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Review

The role of miRNAs in the inflammatory phase of skin wound healing

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Abstract: Wound healing (WH) is a fundamental physiological process to keep the integrity of the skin, therefore impaired and chronic WH is a common and severe medical problem and represent one of the biggest challenges of public health. The resolution of the WH inflammatory phase is characterized by a complex series of events that involves many cellular types, especially neutrophils, macrophages and inflammatory mediators, which are crucial for a correct wound closure. MicroRNAs (miRNAs) play essential roles in wound repair. In fact, miR-142 is linked to inflammation modulating neutrophils' chemotaxis and polarization, while the polarization of M1 toward the M2 phenotype is driven by miR-223 and miR-132 is linked to chemokines and cytokines that activate endothelial cells and attract leukocytes and peripheral cells to the damage site. Thus, understanding the dysregulation of miRNAs in WH will be decisive for the development of new and more effective therapies for the management of chronic wounds.

Keywords: miRNA; wound healing; inflammatory phase; neutrophils; macrophage; inflammatory mediators

1. Introduction

Incomplete wound healing (WH) is a worldwide cause of morbidity and mortality and represent a huge economic burden on our society. WH is a complex process involving more phases, including the hemostatic, inflammatory, proliferative, and remodeling one. The inflammatory phase is a highly ordered process that significantly impacts WH outcomes through neutrophils, macrophages, mast

cells, T cells and keratinocytes release of pro- and anti-inflammatory cytokines. Although the understanding of mechanisms involved in the repair of acute and chronic wounds has made many progresses, there is interest in clarifying and better defining pathological mechanisms. Recently the molecular circuitries controlling WH have reported new significant insights. The identification, characterization and elucidation of the biological roles of microRNAs (miRNAs) and their influence on cellular processes or signaling cascade and tissue homeostasis have offered the strategy for the improvement of skin WH. Several authors report the role of miRNAs' expression levels during WH process, suggesting their potential as regulator factors [1–4]. miR-155, miR-146a, and miR-223 are the most involved in the early inflammatory phases of WH, with immune cells recruitment to the wound site and the promotion of cytokines and growth factors production [5].

In this review, we have provided a brief overview of miRNAs' activity during the inflammatory phase of WH. Mainly, we have discussed miRNAs' differential expression in immune cells, neutrophils and macrophages, involved in the early WH phases. Finally, based on their crucial functions in skin WH, we suggest miRNA's application as innovative wound treatments.

1.1. miRNAs' biogenesis

miRNAs are small non-coding RNAs from 19 to 25 nucleotides (nt) in length which regulate the activity of hundreds of different messenger RNAs (mRNAs) [6]. According to their genome localization, miRNAs can be classified as intronic or intergenic. In the first case, the coding genes share primary regulatory and transcript elements with their host genes; intergenic miRNAs, on the other hand, are encoded starting from a gene located between two exons and which has its own promoter [7].

miRNAs were discovered in 1993 from the analysis of the transcriptional activity of the gene *lin-4,* which is responsible for *Caenorhabditis elegans* larva development. It was observed that the final products of lin-4 transcription were two small RNAs, one of 61 nt and one of 22 nt [8]. Since then, it has been reported and proven that miRNAs are involved in many biological functions, such as cell death and proliferation, by binding sequences in the 3' untranslated region (UTR) or 5' UTR of their target mRNAs. Thus, a loss of function or changes in miRNA's expression may be the cause of human diseases like cancer or immune ones, inflammation and dermatological disorders due to metabolic disruption, vascular deficit or mechanical damage with impaired WH [9–11].

The biogenesis of a miRNA could be divided into canonical and non-canonical pathway [12]. The first starts in the nucleus with the RNA polymerase II/III (Pol II, III) transcription of a primary miRNA (pri-miRNA), hundreds of nucleotides in length, whose main feature is the presence of a stem-loop domain that will be processed by two enzymes, Drosha and Dicer [13]. Specifically, these two enzymes are part of the family of Ribonucleases III, characteristically consisting of domains with dimeric modules with the unique ability to cut the RNA double helices [10]. Thanks to microprocessor complex subunit Dgcr8, Drosha recognizes and binds the pri-miRNA and cuts it at the base of the stem loop, releasing a nucleotide hair-spin structure called precursor miRNA (pre-miRNA), 60–70 nt in length, which is transported, by exportin 5 (XPO5) into the cytoplasm. Here, the terminal loop of the pre-miRNA is recognized and then cut by the complex DICER-TAR RNA binding protein (TRBP), leading to the formation of a miRNA duplex [14]. Of these two filaments, one is degraded, while the other is positioned within an Argonauta protein (Ago protein) generating the RNA-induced silencing complex (RISC). At this point, the mature miRNA is

incorporated into miRISC (miRNA-induced silencing complex) that aims to regulate gene expression by acting on specific mRNAs target [10].

