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Mulholland, E., Dunne, N., & McCarthy, H. (2017). MicroRNA as Therapeutic Targets for Chronic Wound Healing. *Molecular Therapy: Nucleic Acids*, 8, 46-55. DOI: 10.1016/j.omtn.2017.06.003

Published in:

Molecular Therapy: Nucleic Acids

Document Version:

Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:

[Link to publication record in Queen's University Belfast Research Portal](#)

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MicroRNA as Therapeutic Targets for Chronic Wound Healing

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Wound healing is a highly complex biological process composed of three overlapping phases: inflammation, proliferation, and remodeling. Impairments at any one or more of these stages can lead to compromised healing. MicroRNAs (miRs) are non-coding RNAs that act as post-transcriptional regulators of multiple proteins and associated pathways. Thus, identification of the appropriate miR involved in the different phases of wound healing could reveal an effective third-generation genetic therapy in chronic wound care. Several miRs have been shown to be upregulated or downregulated during the wound healing process. This article examines the biological processes involved in wound healing, the miR involved at each stage, and how expression levels are modulated in the chronic wound environment. Key miRs are highlighted as possible therapeutic targets, either through underexpression or overexpression, and the healing benefits are interrogated. These are prime miR candidates that could be considered as a gene therapy option for patients suffering from chronic wounds. The success of miR as a gene therapy, however, is reliant on the development of an appropriate delivery system that must be designed to overcome both extracellular and intracellular barriers.

Introduction to Wound Healing

The largest organ of the human body is the skin; it accounts for approximately 15% of total body weight in adults.^{1,2} The skin acts as a protective barrier against the environment and helps to prevent dehydration. The skin has three distinct layers: (1) epidermis, composed mainly of keratinocyte cells; (2) dermis, which is a collagen-rich tissue containing blood and lymph vessels, fibroblasts, macrophages, and neutrophils; and (3) hypodermis, which is constructed mainly of adipose tissue.³ The development of these layers is a highly coordinated process involving complex gene regulatory networks acting in a temporally resolved integrated way.

A wound is defined as *all manner of tissue damage resulting in the disorder of the original tissue structure and homeostasis*.⁴ The mechanisms involved in the repair of wounds involve some of the most complex biological processes.⁵ The wound healing process in the skin involves three partially overlapping phases: (1) blood clotting and inflammation, (2) new tissue formation, and (3) tissue remodeling

(the longest phase).^{6,7} These processes result in the wound tissue being repaired within a relatively short period. The scar tissue, however, has reduced tensile strength compared to that of healthy skin tissue,⁷ demonstrating approximately 70% of the tensile strength of healthy tissue.⁸

Chronic Wounds

Chronic wounds are the result of multifactorial components within the wound healing process becoming compromised. They can be defined as *wound that is not continuously progressing toward healing⁹ or not healing in a methodical and timely fashion*.⁸ Pathologically, one of the clearest indicators of a chronic wound is their failure to re-epithelialize.¹⁰ The aging population are among those most burdened with chronic wounds, and with low keratinocyte cell turnover, this is a factor affecting their ability to re-epithelialize.¹¹ In general, chronic wounds exhibit reduced mitogenic activity compared to acute wounds, which has been demonstrated not only in keratinocytes but also in skin fibroblasts.¹² It has been shown that fibroblast cells isolated from Venus leg ulcers have reduced cell migration properties in comparison to cells isolated from regular skin.¹²

Overall, factors affecting healing ability are categorized as either local and/or systemic influences. Local refers to causes that directly affect the characteristics of the wound site itself and systemic factors related to the general overall health or disease state of the individual, which affects healing ability. As such, the following sections detail diabetes and infection as examples of a systemic and local factor, respectively, which can influence a wound to become chronically impaired. Differences in microRNA (miR) expression will also be explored within the sections [miR and Wound Healing](#) to [miR Expression in Chronic Wounds](#).

<http://dx.doi.org/10.1016/j.omtn.2017.06.003>.

