**REVIEW ON THE ROLE OF CYTOKINE SIGNALLING ON WOUND HEALING, IMPAIRED WOUND HEALING AND THE ROLE OF OXIDATIVE STRESS IN THE DEVELOPMENT AND PROGRESSION OF IMPAIRED WOUND HEALING.**

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THE ROLE OF CYTOKINE SIGNALLING ON WOUND HEALING

Wound healing is a dynamic process comprising three overlapping, highly orchestrated stages known as inflammation, proliferation and re-epithelialization, and tissue remodeling. This complex process is regulated by numerous cytokines, with dysregulation of cytokine-induced signaling leading to impaired wound healing. Suppressor of cytokine signaling (SOCS) proteins are a family of eight intracellular proteins which may hold the potential to maintain homeostasis during wound healing through their negative feedback inhibition of cytokine signaling.

The process of wound healing is highly complex and requires substantial interaction and coordination between different cell types to succeed in an orderly and timely manner. Issues arising in the coordination or regulation of this process can have severe consequences, in some cases impairing the capacity to complete the process, resulting in wound chronicity.

**Structure of suppressor of cytokine signaling family members.**

All SOCS proteins consist of three structural and functional domains which are: a N-terminal domain with variable length of amino acids sequences, a central SH2 domain with ESS and a SOCS box domain containing a BC box and a Cul box at the C-terminus. Each two SOCS family members could be paired due to their structure and function similarity. SOCS-1 and SOCS-3 both possess a unique KIR which inhibit JAK protein activity. SOCS-4 and SOCS-5 both contain a highly conserved region within their N-terminal domain termed as N-terminal conserved region. SOCS-6 and SOCS-7 share more than 50% amino acid identity in SH2 domain and SOCS box domain.

**Role of cytokines signalling in wound healing**

Cytokines are a class of small proteins involved in both paracrine and autocrine cell signaling. Cytokines include, among others, chemokines (which promote chemotaxis), interferons and interleukins (which are vital for the function of a healthy immune system) and members of the TNF family (which can induce apoptosis). The cytokines which are produced and released following an immune event can initially dictate whether an immune response is necessary and, if so, whether that response is cytotoxic, humoral, cellular mediated or allergic in nature (Borish LC, Steinke JW, 2003). Wound healing is tightly regulated by a large number of cytokines and growth factors through various sophisticated signaling pathways. Throughout the wound healing process cytokines and growth factors act as important mediators of differentiation, proliferation, maturation and various other functions of the cells which contribute to wound closure.

A variety of ECM components, cytokines and growth factors are derived from activated keratinocytes during the proliferation and re-epithelialization phase of wound healing, and act as chemoattractants which can then activate fibroblasts, endothelial cells and lymphocytes, as well as neighboring keratinocytes (Freedberg IM, Tomic-Canic M, Komine M, 2001). Some of these cytokines and growth factors, such as IL-1 and TNF-α, regulate activation of keratinocytes, whereas TGF-α also mediates keratinocyte proliferation. Once the wound has healed, dermal–fibroblast-derived TGF-β acts as a regulator to suppress the proliferation of keratinocytes and to induce synthesis of ECM (Freedberg IM, Tomic-Canic M, Komine M, 2001). IFN-γ was found to strongly and specifically induce the expression of keratin-17 (Jiang CK, Flanagan S, Ohtsuki M, *et al* 1994), a protein expressed in various healthy epithelia that are characterized as contractile tissue. Thus, IFN-γ was suggested to contribute to the contractile nature of keratinocytes in the later stage of wound healing (Freedberg IM, Tomic-Canic M, Komine M, *et al,* 2001). IL-6 derived from fibroblasts, macrophages, endothelial cells and keratinocytes is another essential cytokine which affects granulation tissue formation, re-epithelialization, angiogenesis, cell infiltration and remodeling. Additionally, IL-6 showed enhanced expression in chronic wounds exhibiting aberrant inflammation, suggesting the importance of the precise control of IL-6 expression patterns in normal wound healing (Behm B, Babilas P, Landthaler M, *et al,* 2012). EGFR is expressed in the basal layer and the first suprabasal layer of adult epidermis (Nanney LB, Stoscheck CM, King LE *et al*, 1990). Activation of EGFR through ligand binding could induce keratinocyte proliferation and migration, as well as the degradation of ECM components (Nickoloff BJ, Griffiths CE, Barker JN. 1990). However, evidence showed that deficient expression of the downstream signaling protein, STAT-3, induced by EGFR could lead to impaired keratinocyte migration and remodeling (Sano S, Itami S, Takeda K *et al,* 1999). . In addition, it was found that the expression of EGFR was reduced in chronic wounds, and that keratinocytes at the nonhealing edge of chronic wounds are incapable of responding to EGF stimulation due to the cytoplasmic localization of EGFR (Brem H, Stojadinovic O, Diegelmann RF *et al*, 2007) indicating the essential role of EGFR on pathological wound healing. As a result of extensive studies on angiogenesis, many cytokines and growth factors have since been identified as either proangiogenic or antiangiogenic molecules (Johnson KE, Wilgus TA, 2014). FGF is a potent mitogen for vascular and capillary endothelial cells (Schweigerer L, Neufeld G, Friedman J, *et al* , 1987) and has been shown to stimulate their proliferation, differentiation, migration, invasion and tubule formation ability (Kanda S, Landgren E, Ljungstrom M, *et al*, 1996). Another extensively investigated growth factor, TGF-β, was also identified as a critical mediator of angiogenesis due to the fact that it can stimulate endothelial cell differentiation, migration and capillary tubule formation (Igaz P, Toth S, Falus A, 2001).

