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MiRNA in innate immune responses: novel players in wound inflammation

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Roy S, Sen CK. MiRNA in innate immune responses: novel players in wound inflammation. Physiol Genomics 43: 557–565, 2011. First published December 7, 2010; doi:10.1152/physiolgenomics.00160.2010.—Chronic wounds represent a major and rising socioeconomic threat affecting over 6.5 million people in the United States costing in excess of US $25 billion annually. Wound healing is a physiological response to injury that is conserved across tissue systems. In humans, wounding is followed by an instant response aimed at hemostasis, which in turn provides the foundation for inflammatory processes that closely follow. Inflammation is helpful and a prerequisite for healing as long as it is mounted and resolved in a timely manner. Chronic inflammation derails the healing cascade resulting in impaired wound closure. Disruption of Dicer, the RNase III enzyme that generates functional miRNAs, has a major impact on the overall immune system. Emerging studies indicate that miRNAs, especially miR-21, miR-146a/b, and miR-155, play a key role in regulating several hubs that orchestrate the inflammatory process. Direct evidence from studies addressing wound inflammation being limited, the current work represents a digest of the relevant literature that is aimed at unveiling the potential significance of miRNAs in the regulation of wound inflammation. Such treatment would help establish new paradigms highlighting a central role of miRs in the understanding and management of dysregulated inflammation as noted in conjunction with chronic wounds.

wound healing

BACKGROUND

The process of wound healing is well regulated and for the ease of understanding is divided into specific functional phases: hemostasis, inflammation, proliferation, and remodeling (43, 49, 73, 80). Chronic wounds fail to progress through the normal phases of healing and therefore enter a state of prolonged pathologic inflammation (49). An improved understanding of the molecular mechanisms that regulate and coordinate the inflammatory response in wounds has potential for improved therapeutic intervention of chronic wounds. We first hypothesized in 2007 and reported in 2008 that the understanding of microRNA (miRNA or miR) biology is critically important to develop a comprehensive understanding of the molecular mechanisms that govern wound healing (6, 74, 75, 78, 79). Emerging studies suggest that miRNAs play a significant role in the immune responses (3). Likewise, the endonuclease enzyme Dicer, the RNase III enzyme that generates functional miRNAs, plays a key role in the regulation of the immune system (13). While the role of miRNA in inflammatory response associated with cancer has been extensively studied and reviewed (31, 59, 82, 89), how miRNA may govern wound inflammation remains unexplored. Such information will help understand persistent and unresolved inflammation as seen in chronic wounds. The role of miRNA in processes that drive wound healing such as epithelialization and angiogenesis has been discussed elsewhere (6, 74, 75, 78, 79). In this work, emphasis is directed toward addressing the significance of miRNA in regulating the component biological processes underlying wound inflammation. We first discuss the major miRNAs that are known to regulate the innate immune responses. Next, we address the regulation of key cytokines, chemokines, and growth factors that regulate the inflammatory response following wounding (Fig. 1). Direct evidence from studies addressing wound inflammation being limited, this current work represents a digest of the relevant literature that is aimed at unveiling the potential significance of miRNAs in the regulation of wound inflammation. Such treatment would help establish new paradigms highlighting a central role of miRs in the understanding and management of dysregulated inflammation as noted in conjunction with chronic wounds.

miRNA IN REGULATION OF INNATE IMMUNE RESPONSE

The innate immune response provides primary defense against infection by external pathogens such as bacteria, fungi, and viruses. The presence of invading pathogens is commonly detected by tissue macrophages using receptors known as PAMP (pathogen-associated molecular pattern) receptors (95).