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Diabetes

Diabetes is a systemic factor that can be linked to wound healing and affects approximately 3.2 million people in the UK, which accounts for 6% of the population.¹³ This population is at risk of developing chronic non-healing diabetic foot ulcers. The increase in serum glucose has a significant influence on wound healing due to destructive effects showcased by hyperglycemia on the physiology of the affected cells.¹⁴ For example, sorbitol, a by-product of glucose metabolism, can accumulate within tissues, resulting in renal and vascular complications.¹⁵ Hypoxic regions are prevalent in diabetic wounds and are characterized by an absence of endothelial cells, resulting in reduced angiogenesis and elongation of the inflammatory phase. Diabetic mice models have shown decreased restoration of vasculature in diabetic wounds, with hindered endothelial progenitor cell mobilization and homing and reduced levels of VEGF, compared to a non-diabetic controls in vivo.¹⁶

Non-enzymatic glycosylation associated with hyperglycemia also inhibits the function of structural and enzymatic peptides.¹⁶ Diabetic animal models of wound healing show decreased rates of granulation. Likewise, in humans, the findings were comparable, showing slow wound maturation and a lower count of fibroblast cells compared to non-diabetic wound sites.¹⁴

Several cellular functions are affected in diabetic wounds, such as defective T cell immunity, leukocyte chemotaxis, phagocytosis, bactericidal capacity, and dysfunctions of fibroblasts and epidermal cells. Taken together, such defects contribute to inadequate clearance of infection and delayed healing.¹⁷

Obesity is intrinsically linked to diabetes (type 2) and impaired wound healing. By 2050 in the UK, obesity is projected to affect 60% of adult men, 50% of adult women, and 25% of children.¹⁸ Obese patients have been reported to experience wound complications more frequently in relation to post-surgery healing, such as higher rates of infection.¹⁹ This has been linked to ischemia and hypo-perfusion in the subcutaneous adipose tissue. Skin folds also harbor microorganisms that thrive in moist areas and contribute to infection and tissue breakdown.⁶

Infection is an example of a local factor that is linked to impaired wound healing. Virtually all open wounds experience colonization of microorganisms, which typically has no clinical consequence because no infection is evident. However, infection within a wound site can influence the healing process by prolonging the inflammatory phase. This is due to incomplete clearance of the wound site. Therefore, there is prolonged elevation of cytokines (e.g., interleukin-1 [IL-1] and tumor necrosis factor alpha [TNF- α]), which are pro-inflammatory.²⁰ If the bacterial count within the wound is more than 105 colony-forming units per gram (CFUs/g) of tissue, the wound will not heal, irrespective of the treatment regime (e.g., skin graft placement and primary sutures).²¹ Furthermore, no healing will occur if the β -hemolytic strain of *Streptococcus* is present at the wound site, even at less than 105 CFUs/g.²²

Bacterial biofilms are a huge hurdle to overcome in the healing process for chronic wounds. A biofilm is an intricate community of microorganisms, characterized by massive cell densities that are accompanied by an extracellular polymer matrix, made mainly of polysaccharides and proteins.²³ This matrix is multifunctional, acting both as a physical protector from biological and pharmaceutical antimicrobials. Further to this, it facilitates the adhesion of bacteria cells to surfaces, particularly foreign bodies.

Current Treatments

Gauze has been historically popular for wound dressings. However, the use of gauze promotes desiccation of the wound base, which is not advantageous for efficient healing.²⁴ Furthermore, dry gauze often adheres to the surface of the wound, which can be painful for the patient upon removal. Such gauze dressings are susceptible to complete saturation of wound fluid and so can be ineffective in the protection against bacterial invasion.^{24,25} Films are alternatives to gauze dressings and can be utilized as multifunctional adhesive dressings, as in they can be used as primary dressings directly on the wound or alternatively as a secondary dressing to secure other primary dressings to the wound. Film membranes are thin and semi-permeable, enabling the exchange of oxygen and water vapor to the wound in addition to preventing liquid and microorganism contaminants.

