h-caldesmon, and smoothelin which are not typically expressed in myofibroblasts [47]. Vascular smooth muscle and endothelial cells express the mesenchymal intermediate filament protein vimentin that is shared with fibroblasts and myofibroblasts [46]. Hence, combinatorial staining for the smooth muscle and pericyte marker desmin (which is also expressed in skeletal and cardiac muscle) and endothelial cell-specific markers such as CD31, VE-cadherin and van Willebrand factor is an efficient strategy to identify the predominant cell types populating granulation tissue. Notably, different additional myofibroblast markers may be employed in different tissue, exploiting the heterogeneous origin of this cell phenotype [39].

2.3. Myofibroblast precursors

The question of where myofibroblasts come from is being debated since their discovery in the early 1970s and, despite having been claimed several times since, is still not resolved. Part of the dispute arises from the high probability that there is not only one myofibroblast precursor but a multitude of possible progenitors. From a functional point of view, the concept of various cells being able to activate into myofibroblasts would greatly reduce the response time of any tissue to an insult and augment the sheer number of cells acutely available for tissue repair. In

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