

Let-7c miRNA Inhibits the Proliferation and Migration of Heat-Denatured Dermal Fibroblasts Through Down-Regulating HSP70

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Wound healing is a complex physiological process necessitating the coordinated action of various cell types, signals and microRNAs (miRNAs). However, little is known regarding the role of miRNAs in mediating this process. In the present study, we show that let-7c miRNA is decreased in heat-denatured fibroblasts and that inhibiting let-7c expression leads to the increased proliferation and migration of dermal fibroblasts, whereas the overexpression of let-7c exerts an opposite effect. Further investigation has identified heat shock protein 70 as a direct target of let-7c and has demonstrated that the expression of HSP70 in fibroblasts is negatively correlated with let-7c levels. Moreover, down-regulation of let-7c expression is accompanied by up-regulation of Bcl-2 expression and down-regulation of Bax expression, both of which are the downstream genes of HSP70. Notably, the knockdown of HSP70 by HSP70 siRNA apparently abrogates the stimulatory effect of let-7c inhibitor on heat-denatured fibroblasts proliferation and migration. Overall, we have identified let-7c as a key regulator that inhibits fibroblasts proliferation and migration during wound healing.

INTRODUCTION

Cutaneous wound healing is a complex physiological process necessitating the coordinated efforts of various cell types and signal pathways (Reinke and Sorg, 2012). Several overlapping stages, including hemostasis, inflammation, tissue granulation, re-epithelialization, and remodeling are involved in wound healing (Barrientos et al., 2008). Denatured dermis caused by deep skin burns is strongly associated with functional impairment, cell

metabolism disorders and pathologically morphological changes. In addition, during wound healing, the denatured dermis not only participates in granulation tissue formation but also plays an essential role in skin morphological and functional recovery (Huang et al., 2001). Hence, it is of great importance to promote the proliferation and migration of denatured dermis.

MicroRNAs (miRNAs) are a class of 18-22 nt small noncoding RNAs that bind to the 3'-UTR of the target messenger RNAs (mRNAs) and result in mRNA cleavage and translational repression (Bartel, 2004). Increasing evidence has demonstrated that a cluster of miRNAs is ectopically expressed and involved in skin morphogenesis, skin cancer and cutaneous wound healing (Yi et al., 2006). For example, miR-221 and miR-222 control melanoma progression by down-regulating the cyclin-dependent kinase inhibitor 1b and the c-KIT receptor (Felicetti et al., 2008). Moreover, the high expression of miR-132 facilitates the transition from the inflammatory stage to the proliferative phase during wound healing (Li et al., 2015). Notably, significant down-regulation of let-7c has been identified in the denatured dermis of deep burn patients (Liang et al., 2012). The let-7 miRNA was originally identified in *Caenorhabditis elegans* as a regulator controlling the timing of terminal differentiation (Reinhart et al., 2000). In humans, ectopic expression of let-7 miRNA has been associated with various diseases (Calin et al., 2004; Motoyama et al., 2008; Yu et al., 2007). Experimental evidence using *in vitro* and *in vivo* models has shown that let-7 miRNA regulates the proliferation of multiple human cell types (Johnson et al., 2007). Earlier work also revealed that the endothelial fibroblast growth factors negatively regulate let-7 expression, leading to the activation of TGF β signaling and transition from the endothelium to the mesenchyme (Chen et al., 2012). These observations indicate the possible role of let-7 in the wound-healing process.

Heat shock proteins (HSPs) are a family of highly conserved proteins (Jego et al., 2013). The major hallmark of this family is that they will respond to heat-related pathology, become up-regulated under a variety of cellular stresses and block caspase-dependent apoptosis (Beere, 2004; Hartl, 1996). Prior studies demonstrated that HSPs could not only prevent the irreversible aggregation of the stress-unfolded proteins but also participate in the cell cycle, cytoskeletal rearrangement and cell apoptosis (Nardai et al., 2006), indicating that HSPs contribute greatly to homeostasis. HSP90 and HSP110 were also found to be increased following hyperthermic treatment (Kariya et al., 2014). Moreover, HSP70 is a major stress-inducible HSP,

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which has been reported to be significantly increased in B-lymphocytes under heat stress (Clayton et al., 2005). Gene ablation studies have shown that HSP70 inhibits apoptosis induced by a wide range of lethal stimuli and increases cellular survival (Schmitt et al., 2003), implying that HSP70 may play an important role in resisting heat stimuli. However, further investigation and elucidation of the precise mechanism and function of HSP70 in heat stimuli are needed.

To sum up, previous work has suggested that let-7 may be involved in wound healing, but little evidence is available to support this conjecture. In this study, we confirm that let-7c is involved in wound healing through down-regulating the proliferation and migration of heat-denatured dermal fibroblasts via inhibiting HSP70, and that inhibition of let-7c promotes cell survival, providing a putative therapeutic target for wound healing.