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Multiple families of PAMP receptors have been identified, including the Toll-like receptors (TLRs), nucleotide-binding domain (NOD)-like receptors (NLRs), and the retinoic acid-inducible gene (RIG)-like receptors (RLRs) families (15). The first report linking miRNA with immune responses came from miRNA expression profiling performed in a monocytic cell line treated with the lipopolysaccharide (LPS), a TLR4 ligand (86). The expression of miR-146a, miR-155, and miR-132 were induced in response to LPS stimulation (Fig. 2)(86). miRNAs are known to influence the fate of immune cells (e.g., miR-223) as well as to regulate adaptive immune responses such as antigen presentation (e.g., miR-155) and T-cell receptor signaling (miR-181a) (81). miR-146, miR-155, and miR-21 have been of particular interest for investigations associated with inflammatory and immune responses. These miRNAs are induced by proinflammatory stimuli such as IL-1β, TNF-α, and TLRs (76). In the following section we discuss the expression, regulation well as the targets of miR-146, miR155, and miR-21.

**miR-146**

The miR-146 family is composed of two members, miR-146a and miR-146b, that are located on chromosomes 5 and 10, respectively (95). miR-146 is induced as a general response in myeloid cells through TLR-2, -4, or -5 ligands (e.g., bacterial and fungal components) or following exposure to the pro-inflammatory cytokines (such as TNF-α or IL-1β) (56, 86, 90). This miRNA, however, is not responsive to TLR -3, -7, or -9 activation (e.g., single- or double-stranded RNA and CpG motifs) (95). A detailed list of activators and conditions known to change expression of miR-146 is displayed in Table 1. The miRNA-146 family (miR-146a/b) regulates TLR4 through a negative feedback loop mechanism (35). IRAK1 and TRAF6

**Fig. 1.** Potential role of microRNA (miRNA, also miR) in regulation of wound inflammatory response. The inflammation response to wound is tightly regulated by proinflammatory signals as well as signals that are anti-inflammatory to resolve inflammation. An imbalance between these signals results in chronic inflammation and derails the healing cascade. miRNA have been shown responsive to as well as regulate some of the key mediators of inflammatory response in the course of wound healing. The details of the miRNA or mediators have been discussed in the review, miRNA regulate the expression of the components of the ARE-BPs that are known to regulate cytokine and Cox-2 gene expression. The miRNA and the targets of miR have been presented as filled and open boxes, respectively. AA, arachidonic acid; ARE-BP, AU-rich element binding protein; COX-2, cyclooxygenase-2; DHA, docosahexaenoic acid, EPA, eicosapentaenoic; Lx, lipoxin; PGE2, prostaglandin E2; Pd, protectin; Rd, resolvins; ROS, reactive oxygen species; RNS, reactive nitrogen species; TLR, Toll-like receptor.

**Fig. 2.** Three major miRNA induced as a response to inflammation. Known key activation signal and the targets of miR-146, miR-21, and miR-155 in macrophages. G-CSF, granulocyte colony-stimulating factor; SHIP-1, src homology 2-containing inositol 5-phosphatase 1; SOCS1, suppressor of cytokine signaling; TGFB-R2, transforming growth factor-β receptor 2; TPM1, tropomyosin1.
represent two prominent targets of miR-146a that help it to negatively regulate the release of IL-8 and RANTES (95). In addition to TRAF6 and IRAK-1, IRAK2 has been identified as another target of miR-146a, which regulates IFN-γ production (29). Furthermore, miR-146a silences cyclooxygenase-2 (Cox-2) mRNA in fibroblasts from patients suffering from chronic obstructive pulmonary disease (COPD). Thus, lowering of miR-146a expression may prolong the half-life of Cox-2 in COPD patients (69). Differential expression and/or binding of miR-146a and miR-146b have been implicated in a number of pathological conditions displayed in Table 2. Dysregulated expression of this miR has been associated with metastatic and proliferative response observed in a number of neoplastic disorders such as papillary thyroid carcinoma, cervical, ovarian and breast cancers (61). Elevated expression of miR-146a in tissues is also associated with chronic inflammatory diseases, such as psoriasis (81), rheumatoid arthritis (32, 53) and COPD (69).

