**Abstract:** The aim of the present study was to compare expression of microRNAs (miRNAs) from scar and normal skin areas in patients who suffered acute injuries in the skin.

A total of 9 patients with acute injuries in the skin who received surgical treatment from December 2012 to March 2013 were included in this pilot study. Specimens from the hypertrophic scar and normal skin areas were obtained from the same patient during surgery. To screen for differentially expressed miRNAs, we applied 3 statistical methods, namely the traditional t test, the false discovery rate (FDR), and a novel sure independence screening procedure based on the distance correlation (DC-SIS). We examined the functional trends and metabolic and regulatory pathways for the target genes of the identified miRNAs, and explored interaction of these miRNAs in the implication of scar healing using Ingenuity Pathway Analysis.

DC-SIS identified 18 differentially expressed miRNAs, 4 of which (miR-149, miR-203a, miR-222, miR-122) were also identified by FDR. The target genes of the 4 miRNAs exhibit a variety of biological functions, and are involved in various pathways such as mitogen-activated protein kinase, Wnt signaling, and focal adhesion. We identified 1 network in which 14 out of the 18 differentially expressed miRNAs were involved. Many of the miRNAs in the network target genes were involved in cell proliferation and apoptosis.

In this pilot study, we identified several miRNAs exhibiting differential expression in patients who suffered acute injuries in the skin. Further studies on these miRNAs are needed to validate our findings and explore their roles in the wound healing process of the skin.

**INTRODUCTION**

In adult humans the skin is the largest organ and has various functions including barrier defense, UV protection, thermoregulation, pigmentation, sensation of touch and pain, and regulation of water loss from the epidermis.1 Acute wounds in the skin caused by accidents such as burning or trauma are serious injuries. Wound healing in the skin is a dynamic process in which various types of cells, such as cells involved in acute and chronic inflammation,2 are involved.

MicroRNAs (miRNAs) refer to a class of single-stranded RNAs that are 19 to 24 nucleotides in length. They suppress the expression of target genes by messenger RNA (mRNA) degradation or the blockade of mRNA translation by binding to the 3′-untranslated region of target mRNA.3 One individual miRNA could regulate many genes, and similarly 1 individual gene could also be regulated by more than 1 miRNA.4,5 miRNAs are reported as critical regulators in skin morphogenesis, wound healing, and regeneration by controlling proliferation, differentiation, and apoptosis of skin cells.6 However, little is known about the key miRNAs that are involved in acute wound healing in the skin and their biological targets and functions, partially due to the dynamic interaction between multiple cell types during wound healing.

To identify the critical miRNAs in patients with acute skin injuries, we compared the miRNA expression from scar and normal skin cells of the same patients. We conducted network analysis of the identified miRNAs showing differential expression, and explored their potential target genes and performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of these target genes.

**MATERIALS AND METHODS**

**Participants**

A total of 9 patients were included for this study. All of them received surgical treatment during the period from December 2012 to March 2013 in the Department of Burns and Plastic Surgery of The Third Xiangya Hospital of Central South University in China. Age of these patients ranged from 3 to 43. All of them received no medical or radiological therapy before surgery, and had no history of diabetes, hypertension, liver, or other chronic diseases. Informed consent was obtained from all patients or their closest relatives. This study was approved by the ethical committee of the Central South University.

**Resection of Tissue Specimens**

Specimens from the hypertrophic scar and normal skin areas were obtained from the same patient during surgery. All the specimens were obtained at least 6 months after healing of wound surface. In the surgery, the scar was removed and cut into 2 × 2 cm. Specimens from the normal skin were obtained in...
areas at least 1 cm away from the scar areas. Both types of specimens were immediately put in liquid nitrogen and kept at –80°C.

miRNA Microarray and Hybridization

miRNAs were extracted using the miRcute RNA Isolation Kit (Tiangen Biotech, Beijing, China). Quality control, labeling, and hybridization were performed commercially according to protocols in the µParafo microRNA microarray assay (LC Sciences, Hangzhou, Zhejiang, China). Fluorescence images were collected using a laser scanner GenePix 400B (Molecular Device, Sunnyvale, CA) and digitized using Array-Pro image analysis software (Media Cybernetics, Rockville, MD). Data were transformed by first subtracting the background and then normalizing the signals using a locally weighted regression filter.