Topical antiseptic and antibiotic treatments can be employed to reduce the bioburden of the wound. Limitations associated with topical antiseptics and antibiotics include toxicity to human tissue that can lead to prolonged inflammation and microbial resistance, in addition to the necessity for many reapplications to achieve the desired effects.²⁶ Acetic acid is an example of a topical treatment typically administered at concentrations of between 0.25% and 1.0% (v/v). It is effective against most gram-positive and gram-negative organisms. Acetic acid is an inexpensive product; however, it can cause cytotoxicity in vitro and has limited activity against biofilms. The latter is a particular problem associated with chronic wounds.^{27,28}

The management and treatment of chronic wounds is influenced by many multifactorial elements; however, the current modes of treatments and dressing are largely inadequate for chronic wounds. Therefore, miR replacement or inhibitory therapy is an alternative mode of treatment because miR has the potential to affect an array of downstream gene targets.

miR

miRs are instrumental in the regulation of gene expression through the promotion of mRNA degradation and inhibition of mRNA translation. Intronic miRs are encoded within a gene transcript precursor, sharing the same promoter with the encoded gene transcripts.²⁹ Unidentified promoters transcribe intergenic miR, located in the non-coding regions. Finally, polycistronic miRs derived from primary transcripts contain a diverse range of hairpins, giving rise to different miRs.³⁰

The process of miR biogenesis (Figure 1) is a highly complex process. In mammalian cells, miRs are transcribed by RNA polymerase II.³¹

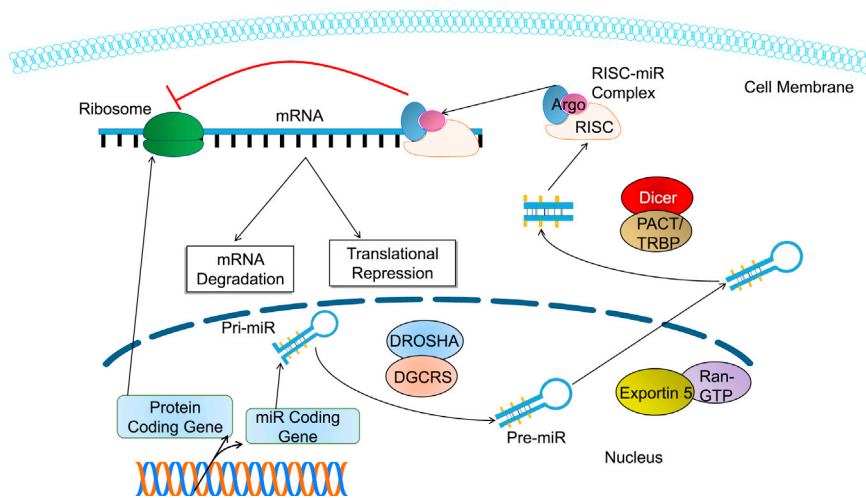


Figure 1. Biogenesis of miR

Biogenesis of miR and the resulting post-transcriptional gene silencing mechanisms. Pri-miR is transcribed within the nucleus and then cleaved by the DROSHA complex to form pre-miR. Exportin 5 transports this pre-miR into the cytoplasm of the cell. There it is further sliced to form mature miR. This miR then binds with the RISC to form a RISC-miR complex, which acts as a translational repressor and degrades mRNA.

vided the optimal miR can be identified, new therapies could be developed.

miR and the Inflammatory Phase

Inflammatory response and resolve is paramount to successful and rapid wound healing.

Due to the tight regulation of pro-inflammatory and anti-inflammatory signaling, disruption of miR biogenesis may result in an imbalance of these signals adversely affecting the healing cascade. Evolving studies demonstrate that miR-21, miR-146a/b, and miR-155 play key roles in regulating the inflammatory process. miR-146 and miR-155 are promoted by TNF- α and IL-1 β , and miR-146 has been shown to silence interleukin-1 receptor-associated kinase 1 (IRAK) and cyclooxygenase-2 (COX2), whereas miR-155 silences Src homology 2 domain-containing inositol 5-phosphatase (SHIP1), suppressor of cytokine signaling 1 (SOCS1), and IL-12.^{36,37}

miR and the Proliferation Phase

Proliferation of keratinocyte cells is critical for re-epithelialization of the wound, ergo miR regulation is vital. For this process to be efficient and progress rapidly, an abundant oxygen and nutrient supply is required and angiogenesis is key. Keratinocytes have been shown to migrate faster upon silencing of SHIP2 and the enhanced activation of the serine-threonine protein kinase AKT signaling pathway.³⁸ miR-205 has been shown to suppress SHIP2, a negative regulator of the AKT pathway and enhance cofilin expression by a reduction in the regulation of phosphor-cofilin, which increases cell motility.³⁸ Another important miR is miR-21, which has been shown to be an inhibitor of phosphatase and tensin homolog PTEN. PTEN inhibits the AKT pathway, thereby activating many cell survival and proliferative pathways.