miR-155

microRNA-155 (miR-155) represents a common target of a broad range of inflammatory mediators including TNF-α, LPS, polyriboinosinic:polyribocytidylic (Pi:PC) acid and IFN-β (56) (Table 1). The transcription factor c/ebp-β is a direct target of miR-155 (97). Silencing of miR-155 in murine macrophages, human monocytic cells, as well as in LPS-treated mice causes marked derepression of the c/ebp-β isoforms and down-regulation of granulocyte colony-stimulating factor (G-CSF). Overexpression of miR-155 in the THP1 monocytic cell line decreases PU.1 protein levels at both mRNA as well as protein levels (44). Using gain- and loss-of-function approaches, miR-155 was observed to repress src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP1) through direct 3′-untranslated region (UTR) interactions. Repression of endogenous SHIP1 by miR-155 occurred following sustained overexpression of miR-155 in hematopoietic cells both in vitro as well as

Table 1. miR-146, miR-155, and miR-21: activators and targets

<table>
<thead>
<tr>
<th>miR</th>
<th>Expression</th>
<th>Activators/Conditions</th>
<th>Cell/Tissue</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-146</td>
<td>up</td>
<td>A TLR4 ligands (e.g., LPS)</td>
<td>Thp-1, monocytic cell line</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>TLR-2, -4, or -5 ligands (e.g., bacterial and fungal components and proinflammatory cytokines)</td>
<td>myeloid cells</td>
<td>56, 86, 90</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>TNF-α or IL-1b #28</td>
<td>senescent cells</td>
<td>5, 14</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>IL-1 receptor signaling &amp; inflammatory cytokines associated with senescence-associated secretory phenotype (SASP)</td>
<td>myeloid cell differentiation</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>down</td>
<td>treated in vitro-differentiating HL-60 cells with high glucose (20 mM, 24 h) compared with low (5.6 mM) glucose</td>
<td>cultured human islets</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>IL-1β</td>
<td>A549 cells, BEAS2B cells and primary human airway epithelial cells macrophages</td>
<td>60</td>
</tr>
<tr>
<td>miR-155</td>
<td>up</td>
<td>inflammatory mediators [e.g., TNF-α, LPS, polyriboinosinic:polyribocytidylic (Pi:PC) acid and IFNβ]</td>
<td>mouse Raw 264.7 macrophages</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>LPS stimulation</td>
<td>mouse Raw 264.7 macrophages</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>ip injection with LPS</td>
<td>C57 BL6 mice</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>up (&gt;100-fold)</td>
<td>maturation of DCs</td>
<td>DCs</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>innate and adaptive immune responses</td>
<td>macrophages and B- and T-cells</td>
<td>87</td>
</tr>
<tr>
<td>miR-21</td>
<td>up</td>
<td>administration of aerosolized LPS to mouse lung</td>
<td>mouse lung</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>IL-6 induces the expression of miR-21 in a STAT3-dependent manner</td>
<td>multiple myeloma cells</td>
<td>39</td>
</tr>
</tbody>
</table>

LPS, lipopolysaccharide; DC, dendritic cell.

miR-21

miR-21 overexpressed in multiple cancer forms and tightly associated with cancerogenesis (30). Elevated expression of miR-21 in tissues is also associated with chronic inflammatory diseases, such as psoriasis (81), rheumatoid arthritis (32, 53) and COPD (69). miR-21 overexpressed in multiple cancer forms and tightly associated with cancerogenesis (30). Elevated expression of miR-21 in tissues is also associated with chronic inflammatory diseases, such as psoriasis (81), rheumatoid arthritis (32, 53) and COPD (69).

