Macrophage functions in wound healing

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Malgorzata Kloc*[1-3], Rafik M. Ghobrial[1-2], Jarek Wosik[4, 5], Aneta Lewicka[6], Sławomir Lewicki[7], Jacek Z Kubiak* [7,8]

[1]The Houston Methodist Research Institute, Houston, Texas 77030, USA; [2]The Houston Methodist Hospital, Department of Surgery, Houston, Texas, USA; [3]The University of Texas, M.D. Anderson Cancer Center, Department of Genetics, Houston Texas, USA; [4]Electrical and Computer Engineering Department, University of Houston, Houston, Texas 77204, USA; [5]Texas Center for Superconductivity, University of Houston, Houston, Texas 77204, USA;
[6]Laboratory of Epidemiology, Military Institute of Hygiene and Epidemiology (WIHE), Warsaw, Poland;
[7]Department of Regenerative Medicine and Cell Biology, Military Institute of Hygiene and Epidemiology (WIHE), Warsaw, Poland;
[8]Univ Rennes, UMR 6290, CNRS, Institute of Genetics and Development of Rennes, Cell Cycle Group, Faculty of Medicine, Rennes, France

*Corresponding Authors
Malgorzata Kloc
e-mail: mkloc@houstonmethodist.org
or
Jacek Kubiak
e-mail: jacek.kubiak@univ-rennes1.fr

Abstract

Macrophages play a crucial role in regeneration and consecutive phases of wound healing. In this review we summarize current knowledge on the ontogeny, origin, phenotypical heterogeneity and functional exchangeability of macrophages participating in these processes. We also describe the genetic, pharmacologic and bioengineering methods for manipulation of macrophage phenotype and functions and their potential for development of the novel, clinically applicable, therapies.

Introduction.

Wound healing especially in the case of larger wounds produces debilitating and disfiguring scars. Scarring results from the prolonged inflammation and the failure to properly transit from the regenerative phase to the resolving phase of the wound healing process (Takeo et al., 2015). Because the excessive scarring often results in the loss of function, there is a great need for development of novel scarless healing strategies (Moore et al., 2018). In addition, certain chronic medical conditions such as diabetes mellitus, rheumatoid arthritis, vascular or arterial diseases and low HGH (human growth hormone) cause slow or delayed wound healing, which results from the failure to transit from the inflammatory phase to the regenerative phase (Frykberg and Banks, 2015; Guo and DiPietro, 2010; Nunan et al., 2014). This also necessitates development of novel wound healing therapies.

Macrophages play a crucial role in the regeneration and wound healing processes. A constantly expanding research on macrophages paints an incredibly complicated picture of macrophage ontogeny and functions. As the scientists like to categorize, the macrophages, depending on the origin, function and the signature molecules produced, have been assigned into several distinct categories. However, it has recently become clear that such a typecasting is limiting and inadequate to describe macrophage heterogeneity and functional exchangeability. While keeping this in mind, in this review we describe different macrophage categories and their functions in regeneration and wound healing process. We retained the classical categories of M0, M1 and M2 macrophages because majority of existing scientific literature refers to these classical macrophage categories.

1. Principle and phases of wound healing

The injury triggers a series of overlapping events: 1. Hemostasis,
2. Defensive/inflammatory phase, 3. Tissue formation/proliferative phase, and
4. Tissue remodeling/resolution/maturation phase.

Hemostasis consists of the formation of the platelet plug/clot and the constriction of blood vessels. This limits blood loss and forms a supporting platform for newly migrating cells. The chemokines released during hemostasis attract inflammatory cells and stimulate resident immune cells, which together start the inflammatory phase of wound healing that mainly depends on the macrophages.

The tissue formation/proliferative phase of wound healing depends mainly on function of fibroblasts and macrophages and consists of angiogenesis, deposition of collagens (fibroplasia) and formation of the granulation tissue. The final remodeling/resolution/maturation phase of wound healing restores more or less original architecture of the tissue and apoptotically removes unneeded blood vessels. This phase depends on variety of the cell types including macrophages that produce matrix metalloproteinases (MMPs) -a family of zinc-dependent proteases, which are involved in extracellular matrix digestion and remodeling.
Many human diseases such as diabetes mellitus and venous stasis disease, lead to impaired/ delayed wound healing or persistent (chronic) wounds. Such wounds do not progress through the normal phases of healing, never enter the regenerative phase of healing but, instead, remain in a permanent stage of inflammation (Guo and DiPietro, 2010; Nunan et al., 2014).

2. Ontogeny of macrophages and the origin of wound macrophages

Macrophages present in the adult mammal derive from two different sources: the extraembryonic yolk sac and the monocytes. Cell fate map and macrophage depletion studies showed that in mice, during early gestation (embryonic day 6.5 - 8.5) the yolk sac produces a subset of erythro-myeloid progenitor cells (EMPs) that subsequently colonize developing fetal organs to become the tissue resident macrophages (Gomez et al., 2015; Ginhou et al, 2010; Hoeffel et al., 2012; Samokhvalov, 2014; Schulz et al., 2012). The resident macrophages are self-renewing, and the population of resident macrophages in the given adult organ is self-sustaining without the input from the monocytes (Hashimoto et al., 2013). In the steady state conditions the resident macrophages are the sentinels maintaining organ homeostasis, but upon infection or injury they may become additionally stimulated to mount an appropriate immune response (Davies et al., 2013; Epelman et al. 2014). Another source of macrophages present in the adult mammal are the blood-circulating monocytes. The monocytes derive from the hematopoietic stem cells (HSCs) located during embryogenesis in the fetal liver, and in the bone marrow of the adult (Epelman et al. 2014; Orkin and Zon, 2008). Upon injury, the blood/bone marrow derived monocytes are recruited into the wound where they transform into the mature macrophages. The macrophages present in the wound comprise of both monocyte-derived and tissue resident macrophages (Brancato and Albina, 2011).