miR and the Remodeling Phase

An important aspect of this phase of wound healing is the deposition of collagen. It has been observed that miR-29a has a direct influence on the expression of collagen at a post-translational level.³⁹ Fetal skin from mammals can heal without scarring. However, during advanced gestation, the transition is to a scarring phenotype.⁴⁰ miR-192, miR-29b, and miR-29c are highly induced in the later phase of this process. Transforming growth factor β (TGF- β) (anti-fibrotic) and proteins such as SMADs that are involved in the pathways for scar-free healing are targets of miR-29b/c. miR-192 also enhances collagen 1- α -2 expression through the targeting of SMAD-interacting protein 1 (SIP1).^{34,41}

Primary miR (pri-miR) is cleaved by the RNase III intra-nuclear enzyme (i.e., Drosha) into many miR precursors (pre-miRs).³² Drosha and DGCR8/Pasha establish the microprocessor complex, which cuts the pri-miR at the ssRNA/dsRNA junction found at the base of the pri-miR hairpins and results in pre-miR production.³¹ The resulting pre-miRs are ~65 nt in length, with characteristic stem-loop hairpin secondary structures.³³ The pre-miR is bound by Exportin 5 in the presence of the co-factor Ran-guanine triphosphatase (Ran-GTP) and is transported to the cell cytoplasm. Once the pre-miR has moved to the cytoplasm, a second RNase III enzyme (i.e., Dicer) processes the pre-miR with the help of several co-factors (e.g., double-stranded RNA-activated protein kinase [PACT] and TAR RNA-binding protein [TRBP]).³⁴ Processing by Dicer leads to a mature miR strand and an opposing strand that degrades. Thus, the miR is incorporated into the RNA-induced silencing complex (RISC).³⁵

miR Function

miR modulates gene expression through association of an Argonaute protein, which loads the miR into the RISC at the 3' end.³⁵ The incorporated miR guides the RISC to its specific mRNA target through base-pair complementarity, which results in the disruption of translation.

Depending on the grade of complementarity between the target mRNA and the miR, two different mechanisms of RISC-mediated gene regulation may ensue. In the case of almost complete complementarity, the RISC cleaves the target mRNA, preventing translation.³³ In the case of imperfect complementarity, translation is suppressed by miR decapping and/or deadenylation (Figure 1).³¹

miR and Wound Healing

Several studies have demonstrated that miR expression is upregulated or downregulated during the overlapping phases of wound healing. Table 1 details key miRs that have been found to be instrumental during the different phases of wound healing. Therefore, pro-

**Table 1. miR Involved in the Normal Wound Healing Process**

	miR	Targets	Reference
Pro-inflammatory	miR-155	SOCS1, SHIP1, IL-12	34,65
	miR-140	PDGF	66
	miR-16	COX2	67
Anti-inflammatory	miR-105	TLR2	68
	miR-21	PDCD4, PTEN	34,69
	miR-125b	TNF- α	70,71
	miR-223	Mef2c	72
	miR-203	TNF- α , IL-24	73
	miR-146a,b	TRAF6, IRAK1, STAT1, TNF- α , COX2	34,71,74
	miR-21	TIMP3, TIAM1	43
Proliferation	miR-31	EMP-1	49,50
	miR-155	KGF, FGF-7	75
	miR-99	IGF1R, mTOR, AKT1	76
	miR-198	DIAPH1, PLAU, LAMC2	77
	miR-184	AKT	38,78–80
	miR-205	SHIP2, Rho-ROCK1	38
	miR-203	RAN, RAPH1	81
	miR-210	E2F3, ISCU1, ISCU2	82
	miR-483-3p	MK2, MK167, YAP1	83
	miR-17-5p	TIMP1	34
Pro-angiogenic	miR-17-92	TSP-1, CTGF	51,84,85
	miR-31	FIH-1, Spred1	86,87
	miR-126	Spred1, PIK2R2	79,88,89
	miR-130a	GAX, HOXA5	90
	miR-210	EFNA3	91,92
	miR-296	HGS	93
	miR-378	Fus-1, Sufu	94
	miR-424	CUL2	47
	miR-92a	Integrin- α 5	34,95
	miR-17	JAK1	96
Anti-angiogenic	miR-15b	VEGF	97
	miR-16	VEGF	97
	miR-20a	MKK3, VEGF	97,98
	miR-20b	HIF-1 α , VEGF	97,99
	miR-221	c-kit	100,101
	miR-222	c-kit	93,94
	miR-320	IGF-1	102,103
Remodeling	miR-503	CCNE1,cdc25A	104
	miR-29a	TAB-1, collagen I and II	39,105
	miR-29b	SMADs, β -catenin	106
	miR-29c	SMADs, β -catenin	107
	miR192/215	E-cadherin, SIP1	41,108