MATERIALS AND METHODS

Cell culture

Normal human skin was collected from patients who had undergone plastic surgery procedures in the China-Japan Union Hospital of Jilin University, with informed consent and approval from the Ethics Committee of the China-Japan Union Hospital of Jilin University. Primary human dermal fibroblasts were obtained as follows: firstly, skin tissues were dissected into 0.1×0.1 cm pieces followed by enzymatic digestion by trypsin-EDTA (Sigma, USA). Subsequently, the isolated cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, USA) containing 10% fetal bovine serum (FBS, Sigma), 100 units/ml penicillin and 100 μ g/ml streptomycin (Invitrogen, USA) in an atmosphere of 37°C with 5% CO₂. Heat-denatured human dermal fibroblasts were obtained by placing the cell culture plates in a water bath at 52°C for 30 s (with 37°C for 30 s as the control group) (De-quan et al., 2008). Primary dermal fibroblasts from passages 5-8 were used throughout the experiments. Human embryonic kidney 293 (HEK-293) cells were also cultured in DMEM supplemented with 10% FBS, 100 units/ml penicillin and 100 μ g/ml streptomycin, and incubated at 37°C with 5% CO₂.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed as previously described (Zhu et al., 2015). Briefly, total RNA was extracted from cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Analysis of miRNA expression was performed using the TaqMan MicroRNA Assay (Thermo Fisher Scientific, USA). The SYBR Green qPCR Master Mix (Thermo Fisher Scientific) was used to quantify mRNA expression. U6 SnRNA and β -actin were used for normalization of the relative levels of let-7c and genes, respectively. Data were calculated based on the $2^{-\Delta\Delta Ct}$ method.

Western blotting

A total of 25 μ g of protein extracted from cells were separated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred to nitrocellulose membranes (Bio-Rad, USA). After blocking with PBS containing 3% nonfat milk for 1 h at 37°C, the membrane was exposed to the primary antibodies, including anti-HSP70 (1:1500), anti- β -actin (1:2000), anti-Bax (1:2000) and anti-Bcl-2 (1:2000) (Santa Cruz Biotechnology, USA) and incubated at 4°C overnight. Subsequently, the horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) was added and incubated for 1 h at 37°C. The protein bands were detected

using an enhanced chemiluminescence detection system (Amersham, UK).

Cell transfection

Transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. In brief, cells were cultured in 24-well plates and transfected with 50 nM of let-7c mimics, let-7c mimics negative control (mimics control), let-7c inhibitor, and let-7c inhibitor negative control (inhibitor control) (GenePharma, China) for 48 h, respectively. Cells were transfected with HSP70 siRNA (siHSP70) and negative control siRNA (siNC) for 48h. Subsequently, the transfected cells were subjected to heat treatment at 52°C for 30 s and then collected for further analysis 24 h later. The oligonucleotide sequences used were as follows: let-7c mimics (5'-UGAGGUAGUAGGU-UGUAGGUGUU-3'), let-7c inhibitor (5'-AACCAUACAACCUAC-UACCUC-3'), mimics control (5'-UUCUCCGAACGUGUCAC-GUTT-3') and inhibitor control (5'-UUUGUACUACACAAAAG-UACU G-3').

Cell proliferation assay

Cell proliferation was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, dermal fibroblasts were cultured in 96-well plates and transfected with let-7c mimics, let-7c mimics control, let-7c inhibitor, and let-7c inhibitor control, respectively. Next, the transfected cells were subjected to heat treatment at 52°C for 30 s and 20 μ l (0.5 mg/ml) of MTT was added to each well, followed by incubation for another 4 h at 37°C. Subsequently, 100 μ l of dimethyl sulfoxide (DMSO, Sigma) was added to dissolve the formazan crystals and the absorbance at a wavelength of 490 nm was determined using an ELISA reader (Bio-Rad, USA).

Transwell migration assay

Cell migration was performed using the transwell migration assay. Briefly, the transfected dermal fibroblasts were subjected to heat treatment and cultured in serum-free DMEM for 24 h prior to the experiment. Next, 2×10^4 dermal fibroblasts were plated into the upper chamber of the transwell (Corning, USA). The lower chamber was filled with DMEM containing 10% FBS. The 48 h post-incubation, the cells on the lower chamber of the filter were counted and recorded.

Dual-luciferase reporter assay

To validate whether let-7c directly targets the HSP70 3'-UTR, we performed a dual-luciferase reporter assay. The 3'-UTR fragments of HSP70 containing the let-7c binding sequences, as well as the mutant 3'-UTR fragments were amplified and sub-cloned into the downstream sites of the firefly luciferase (luc) coding sequence in the pGL3 promoter vector (Promega, USA). Next, 0.8 μ g of the recombinant pGL3 reporter vector and 50 nM of let-7c mimics and let-7c mimics control miRNA, were co-transfected into HEK-293 cells using Lipofectamine 2000 (Invitrogen). The cells were then harvested 48 h post-transfection and the luciferase activities were measured using a dual luciferase assay kit (Promega) following manufacturer's instructions.