**Suppressor of cytokine signaling and essential cytokines/growth factors and receptors involved in wound healing.**

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| **Cytokine/growth factor/receptor** | **Target components in wound healing** | **Functions in wound healing** | **SOCS induced by cytokine/growth factor** | **SOCS that negatively/positively regulates downstream signaling** | **Downstream pathway/molecule by which cytokine/receptor are negatively/positively regulated by SOCS** |  |
| IL-1β | Endothelial cells,macrophages,leukocytes, keratinocytes, fibroblasts | Inflammation, angiogenesis, re-epithelialization, tissue remodeling, induces keratinocyte, neutrophil and fibroblast chemotaxis, induce neutrophil activation | SOCS-2 SOCS-3 | SOCS-3/negative | – |  |
| IL-2 | Fibroblast | Increase fibroblast infiltration and enhance fibroblast metabolism | CIS SOCS-1 SOCS-2    SOCS-3 | CIS/negative SOCS-1/negative SOCS-2/positive    SOCS-3/negative | IL-2R via STAT-5   Through association with SOCS-3 and degradation Association with JAK-1 and IL-2R/binding to calcineurin |  |
| IL-4 | Macrophages, fibroblasts | Enhance collagen synthesis, induces fibroblast proliferation | SOCS-1 SOCS-2 | SOCS-1/negative | Inhibition of activated JAK-1 and STAT-6 |  |
| IL-4R |  |  | SOCS-5 | SOCS-5/negative | Inhibition of STAT-6 |  |
| IL-6 | Endothelial cells,macrophages, keratinocytes, leukocytes, fibroblasts | Inflammation, angiogenesis, re-epithelialization, collagen deposition, tissue remodeling, induce fibroblast proliferation | CIS SOCS-1 SOCS-3 SOCS-5 | SOCS-3/negative   SOCS-5/negative | – |  |
| IL-10 | Macrophages | Inhibits macrophage activation and infiltration, inhibits TNF-α, IL-1 and IL-6 expression | CIS SOCS-3 | – | – |  |
| IFN-γ | Macrophages, keratinocytes | Induces collagenase activity, preventing collagen synthesis and crosslinking | CIS SOCS-1 SOCS-2 SOCS-3 | SOCS-1/negative   SOCS-3/negative | – |  |
| TNF-α | – | Regulates collagen synthesis and degradation, increases vascular permeability and homeostasis, provides metabolic substrates | CIS SOCS-1 SOCS-3 | SOCS-1/negative | – |  |
| EGF | Keratinocytes, fibroblast | Re-epithelialization, increases fibroblast collagenase secretion, inhibits fetal wound contraction | CIS SOCS-2 SOCS-3 SOCS-4 SOCS-5 | SOCS-2/negative    SOCS-4/negative | Association with activated EGFR   Competing docking site with STAT-3 |  |
| EGFR | – | – | SOCS-4   SOCS-5   SOCS-7 | SOCS-4/negative   SOCS-5/negative   SOCS-7/negative | Association with activated EGFR and degradation Association with activated EGFR and degradation Association and degradation |  |
| PDGF | Leukocytes,macrophages, fibroblasts | Inflammation, re-epithelialization, collagen deposition, tissue remodeling, recruits fibroblasts and macrophages, induces collagen synthesis | SOCS-3 |  |  |  |
| HGF | Endothelial cells, keratinocytes | Suppression of inflammation, granulation tissue formation,angiogenesis, re-epithelialization | SOCS-1 SOCS-3 | SOCS-1/negative SOCS-3/negative | Inhibition of STAT-3 activation |  |
| TGF-β | Fibroblasts, keratinocytes, macrophages, leukocytes, endothelial cells, ECM | Inflammation, angiogenesis, granulation tissue formation, collagen synthesis, tissue remodeling, leukocyte chemotactic function | SOCS-3 | SOCS-3/negative |  |  |

ECM: Extracellular matrix; SOCS: Suppressor of cytokine signaling.