Table 2. miR-146, miR-155, and miR-21: dysregulation and health disorders

<table>
<thead>
<tr>
<th>miR</th>
<th>Expression</th>
<th>Disease/Pathology</th>
<th>Cells/Tissues</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-146</td>
<td>up</td>
<td>acute coronary syndrome (ACS) patients</td>
<td>peripheral blood mononuclear cells (PBMC)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>vesicular stomatitis virus (VSV) infection</td>
<td>macrophages</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>human memory T-cells compared to human naive T cells</td>
<td>T-cells</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>T-cell receptor (TCR) stimulation</td>
<td>human primary T-lymphocytes</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>human rheumatoid arthritis</td>
<td>synovial fibroblasts</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>Helicobacter pylori infection</td>
<td>gastric mucosa</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>LPS-induced septic shock and endotoxin shock</td>
<td>B-cells</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>lymphomas of activated-B-cell origin, including Hodgkin’s lymphoma and diffuse large cell B-cell lymphoma</td>
<td>B-cells</td>
<td>87</td>
</tr>
<tr>
<td>miR-155</td>
<td>up</td>
<td>overexpressed in multiple cancer forms and tightly associated with cancerogenesis</td>
<td>cancer cells</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>infarcted region of the ischemia-reperfused heart</td>
<td>cardiac fibroblasts</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>up</td>
<td>allergic airway inflammation</td>
<td>lung</td>
<td>40</td>
</tr>
</tbody>
</table>

1Ref, reference.
in vivo. Primary macrophages from miR-155 knockout mice also demonstrated repressed SHIP1 expression, unveiling a molecular link between miR-155 and SHIP1 (55). miR-155 directly targets transcript coding for several proteins involved in LPS signaling such as the Fas-associated death domain protein (FADD), IkB kinase epsilon, and the receptor (TNFR superfamily)-interacting serine-threonine kinase 1 (Ripk1) (90).

miR-155 has emerged as a central regulator of the immune system (81, 86). A potential role of miRNA-155 in the adaptive immune response was provided from studies using knockout mice (63, 88, 91). These miRNA-deficient animals displayed severe immunodeficiencies, particularly impaired B-cell responses and skewed Th2-helper T-cell responses (63, 88, 91). In addition to the deficiency in adaptive immunity, autoimmune phenotype in the lungs of miR-155 null mice was also observed (63). Increased airway remodeling and leukocyte invasion suggested that miR-155 plays a key role in regulating the response of the immune system to self-antigens (63). miR-155 is encoded within an exon of the noncoding RNA known as bic (B-cell integration cluster). To uncover the significance of miR-155 in regulating immune function in vivo, bic/miR-155 (bic) null mice were developed. These mice fail to generate high levels of class-switched antibody upon immunization with thymus-dependent and thymus-independent antigens. The bic-deficient T-cells show skewed differentiation into the Th2 lineage under a variety of in vitro conditions. Thus, miR-155 emerged as a central regulator of lymphocyte differentiation (91). Thai et al. (88) generated two mutant mouse strains with miR-155 deficiency. In the first strain, a major portion of the bic exon, including miR-155, was replaced by a β-galactosidase (lacZ) reporter, generating a bic/miR-155−/− bic null mice. The second mutant strain was generated using an established knock-in strategy. In these mice, miR-155, together with an enhanced green fluorescent protein (EGFP) reporter, was conditionally expressed in mature B-cells in a Cre-dependent manner. These approaches, using a combined genetic loss- and gain-of-function approach, demonstrated that miR-155 regulates specific differentiation processes in the immune response and that it exerts its functions at least partly by influencing cytokine production (88).

miR-21

miR-21 is likely one of the most studied miRs to date. miR-21 initially was described as an “oncomir” overexpressed in multiple cancer forms and tightly associated with cancerogenesis (Table 2). It has been commonly noted that inflammatory stimuli induce miR-21 (Table 1). A single primary transcript containing miR-21 (pri-miR-21) is transcribed from an evolutionarily conserved promoter that resides in an intron of an overlapping coding gene, TMEM49 (23). The putative miR-21 promoter region contains three AP1 and one PU.1 binding sites (23). Computational analyses predicted transcription repressor NFIB mRNA as a target for miR-21 and the miR-21 promoter itself contains a conserved binding site for the NFIB protein (23, 30). In silico analyses combined with experimental biology approaches have identified numerous target proteins whose expression is regulated by miR-21. Phosphatase and tensin homolog (PTEN) is a phosphatidylinositol-3,4,5-trisphosphate 3 (PI3P)-phosphatase that inhibits phosphoinositide-3-kinase (PI3K) pathway while dephosphorylating (PI3P) and thus prevents Akt activation (85). The first evidence that PTEN is a valid miR-21 target was reported in cholangiocarcinoma cell line Mz-ChA-1 (48). Using laser-capture microdissection technique we demonstrated that miR-21 signal was localized to cardiac fibroblasts of the infarcted region of the ischemia-reperfused heart. PTEN was identified as a direct target of miR-21 in cardiac fibroblasts (66). The tumor suppressor PDCD4 is a proinflammatory protein that promotes activation of the transcription factor NF-κB and suppresses interleukin 10 (IL-10). Transfection of cells with a miR-21 precursor blocked NF-κB activity and promoted the production of anti-inflammatory IL-10 in response to LPS. Transfection with antisense oligonucleotides to miR-21 or targeted protection of the miR-21 site in PDCD4 mRNA displayed the opposite effect (77). This study demonstrated that miR-21 regulates PDCD4 expression following LPS stimulation (77). In addition to PTEN and PDCD4, a number of other targets of miR-21 including tropomyosin (TPM1), sprouty1 and 2, TGF-β receptor (TGFBR2) Cdc25a have been validated (30).

