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Deviations in MicroRNA-21 Expression Patterns Identify a Therapeutic Target for Diabetic Wound Healing

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Abstract

Chronic inflammation plays a major role in impaired healing of diabetic wounds. Mounting evidence highlights the role of controlled, sequential polarization of macrophages in producing the appropriate progression through the stages of wound healing: inflammation (pro- inflammatory stage), proliferation and remodeling (regenerative stage). Non-coding RNAs, including microRNAs, maintain critical roles in regulating normal biological processes, such as wound healing; and are being explored as therapeutic targets for modulating dysfunction in disease states. Interestingly, microRNA-21 (miR-21) has a suggested role in the induction of pro-inflammatory and regenerative stages of healing, but clarity remains elusive on the specific mechanisms determining the direction miR-21 shifts wound healing processes. Findings by Liechty et al. in International Journal of Molecular Science indicate an important role of miR-21, in shaping the wound healing cascade by preferentially inducing M1-like (pro-inflammatory) polarization of macrophages in the early phase of diabetic wound healing. Persistent elevation of miR-21 is suggestive of sustained pro-inflammatory drive, and subsequent wound healing impairment, in the skin of diabetic murine models and diabetic human skin. Differences in the expression patterns of miR-21 during diabetic wound healing identifies the potentially critical role of therapeutic timing, for miR-21 based therapies, in driving positive outcomes for patients.

Keywords: Diabetes • Wound healing • Inflammation • miR-21• Hyperglycemia

Introduction

Normal wound healing progresses sequentially through inflammatory, proliferation and remodeling stages. During the inflammatory stage, M1 (pro-inflammatory) macrophages dominate and are critical to the clearance of pathogens. During proliferative and remodeling stages, macrophages transition to the M2 (pro-remodeling) phenotype and serve to resolve inflammation and repair the wound. Impaired wound healing in diabetes is marked by persistent M1 macrophages and unresolved inflammation, leading to delayed repair. MicroRNAs (miR) post-transcriptionally modulates gene expression with varying downstream effects, including the progression or resolution of each wound healing stage [1]. Exploration of miR-21 has identified roles in both the pro-inflammatory and pro-regenerative stages of wound healing [2-8]. Liechty et al. in International Journal of Molecular Science explored the role of miR-21 in macrophage polarization and persistent inflammation in diabetic wounds [6]. A key finding was observation of differing expression patterns of miR-21 between diabetic and non-diabetic wounds. In non- diabetic wounds miR-21 expression peaked at day 7 (post-injury) and returned closer to baseline by day 14. In diabetic wounds, miR-21 expression steadily increases, remaining significantly higher than non-diabetic expression at day 21. Additionally, the article reported that miR-21 expression can induce M1-like macrophages and contribute to the production inflammatory-mediators. This work identified miR-21 as a potentially important target for genetic-based therapeutics, as inhibiting miR-21 in early stages of wound healing could assist in resolving persistent inflammation and tissue injury.

Hyperglycemia and subsequent miR-21 expression induces proinflammatory-like macrophages

Hyperglycemia and poorly regulated glucose metabolism favors an inlammatory microenvironment by (1) increasing activation of NF-kB, resulting in greater expression of inflammatory cytokines (IL1-▪, TNF▪, IL-6, etc); and (2) enhancing oxidative stress through increased production of Reactive Oxidative Species (ROS) [1,2]. Both contribute to M1 macrophage polarization, which additionally drives inflammation through further production of inflammatory cytokines [9-11]. Liechty et al. found that hyperglycemia increases expression of miR-21. Increased expression of miR-21 was identified as a positive inducer of M1-like macrophages, as evidenced by increased production of M1 marker genes (IL1-▪, TNF▪, IL-6, iNOS) [6]. Overexpression of miR-21 induced NOX2 expression [6], a gene previously linked to macrophage production of ROS [10]. These findings identify miR-21 a signal transducer of hyperglycemia and regulator of the inflammatory response. In normal wound healing, drivers of inflammation eventually subside. Chronic elevation of blood glucose and dysregulation of glucose metabolism induces a chronic "injury" signal, which may contribute to persistent elevation miR-21 and inflammation through prolonged M1 polarization subsequent production of inflammatory cytokines and ROS; leading to stalled wound healing.