Instead, the non-canonical pathway represents an alternative way of miRNAs' production. Although the exact mechanisms are still under study, it can be classified as Drosha or Dicer independent pathways. Between these, the Drosha/Dgcr8 independent one begins with the processing of introns by the spliceosome and continue with the subsequent debranching that leads to the production of the pre-miRNA [12], which arrive in the cytoplasm to be processed like in the canonical pathway (Figure 1).

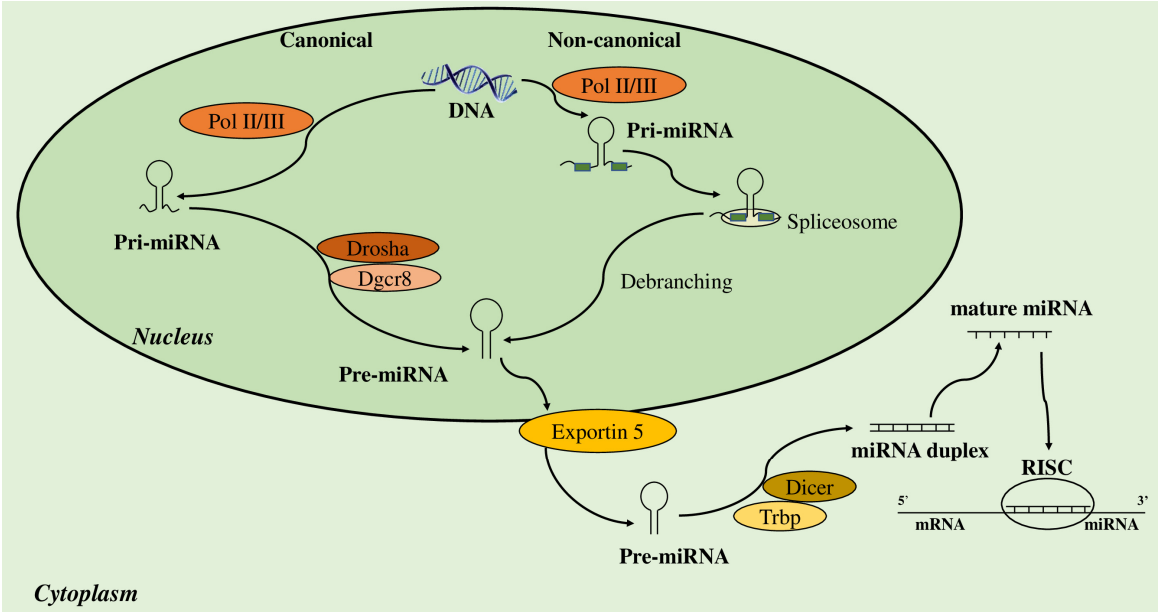

Figure 1. Biogenesis of miRNAs. In the canonical pathway, RNA polymerase II or III transcribes a primary miRNA (pri-miRNA) with a hairpin-like structure. The complex Drosha-Dgcr8 processes the pri-miRNA generating a precursor miRNA (pre-miRNA), which is exported in the cytoplasm by exportin 5 (XPO5) where the pre-miRNA double helix is cut for generating two distinct filaments: one is degraded and the other represents the mature miRNA, that can bind its mRNA target by its incorporation in the RNA induces silencing complex (RISC). In the non-canonical pathway, Drosha independent, pri-miRNA, derived from RNA polymerase II or III transcription, is first processed by the spliceosome and then debranched for intron elimination. The obtained pre-miRNA is transferred in the cytoplasm and performs its functions like the pre-miRNA in the canonical pathway.