AU-RICH ELEMENTS AND ARE-BINDING PROTEINS

The AU-rich elements (AREs), located in 3′-UTR of transcripts, are well-established determinants of RNA stability (19). AREs are prerequisite for regulating the half-life of many cytokines and are central to achieving temporal and spatial regulation of these genes (65). The ARE-binding proteins (ARE-BPs) regulate this process either by decay promotion/destabilization (e.g., tristetraprolin, TTP; AU-rich binding factor 1, AUF1) or by stabilization (e.g., Hu protein R, HuR) (4). Both TTP and HuR are predicted to be miRNA targets (4). TTP destabilizes the expression of class II AREs that are present in many proinflammatory growth factors and cytokines such as TNF-α and GM-CSF (4, 8).

WOUND INFLAMMATORY RESPONSE

Wound-induced inflammatory response constitutes one of the early events that determine the fate and quality of healing (18). Infiltrating leukocytes represent the principal cellular components of the inflammatory response. These cells not only fight infection but also deliver cytokines, chemokines, and growth factors, laying the foundation for tissue repair. Controlled influx of specific populations of blood-borne cells, including leukocytes, marks the early phase of healing (58). Understanding the mechanisms that regulate the inflammatory response in wound repair is the foundation that will help design innovative strategies to address dysregulated inflammation as noted in say chronic ulcers where resolution of inflammation is often impaired. In the following section, we describe how miRNA may regulate wound inflammation.

Chemokines, Cytokines, and Growth Factors

MCP-1. Following wounding, the CC chemokine macrophage chemoattractant protein (MCP-1/CCL2) is one of the major chemo-attractants for monocytes/macrophages. It also helps recruit a subset of T-cells and on mast cells carrying the CCR3 receptor (94). The expression of MCP-1 was highly upregulated (~70 fold) 12h following wounding.
been discussed in an earlier section. The regulation of IL-10 by miR-21 via PDCD4 has a half-life of IL-10 mRNA, which in turn elevates IL-10 expression by competitive binding to the 3′-UTR and variably impose a series of flexible rate-limiting controls to modify the abundance of the TNF-α mRNA and the rate of its translation in response to inflammatory signals 

TNF-α. TNF-α is a potent proinflammatory cytokine produced by activated macrophages known to drive the inflammatory response to wounding. Depending on the concentration, length of exposure, and presence of other cytokines, the effect of TNF-α can be beneficial or deleterious. TNF-α is involved in tissue remodeling, mounting and sustenance of inflammation, cachexia, shock, and cell death (68). Lowering of the functionally available levels of TNF-α, using anti-TNF-α therapy directed at managing activated macrophages, restores diabetic wound healing in ob/ob mice (25). miR-125b targets the 3′-UTR of TNF-α transcripts. Thus, LPS-induced downregulation of miR-125b may be instrumental in bolstering the production of TNF-α. In cells, TLR4 activation downregulates miR-125b expression (90). Also, TNF-α-mediated induction of endothelial adhesion molecules can be regulated by miRNAs that are induced by TNF-α. Specifically, E-selectin and ICAM-1 are targets of TNF-induced miRNAs, miR-31 and miR-17–3p, respectively (84). The posttranscriptional mechanisms gradually and variably impose a series of flexible rate-limiting controls to modify the abundance of the TNF-α mRNA and the rate of its translation in response to inflammatory signals (83). These posttranscriptional controls consist of signaling networks converging on RNA-binding proteins and miRNAs, which in turn target a code of secondary or tertiary ribonucleotide structures located on the TNF mRNA (83).