Statistical Analysis

We used 3 methods to screen for differentially expressed miRNAs. First, we applied the traditional t test and set statistical significance level at a P value ≤ 0.05 (criterion 1). We then used false discovery rate (FDR) to adjust for multiple testing and consider a change to be significant if the corresponding q-value is ≤ 0.05 (criterion 2). Finally we applied a newly developed statistical screening method, a sure independence screening based on the distance correlation (DC-SIS; criterion 3). As a novel statistical method for feature selection for ultrahigh-dimensional data, DC-SIS is model free since it makes no assumption (eg, linear model) for the response (eg, miRNA expression) and the predictors (eg, presence of scar or not). DC-SIS therefore is robust to model misspecification, and the sure independence feature it bears ensures that all truly important variables can be selected with sufficient sample size. DC-SIS therefore is more flexible and reliable in selecting important predictors than conventional statistical methods such as the t test. Because our sample size is limited, to reduce the possibility of missing important miRNAs, we chose our model size to be 6[n/log(n)], where n is the sample size and [n/log(n)] denotes the integer part of n/log(n).

Statistical analyses were performed using the program R (www.R-project.org) and SAS version 9.3 (SAS Institute Inc, Cary, NC).

Function Annotation and Pathway Analyses

We examined the functional trends and metabolic and regulatory pathways for the target genes of the top-hit miRNAs identified in our study (see below for details). Biological targets of the miRNAs were predicted using the online web server TargetScan (http://www.targetscan.org/). Functional GO analysis and KEGG analysis were performed using the online gene set analysis toolkit WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt). GO analysis includes 3 parts, namely the biological process, molecular function, and cellular component. Gene overrepresentation was identified using hypergeometric distribution. Multiple testing was adjusted for using the FDR. For simplicity, we focus on the top 10 most significant GO categories and KEGG pathways.

Ingenuity Pathway Analysis

To further explore the relationship of the 18 identified miRNAs, we used Ingenuity Pathway Analysis (IPA, QIAGEN, Redwood City, CA, http://www.qiagen.com/ingenuity) to construct molecular networks to better understand the interaction of these miRNAs in the implication of scar healing. IPA constructs networks based on Ingenuity Knowledge Base (IKB), a repository of millions of expert-curated findings of biological and chemical interactions and functional annotations. Each individual interaction in the network was supported by findings in Ingenuity Knowledge Base. Each identified network has a p-score (−log10(P value)), where the P value was calculated using Fisher exact test, representing the probability of finding no less than the number of focus miRNAs (out of all the 18 miRNAs).

RESULTS

Differentially Expressed miRNAs

A total of 513 miRNAs were interrogated and included in the final analyses, and we found that 170 (33.1%) had significant changes with corresponding P values ≤ 0.05 using the t test. Specifically, 58 miRNAs (34.1%) exhibited elevated expression, and 112 (65.9%) exhibited lowered expression (Table 1).

After adjusting for multiple testing using FDR, we found that only 4 miRNAs (miR-149-5p, miR-203a, miR-222-3p, miR-122-5p) had significant change in expression. Specifically, none exhibited elevated expression, and all 4 miRNAs exhibited lowered expression (Table 1).

DC-SIS feature screening identified 18 miRNAs, all of which had small P values of change in expression using paired t test (Table 2). Specifically, 4 miRNAs (22.2%) exhibited elevated expression, and 14 miRNAs (77.8%) exhibited lowered expression (Table 1). All 4 miRNAs identified by FDR were also identified by DC-SIS, and hence are those showing significant changes in expression as identified by all the 3 criteria (Table 2).