Although the degree of co-operation between these two macrophage groups and their relative input to the wound healing are still vague, it has been shown that certain processes, such as fibrotic response during liver injury, depend mainly on the macrophages derived from the blood-circulating monocytes (Seki et al., 2009).

2. Monocytes and macrophages

There are at least two different types of monocytes differing in the signature molecules and the timing/sequence of arrival to the wound site. First type are the inflammatory monocytes: CX3CR1^{low}, CCR2^{high}, Ly6C^{high} (in mice), CD14^{++} CD16^{-} (in humans), and the second type: CX3CR1^{high}, CCR2^{low}, Ly6C^{low} (in mice), CD14^{low} CD16^{+} (in humans). The first to arrive to the injury site are the inflammatory monocytes CX3CR1^{low}, CCR2^{high}, Ly6C^{high}, followed by the CX3CR1^{high}, CCR2^{low}, Ly6C^{low} monocytes (Fig. 1; Brancato and Albina; 2011; Daley et al., 2010; Nahrendorf et al., 2007;). The CX3CR1 is a receptor for fractalkine ligand CX3CL1, a transmembrane protein and chemokine involved in migration of immune cells. The CCR2 is a receptor for monocyte chemo attractant protein-1 (CCL2), a chemokine mediating monocyte chemotaxis; and the Ly6C is a 14kD cell surface phosphatidylinositol-anchored glycoprotein expressed on macrophage precursors. Both CX3CR1 and CCR2 are necessary for the recruitment of blood monocytes to the wound. Studies in the mouse model of excisional skin wound healing showed that CX3CR1 knockout mice and the wild-type mice infused with anti-CX3CR1-neutralizing antibody had impaired wound healing, which was caused by the reduction of macrophage number, reduced collagen deposition and impaired neovascularization of the wound (Ishida et al., 2008). Studies in the liver injury mouse model showed that the CCR2 knockout causes impairment in the recruitment of monocytes and collagen production/injury-related fibrosis (Seki et al., 2009). Studies from our laboratory showed that the macrophage specific knockout of small GTPase RhoA inhibited expression and recycling of CX3CR1 receptors in the macrophages, which in turn prevented their movement to the transplanted heart and inhibited chronic rejection in mouse transplantation model (Liu et al., 2017a). These findings suggest that targeting of CX3CR1/fractalkine via RhoA pathway inhibition may be also useful for the manipulation of macrophage recruitment into the wound site.

Classically, mouse macrophages are divided into three phenotypically and functionally distinct categories; M0 naïve/unstimulated macrophages, pro-inflammatory/ antimicrobial M1, and anti-inflammatory/prohealing/alternatively activated M2

macrophages. The M1 and M2 phenotype can be created *in vitro* by an exposure of M0 macrophages to IFNγ/LPS or IL-4/IL-13, respectively. In rodents, M1 macrophages produce signature bactericidal molecule the inducible nitric oxide synthase (iNos), while the M2 macrophages produce arginase enzyme (Arg-1; Wu et al., 2016). Because arginase degrades arginine to ornithine, which is a precursor of collagen component proline, mouse M2 macrophages were always believed to be responsible for collagen deposition/fibrosis and tissue repair/wound healing. However, the studies on macrophages isolated from the wounds (Brancato and Albina, 2011; Daley et al. 2010,), and the fact that human M2 macrophages do not produce arginase (Munder et al. 2005; Raes et al, 2005) indicate that the *in vivo* reality is much more complex. Study in sponge mouse wound model showed that murine wound does not contain IL-4 and IL-13, the cytokines considered necessary for differentiation of M2 macrophages and that wound macrophages exhibit a complex phenotype, which includes M1 and M2 traits and changes as the wound matures (Daley et al. 2010). Table 1 summarizes the difference between molecules produced by mouse, rat and human macrophages.

Macrophages play many roles in the defensive/inflammatory and remodeling/resolution/maturation phases of wound healing process. Depending on their function in the wound healing process macrophages can be divided into three different categories: pro-inflammatory, tissue repair and anti-inflammatory/resolving macrophages (Wynn and Vanella, 2016; Table 2). These macrophages:

1. Phagocyte and destroy bacteria,
2. Produce enzymes that digest necrotic tissue,
3. Phagocyte and remove cell debris, dead cells and necrotic tissue,
4. Produce chemokines/growth factors such as PDGF, TGF-β1, IGF-1, and VEGF-α that promote cell proliferation and blood vessel development, and promote cell immigration, proliferation and survival,
5. Attract endothelial cells and promote angiogenesis,
6. Attract fibroblasts that produce collagen and extracellular matrix, which form the structural scaffold (stroma) for the newly build tissue,
7. Synthesize many components of extracellular matrix, and
8. Synthesize matrix-remodeling enzymes.