IL-10. IL-10 is recognized as a major suppressor of the inflammatory response (50). IL-10 downregulates the expression of proinflammatory genes. In fetal wounds, IL-10 plays a major role in subduing the expression of proinflammatory cytokines resulting in minimized matrix deposition and scar-free healing (36). An imbalance featuring increased levels of the proinflammatory cytokines TNF-α and IL-6 and a decreased level of IL-10 is noted in diabetic wounds. These observations point towards IL-10 insufficiency as being a key factor underlying the exaggerated and sustained inflammatory response commonly noted in diabetic wounds (33). Of note, IL-10 inhibits the LPS-inducible expression of miR-155 (47), while miR-21 or miR-146a remain unaffected. IL-10 inhibits the transcription of miR-155 from the BIC gene in a STAT3-dependent manner. Such inhibition of miR-155 upregulates the abundance of miR-155 target protein such as SHP1. Through its inhibitory effect on miR-155, IL-10 promotes the expression of anti-inflammatory genes (47). In macrophages stimulated with TLR ligand, miR-466l can upregulate both the miRNA and protein expression of IL-10 via competitive binding to the 3′-UTR contain AREs. The RNA-binding protein TTP mediates rapid degradation of IL-10 mRNA. Thus, the net effect of the binding of miR-466l to IL-10 AREs is to prevent IL-10 mRNA degradation mediated by TTP, resulting in extended half-life of IL-10 mRNA, which in turn elevates IL-10 expression (41). The regulation of IL-10 by miR-21 via PDPCD4 has been discussed in an earlier section.

TGF-β1 Signaling

As a physiological response to wounding, TGF-β1 is released in large amounts from platelets. It serves as a chemottractant for neutrophils, macrophages, and fibroblasts. These cell types further augment TGF-β1 levels in the wound environment via a feed-forward loop (94). In addition to the functionally active form, latent TGF-βs are also produced and sequestered within the wound matrix, allowing sustained release upon action of proteolytic enzymes. The TGF-β superfamily encompasses a diverse range of proteins, many of which play important roles during development, tissue homeostasis, disease processes, and repair (94). Signaling via active TGF-β is mediated by a heterotetrameric complex of two trans-membrane receptor serine/threonine kinases, consisting of a type II ligand binding receptor (TβRII) and a type I signaling receptor (TβRI) (93). Transcriptional regulators Smad2 and Smad3 are direct substrates of TGFβRI. Upon activation, the phosphorylated Smads2 and 3 undergo conformational change, which allows them to bind to cytoplasmic Smad4, a shuttle to the nucleus, resulting in induction of TGF-β gene expression (93).

Full-thickness incisional wounding in Smad3-null mice show accelerated closure, characterized by an increased rate of re-epithelialization and reduced inflammation (2), miRNA biogenesis by ligand-specific SMAD proteins is now known to control vascular smooth muscle cell phenotype (17). Smads play a regulatory role in the processing of miRNA into the nucleus (28). Receptor-activated Smads regulate the processing of a subset of miRNAs, particularly miR-21 (37). In breast cancer, miR-155 play an important role in TGF-β-induced epithelial-mesenchymal transition (EMT), cell migration, and invasion by targeting RhoA (34). Among miRNAs that directly target molecules in TGF-β signaling pathway, miR-128a negatively target TGFβRI protein expression by binding to the 3′-UTR region of the gene (45).