Thus, miRNAs play a key regulatory function over a great number of target genes, like those with a role in cellular proliferation and differentiation, fat acids metabolism and differentiation of the hematopoietic cell line [8]. This is possible thanks to the extensive extracellular miRNA's delivery in biological fluids, like plasma and serum, where they don't lose their structure and properties. In particular, miRNAs can be delivered in exosomes or in association with proteins, such as argonaute RISC catalytic component 2 (AGO2), and then they reach their target. Although is not precisely clear how circulating miRNAs' uptake occurs, it has been described hypothetical pathways through

endocytosis, fusion with cell plasmatic membrane or micropinocytosis [15]. This is also one of the main problems for the development of specific formulations based on miRNA's delivery [16].

1.2. Wound healing overview

Skin is the largest organ in the human body and the first barrier against pathogenic infiltration. Every day there is a high risk of damage to skin integrity, which can occur either accidentally or because of surgery or pathological conditions [17]. In healthy subjects, skin integrity is re-established spontaneously without complications, following the normal well-regulated process. Nevertheless, there are many factors which can act during the different phases of the healing process, avoiding that the wound does not close or become chronic, at the same time increasing the possibilities of infection, as in the case of diabetic ulcers [18], trauma or venous pathology [19].

In fact, the most serious consequences of impaired WH could be sepsis or amputation of the damaged area involved and could affect not only people's quality of life, but also the economy of the health system, with high costs of treatments and hospitalizations. Thus, the necessity to study wound healing process and to develop new drugs and technologies to improve patients' well-being is evident.

WH is defined as a complex and dynamic biological process consisting of a series of highly coordinated cellular events with the final issue of promoting tissue repair [20]. It consists of interdependent and overlapping phases starting with hemostasis and proceeding on with inflammation, proliferation, and remodeling [21]. In each phase of wound repair, a close collaboration between various cell types such as immune and endothelial cells, keratinocytes, and fibroblast, as well as mediators, including cytokines, chemokines and matrix molecules, is required [22] (Figure 2).

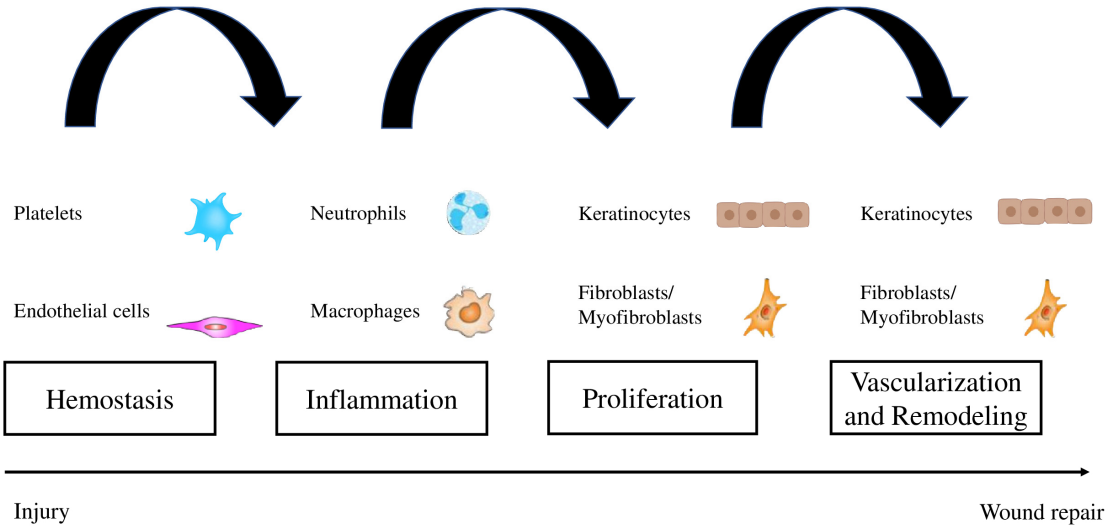

Figure 2. The phases of WH and their cellular components. Wound repair begins with hemostasis, where a platelet plug prevents blood loss. During inflammatory phase neutrophils remove debris and prevent infection. Monocytes arrive later and differentiate into tissue macrophages to clear remaining cell debris and neutrophils. In the proliferative phase, keratinocytes migrate to close the wound gap, fibroblasts replace the initial fibrin clot with granulation tissue and myofibroblasts cause overall wound contraction. Finally, in the remodeling phase, the deposited matrix is arranged by fibroblasts.