Below we describe recent discoveries on these functions of macrophages and their potential application in development of novel wound healing strategies.

4. Role of macrophage phagocytosis in tissue remodeling and wound healing

Madsen et al. (2013) developed an assay to visualize collagen turnover *in situ* and to identify cell types and molecules involved in degradation and phagocytosis of debris. They found that the collagen introduced into the mice dermis was phagocytosed (endocytosed) by M2-like macrophages, and degraded by lysosomal pathway, and that a deletion of the collagen receptors, such as urokinase plasminogen activator receptor-associated protein (Endo180), or the mannose receptors (Mrc1 and Mrc2), inhibited collagen degradation. This study indicates that collagen is internalized by M2-like macrophages via the receptor-mediated pathway. Because the collagen removal and turnover are crucial for tissue remodeling during wound healing this information can potentially be applied for development of novel wound healing strategies. Wang et al. (2017) studied macrophage functions in diabetic wound healing. Diabetic wound accumulates high levels of Advanced Glycosylation End Products (AGEs). Macrophages express high level of AGEs receptor RAGE. Authors showed that topical application of anti-RAGE antibody to diabetic mice wounds improved wound healing. Blocking AGE-RAGE signaling induced phenotypic switch of macrophages from M1 (pro-inflammatory) to M2 (pro-healing) and increased quantity of neutrophils phagocytized by macrophages. This study indicates that inhibition of AGE-RAGE signaling pathway improves macrophages function/phagocytosis in the early inflammatory phase and improves wound healing. Magatti et al, (2017) studied the effect human amniotic mesenchymal cells (hAMTCs) that are used in regenerative medicine applications on the monocytes and macrophages in the rodent model of wound healing. They found that hAMTCs and their condition medium (CM) induce differentiation of myeloid cells and monocytes into macrophages. In addition hAMTCs or CMs treatment of monocytes under M1 polarization conditions shifted them from M1 towards M2-like phenotype. These M2-like macrophages expressed CD14, CD209, CD23, CD163 and PM-2 K, had higher phagocytic activity, increased production of IL-10, and lower production of pro-inflammatory cytokines. In contrast, the monocytes under M2 polarization condition treated with hAMTCs or CM remained as the M2. However these M2 had enhanced anti-inflammatory profile. They had lower expression of the co-stimulatory molecule CD80, decreased phagocytosis and secretion of inflammatory chemokines. In addition this study showed that macrophages exposed to CM improve tissue regeneration/repair in wound-healing models. This study indicates that amniotic

mesenchymal cells and factors released by these cells have a potential to be used to manipulate macrophage phenotype and function in wound healing.

5. Production of chemokines/growth factors

He et al. (2018) investigated the effect of conditioning media derived from M0, M1 and M2 macrophages on bone marrow mesenchymal stem cells (BMMSCs). They found that medium derived from naïve M0 macrophages induced differentiation of BMMSCs into osteogenic cells, while medium derived from M1 macrophages supported proliferation and adipogenic differentiation of BMMSCs, increased production of extracellular matrix components, such as fibronectin, COL-1 and integrin β1 and promoted formation of the cell sheets. The medium derived from M2 macrophages supported osteogenesis differentiation and also enhanced capacity of stem cells to form cell sheets. These data indicate that each macrophage subtype has a unique effect on stem cells and that modulation of macrophage phenotype following the injury may serve as an effective method to stimulate stem cells during wound healing process. Quiros et al. (2017) studied the role of macrophages in the wound healing following intestinal mucosal injury. Using conditional deletion of IL-10 in mouse model they found that macrophage-derived interleukin 10 (IL-10) was rapidly induced after injury, and was necessary for the wound closure. In addition, macrophage-derived IL-10 activated epithelial cAMP response element-binding protein (CREB) and subsequent synthesis and secretion of the pro-repair WNT1-inducible signaling protein 1 (WISP-1), which induces epithelial cell proliferation and promotes wound closure. These findings define macrophage–derived IL-10 as a major factor in regulating cell proliferation via CREB/WISP-1 signaling pathway during wound healing.

6. Promotion of cell proliferation, angiogenesis and production of extracellular matrix

Gindele et al. (2017) developed an *in vitro* mechanical scratch injury model in primary human small airway epithelial cells that recapitulates epithelial wound healing, and investigated the role of macrophages sub-types in this process. They found that M1 macrophages promoted focal adhesion kinase (FAK, which is a part of multi-protein structures (focal adhesions) that link the extracellular matrix (ECM) to the cell cytoskeleton) expression and that M1 and M2 macrophages induced epithelial de-differentiation. They also found that there were different subtypes of M2

