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REVIEW ARTICLE

Mechanotransduction of Wound Healing

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ABSTRACT

Skin injury caused by mechanical force is a phenomenon mankind faces pretty often. The mechanism through which our body responds towards wound healing and skin regeneration is highly complex and requires the involvement of multiple gene regulatory pathways. A better understanding of the proteins and secondary messengers involved in those pathways can help in the development of exact therapeutic mechanisms that can contribute towards improvement in the treatment of scar formation. Here, we describe very briefly the multiple stages of wound healing and the important signaling pathways involved in wound healing.

Keywords: hypertrophic scarring, Mechanotransduction, Wound Healing
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INTRODUCTION

The commencement of biological processes occurs through meticulous and close interplay of multiple signaling pathways which are precisely intertwined with each other. Once morphological features of any tissue are damaged due to any external disturbance, we call it '*wound*'. The defense displayed by the cell in terms of physical or genotypical changes to maintain the integrity of the skin is termed '*healing*'. Healing the injured skin occurs in a variety of ways.[1,2] Healing can occur either through fibrotic scar tissue which can cause permanent defective structure or hypertrophic scarring (HTS). HTS can't be eradicated easily. However, the development of deep insights into the signaling pathways leading to HTS can potentially provide ways for the reduction of scars and skin regeneration. The defensive response requires multitude of molecules bringing together numerous cell types to work in coordination and sequential manner. These pathways in turn cause changes in physiological features, and structure of the skin, and eventually lead to regeneration of the wound area. The mechanotransduction phenomenon is described by the process of alteration of function, migratory and signaling process of the cell in response to mechanical stress. Extracellular matrix and extracellular fluid act as intermediates for information transmission into the cells and control constant remodeling. We aim to report the mainstream mechanisms starting from mechanical stress triggers to the activation of intracellular signaling pathways contributing to towards wound healing. [3,4]

STAGES OF WOUND HEALING

The complicated process of wound healing has been classified primarily through four different stages, which are hemostasis, inflammation, proliferation, and tissue remodeling. Instantaneously after the occurrence of injury or cut the healing process begins with the diminution of blood flow which is caused through accumulation of platelets. The adherence of platelets at the site of blood vessels leads to clotting which not only prevents bleeding but also activates beneficial proteins which kickstarts a variety of signaling pathways.[5,6]

This first response to the accumulation of platelets at the subendothelial matrix is called homeostasis. Accumulation of platelets in turn mediates the release of some specific growth factors like platelet-derived growth factor (PDFG), transforming growth factor – β (TGF-β), epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). The coagulation complex so formed activates a factor called 'X' which activates prothrombin to thrombin. Thrombin activates fibrinogen to fibrin which can form polymers thus giving rise to cross-linked mesh-like structures stabilizing the growing platelet plug.[7,8]

The inflammatory phase occurs after 24 hours of the post-wounding phase. Neutrophils and monocytes (which are further differentiated into macrophages) are recruited as responders to the site of injury following the signals of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). The inflammatory response executed by the neutrophils is primarily through Reactive Oxygen Species (ROS). Furthermore, they are armed with various antimicrobial agents, proteases, cytokines, and ***chemotaxins*** to influence the inflammatory phase. Inflammatory cytokines include tumor necrosis factor (TNF-α), interleukin (IL-6), and monocyte chemoattractant protein (MCP-1).[9,10]

Three key major events take place during proliferation which are re-epithelization, angiogenesis, and the formation of granulation tissue. To begin with, there is active proliferation of keratinocytes at the wound edges.

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Furthermore, the epithelial cells go for differentiation to fibroblast-like cells and dermo-epidermal junctions are reconstructed. In the process of angiogenesis, the vascular system is restored through activation of growth factors, e.g., TGF-β, PDGF, bFGF, Release of growth factors promotes the formation of pre-mature vessels. Granulation of tissues occurs through the replacement of fibrin matrix. The fibrin matrix is replaced with fibroblasts associated with macrophages, type III collagen and sprouts of capillaries. Other than fibroblasts, myofibroblasts also become abundant and lay down a disorganized extracellular matrix (ECM) composed of collagen, fibronectin, hyaluronic acid, and proteoglycans which finally leads to wound closure.[11,12]

Upon completion of the job of wound healing remodeling occurs which leads to apoptosis of the cells or exit from the site of injury. Remodeling leads to biological scanning of the wound area causing the formation of fibrin clot and further development of a mature scan. The ECM gradually forms a denser layer through the synthesis of collagen. A balance between collagen formation and degradation is mediated by anti-inflammatory macrophages, fibroblasts, and keratinocytes.[13,14]