Functional and Network Analysis

We explored functions of the target genes for the 4 miRNAs found to be differentially expressed by FDR. The GO analysis of the biological process of the target genes for miR-149-5p indicates that 164 (36.9%) of the genes are involved in cell signaling (P for enrichment = 5.28 × 10−5) and 166 (37.3%) in cell communication (P = 7.72 × 10−5; Table 3A). One hundred ninety-five (22.5%) of the target genes for miR-203a are involved in the negative regulation of cellular process (P for enrichment = 9.66 × 10−5), and many target genes are involved in the regulation of biosynthetic or cellular metabolic process (Table 3B). Ninety (20.2%) of the target genes for miR-222-3p are involved in nervous system development (P for enrichment = 1.30 × 10−5), and about one-third in cellular metabolic or biosynthetic process (Table 3C). The GO analysis of the biological process of the target genes for

### TABLE 1. Summary of Differentially Expressed miRNAs

| Method | Up, n (%) | Down, n (%) | Total, n (%) |
|--------|-----------|-------------|--------------|
| t test | 58 (34.1) | 112 (65.9) | 170 (33.1) |
| FDR    | 0 (0)     | 4 (100)    | 4 (0.8)     |
| DC-SIS | 4 (22.2)  | 14 (77.8)  | 18 (3.5)    |

DC-SIS = sure independence screening based on the distance correlation, FDR = false discovery rate, miRNA = microRNA.

Expression was measured for scar and normal skin cells from the same patients.
miR-122-5p failed to find significant enrichment of target genes involved in any biological process. The corresponding molecular function analysis indicates that 2 genes are involved in glucosamine-6-phosphate deaminase activity (P = 2.18 x 10^-5; Table 3D).

The KEGG pathway analysis of the target genes for miR-149-5p indicates that 14 genes (3.1%) are involved in antigen processing and presentation, inflammation, proliferation, and maturation (P = 2.00 x 10^-4; Table 4A). Twenty-three (2.6%) target genes for miR-203a are involved in insulin signaling pathway (P = 6.72 x 10^-3), and 9 (2.0%) in lysosome (P = 6.21 x 10^-4; Table 4A). Twenty-three (2.6%) target genes for miR-203a are involved in insulin signaling pathway (P = 6.72 x 10^-3), and 9 (2.0%) in lysosome (P = 6.21 x 10^-4; Table 4A). Twenty-three (2.6%) target genes for miR-203a are involved in insulin signaling pathway (P = 6.72 x 10^-3), and 9 (2.0%) in lysosome (P = 6.21 x 10^-4; Table 4A). Twenty-three (2.6%) target genes for miR-203a are involved in insulin signaling pathway (P = 6.72 x 10^-3), and 9 (2.0%) in lysosome (P = 6.21 x 10^-4; Table 4A).

The target genes of the 4 miRNAs exhibit a variety of biological functions in wound healing. miR-31 is highly expressed during wound healing, and transgenic mouse model with elevated miR31 levels showed aberrant wound healing and hair loss.10 In our study, we found that miR-31 is among the top-hit miRNAs (Table 2), and is upregulated in scar cells. miR-21-5p is also among top-hit miRNAs and upregulated, consistent with a previous study showing that miR-21 promotes keratinocyte migration and reepithelialization during wound healing in mice through tissue inhibitor of metalloproteinases 3 and T-cell lymphoma invasion and metastasis 1.11 In our study, miR-21-5p is also an important hub in the network we constructed, and this further implies that it might have important functions in wound healing.

Wound healing is a complex process that involves hemostasis, inflammation, proliferation, and maturation.9 Previous research has identified multiple miRNAs exhibiting significant change in expression in the presence of acute injuries and explored their function in wound healing. miR-31 is highly expressed during wound healing, and transgenic mouse model with elevated miR31 levels showed aberrant wound healing and hair loss.10 In our study, we found that miR-31 is among the top-hit miRNAs (Table 2), and is upregulated in scar cells. miR-21-5p is also among top-hit miRNAs and upregulated, consistent with a previous study showing that miR-21 promotes keratinocyte migration and reepithelialization during wound healing in mice through tissue inhibitor of metalloproteinases 3 and T-cell lymphoma invasion and metastasis 1.11 In our study, miR-21-5p is also an important hub in the network we constructed, and this further implies that it might have important functions in wound healing.