macrophages with different expression profile: M2a macrophages inhibited cell proliferation and expression of fibronectin (possibly via the retinoic acid pathway). The M2b and M2c macrophages inhibited deposition of fibronectin (possibly via MMP expression. These data indicate that different macrophage subtypes have different impact on wound healing. Recently Yeh et al. (2017) studied the effect of Artocarpin (ARTO), a prenylated flavonoid purified from the plant *Artocarpus communis*, on the wound healing in mouse model. Using immmunostaining and microarray analysis of cytokine production in neutrophils and macrophages, they showed that ARTO increases collagen synthesis, re-epithelialization, angiogenesis and enhances wound healing. This study indicates that ARTO has a potential to be used as a novel therapeutic for the enhancement of wound healing. In another study Yu et al. (2017) investigated the role of macrophages and retinoic acid (RA) in the wound healing after laser resection of the prostate. They found that treatment with retinoic acid enhanced function of anti-inflammatory M2 macrophages and promoted stromal cell activation and angiogenesis. They also found increased production of arginase 1 (Arg1), which is the marker of anti-inflammatory M2 macrophages. This study indicates that retinoic acid improves healing process through its stimulatory effect on macrophages. Chaqour et al. (2018) studied the effect of Abscisic acid (ABA)- a plant hormone, (involved in plant stress response and plant buds dormancy) on the retinal and fetal angiogenesis, using a 3D sprouting assay methodology in retinal vasculature mice model. They showed that ABA inhibits development and regeneration of blood vessels and speeds up macrophage-induced programmed regression of fetal blood vessels. They also showed that ABA affects macrophage phenotypes and promotes development of pro-inflammatory M1 macrophages that express anti-angiogenic molecules. The fact that ABA inhibits angiogenesis and changes macrophage phenotype can be useful in managing the vasoproliferation during the wound healing process.

8. Macrophages' matrix remodeling enzymes

The production and secretion of matrix metalloproteinases (MMP) by different macrophage subtypes plays a major role in wound healing processes. Roch et al. (2014) studied the MMPs expression pattern in different subtypes of human macrophages: naïve, unpolarized M0 macrophages, M1 pro-inflammatory and M2 ant-inflammatory macrophages. They showed that all three types of macrophages expressed various MMPs, but the M1 showed the highest expression of MMP-1,

MMP-3, and MMP-10, and the M2 showed the highest expression of MMP-12. They also compared the enzymatic activity of MMPs in different macrophage subtypes and found that MMPs from the M2 macrophages had the highest enzymatic activity.
In another study Duffield et al. (2005) showed, using a model of reversible liver injury induced by carbon tetrachloride, that selective depletion of CD11b+ macrophages impairs matrix degradation and prevents restoration of normal liver tissue architecture. These data indicate that macrophage subtype composition of the wound influences the outcome of extracellular matrix remodeling. In recent studies Parasa et al. (2017) used newly developed experimental human lung tissue model containing human monocytes and differentiated macrophages to study how MMPs inhibition influences tuberculosis granuloma formation, which is similar to the granulation tissue formed during proliferative phase of wound healing To inhibit MMP activity they used a global MMP inhibitor, 200 nM Marimastat (Merck Millipore). Results from this study suggest that macrophage-derived MMP activity contribute to granuloma formation. The study of the role of Metaloproteinase 9 (MMP-9) in mouse heart wound healing caused by myocardial infraction (MI) showed that macrophage-derived MMP-9 enhanced collagen accumulation and tissue remodeling and improved healing of the cardiac wound (Meschiari et al., 2017). These studies indicate that targeted intervention with macrophages' MMPs may change the outcome of the wound healing process.
Table 2 shows the summary of the molecules produced by inflammatory, tissue repair and anti-inflammatory/resolving macrophages, which, on the basis of extensive literature search, could be a potential targets for therapeutic intervention in delayed/ chronic wound healing and excessive scarring.

9. Macrophage mitochondria, respiration and accelerated wound healing

Macrophages are not only very heterogeneous phenotypically and functionally, but also differ in the mode of respiration/energy production by mitochondria. The classical understanding had been that M1 macrophages rely mainly on aerobic glycolysis, while M2 macrophages use oxidative phosphorylation (OXPHOS). This was supported by the studies showing that inhibition of oxidative metabolism reverses M2 to the M1 phenotype (Rodríguez-Prados et al., 2010; Vats et al., 2017). However, recent observations and experiments indicate that both glycolysis, and OXPHOS shape M1 and M2 phenotypes (Huang et al., 2016; Galván-Peña et al., 2014; Mehta et al., 2017). It is known that M1 and M2 macrophages differ in shape,

the M1 are round and M2 are elongated. It has been shown that cell elongation and migration depends on the translocation of mitochondria to the most energy-demanding regions of the cell, where the actin cytoskeleton undergoes the most intense reorganization (Boldogh and Pon, 2006; Cunniff et al., 2016; Schuler et al., 2017), and that a disruption of macrophage mitochondria and ATP/ADP homeostasis reverses M2 to the M1 phenotype (Chen et al., 2018c). Mitochondria, besides producing energy also produce the reactive oxygen species (ROS), which are the source of oxidative stress and bactericidal activities of M1 macrophages (Hall et al., 2013; Mills and O'Neill. 2016). There is also evidence that a dysregulation of macrophage functions involves elevated production of ROS, and disruption of Protein-S-glutathionylation signaling pathway (Short et al., 2016). Recently, Demyanenko et al. (2017) showed that the excessive production of ROS is responsible for the inferior healing of diabetic wounds, and that oral administration of mitochondria-targeted antioxidant 10-(6-plastoquinonyl) decyltriphenylphosphonium (SkQ1), increased macrophage infiltration and improved inflammatory and regenerative phases of wound healing in the diabetes II mouse model. These data indicate that reprogramming of macrophage metabolism may be useful in manipulating the outcome of the wound-healing process.