The hemostasis phase begins immediately after the damage and its main goal is to stop bleeding at wound site, through the immediate vasoconstriction and activation of platelets [23]. In fact, the breakdown of the vessels precedes the exposure of proteins of the sub-endothelial matrix, such as fibronectin and collagen. The platelets recognize and bind endothelial proteins and activate the coagulation cascade that finally generates a scar that allows the block of bleeding [24]. Furthermore, platelets secreting chemokines and specific growth factors, such as C-X-C motif chemokine ligand 4 (CXCL4) and transforming growth factor beta (TGF-β), attract other cell types, including fibroblasts and keratinocytes, promoting the subsequent stages of tissue repair [25].

The second stage of WH is the inflammation, which can be divided into an early phase, with neutrophils' recruitment, and a late phase, with the involvement of monocytes/macrophages [26]. The immune system cells collaborate following multiple signal molecules, including damage-associated molecular patterns (DAMPs) [27], chemokines and growth factors released by platelets, like CXCL8 and TGF-β, pathogen-associated molecular patterns (PAMPs) and bacterial endotoxins. Once the neutrophils arrive at the site of damage, they carry out many functions acting as phagocytic agents for microorganisms' debris and necrotic tissue, and releasing antimicrobial peptides (AMP), proteinases, and reactive oxygen species (ROS) [28]. Moreover, neutrophils act as chemoattractant for other cells involved in the inflammatory phase, through the release of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-6, which amplify the inflammatory response, vascular endothelial growth factor (VEGF) and IL-8, pivotal for an adequate repair response [28,29].

In the late inflammatory response, neutrophils are eliminated by extrusion or apoptosis and replaced by macrophages, recalled by chemotactic factors such as TGF-β and protein 1 chemotactic for monocytes (MCP-1) [30,31]. Macrophages have a dual role in the WH inflammatory phase, based on the differentiated phenotype. With a pro-inflammatory phenotype (M1, classically activated) they are responsible for neutralization of pathogens and production of inflammatory cytokines such as IL-1, IL-6 and TNF-α, ROS and growth factors like platelet-derived growth factor (PDGF), TGF-β, fibroblast growth factor (FGF), and VEGF [27]. As anti-inflammatory phenotype (M2, alternately activated) [21] macrophages are involved in keratinocytes, fibroblasts, and endothelial cells activation, useful for the later stages of WH, with release of anti-inflammatory cytokines (IL-4, IL-10, IL-13) and growth factors [27].

The third phase of WH is the proliferative one, which begins between the second and third day, continue for up to two weeks and involves different cell types with the final aim to form new connective or granulation tissue. Fibroblasts, attracted by factors as PDGF and TGF-β, arrive at wound site and produce matrix proteins, like hyaluronan, fibronectin, proteoglycans and type 1 and type 3 procollagen [28]. In addition, fibroblasts synthesize collagen that acts as a scaffold for the creation of a new matrix and, after differentiation into myofibroblasts, regulate the contractility of the wound. Keratinocytes, releasing metalloproteinases (MMPs) and extracellular matrix proteins (ECM), participate in the reconstitution of the basement membrane and in the epidermis' regeneration. Fibroblasts and keratinocytes, as principal actors of WH, present an active interplay with reciprocal activation [32]. The production of soluble mediators by keratinocytes is regulated by interactions with fibroblasts, which can modulate keratinocytes vitality, proliferation, and differentiation. Moreover, the cellular crosstalk is reported as critical for adequate skin homeostasis [33].

During the angiogenesis process, the formation of new blood vessels is induced by metabolic demands of the highly proliferative healing tissue. The formation of new vessels is the response to

hypoxia and angiogenic factors such as FGF, VEGF and PDGF, while microvascular endothelial cells proliferate intensely and migrate to the damage site, sprouting new vessels [27,28].

The last phase of WH, named the remodeling, is responsible for the definitive repair of the damage and for the formation of new epithelial tissue. During the remodeling step, which can take two years, a delicate balance between the individual events is necessary. In fact, in this phase, are involved the establishment of a new matrix and granulation tissue, with the production and degradation of collagen (mainly type III collagen) managed by MMPs and the formation of fibrils by proteoglycans, while elastin regulates their plasticity determining the formation of a fully matured scar [34].