Wound healing involves multiple steps and a variety of cells and events. Cell differentiation and proliferation is essential for tissue remodeling and recovery of physiologic functions.12 We identified multiple miRNAs involved in cell differentiation. One miRNA is miR-203, which has potent antiproliferative function and regulates the balance between stem cell proliferation and terminal differentiation in skin cells, possibly by targeting p63 when stem cells in the epidermis are proliferating and differentiating into stratified epithelium.13 miR-203 can be considered as a “skin-specific miRNA” as it has the highest level of expression in the skin.14 It induces cell differentiation or cellular metabolism, and nervous system development, and are involved in multiple pathways such as MAPK, Wnt signaling, and focal adhesion. We identified 1 network, comprising 14 focus miRNAs, which plays an important role in cell death and survival.

**TABLE 2. Information of the 18 miRNAs Identified by DC-SIS**

| miRNA        | Chr | Case      | Control | P     | q      | Rank |
|--------------|-----|-----------|---------|-------|--------|------|
| hsa-miR-31-5p| 9   | 2,429     | 65      | 0.001 | 0.063  | 1    |
| hsa-miR-149-5p| 2  | 36        | 290     | 0.000 | 0.028  | 2    |
| hsa-miR-203a | 14 | 10,854    | 30,966  | 0.000 | 0.028  | 3    |
| hsa-miR-222-3p| X  | 2,361     | 7,253   | 0.000 | 0.045  | 4    |
| hsa-miR-224-5p| X  | 234       | 464     | 0.001 | 0.063  | 5    |
| hsa-miR-21-5p| 17 | 27,080    | 7,018   | 0.010 | 0.138  | 6    |
| hsa-miR-122-5p| 18 | 23        | 47      | 0.000 | 0.049  | 7    |
| hsa-miR-221-3p| X  | 2,578     | 7,264   | 0.001 | 0.056  | 8    |
| hsa-miR-141-3p| 12 | 553       | 3,269   | 0.005 | 0.136  | 9    |
| hsa-miR-200b-3p| 1  | 1,175     | 4,241   | 0.001 | 0.087  | 10   |
| hsa-let-7d-3p | 9  | 54        | 68      | 0.006 | 0.136  | 11   |
| hsa-miR-28-3p | 3  | 207       | 316     | 0.007 | 0.138  | 12   |
| hsa-miR-376c-3p| 14 | 6,882     | 1,481   | 0.010 | 0.138  | 13   |
| hsa-miR-328   | 16 | 17        | 39      | 0.003 | 0.129  | 14   |
| hsa-miR-127-3p| 14 | 1,310     | 341     | 0.015 | 0.140  | 15   |
| hsa-miR-224-3p| X  | 52        | 130     | 0.006 | 0.136  | 16   |
| hsa-miR-652-3p| X  | 40        | 205     | 0.006 | 0.136  | 17   |
| hsa-miR-362-5p| X  | 93        | 180     | 0.005 | 0.136  | 18   |

miRNA in bold indicates significant miRNAs identified by FDR. Chr = chromosome; DC-SIS = sure independence screening based on the distance correlation; FDR = false discovery rate, miRNA = microRNA, q = q values obtained using FDR, rank = rank of importance obtained using DC-SIS.