miR Expression in Chronic Wounds

It has also been observed that many miRs expressed during the normal wound healing process are dysregulated during chronic wound healing.³⁴ A recent study investigating the expression of miRs in rodents induced with diabetes showed that 18 miRs were up-regulated and 65 miRs were downregulated compared to control wound healing groups (i.e., non-diabetic rodents). Therefore, many different miRs could be used as a therapeutic in the healing of chronic wounds.⁴² On the basis of the amalgamation of these observations, the following sections explore several specific miRs and show how they could be exploited for advanced wound healing applications.

miR-21

Murine studies have demonstrated that miR-21 plays an important role in keratinocyte migration and re-epithelialization during wound healing. miR-21 has been found to be upregulated during wound healing, coincident with TGF- β temporal expression. Consistently, knockdown of endogenous miR-21 using a specific antagomir dramatically delayed re-epithelialization, possibly due to the reduced keratinocyte migration. Likewise, Yang et al.⁴³ found that through the utilization of wound scratch healing assays, transfection with miR-21 significantly increased keratinocyte cell migration by 62% compared to scrambled controls. TIMP3 and TIAM1 are regulated by miR-21 in vitro and in vivo. TIMP3 inhibits the activation of the EDK pathway with downstream activation of ERK1/2, thus promoting proliferation. Therefore, it can be postulated that miR-21 promotes keratinocyte migration and boosts re-epithelialization during skin wound healing.

miR-21 has also been found to be a key regulator of vascular smooth muscle cell proliferation and apoptosis via BCL2 activation and PTEN suppression. It also regulates MMP-2 via the PTEN pathway as a result of myocardial infarction.⁴⁴ Inhibition of the PTEN pathway also activates many downstream targets, such as mTOR, which regulates protein synthesis, glucose homeostasis, autophagy, and proliferation (Figure 2).⁴⁵ This suggests that introduction of miR-21 within the diabetic chronic wound site could have potential as a therapeutic by increasing the extent of cell migration.⁴⁶

miR-424

In normal wound healing, miR-424 is upregulated in response to hypoxic conditions (Figure 3).⁴⁷ It works through the stabilization of transcription factor HIF- α . In normoxic conditions, oxygen targets HIF for degradation by post-translational hydroxylation at specific prolyl residues domains (PHD) within these subunits. This increases the affinity for the ubiquitin ligases for proteolytic destruction by the ubiquitin/proteasome pathway. The oxygen-dependent hydroxylation process, however, is suppressed during hypoxia, leading to stabilization of HIF-1 α and subsequent attachment to its constitutive partner HIF-1 β to induce transactivation. Stabilization of HIF has been classically known to induce the transcription of coding genes (e.g., vascular endothelial growth factor [VEGF], erythropoietin, and nitric oxide synthase-2).⁴⁸