TLR Signaling

Inflammatory cells, including macrophages and neutrophils, recognize invading microbial pathogens primarily through TLRs (1). Based on the adaptor molecules recruited to the TLR intracellular domain after ligand engagement, the TLR-activated signaling events are largely defined as myeloid differentiation primary response gene 88 (MyD88)-dependent or TIR-domain-containing adapter-inducing IFN-β (TRIF)-dependent (57). MyD88-deficient mice exhibit severely impaired wound healing phenotype characterized by delayed granulation tissue formation and compromised blood vessel formation (42). The impairment of wound healing in these animals is not associated with increased complication caused by infection. Thus, in addition to the host pathogen response, signaling through the MyD88-dependent pathway regulates infection-independent processes in wound healing (42). MyD88 signaling involves recruitment and activation of IRAK4, IRAK1, and TRAF6, leading to the activation of the NF-kB, AP1, and MAP kinase pathways (57). Expression profiling of miRNAs in human monocyte cell line THP1 treated with LPS (TLR4 agonist) identified miR-146, miR-155, and miR-132 as LPS responsive (86). miR-146a negatively regulates TLR signaling via targeting TRAF6 and IRAK-1, IRAK2 (86). TLR-signaling responsive miRs-146a and 155 have been discussed elsewhere in this work.
**NADPH Oxidase and Reactive Oxygen Species Production**

In its molecular form, oxygen is required for oxidative metabolism-derived energy synthesis, protein synthesis, and the maturation (hydroxylation) of extracellular matrices such as collagen (73). In a wound setting, large amounts of molecular oxygen are partially reduced to form reactive oxygen species (ROS). Oxygen free radicals such as superoxide anion as well its nonradical derivative hydrogen peroxide (H₂O₂) are released during the inflammatory response (73). Among driving other key processes, ROS drives endothelial cell signaling required for successful angiogenesis. We provided first evidence demonstrating that miRNA may regulate ROS-driven redox signaling (78). Arrest of the miRNA biogenesis enzyme dicer led to lower inducible production of ROS in human endothelial cells when activated with phorbol ester, TNF-α, or vascular endothelial growth factor (78). NADPH oxidases represent a major source of superoxide anion radicals at the wound site. NADPH oxidases in phagocytic cells help fight infection. The p47phox component of NADPH oxidase complex has been identified as one of the targets of miRNA that regulates the ROS production and the angiogenic properties of endothelial cells (78). Microarray analysis of hormone refractory cell lines identified hsa-miR-128a as being hormone-responsive (45). Regulation of ROS by miR-128a via the specific inhibition of the Bmi-1 oncogene has been recently demonstrated (92).

**Lipid Mediators**

Lipid mediators such as eicosanoids consist of a family of biologically active metabolites, including prostaglandins (PG), prostacyclin (PC), thromboxanes (TX), leukotrienes (LT), and lipoxins (LX) (26). Free arachidonic acid may be metabolized through the COX pathway, involving COX-1 and COX-2, along with terminal synthases, to generate PG, PC, and TX. Eicosanoids are well known to initiate, amplify, and perpetuate inflammation in both acute as well as chronic wounds (7). The ω-3 polyunsaturated fatty acids eicosapentaenoic (EPA; i.e., ω-3, C20:5) and docosahexaenoic acid (DHA; i.e., ω-3, C22:6) are transformed, in a manner equivalent to arachidonic acid metabolism, by COX-2 and LOX enzymes to generate novel classes of endogenous lipid autacoids with anti-inflammatory and protective activities (26). Induction of COX-2 represents one of the earliest responses following cutaneous injury (58). Conserved ARE in the promoter of COX-2 gene has been identified (98). Rapid degradation of COX-2 mRNA has been attributed to AREs at 3’-UTR of COX-2 (46). ARE-BP HuR has been shown to bind the COX-2 3’-UTR and stabilize the transcript (46). miR-101a and miR-199a appear to control COX-2 expression in the mouse uterus during embryo implantation (9).