10. Molecular mechanisms of macrophage polarization and cytoskeleton reorganization as a key to understand and potentially modify macrophages functions in regeneration/wound healing

All eukaryotic cells, including immune cells such as macrophages, contain actin filament cytoskeleton, which is responsible for cell shape, movement, interaction with, and the attachment to, the substrate, matrix degradation and receptor recycling. In addition, actin filaments participate in the structural organization and functions of cellular organelles, such as Golgi complex, matrix degradation organelles, the podosomes, and the formation and transport of phagocytic and exocytic/endocytic vesicles. The master regulator of actin cytoskeleton is the small GTPase RhoA and its downstream effector ROCK p160 kinase. The RhoA pathway is activated by the guanine exchange factors GEFs and is reciprocally regulated by GTPase Rac1 and mTOR pathways (Chen et al.2017; Gulhati et al., 2011; Gordon et al., 2014; Linder et al., 2015). Recent studies from our laboratories showed that interference with RhoA pathway modifies macrophage phenotype and function in rodent cardiac

transplantation model. We showed that macrophage specific deletion of RhoA, inhibition of ROCK or inhibition of RhoA activator GEFs (Chen et al., 2017), elongates macrophages (they switch into M2 pro-healing phenotype), disrupts their Golgi complex and receptor recycling pathway, and influences extracellular matrix degradation. All these changes prevented macrophages from entering the transplanted hearts and inhibited chronic (long term) rejection (Chen et al., 2018a,b,d; Liu et al, 2016a-d, Liu et al. 2017a,b; Wu et al., 2016). We also showed that interference with the RhoA pathway disrupted recycling and expression of CX3CR1 (fractalkine) receptor in monocytes and macrophages and prevented their movement into the graft (Fig. 2; Liu et al., 2017a). Because CX3CR1 plays an indispensable role in the recruitment of monocytes/macrophages into the wound, these results suggest that RhoA pathway inhibition has a potential to be used for the manipulation of macrophage content/function also in the wound-healing process. We also showed recently that macrophage phenotype could be manipulated mechanically by the application of magnetic field/gradient (Wosik et al., 2018). This study showed that macrophage exposed to the strong magnet become extremely elongated M2-like mimicking the RhoA-interference phenotype. This suggests that application of magnetic field to the wound can potentially also influence the immunological response in the wound and change the outcome of the wound healing process.

11. The limitations of wound healing research and therapies, potential future directions, and the novel methods promoting the pro-wound healing activity of macrophages.

Various animal models, including rodents and pigs, are used to study the wound-healing process. Unfortunately, because of their anatomical, physiological, cellular, immunological and molecular interspecific differences, the results from these models are difficult to translate into human wound healing. For example, rodent skin has dense fur, which accelerates healing because the stem cells derived from the hair follicles participate in wound re-epithelialization (Dorsett-Martin, 2004). Although the skin of pigs is furless and anatomically more similar to human skin, it does not have eccrine sweat glands, which participate in wound repair in humans (Rittié et al., 2013). In addition, the wound healing therapies developed in animal models are often inapplicable for humans because the drugs that are used are not approved for human use. Thus, there is an urgent need to study the wound healing process

directly in humans. Until recently, this was, with the exception of a very limited number of clinical trials, nearly impossible. Fortunately, the development of novel, highly sensitive molecular technologies and wide-scale analytic platforms, such as RNA-sequencing (RNA-seq), microarrays and quantitative reverse-transcription PCR (qRT-PCR), allows the study of the molecular events during different phases of the wound healing process directly in humans using very small biopsies of patient skin (Nuutila et al., 2012, 2014). For novel therapeutic approaches, one emerging strategy is to locally change the wound healing balance from the scarring to regeneration phase using transforming growth factor (TGF)-beta, which induces embryonic-like healing processes in adult mammals (Reth et al., 2008). The phase II efficacy trial using local administration of recombinant TGF-beta3 polypeptide called Juvista (from Renovo Ltd.) to treat human wounds showed significant improvement in healing and scar reduction in the majority of the patients in the trial (Occleston et al., 2018; Reth et al., 2008;). Another drug-free approach is to use locally applied devices that produce static or gradient magnetic fields to accelerate wound healing. Several clinical studies have shown that magnetic resonance therapy significantly accelerates wound healing (Brizhik et al., 2016). In addition, our recent study showing that the magnetic field gradient induces macrophage changes similar to those induced by RhoA interference (Wosik et al., 2018) suggests that the application of a magnetic field to the wound has the potential to change the wound-healing outcome without pharmacological treatment or genetic manipulation. This would be a very important innovation because the experimental pharmaceutical interference with the RhoA pathway that is effective in rodent models is not applicable for human clinical use. Thus, the physical induction of a RhoA-interference-mimicking phenotype could resolve this problem. Another approach to accelerate wound healing lies in the improvement (speeding up) the proliferative phase of healing. This can be experimentally achieved by ectopic activation of cell cycle-regulating enzymes (e.g. ERK1/2 MAP kinases; Escuin-Ordinas et al. 2016) or by the use of mesenchymal stem cells (Balaji et al. 2012). Both treatments may, theoretically, stimulate wound macrophages, but so far there is no experimental data on this subject.

