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

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Statistical Analysis C
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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

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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% CO_2. 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 $2^{-\Delta CT}$ method.

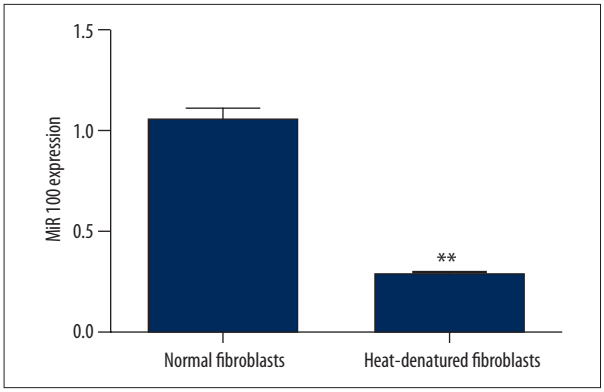

Figure 1. miR-100 expression was significantly downregulated in burn-denatured fibroblasts.

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 2×10^5 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, 1×10^5 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.

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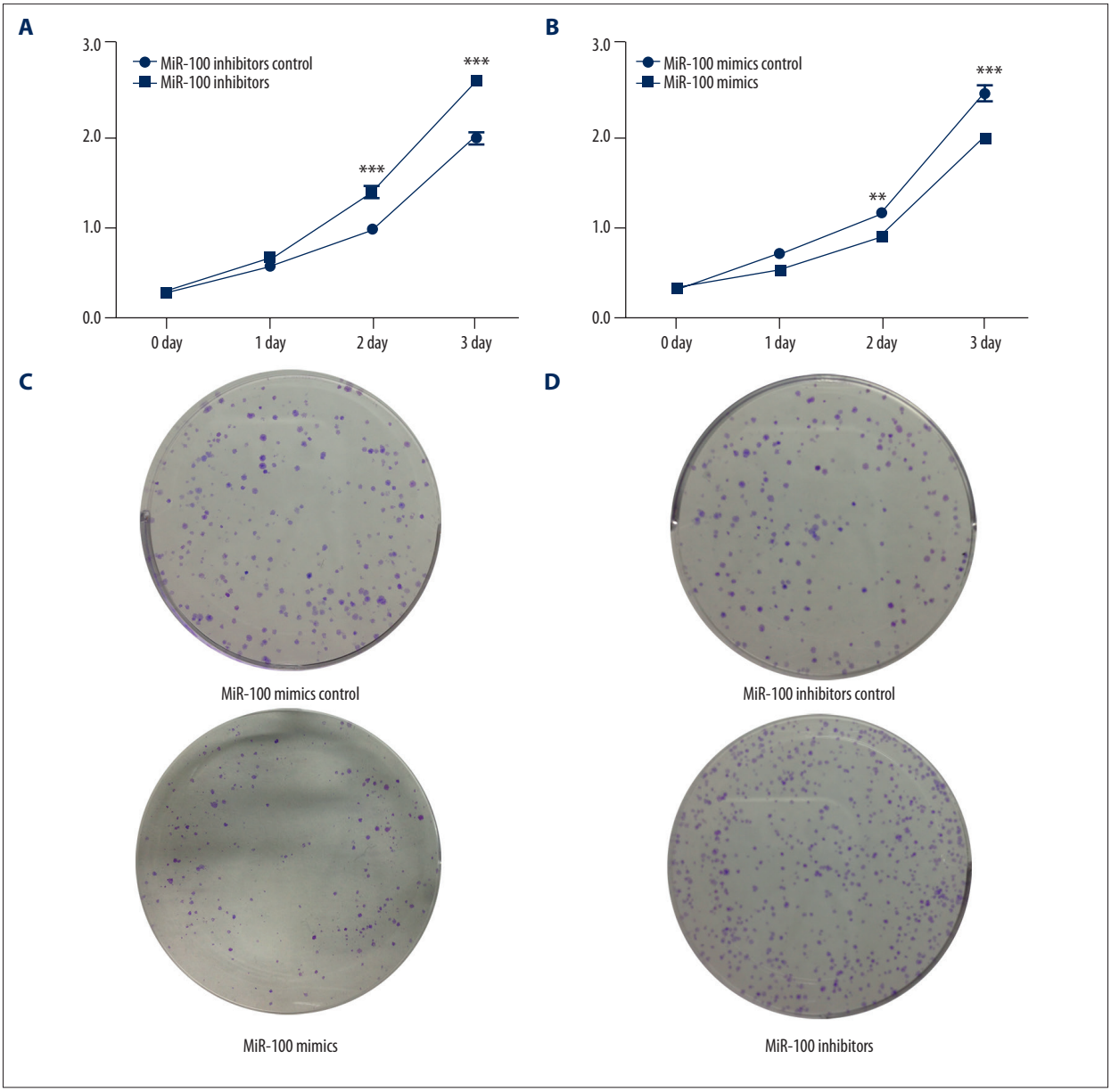