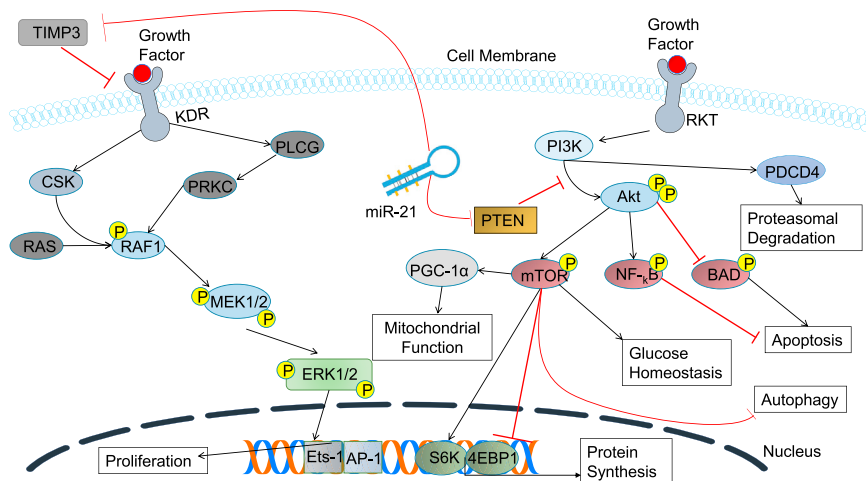


Figure 2. Therapeutic Wound Healing Targets of miR-21

Schematic diagram of miR-21 targets TIMP3 and PTEN. By inhibiting PTEN, miR-21 promotes the activation of many cell survival pathways. The Akt pathway functionality increases; thus, the mTOR pathway, for example, is heightened, which regulates protein synthesis, mitochondrial function, and glucose homeostasis. $\text{NF-}\kappa\text{B}$ inhibits apoptosis and Akt inhibits BAD, which is a pro-apoptotic pathway.

With regards to miR-424, this results in transactivation genes that govern angiogenesis and metabolic pathways. miR-424 cleaves to the CUL2 3' UTR and inhibits CUL2 expression. A drop in CUL2 levels leads to the destabilization of VCBCR U3-ligase complex, which leads to the stabilization and nuclear translocation of HIF-1 α in endothelial cells. In vitro wound scratch assays found that miR-424 significantly increased cell migration, with endothelial cells transfected with miR-424, decreasing the wound width by 40% compared to a 16% decrease in the control group. Furthermore, angiogenesis tubular formation assays with endothelial cells transfected with miR-424 also showed increased tube establishment.⁴⁷ This miR (mu-miR-322), which is the rodent homolog of miR-424, has been demonstrated to downregulate in a diabetic rodent model.⁴² However, in rodent models with ischemia, it has been shown that this miR is significantly upregulated.⁴⁷ If there are prolonged periods of hypoxia, the natural response of cells is the initiation of apoptosis. Angiogenesis is crucial for rapid wound healing because it provides

nutrients and oxygen to the wound site. With angiogenesis being compromised in patients with diabetes, it is crucial that it is stimulated to progress healing. Furthermore, fibroblasts that are exposed to prolonged periods of hypoxia may not participate in the formation of extracellular matrix (ECM), which again causes delays in healing. miR-424 is, therefore, a promoter of angiogenesis, validated by in vitro and in vivo rodent models.

miR-31

Human wound healing studies have demonstrated that miR-31 upregulation occurs in keratinocytes at the wound edge during proliferation. It has also been shown that overexpression of miR-31 increases proliferation, leading to an increased rate in re-epithelialization. Given that a low rate of re-epithelialization is typical of chronic wounds, miR-31 may be able to aid in the rapid closure of the wound.⁴⁹

Both TGF- β 1 and TGF- β 2 have been found to be upregulated in wounds and promote keratinocyte migration. TGF- β 1 and TGF- β 2 receptor binding results in the activation of downstream SMAD proteins, which in turn induce the expression of TGF- β

