

## Supplemental Materials for

# miR-21 Promotes Keratinocyte Migration and Re-epithelialization During Wound Healing

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## Material and Experimental Design

*Cell culture and transfection.* HaCaT cells were cultured in Dulbecco's Modified Eagle Medium supplemented with %1 MEM Non-Essential Amino (Hyclone) and 10% fetal bovine serum (Hyclone) at 37°C and 5% CO<sub>2</sub>. HaCaT cells were transiently transfected with miR-21 mimic, scrambled miRNA, miR-21 antisense oligonucleotides or scrambled oligonucleotides (Genepharma).

*Cell migration and proliferation assay.* Cell migration assay was performed using a scratch wound-healing format. 80-90% confluent keratinocytes in a 6-well plate were treated with 10 µg/ml mitomycin C (Sigma) for 2 hours in order to remove the influence of cell proliferation. Cells were then scratched, and treated with 5 ng/ml TGF-β1. Cell migration was monitored by microscopy. The images acquired for each sample can be further analyzed quantitatively. For each image, distances between one side of scratch and the other can be measured. By comparing the images from time 0 to the last time point (12 or 24 hours), we obtain the distance of each scratch closure on the basis of the distances that are measured by software. In 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay, 2×10<sup>3</sup> cells per well were seeded into a 96-well plate, and after a 4 hours culture, cell growth was measured at 0, 1, 2, 3, 5 days.

*Luciferase activity assay.* Full length of TIMP3 mRNA 3'UTR region was amplified using specific primers: sense: 5'-CCGCTCGAGATGCCAGAAAGAAT GAGG-3'; anti-sense: 5'-CGACGCGTTAGCCAGAAGCCAAACAC-3', and cloned into pGL3-CM to generate luciferase reporter vector. The method for luciferase assay was described previously [22].

*Northern blot.* Total RNA was isolated using TRIzol reagent (Invitrogen) based on the suggested protocol. Northern blot analysis was performed as described [22] using 20 µg total RNA from each sample. Probes were synthesized in Invitrogen Biotechnology Co., Ltd (Beijing) as following: miR-21: 5'-TCAACATCAGTCTGATAAGCTA-3'; U6: 5'-CGAATTTGCGTGTTCATCCTTGCG-3'; 5s rRNA: 5'-CGACCCTGCTTAGCTTCCGA-3'. Probes were labeled with <sup>32</sup>P γ-ATP (Perkin Elmer) using T4 polynucleotide kinase (New England Biolabs).

*In vivo wound-healing experiments.* Littermates of C57BL/6 mice at 7-8 weeks of the same sex were used. Four 4-mm full-thickness cutaneous biopsy punch wounds were made on either side of the mouse. The wounded tissues were collected at 3 days after injury.

*Inhibition of miR-21 in vivo.* Skin wound-healing model and the method of administration of Pluronic gel were described in previous studies [23]. 10 µl Pluronic gel F127 (Sigma) containing 1µg miR-21 AS or scramble AS were administered immediately to each punch wounds of C57BL/6 mice. Repeated administration was given 24 hours later. Wound edges were collected at 0, 1, 2 and 3 days after injury for isolation of RNA or histological analysis.

*Immunofluorescence and immunohistochemistry analysis.* Skin tissues were fixed in 4% PFA at 4°C overnight, embedded in paraffin, sectioned at 5 µm. Skin tissues snap-frozen in OCT were used for immunofluorescence (Thermo Lifesciences). For BrdU labeling, mice were injected intraperitoneally with 100

$\mu\text{g/g}$  body weight of BrdU (Sigma) 2 hours before sacrifice. The primary antibodies used were K14 (1:1000, COVANCE) and BrdU (1:300, Abcam).

*Real-time PCR.* Real-time PCR was performed with Roche LightCycler 1.5 system using a SYBR Green assay. Expression values were normalized to GAPDH expression. The primer sequences were as follows: TIMP3 (human): 5'-CAACTCCGACATCGTGATCCG-3' and 5'-GAAGCCTCGGTACATCTTCATC-3'; Timp3 (mouse): 5'-AACTCCGACATCGTGATC-3' and 5'-CAGCAGGTACTGGTATTTGT-3'; TIAM1 (human): 5'-TCTGGGATAGACCACAACA-3' and 5'-CAGAGTGGATGGCGGTGA-3'; Tiam1 (mouse): 5'-AGGCAGCAGCACTCATCC-3' and 5'-TGGCGACCTTGTTTCATAG-3'; GAPDH (human): 5'-TGAAGGTCGGAGTCAACGGAT-3' and 5'-CTGGAAGATGGTGATGGGATT-3'; GAPDH (mouse): 5'-TGCCCAGAACATCATCCCT-3' and 5'-GGTCCTCAGTGTAGCCCAAG-3'.

*Statistical analysis.* All values were expressed as mean $\pm$ SEM. Statistical analysis was performed by 2-tailed Student's *t* test, except for paired *t* test in Figure 2C and 2D. Results were considered statistically significant at  $P < 0.05$ .

**Figure S1:** The effect of miR-21 knockdown on the proliferation of HaCaT cell. MTT assay showed that knockdown of miR-21 with antisense oligonucleotides (miR-21 AS) did not show any effect on the proliferation of HaCaT cell.