Figure 2. miR-100 inhibited the growth of burn-denatured fibroblasts. Upregulation of miR-100 inhibited the growth of burn-denatured fibroblasts as shown by CCK8 assay (**A**) and colony formation assay (**C**). Downregulation of miR-100 promoted the growth of burn-denatured fibroblasts as shown by CCK8 assay (**B**) and colony formation assay (**D**).

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).

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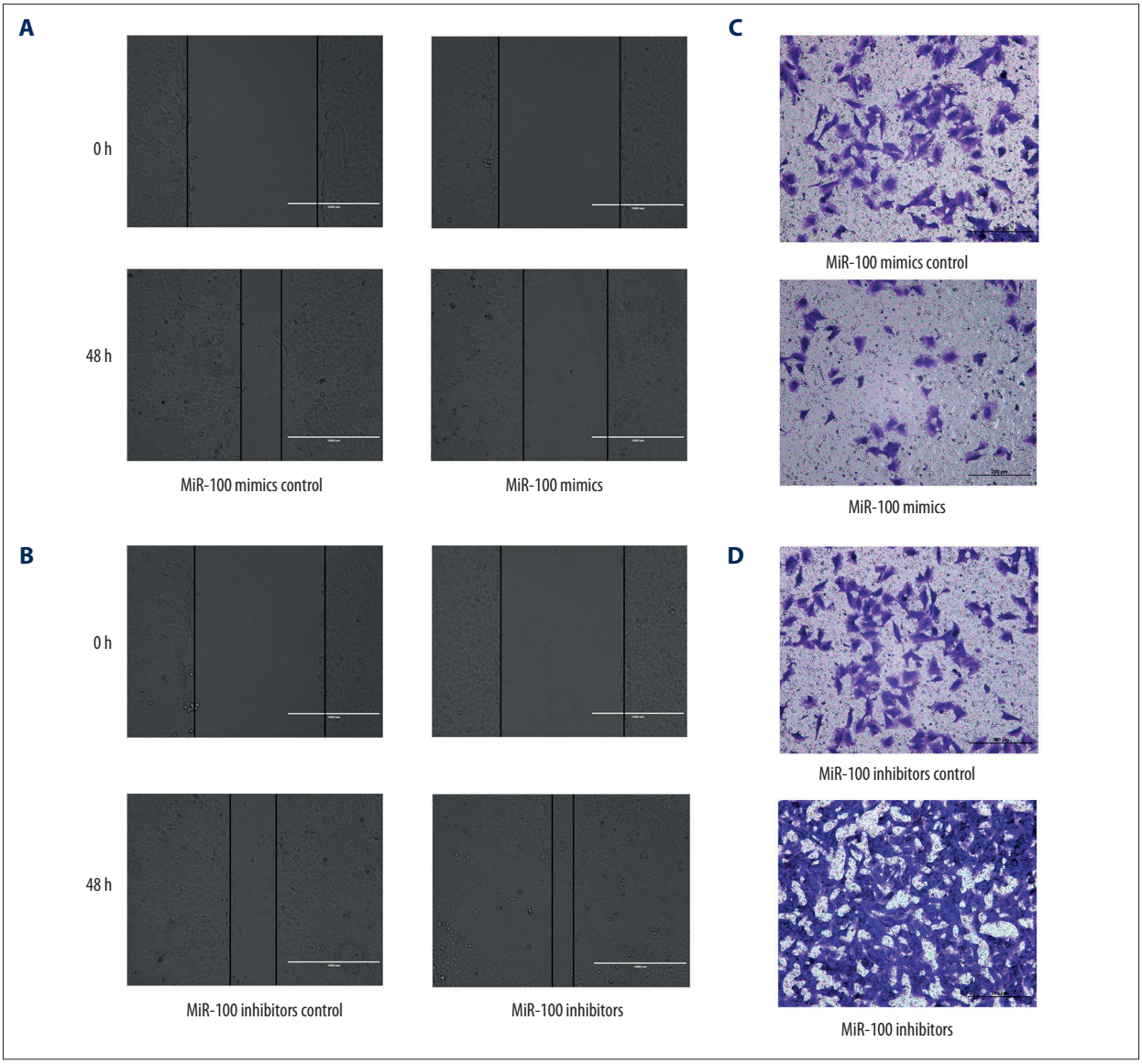