For a complete and healthy WH, multiple processes and mechanisms can be involved and require critical coordination. The crosstalk between keratinocytes and immune cells represents one of the main events that can be reported to acute or chronic wound healing disorders developments, together with DNA repair, mitochondrial function, cell cycle, proteolysis, and cellular metabolism. Thus, effective wound repair needs the coordinated action of many different cell types and one of the most representative mechanisms, responsible for the failure of WH, is an out-of-control of the inflammatory response, that is self-sustaining.

2. Role of miRNAs in cells and mediators' activity

Inflammation is a crucial part of the healing process, allowing the defense against damage by pathogenic microorganisms and regulating the production of cytokines and the correct sequence of the other phases of the repair process. Unregulated inflammation with abnormal cytokines production and over-activity of immune cells are, therefore, a crucial part of the healing process. Immediately after injury, neutrophils are the first cells to infiltrate the wound site to destroy foreign particles. Subsequently, macrophages intervene and are attracted to the wound site to eliminate bacteria and tissue debris. In both these phases the multiple mediators of the inflammatory phase are present, like cytokines and chemokines [35,36].

miRNAs are reported to be involved in the modulation of the complex balance in the inflammatory phase, by controlling cell signaling pathways and mediators production.

Considering the main protagonists of the inflammatory phase in WH, below we report miRNAs' classes in correlation with them, defining their role and potential (Figure 3).

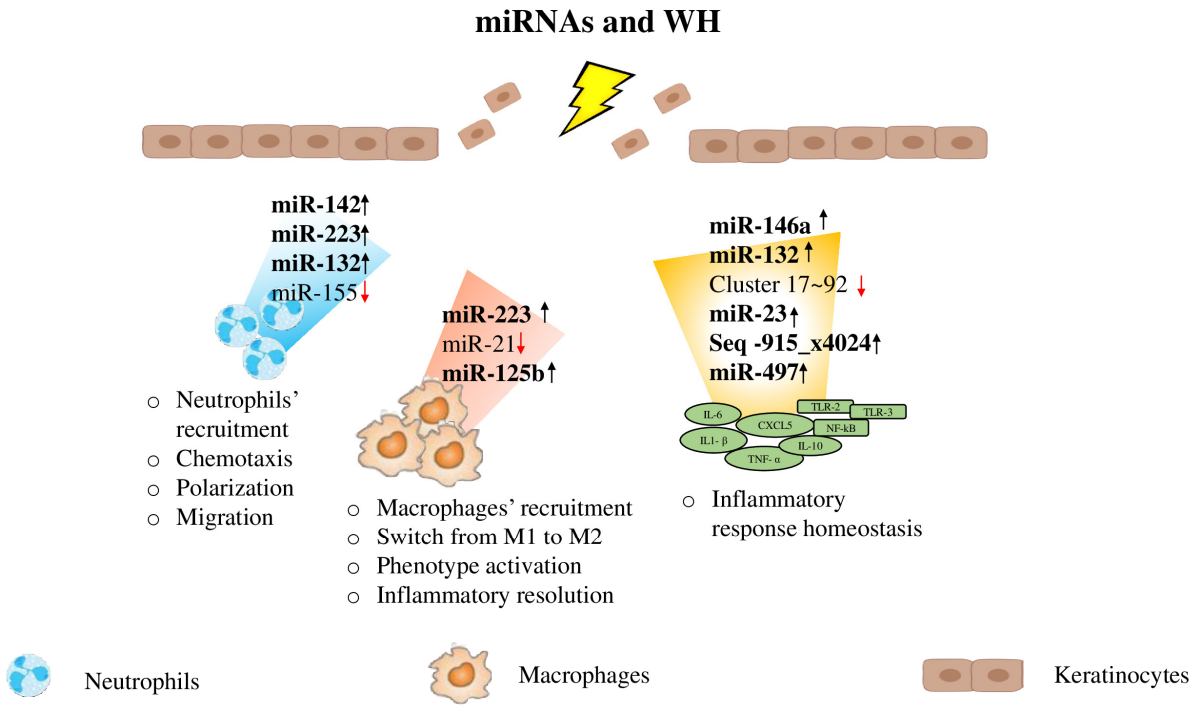

Figure 3. Interaction between miRNAs and WH. The inflammatory phase of WH is regulated by several miRNAs, which perform their role modulating the activity of specific cells such as neutrophils, macrophages, and inflammatory mediators.