SIGNALING PATHWAYS

Extrinsic mechanical cues are the driving force to initiate intracellular biochemical pathways through the transmission of signals via mechanical forces. The interfacial mechanosensitive proteins perceive the mechanical forces from the outside environment and integrins and cadherins are the most common responsive proteins present at the interface. Furthermore, the cellular response is primarily executed by a highly dynamic cytoskeleton generated through the mechanical properties of actin filaments, microtubules, or intermediate filaments. Mechanosensing proteins also include ion channels, catenin complex and cell adhesion molecules. All the mechanosensing elements trigger signaling pathways causing a cascade of responses.[15,16]

Integrins-FAK Signaling

One of the key mediators of skin mechanobiology which is activated through cutaneous injury is the focal adhesion kinase (FAK), which is a non-receptor cytoplasmic tyrosine kinase. Upon induction of mechanical stress there occurs phosphorylation of FAK protein at tyrosine-397 residue. The activation of inflammatory pathways occurs through the transfer of signals from the mechanical stress perceived by FAK. Accumulation of fibroblasts at the injury site causes secretion of profibrotic cytokines that in turn lead to increased collagen synthesis. FAK pathway has a significant role in wound closure and that has been demonstrated in FAK knock-out cells which showed delayed wound healing.[17,18]

Wnt/β-catenin signaling pathway

The Wnt/β-catenin signaling pathway is activated in the kinds of cells or tissues having high turnover numbers for example epithelium or epidermis. Activation of the Wnt/β-catenin pathway triggers the production of the huge amount of β-catenin which accumulates at the cytoplasm. The high amount of β-catenin further translocates to the nucleus and primarily binds to T-cell factors (TCF) and activates transcription.

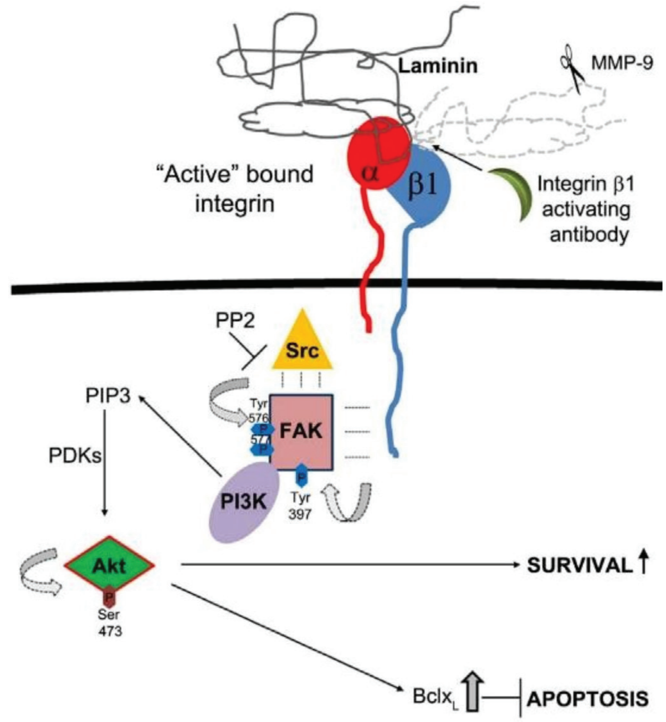

Figure 1: Steps of activation of FAK-protein and downstream signaling pathway which eventually cause activation of Akt signaling and finally blocks apoptosis and promotes survival.

Loss of β-catenin leads to weakening of tight junctions and further loss of cellular response towards mechanical stimuli. Further, Wnt/β-catenin is linked directly with TGF-β signaling, fibroblast upregulation, production of reactive oxygen species (ROS) and DNA damage.[19,20]

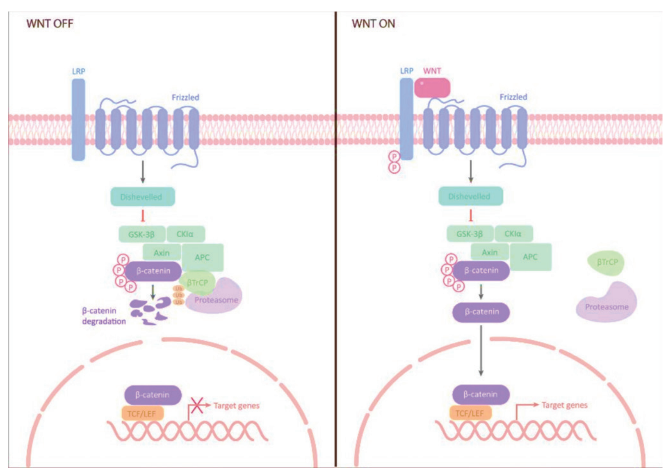

Figure 2: Wnt/β-catenin signaling pathway. Wnt is attached to the frizzled receptor and causes release of β-catenin which eventually travels to the nucleus acting as an upstream regulator of certain genes.