**When is wound healing referred to as ‘impaired’? And why?**

Chronic wounds are, by definition, wounds that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation (Menke *et al*., 2007). As a result, the healing process is delayed, incomplete, and does not proceed in a coordinated manner, subsequently resulting in poor anatomical and functional outcome (Menke *et al*., 2007). These wounds cause a major disability and are characterized by chronicity and frequent relapse. The differential diagnosis of the underlying etiology of a nonhealing wound is large, but most (~70%) ulcers are caused by ischemia, secondary to diabetes mellitus, venous stasis, and pressure (Menke *et al*., 2007).

There are no large-scale, population-based studies that examine the prevalence and economic cost of chronic wounds in the United States (Menke *et al*., 2007). The prevalence of the 3 major types of nonhealing wounds is estimated to be between 3 and 6 million in the United States, with patients 65 years and older accounting for 85%. Nonhealing wounds result in enormous health care expenditures with the total cost being estimated at more than $3 billion per year (Menke *et al*., 2007). None of the financial estimates take into account the amount of lost work time, decreased productivity, disability payments, nor the cost of rehabilitation (Menke *et al*., 2007).

In addition, the resultant psychosocial damage incurred by patients and their significant others, friends, and families is incalculable. Unfortunately, nonhealing wounds are prone to complications that not only effect the time to healing completion but also have a negative impact on the patients themselves (Menke *et al*., 2007). The complications of chronic wounds include functional limitations, infections, and malignant transformation. Functional limitations include gait changes and difficulty ambulating. Many patients have chronic pain that decreases their quality of life. Another large category of complications is related to infections (Menke *et al*., 2007). Cellulitis, abscess formation, osteomyelitis, gangrene, and even sepsis all may occur as a result of an infected wound. Furthermore, chronic wounds have the potential for malignant transformation (i.e., Marjolin’s ulcer). Lastly, foot ulcers are one of the most common causes of nontraumatic amputation (Menke *et al*., 2007).

ROLE OF OXIDATIVE STRESS IN THE DEVELOPMENT AND PROGRESSION OF IMPAIRED WOUND HEALING.

A delicate balance between the positive role of ROS and their deleterious effects is important for proper wound healing (Sanchez *et al*., 2018). Whereas production of ROS is essential to initiate wound repair, excessive amount of ROS generation is deleterious in wound healing. Ongoing oxidative stress, associated with lipid peroxidation, protein modification and DNA damage has been shown to impair wound healing processes via increased cell apoptosis and senescence (Sanchez *et al*., 2018). In physiological conditions, low levels of ROS production by NOX activation in neutrophils and macrophages are responsible for respiratory bursts during phagocytosis of the inflammatory phase (Sanchez *et al*., 2018). In contrast, as chronic inflammation develops in pathological conditions, NOX activation is exacerbated, which may lead to excessive production of ROS production, further accelerating inflammation and oxidative stress cellular damage (Sanchez *et al*., 2018). Clinical studies suggest that non-healing wounds are maintained in highly oxidizing environment, which lead to impaired wound repair (Sanchez *et al*., 2018). Clinical conditions such as tissue hypoxia and hyperglycemia are typically associated with highly oxidizing environments (Sanchez *et al*., 2018).

Hypoxic Wound

Whereas generation of ROS during the normal wound healing is related to NOX activation, the presence of hypoxia stimulates oxidant production by the electron transport chain (ETC) of the mitochondria mainly via complexes I and III (Sanchez *et al*., 2018). This observation is paradoxical, in the sense that superoxide is a product of the one-electron reduction of O2, which is reduced in hypoxia. ETC-derived ROS are transferred across the inter-membrane space to reach the cytosol where they act as second messengers (Sanchez *et al*., 2018). During hypoxia, mitochondria augment the release of ROS in the cytosol, which appears counter intuitive as O2 tension is reduced in the mitochondrial compartment (Sanchez *et al*., 2018). Hypoxia-induced mitochondrial ROS release has been shown to activate cell protection signaling through transcriptional and post-translational mechanisms (Sanchez *et al*., 2018).

In line, low oxygen levels leading to mitochondrial ROS production activate prolyl-4-hydroxylases. Prolyl-4-hydroxyases can induce hypoxia-inducible factor 1 (HIF-1) activation, which is involved in regeneration of lost or damaged tissue in mammals (Sanchez *et al*., 2018). In the microenvironment of early wounds, ischemia due to vascular disruption and high O2 consumption by immune competent cells can favor O2 depletion and hypoxia. Moreover, pathological conditions, such as diabetes, impair microvascular blood flow, thus aggravating tissue oxygenation, whereas temporary hypoxia after injury can be beneficial for wound healing, prolonged or chronic hypoxia delays wound healing (Sanchez *et al*., 2018). Impaired wound repair in hypoxic tissue has been related to the combination of mechanisms that increase ROS production and reduce antioxidant defenses (Sanchez *et al*., 2018).

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