Diabetic wounds are marked by unresolved elevation of miR-21 expression

In non-diabetic wounds, the initial phase of wound healing involves activation of M1 macrophages and release of inflammatory cytokines to clear debris and pathogens from the site of tissue injury [11]. This is a critical, normal process that transitions to a pathologic state when it persists unmitigated, as seen in diabetic wounds. Hyperglycemia produced overexpression of miR-21, which has been demonstrated to induce M1-like polarization of macrophages and release of inflammatory cytokines and ROS [6,7]. Conversely, miR-21 has also been identified as a mediator in the resolution of inflammation and promotion of M2 macrophage phenotype transition [12]. Combined, these findings suggest that depending on conditions (microenvironment, expression levels, etc.) miR-21 can either lead to enhanced inflammation or resolved inflammation and have been

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identified as a potential "switch" between the pro- inflammatory and pro-regenerative stages of wound healing [4]. Data reported by Liechty et al. demonstrated that though diabetic wounds had persistent elevation of miR-21, it did not achieve the peak level observed in non-diabetic wounds prior to the return near baseline [6]. It is possible that miR-21 expression level determines whether M1 polarization persists or transition to the M2 phenotype occurs. At "subthreshold" expression levels—levels below the critical amount necessary for M2 transition, miR-21 drives M1 activation leading the production of ROS and inflammatory cytokines which further induces miR-21 expression [4,13]. At "threshold" level, miR-21 enhances inhibition of PTEN, leading to increased production of 1L-10 [12,14]. It also enhances inhibition of PDCD4, further activating the PIK3-Akt-mTOR pathway [15,16].

Both conditions favor M2 transition and resolution of inflammation, presenting a mechanism by which miR-21 can turn off inflammation and produce M2 macrophages. In diabetic wounds, miR-21 can remain elevated but "subthreshold" leading to persistent M1 polarization and inflammation (Figure 1).

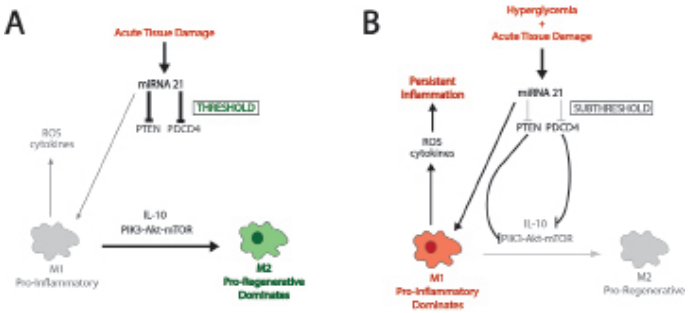

Figure 1. Schematic of miR-21 "switch" theory in macrophage polarization during later stages of wound healing. In non-diabetic wounds miR-21 accumulation (threshold) turns off the inflammatory response, in favor of M2 macrophage polarization and tissue repair. In diabetic wounds hyperglycemia is a chronic driver miR-21 accumulation, but remains below switching threshold (subthreshold), leading to persistent M1 polarization and inflammation.

Conclusion

Described above are potential mechanisms, mediated by miR-21 expression, contributing to persistent inflammation observed in diabetic wounds. Liechty et al. reported a time-course of miR-21 expression, observing differences including both an early and a late phase elevation, when compared to diabetic wounds. In the absence of a functioning "off-switch" for M1 polarization and inflammation, the development of targeted therapeutics to modulate miR-21 expression could be valuable in mitigating impaired wound healing in diabetic patients, but the time course for delivery will be an important consideration.

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