Below we describe several emerging novel therapeutic approaches, which promote pro-wound healing activity of macrophages. These methods rely either on the stimulation of the pro-healing M2 phenotype of the wound macrophages using

environmental changes, anti-apoptotic factors, growth factors or supporting cells (fibroblasts or mesenchymal stem cells) or on the depletion, silencing or switching the phenotype of the pro-inflammatory M1 macrophages present in the wound (Goren et al., 2007; Guo et al., 2016; Ferrer et al. 2017; Ishida et al., 2008; Krzyszczyk et al., 2018).

For example, the suppression/silencing of the pro-inflammatory M1 macrophages and the improvement of wound healing in diabetic mouse was achieved through the co-administration of neutralizing monoclonal (anti-F4/80 and anti-TNF-α) antibodies (Goren et al., 2007; Krzyszczyk et al., 2018). Another study showed that the inhibition of apoptosis through the blocking of Interferon Regulatory Factor 8 (IRF8) abrogates macrophage polarization into pro-inflammatory M1 phenotype and improves cutaneous wound healing in diabetic mice (Guo et al., 2016). Another approach is to silence the wound macrophages through the increased whole body oxygenation in the hyperbaric chambers (Benson et al.,2003; Krzyszczyk et al., 2018).

The injection of mesenchymal stem cells (Lu et al; 2011; Zhang et al. 2010; Krzyszczyk et al., 2018) or mesenchymal stem cell-conditioned media (Chen et al., 2008) increased the number of macrophages and endothelial progenitor cells in the wound and improved mouse and human wound healing. The injection of the *in vitro* produced pro-healing M2 macrophages or the blood-derived macrophages from the young healthy humans into the wound, accelerated mouse and human wound healing (Jetten et al., 2014; Krzyszczyk et al., 2018; Zuloff-Shani et al., 2010). Ferrer et al., (2016) showed that human dermal fibroblasts (dFb) promote macrophage polarization into pro-healing M2 phenotype and accelerate wound healing in mice. Another, biomaterial supplementation, approach is to improve macrophage infiltration and promotion of M2 phenotype through the implantation of the hydrogels (Blakney et al.,2012; ; Krzyszczyk et al., 2018) or the microdelivery of extracellular matrix components to the wound (McWhorter et al., 2013; Cha et al., 2017; Krzyszczyk et al., 2018).

Because the macrophages are crucial players in all phases of wound healing, a full understanding of their roles and mechanisms involved, may lead in the future to a completely new approaches in wound healing therapies.

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Table 1.

Molecules produced by rodent and human macrophages

Molecule	Mouse	Rat	Human
Arginase-1	+	+	-
IL-1	+	ND	+
IL-6	+	+	+
iNOS	+	+	+
Mannose Rceptor-1	+	ND	+
Relm-α (Fizz1)	+	ND	-
ROS	+	-	+
TGFβ	+	ND	+
TNFα	+	+	+
VEGF	+	ND	+

+ Confirmed expression; ND - no data available; **-** Confirmed lack of expression. IL- Interleukin; iNOs- inducible nitric oxide synthase; Relm-α (Fizz1)- Resistin-like molecule alpha 1; ROS- Reactive oxygen species; TGFβ-transforming growth factor beta; TNFα- Tumor necrosis factor alpha; VEGF-Vascular endothelial growth factor.

Table 2.

Molecules produced by macrophages involved in inflammation, tissue repair and regeneration (resolving) as possible targets for therapeutic interventions in delayed wound healing and excessive scarring

Pro-inflammatory macrophages	Tissue repair macrophages	Resolving macrophages
STAT1	STAT6	STAT3
IRF5	IRF4	ATF3
iNOs	Arg-1	Arg-1
TNFα	LXR	GR
IL1-β	PPARg	IL-10
IL-6	TGFβ	TGFβ
CSF1R	TGFα	SOCS3
CX3CR1	Gal-3	SOCS1
	Activin	
	FGF	
	AREG	
	PDGF	
	IGF-1	
	VEGFα	
	CCL1	
	CCL22	
	Wnt	