YAP/TAZ PATHWAY

Another two major downstream regulators that contribute towards mechanotransduction of signaling and arrangement of the cytoskeleton are Yes-associated protein (YAP) and transcriptional co-activator with

PDZ-binding motif (TAZ). The cues that activate YAP/TAZ signaling pathways are the Hippo-kinase pathway, Wnt-signaling, G-protein coupled receptors (GPCR) and mechanical forces. The significant mechanical inputs are primarily ECM stiffness, cell attachment or detachment, and cellular tension. In response to mechanical stress. The YAP/TAZ proteins dephosphorylate themselves from their inactive complex and translocate to the nucleus by coupling themselves with DNA-binding transcription factors like TEADs. Localization into the nucleus causes cell proliferation and inhibition of cell differentiation. Further, the absence of mechanical forces promotes the export of YAP/TAZ from the nucleus. Knockdown studies have shown that YAP/TAZ localization is important for the progression of wound regeneration and the growth of tissues at the site of the wound.[21,22]

PI3K/Akt signaling

Mechanical stress activates phosphatidylinositol 3-kinase (PI3K)/ Akt signaling pathway which is involved in a broad range of regulatory processes, like cell proliferation, metabolism, motility, secretion, survival and apoptosis. Proto-oncogene Akt is a Serine-threonine protein kinase that gets activated upon release of PI3K. Akt is phosphorylated through PDK or mTOR2 which are activated through secondary messengers like phosphatidylinositol triphosphate (PIP3) released by activation of PI3K. Phosphorylation of Akt causes activation or repression of certain factors. The resultant of Akt phosphorylation is the promotion of cell growth, translation and differentiation and the prevention of apoptosis. Three classes of PI3K are expressed by human cells out of which, Class I is most widely known. The PI3K is a heterodimer of catalytic and regulatory subunit. Activation of PI3K causes the release of PIP2 which gets converted to PIP3 which acts as an activator of Akt pathway.[23,24]

Rho GTPase

Rho GTPases are small GTPases acting as molecular switches activated by Rho guanine exchange factors (GEFs) and repressed by Rho GTPase activating proteins (GAPs). Rho GTPases regulate many processes like cytoskeleton remodeling, cell growth and proliferation, cell motility and morphology. Rho A, Rac1 and Cdc42 produce contractile forces by coordinating myosin II activity by Rho kinase (ROCK) stimulation. Inhibition of Rho GTPase signaling causes a reduction of collagen synthesis, fibroblast contractibility and prevents excessive scarring.[25,26]

STRATEGIES TO MINIMIZE SCAR FORMATION

The eventual goal of wound healing research lies in reducing the scar formation process. Primarily, minimizing the tension across the wound area potentially contributes towards a reduction in scar formation. The primary process of revising poorly healed scars can be classified into several steps. To start with, de-epithelialization of the scar is an important step followed by undermining one side of the wound and anchoring the other end through subcuticular sutures. Now, the tension generated in this process is crucial for the procedure of minimization of the scar. The application of tension is manipulative and controlled based on the location, size, shape, blood supply, and direction of the wound. So, by maintaining a balance between the insertion of tension and other factors the effect of scar formation can be minimized.

CONCLUSIONS

The exact pathogenesis of many fibrotic diseases requires corrective treatment which is possible through proper study utilizing animal models. Understanding pathways causing scar formation is important to develop a cure for it. A growing number of studies have provided insights into the pathways contributing to wound scar formation and skin regeneration. A comprehensive understanding of these pathways can contribute to develop novel therapeutics that can reduce scar formation in many fibrotic diseases. Different signaling pathways discussed in this review article affect various stages of wound healing and targeting effective stages of these pathways can lead to improved scarring.

FUTURE PERSPECTIVES

For this expanding field the future lies in a detailed understanding of mechanical force and its influence on aspects of repair and regeneration. Mechanical forces could alter phenotypical responses to disease and dysfunction. A better understanding of mechanotransduction pathways can help in developing target molecules. Target molecules can act as suitable pharmaceutical agents preventing excessive scarring and fibrosis. Also, information about mechanotransduction pathways can lead to develop devices that can contribute to improving wound healing.

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