2.1. miRNAs and neutrophils

Inflammation, as the first stage of WH, begins immediately after the trauma and is completed in a few hours. The normal progression of this initial phase is critical and determines the recruitment of neutrophils. A significant amount of evidence supports that, neutrophils play an important role in the early inflammatory phase by mediating proper healing [37]. Particularly, some studies have revealed an essential role of miR-142 family, which is already known for being involved in immunity and in cancer [38] and for the pro-inflammatory cytokines production, with main target the interleukin 1 receptor associated kinase 1 (IRAK1) [39]. Indeed, miR-142 could be described as a modulator of neutrophils' chemotaxis and polarization, during the WH inflammatory phase [40]. Besides, in knockout 6- to 12-week-old mice, it has been found that the presence of miR-142 helps to regulate neutrophils' polarization and migration speed at the site of the damage and to counteract the infection, mostly in presence of *Staphylococcus aureus* [41].

Additionally, miR-223 is normally expressed in the site of the wound and it was suggested to help fighting against *Staphylococcus aureus* and to be a key regulator of neutrophils' activity. Using knockout mice, it has been underlined that miR-223 is over-expressed in neutrophils at wound site, regulate their migration and, consequently, the timing of the inflammatory phase. The lack of miR-223 is responsible of a significant decrease in neutrophils' arrival, after 3 hours from the damage, with an increased production of IL-6 and an excessive macrophage infiltration [42]. Thus, in presence of infection by *Staphylococcus aureus*, miR-223 controls the infection and reduce the inflammation. In this way, over-stimulated and recruited neutrophils support and promote wound resolution [42].

Besides, Li et al. [43] have described as miR-132, expressed in epidermal keratinocytes, is associated with the regulation of the inflammatory phase in WH, by acting on Nuclear Factor-kappa B (NF-kB) pathway and the relative neutrophils' activation. The functional expression of miR-132 is linked to the induction of chemokines and cytokines that could activate endothelial cells and attract leukocytes and peripheral cells to the damage site. It has been shown that the chemokines CXCL1, CXCL5, CXCL8 and Chemokine (C-C motif) ligand 20 (CCL20), mostly expressed by keratinocytes, attract neutrophils, necessary to amplify the inflammatory phase [43]. Moreover, growth factors and cytokines, including TGF-β1 and TGF-β2, are responsible for the induction of miR-132 and they are in turn up-regulated in the inflammatory phases of WH [44], with the resulting anti-inflammatory effects and the regulation and activation of neutrophils [45].

On the contrary, a decrease in the induction and activity of miR-155, considered a "master of inflammation" [46] could improve the resolution of the inflammatory phase and consequentially an optimum wound healing. Neutrophils have a critical role against bacterial infections, and it is reported that miR-155 negatively controls neutrophils' migration toward chemotactic stimulation [47]. Wang et al. [48] and Yang et al. [49] have characterized miR-155 mechanism of action. The absence or the reduction of miR-155 levels result in a decreased recruitment of neutrophils in the site of the wound. More in details, the diminished recall of immune cells leads to an inverted trend of the amount of signal molecules released, thus a reduction of the levels of pro-inflammatory, like IL-1β, TNF-α and CCL2, in comparison with the anti-inflammatory one, such as IL-10. The inhibition of miR-155 was seen to be involved also in wound retraction and healing of diabetic mice, where it works directly on neutrophils' recall, modulating the inflammatory phase and promoting a better closure [50,51].

2.2. miRNAs and macrophages

Macrophages, together with neutrophils, are the principal leucocytes involved in the regulation of the inflammatory process of WH and once arrived in the damage site, they perform several functions, including phagocytosis and production of cytokines and growth factors [52]. Macrophages' function, besides, during this phase, is regulated by several miRNAs.

The studies of Dang et al. [53] and Meng et al. [54] reported that miR-223 acts as a potent regulator of the inflammatory response by driving the polarization of M1 toward the M2 phenotype, leading to the resolution of the inflammatory phase and promoting angiogenesis and granulation.