Activin (Bamberger et al. 2005; Fumagalli et al. 2007); AREG, Amphiregulin (Chiarini et al., 2016; Zaiss et al., 2025); Arg1, Arginase-1 (Campbell et al. 2013; Cash et al, 2014); ATF3, Activating transcription factor 3 (Landen et al. 2016); CCL, Chemokine (C-C motif) ligand (Balaji et al., 2015); CSF1R, Macrophage colony-stimulating factor 1 receptor (Balaji et al., 2015); CX3CR1, fractalkine receptor (Balaji et al., 2015); FGF, Fibroblast growth factor (Eming et al., 2014); Gal-3, Galectin 3 (Dings et al. 2018); GR, glucocorticoid receptor (Landen et al. 2016); IGF, Insulin-like growth factor 1 (Balaji et al., 2014); IL, Interleukin (Chiarini et al, 2016; King et al. 2014); IRF, Interferon regulatory factor (Serra et al., 2017); LXR, Liver X receptor (DeLeon-Pennell et al. 2018); NOs, Nitric oxide synthase(Landen et al. 2016); PDGF, Platelet-derived growth factor (Eming et al., 2014); SOCS, Suppressor of cytokine signaling (Feng et al. 2016); STAT1-3, Signal transducer and activator of transcription 1-3 (Song et al.; 2017; Zhou et al., 2016); TGF, Transforming growth factor (Pakyari et al., 2013; Sun et al., 2015); TNFα, Tumor necrosis factor α (Ashcroft et al., 2012); VEGFα, Vascular endothelial growth factorα (Eming et al.2014; Wilgus et al., 2008); Wnt, Wingless type proto-oncogene (Shi et al.2015; Sun et al., 2015).

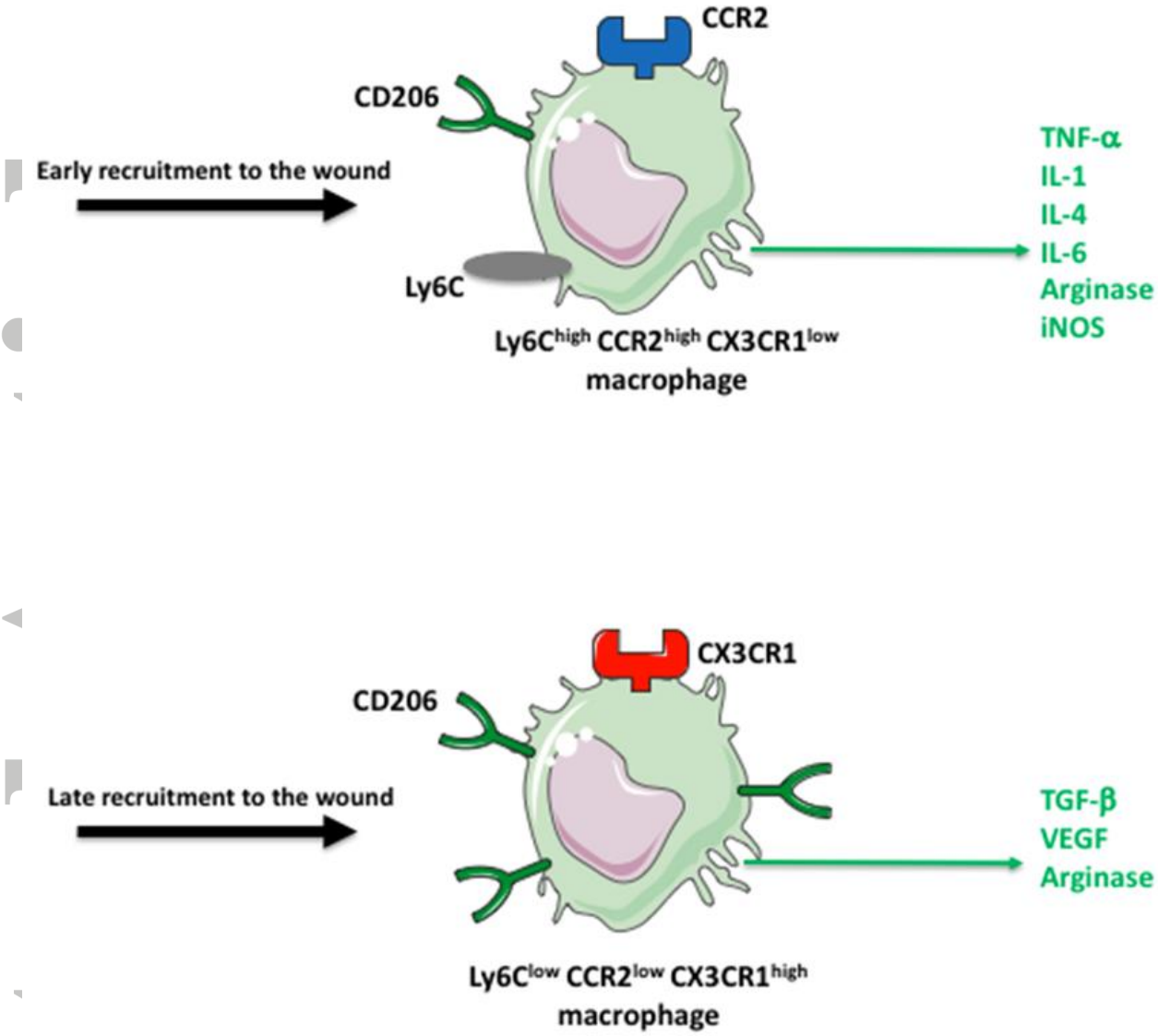

Fig. 1. Markers of wound healing mouse macrophages
In mouse there are two main types of wound healing macrophages, which differ in the timing of the arrival to the wound, and the receptors and cytokines they express. The early recruited macrophages express high level of CCR2 (monocyte chemoattractant protein-1 (CCL2) receptor and low level of CX3CR1 (fractalkine) receptor, high level of Ly6C (lymphocyte antigen 6 complex, locus C) and a moderate level of the mannose receptor (MR/CD206). The early macrophage produce high levels of TNF-α, IL-1, IL-4, IL-6, arginase enzyme, and inducible nitric oxide synthase iNos. The late macrophages express high level of CX3CR1, low level of CCR2, low level of Ly6C and a high level of CD206, and produce high level of TGF-ß, VEGF and arginase (Brancato and Albina, 2011)