Figure 3. miR-100 inhibited the migration of burn-denatured fibroblasts. Upregulation of miR-100 inhibited the wound healing (**A**) and migration (**C**) of burn-denatured fibroblasts. Downregulation of miR-100 promoted the wound healing (**B**) and migration (**D**) of burn-denatured fibroblasts.

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

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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.

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.

References:

1. Gurtner GC, Werner S, Barrandon Y et al: Wound repair and regeneration. Nature, 2008; 453: 314–21
2. Huang XY, Yang XH, Lei SR et al: With the preservation of denatured dermis and autoskingrafting to repair of deeply burned hands. Zhonghua Shao Shang Za Zhi, 2001; 17: 60–61
3. Yang XH, Huang XY, Lei SR et al: Long-term result of repair of deeply burned hands with large sheet of split-thickness autoskin grafting with the preservation of denatured dermis. Zhonghua Shao Shang Za Zhi, 2005; 21: 27–29
4. Nelson P, Kiriakidou M, Sharma A et al: The microRNA world: Small is mighty. Trends Biochem Sci, 2003; 28: 534–40
5. Macfarlane LA, Murphy PR: MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics, 2010; 11: 537–61
6. Berardi E, Pues M, Thorrez L et al: miRNAs in ESC differentiation. Am J Physiol Heart Circ Physiol, 2012; 303: H931–39
7. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004; 116: 281–97
8. Bartels CL: MicroRNAs: Novel biomarkers for human cancer. Clin Chem, 2009; 55: 623–31
9. Zhang X, Yang J, Zhao J et al: MicroRNA-23b inhibits the proliferation and migration of burn-denatured fibroblasts by targeting Smad3. PLoS One, 2015; 10: e0131867
10. Chen P, Xi Q, Wang Q et al: Downregulation of microRNA-100 correlates with tumor progression and poor prognosis in colorectal cancer. Med Oncol, 2014; 31: 235
11. Oliveira JC, Brassesco MS, Morales AG et al: MicroRNA-100 acts as a tumor suppressor in human bladder carcinoma 5637 cells. Asian Pac J Cancer Prev, 2011; 12: 3001–4
12. Proksch E, Brandner JM, Jensen JM: The skin: An indispensable barrier. Exp Dermatol, 2008; 17: 1063–72
13. Cubison TC, Pape SA, Jeffery SL: Dermal preservation using the Versajet hydrosurgery system for debridement of paediatric burns. Burns, 2006; 32: 714–20
14. Fan PJ, Huang XY, Yang XH: Pathological experimental study of autologous skin transplant on retained denatured dermis. Zhong Nan Da Xue Xue Bao Yi Xue Ban, 2008; 33: 596–600
15. Zhao YH, Yang HG, Deng HT et al: Influence ofthe depth of retained denatured dermis on the survival rate of grafted skin in burn swine with deep partial-thickness burn. Zhonghua Shao Shang Za Zhi, 2013; 29: 365–70
16. Liu Y, Huang XY, Yang XH: Experimental study of autologous skin grafting on retained denatured dermis for the treatment of partial thickness burn wound. Zhonghua Shao Shang Za Zhi, 2005; 21: 14–16
17. Li D, Huang X, Yang X: Morphology of human skin fibroblasts after heat injury *in vitro*. J Clin Rehabil Tissue Eng Res, 2008; 12: 2056–60
18. Mukhopadhyay P, Brock G, Appana S et al: MicroRNA gene expression signatures in the developing neural tube. Birth Defects Res A Clin Mol Teratol, 2011; 91: 744–62
19. Bartel DP. MicroRNAs: Target recognition and regulatory functions. Cell, 2009; 136: 215–33
20. Yang Z, Wu J: Small RNAs and development. Med Sci Monit, 2006; 12(7): RA125–29
21. Xu S, Jin C, Shen X et al: MicroRNAs as potential novel therapeutic targets and tools for regulating paracrine function of endothelial progenitor cells. Med Sci Monit, 2012; 18: HY27–31

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