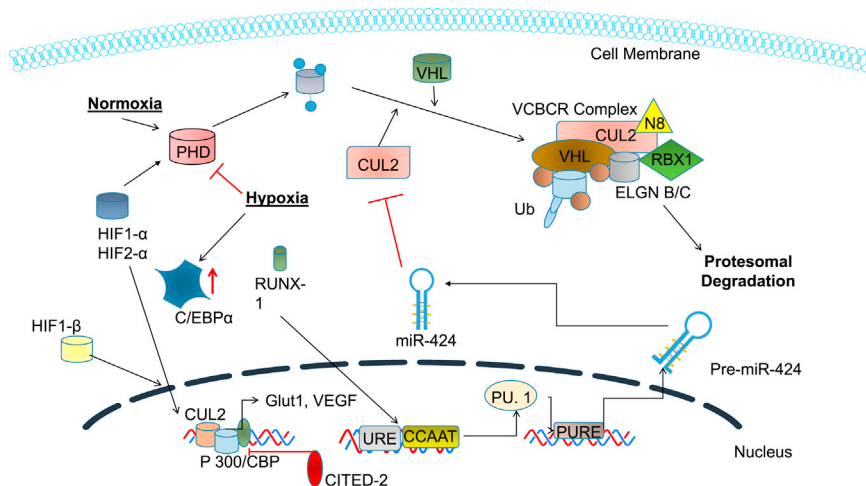


Figure 3. Therapeutic Wound Healing Targets of miR-424

Schematic diagram of miR-424 targeting CUL2. By targeting CUL2, the levels on HIF-1 increase within the cell and its proteasomal degradation is inhibited. Elevated HIF-1 increases the transcription of VEGF and Glut1.

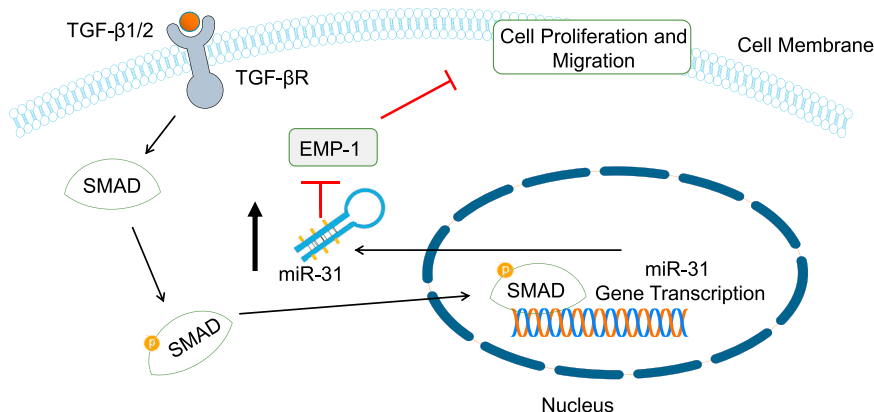


Figure 4. EMP-1 as a Therapeutic Wound Healing Target of miR-31

Schematic diagram of miR-31 targeting EMP-1. EMP-1 inhibits migration and proliferation of cells; thus, its inhibition will result in an increase of these functions.

target genes. Epithelial membrane protein-1 (EMP-1) has been identified as a suppresser of keratinocyte cell proliferation and migration (Figure 4). miR-31 expression is regulated by TGF-β2 and is a direct target of EMP-1. Silencing of EMP-1 expression results in a decreased level of miR-31 in keratinocytes; this suggests that a high level of EMP-1 during inflammation may trigger miR-31 expression.⁴⁹

Another target of miR-31 is factor-inhibiting HIF-1 (FIH-1) (Figure 5), which is an asparaginyl B-hydroxylase enzyme. It has been shown that FIH-1 regulates HIF-1 and subsequently VEGF, thus having a direct effect on the rate of angiogenesis. Upregulation of miR-31 would therefore increase the levels of VEGF through the silencing of FIH-1.⁵⁰

Furthermore, it has been demonstrated that miR-31 is downregulated in wounds within the diabetic rodent model.⁴² Taken together, all the evidence indicates that delivery of miR-31 is a candidate therapy for the inflammatory and proliferative phases of wound healing.

diabetic wounds.^{52,53} In vivo studies using eNOS knock-out mice have demonstrated delayed closure of wounds⁵⁴ and a reduced rate of angiogenesis.⁵⁵ Thus, the silencing of miR-221 and miR-222 could serve as a potential therapeutic to target infection in chronic wound healing.