**DISCUSSION**

In this article, we conducted a pilot study to compare the miRNA expression of the scar and normal skin cells obtained from 9 patients suffering acute skin injuries. We identified 4 miRNAs showing significant change in miRNA expression. The target genes of the 4 miRNAs exhibit a variety of biological functions, including cell signaling and communication, biosynthetic or cellular metabolism, and nervous system development, and are involved in multiple pathways such as MAPK, Wnt signaling, and focal adhesion. We identified 1 network, comprising 14 focus miRNAs, which plays an important role in cell death and survival.
| Biological Process | No. of Genes | P     | Molecular Function | No. of Genes | P     | Cellular Component | No. of genes | P     |
|--------------------|--------------|-------|--------------------|--------------|-------|--------------------|--------------|-------|
| (A) hsa-miR-149-5p |              |       |                    |              |       |                    |              |       |
| Signaling          | 164          | $5.28 \times 10^{-5}$ | Binding          | 317          | $5.90 \times 10^{-3}$ | Cell   | 366  | $2.29 \times 10^{-2}$ |
| Single-organism process | 231  | $5.00 \times 10^{-3}$ | Protein binding  | 219          | $1.40 \times 10^{-3}$ | Cell part| 366  | $2.29 \times 10^{-2}$ |
| Multicellular organismal process | 176  | $7.17 \times 10^{-2}$ | Chromatin binding | 15           | $2.36 \times 10^{-1}$ | Endomembrane system | 64           | $2.29 \times 10^{-2}$ |
| Cell communication | 166          | $7.72 \times 10^{-5}$ | Protein domain–specific binding | 29 | $5.90 \times 10^{-3}$ | Organelle membrane | 80 | $2.29 \times 10^{-2}$ |
| Single-organism signaling | 164 | $5.28 \times 10^{-5}$ | SNAP receptor activity | 4 | $9.57 \times 10^{-2}$ | Neuron projection | 29 | $2.29 \times 10^{-2}$ |
| Single-multicellular organism process | 176  | $1.54 \times 10^{-2}$ | Sequence-specific DNA binding RNA polymerase II transcription factor activity | 5 | $2.36 \times 10^{-1}$ | Vesicle | 39 | $2.29 \times 10^{-2}$ |
| Cell–cell signaling | 55           | $2.00 \times 10^{-4}$ | Prenyltransferase activity | 3 | $2.15 \times 10^{-1}$ | Neuron spine | 12 | $2.29 \times 10^{-2}$ |
| Tube development | 26           | $1.21 \times 10^{-2}$ | PDZ domain binding | 8           | $9.57 \times 10^{-2}$ | Dendritic spine | 12 | $2.29 \times 10^{-2}$ |
| Transmission of nerve impulse | 36  | $1.71 \times 10^{-2}$ | Calmodulin-dependent cyclic nucleotide phosphodiesterase activity | 2 | $1.04 \times 10^{-1}$ | Cytoplasmic vesicle | 38 | $2.29 \times 10^{-2}$ |
| Synaptic transmission | 33   | $1.71 \times 10^{-2}$ | Voltage-gated cation channel activity | 8 | $2.36 \times 10^{-1}$ | Juxtaparanode region of axon | 3 | $2.29 \times 10^{-2}$ |
| (B) hsa-miR-203a |              |       |                    |              |       |                    |              |       |
| Regulation of cellular process | 439  | $2.00 \times 10^{-4}$ | Protein binding  | 414          | $6.28 \times 10^{-6}$ | Cell part| 711  | $1.71 \times 10^{-6}$ |
| Negative regulation of cellular process | 195  | $9.66 \times 10^{-5}$ | Phospholipid binding | 44 | $1.30 \times 10^{-3}$ | Intracellular | 639  | $1.37 \times 10^{-7}$ |
| Regulation of biosynthetic process | 217  | $3.00 \times 10^{-4}$ | Sequence-specific DNA binding RNA polymerase II transcription factor activity | 27 | $1.30 \times 10^{-3}$ | Intracellular part | 619  | $1.71 \times 10^{-6}$ |
| Regulation of cellular metabolic process | 278  | $9.66 \times 10^{-5}$ | Kinase activity | 66           | $3.00 \times 10^{-4}$ | Lamellipodium | 19  | $6.41 \times 10^{-5}$ |
| Regulation of primary metabolic process | 270  | $2.00 \times 10^{-4}$ | Phosphotransferase activity, alcohol group as acceptor | 62 | $3.00 \times 10^{-4}$ | Membrane-bounded organelle | 498  | $3.69 \times 10^{-5}$ |