Moreover, it has been reported a critical role of miR-21 in macrophage polarization, pointing out its implication in the engulfment of apoptotic cells [55] and in macrophages switch from a M1 to M2 phenotype. In fact, the inhibition of miR-21 directs macrophages toward the M1 phenotype, suggesting that sufficient miR-21 in macrophages is essential for their M2 polarization [56]. miR-21 is widely expressed in wound site and possesses a key role not only in the inflammatory phase, where is responsible to regulate macrophages' arrival [57], but also in the last phases of collagen deposition and contraction of the wound [58]. In diabetic conditions, there is an over-expression of miR-21 in WH inflammatory phase, and it has been described a high frequency of M1 macrophages, suggesting miR-21 role in this phenotype induction [59]. In miR-21 M1 polarized macrophages, IL-1β, TNF-α, iNOS, IL-6, and IL-8 are up-regulated [59], providing evidence that miR-21 is involved in the regulation of inflammation. In fact, dysregulation of miR-21 may justify the irregular inflammation and permanent M1 macrophages polarization seen in diabetic wounds. For all these

reasons, it is suggested the direct involvement of miR-21 in the inflammatory response and its impaired regulation promotes a persistent inflammation in diabetic wounds.

Also, miR-125b is implicated in macrophages' regulation and the activation of the M1 phenotype, which trigger the inflammatory pathway [60]. At the same time, miR-125b has been recognized as a key regulator of TNF-α since it is able to bind the cytokine's 3'UTR, acting at post-transcriptional levels and stopping the production [61]. Moreover, a fine regulation has also been demonstrated by miR-155, which performs numerous functions towards immune cells and towards macrophages, as regards the phenotypic differentiation process towards M2 [46].

2.3. miRNAs and mediators in wound healing

Persistent inflammation is responsible of the impaired healing. In recent studies, it has been observed how some miRNAs act as modulators of pro-inflammatory mediators' expression and how they may be involved in sustained inflammation and impaired healing. Among them, miR-146a is considered a negative regulator of the immune response: specifically, it results up-regulated in epidermal keratinocytes stimulated by toll like receptor 2 (TLR-2) [62], a class of receptors well known in many inflammatory diseases [63,64]. In fact, during an inflammatory state with the presence or not of infection, TLR-2 receptors are extensively activated on the surface of many immune cell types, including macrophages, as part of the innate immune system. After recognizing and binding their specific ligand, these receptors start an intracellular cascade of events that culminate with the activation of NF-kB pathway and the releasing of pro-inflammatory molecules [65]. This induces an up-regulation of miR-146a, in epidermal keratinocytes, which works specifically as a negative feedback regulator of TLR-2 receptors in the same cells, targeting IRAK1, CCL5 and the caspase domain-containing protein 10 (CARD 10), which are part of the response of NF-kB, clearly promoting the inflammation resolution [66].

The NF-kB pathway is also the principal target of miR-132, which has the specific function to reduce and alter the transcription factor [43]. This cascade mechanism can be controlled by the down-regulation of Heparin Binding EFG-like growth factor (HB-EFG) and reduction of the pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-8 and chemokines, like CXCL5, CXCL1, CCL20 and CXCL1 transcription, all performed by miR-132 [43].

Additionally, a high anti-inflammatory activity was reported for the miR17~92 cluster [67]. Among this cluster, it has been observed that miR-19a/b and miR-20a are specifically down-regulated in the epidermis and, in consequence, their higher expression could improve wound repair. Results in human primary keratinocytes showed the ability of these miRNAs to inhibit pro-inflammatory cytokines and chemokine production induced by TLR-3 expressed on macrophages' membrane, also defining the reduction of the receptor ability to express its function on keratinocytes in the site of the wound and therefore reducing neutrophils' recruitment [68].

Moreover, miR-17, another member of the cluster 17~92, could act as an anti-inflammatory agent: its over-expression stops the pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) production from macrophages and their migration, concurrently [69].

Besides, the same mechanism has been observed for miR-23, whose main function is to decrease the inflammatory state not only hindering the production of pro-inflammatory cytokines (such as IL-6, IL-1β, TNF-α, CCL2) and promoting the production of anti-inflammatory ones, like IL-10, but also targeting apoptotic signal-regulating kinase 1 (ASK1). Overall, this leads to the resolution of the inflammatory phase and facilitate wound closure [70].