Modes of Upregulating/Downregulating miR

For the introduction of miR into cells, many types of cargo could be utilized, for example, plasmid DNA coding for specific miRs to upregulate expression or antagomirs for silencing of expression.^{56,57} For this, numerous genetic delivery systems are currently utilized in research, with viral vectors being the most efficient.⁵⁸ However, there are apprehensions surrounding viral vectors as genetic carriers due to mutagenesis, toxicity, and its limited capacity for genetic cargo.⁵⁹ Examples of non-viral options include polymers of cationic nature, liposomes, and peptides, which exhibit the ability to not only package genetic cargo, but also deliver it to the nucleus of cells.⁶⁰ Liposomes are composed of a membrane composed of lipids, in which nucleic acid can be encapsulated within. Liposomes can take three forms: anionic, neutral, and, cationic, with cationic being the most frequently

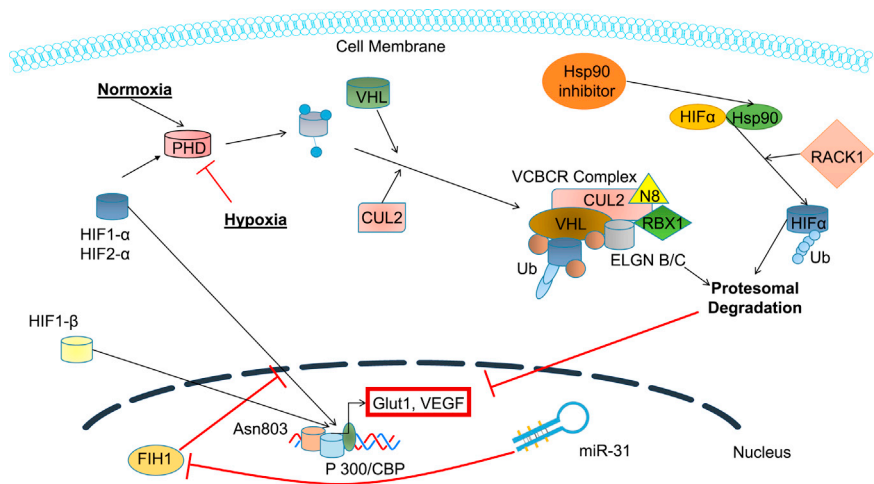


Figure 5. FIH-1 as a Therapeutic Wound Healing Target of miR-31

Schematic diagram of miR-31 targeting FIH-1. Thus, increasing the levels of HIF-1 within the cells. This leads to an increase in the levels of VEGF, thus increasing angiogenesis.



utilized for nucleic acid delivery due to its efficacy in interacting with cell membranes.⁶¹ There are many commercial options of cationic liposomes, such as Lipofectamine RNAi-MAX (Invitrogen)⁶² or SiPORT (Invitrogen).⁶³ In vitro assays looking at the efficiency of liposomes at transfecting non-small-cell lung cancer cells with miR-29b found that there was a 5-fold increase in their expression compared to non-treated cells, validating their power as an miR delivery system.⁶⁴ For wound healing, the ideal non-viral delivery vector must be non-immunogenic and non-toxic so as not to compromise the already delicate wound tissue.

Conclusions

Utilization of miR presents an attractive proposition for the development of therapeutic targets that could act on various pathways associated with chronically impaired wound healing. With varying levels of many miRs during the different phases of wound healing, there are several possible targets that could be employed.

Examples of prime miR candidates for wound healing include miR-21 and miR-31. miR-21, a target for the proliferation and inflammatory phase, could stop inflammation by targeting PDCD4 and promote proliferation and cell survival by activation of the mTOR pathway. miR-31 targets F1H-1; this increases the levels of VEGF intracellularly, which is a key promoter of angiogenesis. A significant advantage of using miRs such as these is that they can be detected in blood serum. With dose and response correlation being a prevalent issue within gene therapy options, detection of miRs as biomarkers in serum will provide translational information with regards to the successful delivery of the therapeutic.

However, the rate-limiting factor in the implementation of an miR therapy is a delivery system. This delivery system must be designed to overcome extracellular and intercellular barriers to ensure the successful delivery of the therapeutic cargo to the desired targets without evoking an immune response. Because it is of principal importance to maintain an optimum healing environment, it would be prudent to incorporate a gene therapy delivery system as part of a dressing instrument. Adopting such a system could confer multi-functionality with the dressing, ensuring a moist and protected micro-environment in addition to the efficacious delivery of the miR gene therapy. It can be rationalized, therefore, that miR holds the potential to become a momentous third-generation nucleic acid therapeutic for the treatment and management of chronic wounds, provided the optimal targeting and delivery system can be designed.

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