| Regulation of cellular macromolecular biosynthetic process | 204  | $3.00 \times 10^{-4}$ | Protein, kinase activity | 55 | $3.00 \times 10^{-4}$ | Intracellular membrane-bounded organelle | 498  | $3.46 \times 10^{-5}$ |
| Regulation of RNA metabolic process | 195  | $3.00 \times 10^{-4}$ | Sequence-specific DNA binding | 56 | $1.30 \times 10^{-3}$ | Nucleus | 338  | $5.81 \times 10^{-6}$ |
| Transcription from RNA polymerase II promoter | 108  | $3.00 \times 10^{-4}$ | Protein serine/threonine kinase activity | 43 | $3.00 \times 10^{-4}$ | Nucleus part | 197  | $4.06 \times 10^{-6}$ |
| Regulation of transcription from RNA polymerase II promoter (C) hsa-miR-222-5p | 98   | $2.00 \times 10^{-4}$ | Receptor signaling protein serine/threonine kinase activity | 11 | $1.30 \times 10^{-3}$ | Nuclear lumen | 176  | $1.66 \times 10^{-5}$ |
| Multicellular organismal development | 157  | $2.77 \times 10^{-7}$ | Binding | 334          | $1.67 \times 10^{-5}$ | Cell      | 370  | $2.00 \times 10^{-4}$ |
| Regulation of metabolic process | 175  | $4.80 \times 10^{-7}$ | Nucleic acid binding transcription factor activity | 54 | $1.67 \times 10^{-5}$ | Synapse   | 25   | $5.40 \times 10^{-3}$ |
| Regulation of cellular process | 250  | $3.99 \times 10^{-7}$ | Chromatin binding | 23 | $2.00 \times 10^{-4}$ | Cell part | 370  | $2.00 \times 10^{-4}$ |
| System development | 139         | $4.80 \times 10^{-7}$ | Protein binding | 229          | $2.51 \times 10^{-5}$ | Intracellular | 325  | $2.70 \times 10^{-3}$ |
| GO Term                                           | Value | Description                                                                 |
|--------------------------------------------------|-------|----------------------------------------------------------------------------|
| Regulation of primary metabolic process          | 162   | $4.80 \times 10^{-7}$ Sequence-specific DNA binding transcription factor activity |
| Regulation of cellular metabolic process         | 167   | $1.87 \times 10^{-7}$ Nucleic acid binding                                  |
| Nervous system development                       | 90    | $1.30 \times 10^{-8}$ Kinase inhibitor activity                            |
| Regulation of macromolecular metabolic process   | 160   | $1.97 \times 10^{-7}$ DNA binding                                           |
| Regulation of macromolecular biosynthetic process| 130   | $4.80 \times 10^{-7}$ Protein kinase inhibitor activity                      |
| Regulation of cellular macromolecular biosynthetic process | 127   | $4.80 \times 10^{-7}$ Sequence-specific DNA binding                        |
| (D) hsa-miR-122-5p Growth                        | 19    | $5.88 \times 10^{-2}$ Sequence-specific DNA binding transcription factor activity |
| Multicellular organism growth                    | 7     | $5.04 \times 10^{-2}$ Glucosamine-6-phosphate deaminase activity            |
| Cellular macromolecular metabolic process        | 88    | $5.04 \times 10^{-2}$ Phosphatase activity                                 |
| Macromolecular modification                      | 43    | $5.04 \times 10^{-2}$ Carbohydrate phosphatase activity                    |
| Protein modification process                     | 43    | $5.04 \times 10^{-2}$ Protein tyrosine/threonine phosphatase activity       |
| Hexose phosphate transport                       | 2     | $5.88 \times 10^{-2}$ Sugar-phosphatase activity                           |
| Cellular protein modification process            | 43    | $5.04 \times 10^{-2}$ Sugar-terminal-phosphatase activity                  |
| Protein glycosylation in Golgi                   | 2     | $5.88 \times 10^{-2}$ Anion:cation symporter activity                     |
| Retinal pigment epithelium development           | 2     | $5.04 \times 10^{-2}$ Glucose-6-phosphatase activity                      |
| Production of siRNA involved in RNA interference | 2     | $5.04 \times 10^{-2}$ Sodium: dicarboxylate symporter activity             |

