miR-100 Inhibits the Growth and Migration of Burn-Denatured Fibroblasts

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Background: Burn-denatured dermis is able to regain the function and shape of normal dermis; however, the potential mechanisms are still vague. The aim of this study was to investigate roles of miR-100 involved in the growth and migration of burn-denatured fibroblasts.

Material/Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to assess the expression of miR-100. Transient transfection of miR-100 mimics and inhibitor was used to up-regulate or down-regulate the expression of miR-100. Cell proliferation and colony formation assay were used to test the cell growth, and wound healing assay and transwell migration assay were used to evaluate the cell migration.

Results: miR-100 expression was notably downregulated in the burn-denatured fibroblasts compared to normal controls. Functionally, transfection of miR-100 inhibitors improved the growth and migration abilities of burn-denatured fibroblasts. In contrast, upregulation of miR-100 inhibits the growth and migration of burn-denatured fibroblasts.

Conclusions: Based on these observations, we concluded that miR-100 can inhibit the growth and migration of burn-denatured fibroblasts.

MeSH Keywords: Cell Migration Assays • Cell Proliferation • MicroRNAs

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/897443
Background

In the process of burn wound healing, the dermal layer offers important support and maintains the physical properties of the skin [1]. Denatured dermis helps to restore normal skin morphology and function. Denatured dermis is associated with a dynamic process involving pathological changes in the metabolic ability of cells, depression of functions, and morphological changes [2]. It has been reported that a novel treatment using large sheets of split-thickness auto-skin grafting can reduce damage caused by deep partial-thickness burns [3]. However, the potential underlying mechanisms by which denatured dermis can remodel the structure in wound healing are still elusive.

Fibroblasts are one of the major cell types in the denatured dermis; they can synthesize extracellular matrix (ECM) and accelerate wound healing. MicroRNAs are short noncoding RNAs, which are highly constant between species [4]. At the post-transcriptional level, they can inhibit target gene expression [5]. MicroRNAs regulate gene expression mainly by binding to the 3' untranslated region, directly resulting in mRNA degradation or translation blocking [6–8]. Moreover, accumulating evidence has demonstrated that miRNAs are crucial in the different stages of wound healing. For example, Xipeng Zhang et al. reported that mRNA-23b can inhibit the cell growth and migration of burn-denatured fibroblasts [9]. In many different types of cancers, dysregulated expression of miR-100 has been found to act as either an oncogene or a tumor suppressor [10,11]. Inspired by these research achievements, in the present study we explored miR-100 in burn-denatured fibroblasts.

Material and methods

Primary Cell Culture

Normal human skin was harvested from surgeries. The skin was digested with trypsin. Obtained fibroblasts were cultured in EMEM with 10% calf serum at 37°C in a humidified atmosphere with 5% CO₂. Heat injury of fibroblasts and their controls were treated as previously noted [9].

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cells with the mirVana™ miRNA Isolation Kit (Applied Biosystems). The expression level of miR-100 was detected by qRT-PCR on the 7500 Fast Real-time PCR system (Applied Biosystem) and finally calculated with the 2⁻ΔΔĈT method.

Transfection

The miR-100 mimics and inhibitor were provided by GenePharma (Shanghai, China). Cells transfection were established as the manufacturer’s instructions illustrated and qRT-PCR was used to test the final transfection efficiency.

Cell proliferation and Colony formation assay

Proliferation assays were performed using CCK8 (Dojindo, Japan). Cells were seeded in 96-well plates in triplicate at approximately 2000 cells per well and cultured in the growth medium. According to the instructions, cells were then treated with the CCK8 reagent and the numbers of cells per well were measured by the absorbance (450 nm) at the indicated time points. For further clarification, colony formation assay was done; 1000 cells per well were put into 6-well plates and incubated for 14 days. The colonies were observed using a phase-contrast microscope.

Wound healing assay

Wound healing assay was performed to test the cell migration ability [9]. Cells were cultivated at 2×10⁵ cells in 6-well plates until they grew to 90% confluence. We used 20-μL pipette tips to scratch the wounds. Cells were photographed after culturing for 0, 24, or 48 h. This assay aimed to mimic the burn healing, but cannot represent the real healing situation.

Transwell migration assay

For the migration assay, 1×10⁵ cells were resuspended in EMEM without serum and added to the upper chambers (Corning Costar, NY, USA). To attract cells, EMEM containing 10% calf serum was added to the lower chamber. After 48 h, cells on the outer surface were stained with 0.5% crystal violet. Finally, an inverted microscope was used to count and photograph the migrated cells.

Figure 1. miR-100 expression was significantly downregulated in burn-denatured fibroblasts.
**Statistics**

SPSS 19.0 statistical software was used to analyze the data. The t test or one-way ANOVA were used to analyze the data, as appropriate. Statistical significance was defined as P<0.05.

**Results**

**MiR-100 was downregulated in burn-denatured fibroblasts**

To investigate the expression of MiR-100 in burn-denatured fibroblasts, qRT-PCR was performed. Interestingly, when compared with normal fibroblasts, miR-100 was notably downregulated in burn-denatured fibroblasts (Figure 1).
MiR-100 inhibits the growth of burn-denatured fibroblasts

To study the potential roles of miR-100 in the recovery of heat injury, mimics, or inhibitors were transfected into the cells. Interestingly, by CCK8 proliferation assay and colony formation assay, it was found that downregulation of miR-100 promoted the proliferation (Figure 2B, 2D), but upregulation of miR-100 showed markedly decreased cell growth (Figure 2A, 2C).

2 MiR-100 inhibits the migration of burn-denatured fibroblasts

We next performed the wound healing assay to evaluate the role of miR-100 in cell migration. As Figure 3A, 3B indicates, miR-100 significantly decreased the migration ability of burn-denatured fibroblasts, but the specific inhibitors improved that ability. To further confirm the role of miR-100 in the migration of burn-denatured fibroblasts, we performed a transwell migration assay after miR-100 mimics and inhibitors were transfected. Consistent with the wound healing assay results, the results showed that when compared with the control, the miR-100...
inhibitor group had better migration ability (Figure 3D) and the ability was notably decreased in the mimics group (Figure 3C).

**Discussion**

The dermis has been documented to have an important function in burn wound healing; it can provide essential elements for the skin because it is enriched with collagen and appendages [12]. Therefore, after excising the eschar, the amount of dermis remaining can determine the degree of damage after severe burns [13,14]. Animal studies have shown that the function and shape of the burned dermis, including fibroblasts, may gradually recover to normal with the help of the skin. Furthermore, it may also help alleviate scar formation [15–17]. We found that during the recovery from heat denaturation, the growth and migration ability of fibroblasts increased. However, the mechanism by which fibroblasts work after severe injury from burns is still complicated and unclear.

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miRNAs have a vital role in regulating cellular activities, including proliferation and differentiation [18–21]. They can profoundly regulate the expression of massive target genes that encode proteins, which may finally lead to change in biological function. Accumulating evidence has demonstrated that miRNAs are crucial in the processes of burn healing.

In the current study, we verified that miR-100 expression was significantly downregulated in burn-denatured fibroblasts. Functionally, inhibition of miR-100 promoted the growth and migration of burn-denatured fibroblasts, but upregulation of miR-100 inhibits proliferation and migration of burn-denatured fibroblasts. The mechanism by which this occurs is unclear, and we intend to investigate this in further research.

**Conclusions**

Based on these findings, we conclude that miR-100 can inhibit the growth and migration of burn-denatured fibroblasts, which may be useful for treating skin burns.

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