Furthermore, recently, in fetal keratinocytes, it has been discovered a new miRNA sequence, Seq-915_x4024, which seems to have a great potential as negative regulator of WH inflammatory phase. In fact, thanks to *in vivo* analysis, Seq-915_x4024 has been shown to suppress the production of pro-inflammatory mediators, like TNF-α, IL-6, IL-8, CXCL1 and CXCL5, and to prevent leukocytes' migration in the site of the damage. Thus, the obtained resolution of the inflammatory state allows to an improved proliferation of keratinocytes and fibroblasts, which will culminate in wound closure [71].

Finally, miR-497 seems to have great potential as anti-inflammatory agent. This miRNA has been tested in the diabetic mouse models of wounds through intradermal injections in the site of injury, suggesting the possible ability of this treatment in leading the acceleration of wound closure, with a decreased expression of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α, also highlighting the clear therapeutic potential of this miRNA [72].

3. Future perspectives

Due to role of miRNAs in the regulation of inflammatory response, it is possible to hypothesize that the action of a single miRNA on different genes and cells determines complex and highly regulated cascade of events, which requires more knowledge about their functions and mechanism of action. To date, some miRNAs have been suggested as valuable indicators in a specific clinical context and to act as screening tools, facilitating patient management and treatment process. The knowledge gained is based on large screening studies or studies based on in vitro cell systems, with the need for clinical verification. Till now only few miRNAs are involved in clinical trials, such as miR-34 for treating multiple type of cancer [73]. Moreover, miR-132 has been already tested for WH, delivered through liposome formulations [74] or poly lactic (glycolic acid) (PLGA) nanoparticles, able to accelerate the vascularization and remodeling phase [75]. The success of using miRNAs as a gene therapy is dependent on the development of a proper delivery system that must be designed to affect both extra-cellular and intra-cellular barriers. Given that their expression is increased or otherwise altered in many pathologies, several approaches have already been put in place to inhibit or enhance their activity, including small molecular inhibitors of specific miRNAs (SMIR), which can bind and inhibit pri-miRNA, pre-miRNA or RISC complex. In this sense, promising alternative delivery approaches are viral and lipid vectors, nanoparticles and structures such as exosomes. The advantageous application is supported by carrying modified miRNAs directly at the target site, avoiding the risk of degradation and subsequent urinary excretion, with high efficiency of action [76]. Other advantages are the ability to target multiple genes of one pathway simultaneously, with the use of miRNAs mimics, synthetically obtained, which made possible to increase the levels of a specific miRNA that helps to counteract the pathological condition or to decrease the levels of the pathological ones, with the help of antagomiRs. Thus, to become an important third generation therapeutic nucleic acid for the treatment and management of impaired wounds, it will be necessary to design the optimal targeting and delivery system, along with identification of new miRNAs' targets. However, the elimination of anti-miR or miR-mimetic from plasma, which occurs within hours by uptake into tissues, and the extremely poor efficiency of the internalization and release of anti-miRs, may be a limiting factor.

A better understanding and investigation of miRNAs potential are required for the concrete availability in human disease treatments. Knowledge of miRNAs' function in WH regulation will

help to develop more effective therapies and drug. Recently the feasibility of miRNAs' analysis, in non-invasive biological samples (such as saliva), has opened a new perspective for a better and broader spectrum application of the predictive power of miRNAs. Define the role of miRNAs in WH can provide a useful and necessary step for the application of a "miRNAs chimera" into a clinical use.

4. Conclusions

miRNAs are emerged as regulators of many biological processes, including cell cycle, apoptosis, proliferation and migration, also in disease conditions. miRNAs' de-regulation may lead to the alteration of gene networks in diseases such as metabolic disorders, non-healing conditions and cancers. Thus, miRNAs represent a possible dual role as biomarkers and therapeutic targets. Several clinical trials demonstrate their effectiveness in treatment of different types of pathologies, by the modulation of inflammatory response. Given the multiplicity of functions performed by miRNAs in the different physiological mechanisms, they can be considered as key regulators of WH, mostly in the transition from the inflammatory to the proliferative phase, with reference to the regulation of gene expression and the activity of macrophages, neutrophils, leukocytes and inflammatory mediators. This review underlines that miRNAs play an important role in the control and resolution of inflammatory phase of WH. In the future, miRNAs may represent an attractive suggestion for the development of new therapeutic targets.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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