**Differentiation of the Inflammatory Leukocytes**

miRNAs play important roles in normal or malignant hematopoiesis, including the differentiation of hematopoietic stem cell (HSC), their self-renewal, and the function of immune cells (24). Early expression profiling studies demonstrated that the expression of specific miRNA such as miR-181, miR-223, and miR-142s were limited to cells of the bone marrow, spleen, and thymus (Fig. 3). Of these, miR-181 was limited to B-cells, whereas miR-223 expression was isolated to myeloid cells (11, 81). Despite its preferential expression in myeloid cells, ectopic expression of miR-223 in hematopoietic progenitor cells did not have a profound effect on myeloid differentiation (81). Later studies proved miR-223 to be an essential modulator of myeloid differentiation in human. Overexpression of miR-223 doubled the cells committed to the granulocyte-specific lineage in a granulocyte differentiation model, whereas knock-down of miR-223 had the opposite effect (20). miR-223 is involved in an autoregulatory feedback loop to control its own expression and enhance granulocytic differentiation (81). The relative levels of PU.1 and C/EBPα determine cell fate between mono-

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**Fig. 3.** Overview of major miRNAs in myeloid cell differentiation. This illustration summarizes the known changes in miRNA expression during differentiation of granulocytic and monocytic cells. DC, dendritic cell; HSC, hematopoietic stem cell; mφ, macrophage; MPC, myeloid progenitor cell.
cyte and granulocyte as end-products (62, 72). PU.1 activates the transcription of miR-424 stimulating monocyte differentiation through miR-424-dependent translational repression of NFIA transcription factor. Ectopic expression of miR-424 in precursor cells enhances monocyte differentiation. These data point underscore the significance of miR-424 in controlling the monocyte/macrophage differentiation program (64). Down-regulation of miRNAs 17-5p-20a-106a regulate monocytopoiesis through targeting of AML1 and upregulation of M-CSF receptor (21). miRNAs, specifically miR-21, miR-155, miR-424, and miR-17–92, and their transcriptional regulatory control are directly implicated in monocytopoiesis (71).

miRNA AS BIOMARKERS AND THERAPEUTIC TARGET IN INFLAMMATION

Circulating miRNAs as Prognostics and Therapeutic Biomarkers

Numerous biological molecules from tissues of the body can be found in human serum. The simplicity of obtaining a blood sample and ease of testing makes the research of finding specific biomarkers for prognostic/therapeutics purposes very tempting. A small number of serum-based biomarkers for a specific disease, such as alpha-fetoprotein (AFP) for liver cancer or C-reactive protein (CRP) for inflammation, have been used for diagnosis for several years (96). The potential of using circulating miRNA levels as diagnostic markers has been reported for cancers (70, 96). Studies have compared the expression levels of miRNAs in plasma or serum from cancer cases to healthy control populations. For example, miR-17-3p and miR-92 expression levels in plasma were found to be elevated in colorectal cancer patients (54, 70). Likewise, in the sera of lung cancer patients, miRNA profile was significantly different compared with healthy subjects, with 28 miRNAs missing and 63 new mRNA species detected. Two highly expressed miRNAs in lung cancer, miR-25 and miR-223, were analyzed by qRT-PCR and confirmed for their ability to serve as blood-based biomarkers for lung cancer in an independent trial of 75 healthy donors and 152 cancer patients (12). miRNAs have been suggested to have high value as biomarkers because of highly sensitive PCR detection methods and low complexity compared with protein biomarkers (96).

miRNA in Therapeutics

The potential to therapeutically regulate miRNA levels at the accessible skin wound site make miRNA-based therapies attractive in wound care. miRNA-based therapies may be also used to address chronic inflammation. To date, the main RNA inhibition agents used in preclinical and clinical studies include antisense oligonucleotides, ribozymes, and the DnAZymes, small interfering RNAs and short hairpin RNAs, and anti-miRNA agents such as antisense oligonucleotides, locked nucleic acids, and antagonims (99). At present several clinical trials are under way to test the therapeutic efficacy of miRNA-based therapies. In 2011, several such trials evaluating efficacy for cancer treatments are expected to be launched (70). Within the next several years, it is anticipated that miRNA-based therapeutics, alone or in combination with other modalities, will be clinically useful treatments for various cancers and inflammatory disorders (70).

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miRNA IN WOUND INFLAMMATION