GO = Gene Ontology, PDZ = Postsynaptic density 95, PSD-85; Discs large, Dlg; Zonula occludens-1, ZO-1, PML = Promyelocytic Leukemia, siRNA = Small interfering RNA.
TABLE 4. Top 10 Enriched KEGG Pathways for Targeted Genes of hsa-miR-149-5p, hsa-miR-203a, hsa-miR-222-3p, and hsa-miR-122-5p

| KEGG Pathway                          | No. of Genes | Adjusted P |
|---------------------------------------|--------------|------------|
| (A) hsa-miR-149-5p                    |              |            |
| MAPK signaling pathway                | 14           | 6.74 × 10^−5 |
| Lysosome                              | 9            | 2.00 × 10^−4 |
| Pathways in cancer                    | 12           | 2.30 × 10^−3 |
| Metabolic pathways                    | 26           | 2.50 × 10^−3 |
| Citrin signaling pathway              | 8            | 7.60 × 10^−3 |
| SNARE interactions in vesicular transport | 4         | 7.60 × 10^−3 |
| Endocytosis                           | 8            | 0.0143     |
| Neurotrophin signaling pathway        | 6            | 0.0209     |
| Glycosaminoglycan                     | 3            | 0.0200     |
| biosynthesis—heparan sulfate          | 6            | 0.0200     |
| Axon guidance                         |              |            |
| (B) hsa-miR-203a                      |              |            |
| Insulin signaling pathway             | 23           | 6.72 × 10^−13 |
| Focal adhesion                        | 25           | 2.01 × 10^−11 |
| Pathways in cancer                    | 31           | 3.51 × 10^−11 |
| MAPK signaling pathway                | 24           | 3.39 × 10^−8  |
| ErbB signaling pathway                | 14           | 4.64 × 10^−8  |
| Adipocytokine signaling pathway       | 12           | 1.89 × 10^−7  |
| Endocytosis                           | 19           | 4.37 × 10^−7  |
| Colorectal cancer                     | 11           | 5.56 × 10^−7  |
| GnRH signaling pathway                | 13           | 1.53 × 10^−6  |
| Pancreatic cancer                     | 11           | 1.64 × 10^−6  |
| (C) hsa-miR-222-3p                    |              |            |
| Pathways in cancer                    | 17           | 4.44 × 10^−6  |
| ErbB signaling pathway                | 9            | 1.17 × 10^−5  |
| Wnt signaling pathway                 | 11           | 1.26 × 10^−5  |
| MAPK signaling pathway                | 13           | 9.29 × 10^−5  |
| Focal adhesion                        | 11           | 0.0001      |
| Axon guidance                         | 9            | 0.0001      |
| T-cell receptor signaling pathway     | 8            | 0.0002      |
| Neurotrophin signaling pathway        | 8            | 0.0005      |
| Bacterial invasion of epithelial cells| 6            | 0.0006      |
| Renal cell carcinoma                  | 6            | 0.0006      |
| (D) hsa-miR-122-5p                    |              |            |
| Glycolysis/glucose metabolism         | 3            | 0.0123      |
| Insulin signaling pathway             | 4            | 0.0123      |
| Epithelial cell signaling in Helicobacter pylori infection | 3 | 0.0123 |
| Metabolic pathways                    | 12           | 0.0123      |
| CAMs                                  | 4            | 0.0123      |
| Pathways in cancer                    | 6            | 0.0123      |
| Antigen processing and presentation   | 3            | 0.0146      |
| MAPK signaling pathway                | 5            | 0.0156      |
| Citrate cycle (TCA cycle)             | 2            | 0.0213      |
| Endocytosis                           | 4            | 0.0250      |

CAM = cell adhesion molecule, GnRH = gonadotropin-releasing hormone, KEGG = Kyoto Encyclopedia of Genes and Genomes, MAPK = mitogen-activated protein kinase, SNARE = soluble Nethylmaleimide-sensitive factor attachment protein (SNAP) receptors, TCA = tricarboxylic acid cycle.