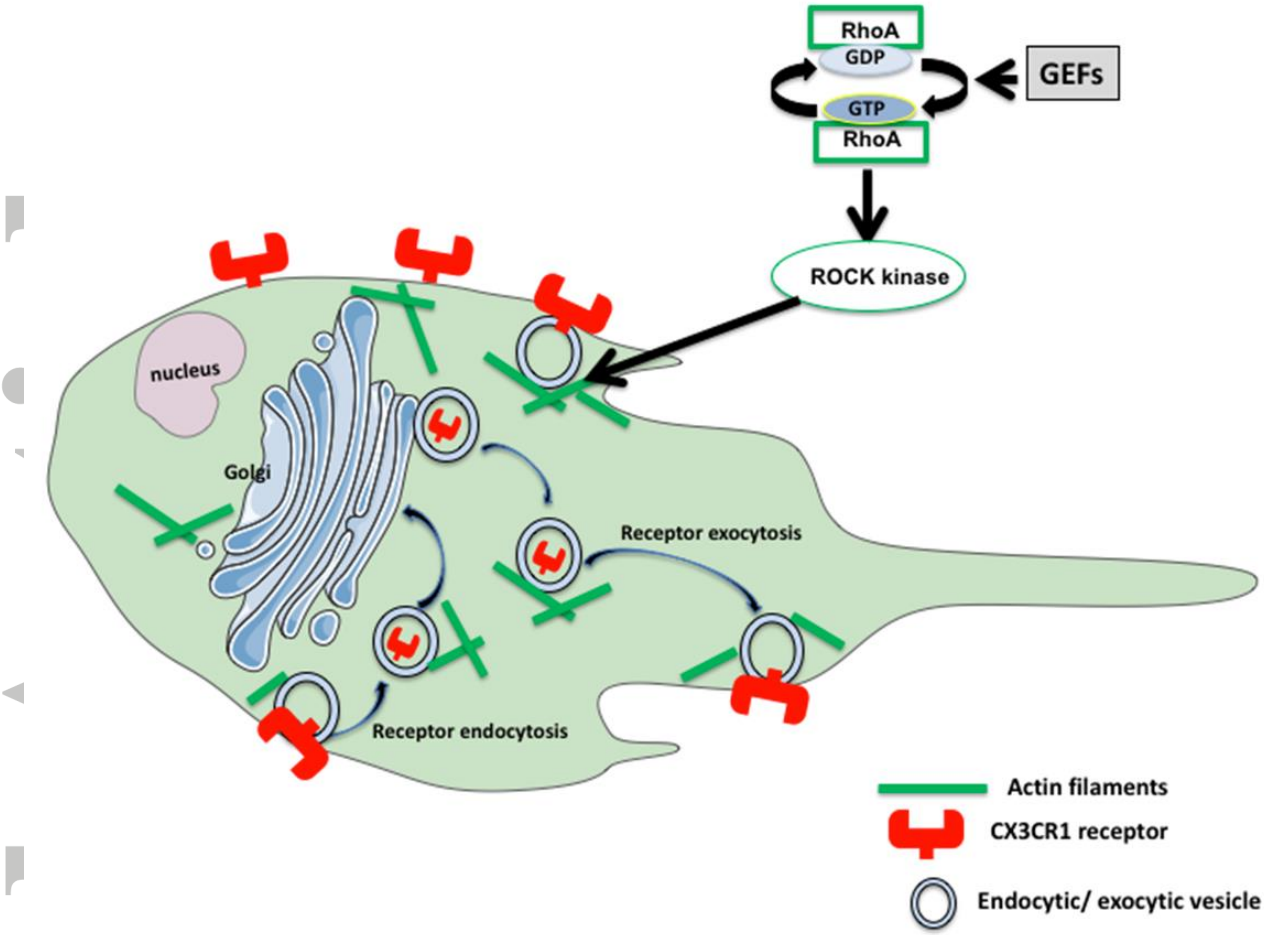

Fig. 2. Receptor recycling in the macrophages depends on the actin and the RhoA pathway

In eukaryotic cells, including mouse and human macrophages, the Golgi complex produces the exocytic vesicles, which bring the receptors from Golgi to the cell membrane and the endocytic vesicles, which recycle the used receptor from the membrane to the cell interior and back to the Golgi. The organization of the Golgi, the formation of exocytic/endocytic vesicles and the process of exo- and endocytosis depend on actin filaments. The actin filaments organization and polymerization is in turn, regulated by small GTPase RhoA and its downstream effector ROCK kinase. The upstream regulators of RhoA are the Guanine nucleotide exchange factors (GEFs). Our studies showed that a deletion of RhoA, inhibition of ROCK or RhoA specific GEFs disorganize actin, Golgi complex and disrupts receptor recycling in mouse macrophages (Chen et al. 2017a; Liu et al. 2016ab; Liu et al. 2017ab;).