The other miRNA, miR-222, can stimulate cell proliferation via coordinating competency for initiation of S phase with growth factor signaling pathways.16 When elevated, it can prevent cycle exit and represses “stemness” in epidermal progenitors, suggesting its involvement in keratinocyte differentiation.13,15 The other miRNA, miR-222, can stimulate cell proliferation via coordinating competency for initiation of S phase with growth factor signaling pathways.16 When elevated, it can prevent quiescence and induce precocious S-phase entry, and hence trigger cell death.16 (we observed downregulation of miR-222 in patients suffering acute skin injuries). These results highlight the critical roles of miRNAs in regulating wound healing of the skin after acute injuries, and also call for future studies to carefully examine the exact mechanism through which they function in scar formation and skin remodeling.

We found that MAPK is a pathway in which the target genes of multiple top-hit miRNAs (miR-149 and miR-222; Table 4A and C) are involved. MAPKs participate in directing cellular responses to various stimuli such as heat shock,17 and regulate gene expression,18 cell differentiation, proliferation,19 and survival.20 Application of MAPK inhibitor promotes wound healing by stimulating early generation of connective tissues in the wound area.21 Whether and how the identified miRNAs influence wound healing in the skin by regulating MAPK pathways warrants further investigation.

The TP53 is a tumor suppressor gene, and is essential in the regulation of cell proliferation.22 Previous research found that transient inhibition of TP53 can accelerate wound healing by promoting leukocyte recruitment, increasing cell proliferation, and reducing apoptotic cell death.23 miR-21 targets multiple components of TP53 (Figure 1), and its downregulation can lead to repression of growth, increased apoptosis, and cell cycle arrest.24 Consistent with these findings, we found that miR-21 exhibited upregulation in patients with scar (Table 2), implying that miR-21 might be implicated in cell proliferation and skin regeneration in patients with scar following serious injuries in the skin.

Wnt signals are essential for the development and homeostasis of the skin, and Wnt signaling is involved in multiple processes of wound healing following acute injuries.25 It is implicated in the reconstitution of the dermal compartment and in the construction of epithelial structures.26 Augmentation of endogenous Wnt signaling could result in improved skin wound healing, suggesting the potential of applying liposomal Wnt3a for the therapeutic treatment of cutaneous wounds; however, the underlying mechanism remains poorly understood.27 miR-222-3p targets multiple genes involved in Wnt signaling, such as DKK2 (dickkopf WNT signaling pathway inhibitor 2), AXIN2 (axin 2), and FRAT2 (frequently rearranged in advanced T-cell lymphomas 2). How miR-222-3p regulates its target genes involved in Wnt signaling, thereby influencing wound healing and scar formation in the skin, deserves further investigation.

Since 1 miRNA can target many genes sharing the same seed sequence simultaneously, miRNAs have their advantages as therapeutic targets. Currently, miRNA manipulation can be achieved by either increasing the expression of a particular miRNA with synthetic miRNA mimics or blocking its action with chemically engineered oligonucleotides called antagonirs.28 The identified miRNAs, if confirmed by future studies, may shed light on the development of efficient therapies for wound healing of the patients suffering acute skin injuries. Our study has some limitations. With limited sample size, we have limited power in detecting miRNAs showing significant changes in expression. However, we believe that the miRNAs identified by all of the 3 statistical methods represent potential markers in wound healing and deserve further investigation. Our study is cross-sectional, and, therefore, we cannot analyze trajectories of miRNA expression during the wound healing process after acute skin injuries. In this study, we focused on the hypertrophic scar, which is different from keloids in that a hypertrophic scar can occur anywhere where there is a traumatic injury or operative incision.29 Although skin
tissue varies greatly with age, gender, and location of body, in this study normal skin specimen was taken from around the scar area of the same patient to minimize confounding factors.

In summary, in this pilot study, we screened for miRNAs exhibiting differential expression in patients suffering acute injuries in the skin. We identified 4 miRNAs showing significant changes in expression. Their target genes are involved in a variety of biological functions, particularly cell signaling and communication, biosynthetic, or cellular metabolism. These target genes are involved in multiple pathways such as MAPK, Wnt signaling, and focal adhesion. We also constructed a network showing the biological and chemical interaction of the focus miRNAs. The exact functions of the identified miRNAs remain unknown and deserve further investigation. Future studies with larger sample size are also needed to validate the findings of this pilot study.

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