Statistical analysis

All quantitative data are presented as the mean \pm SD. For statistical analysis, one-way analysis of variance (ANOVA) among multiple groups, and two-tailed Student's *t*-test were performed between two groups. *p* < 0.05 was considered statistically significant.

RESULTS

Let-7c is down-regulated in heat-denatured dermal fibroblasts

To assess the potential role of let-7c in heat-denatured dermal fibroblasts, we detected the level of let-7c expression by qRT-PCR at different time points following heat damage. As shown in Fig. 1A, compared with the intact dermal fibroblasts, the basal level of let-7c in the heat-denatured fibroblasts was significantly decreased at 12 h post-heat damage, reaching its lowest point after 24 h, after which it gradually increased to basal levels at 48 h.

To further analyze and thus identify any link between the dynamically altered levels of let-7c and HSP70 expression, the total protein levels of HSP70 at different time points post heat damage were determined. Compared with the untreated dermal fibroblasts, HSP70 expression was significantly increased after 12 h and peaked at 24 h post heat damage, then gradually decreased after 48 h (Figs. 1B-1D). Overall, these results suggest that let-7c and HSP70 may be involved in the response to heat stimuli.

Down-regulation of let-7c promotes the proliferation and migration of heat-denatured dermal fibroblasts

To further investigate the biological role of let-7c in heat-denatured fibroblasts, we examined its effects on the proliferation and migration by manipulating let-7c levels using let-7c mimics (to overexpress) or inhibitor (to reduce let-7c activity) in heat-denatured dermal fibroblasts. The transfection efficiencies of the let-7c constructs were confirmed by qRT-PCR. As shown in Fig. 2A, let-7c expression was significantly up-regulated and down-regulated post transfection with let-7c mimics or inhibitor, respectively. However, the expression of let-7a, let-7d and let-7g remain unchanged, suggesting that the effect was specific to transfection with let-7c mimics and inhibitor. Cell proliferation and migration were assessed using the MTT and transwell migration assays, respectively. As shown in Fig. 2B, the MTT assay showed that let-7c inhibition induced an increase, whereas overexpression of let-7c induced a decrease in cell proliferation of dermal fibroblasts transfected with let-7c inhibitor or mimics for 48 h relative to the control miRNA. Meanwhile, transwell migration assays revealed that the migration capacity increased strikingly in the let-7c inhibitor-transfected dermal fibroblasts, whereas it was significantly decreased in the let-7c mimics-transfected dermal fibroblasts (Fig. 2C). All of these results demonstrate that let-7c is a negative regulator of dermal fibroblast proliferation and migration.

HSP70 is a target gene of let-7c

MiRNAs function by targeting the 3'-UTR of their target genes. Thus, identifying the target genes of let-7c is of great importance. We performed an analysis using two independent databases, miRanda and TargetScan. Interestingly, we found that among the more than 100 computationally predicted targets of let-7c miRNA, HSP70, which was extensively associated with heat stimuli stress, was predicted as the target gene of let-7c in both databases (Fig. 3A). To validate whether HSP70 was a direct target gene of let-7c, we performed a dual luciferase reporter assay. As shown in Fig. 3B, co-transfection of HEK-293 cells with let-7c mimics and pGL3-HSP70-3'-UTR (the luciferase reporter plasmid containing the 3'-UTR of wild type HSP70) resulted in significantly lower relative luciferase activity than co-transfection with the let-7c mimics control and pGL3-HSP70-3'-UTR. However, co-transfection of the HEK-

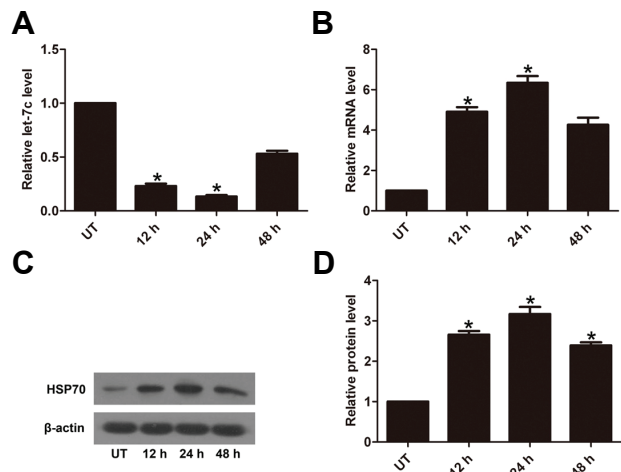


Fig. 1. Let-7c and HSP70 expression in human dermal fibroblasts at different time points (12, 24, 48 h) post-heat damage. Analysis of the level of let-7c (A) and HSP70 mRNA (B) by qRT-PCR. (C) Western blotting analysis of the expression of HSP70 protein. (D) Quantification analysis of relative protein level of HSP70 using Image-Pro Plus 6.0 software and normalized to β -actin. * $p < 0.05$ vs. untreated dermal fibroblasts (UT).

293 cells with the let-7c mimics and pGL3-HSP70-Mut-3'-UTR (the luciferase reporter plasmid containing the mutated nucleotides in the 3'-UTR of HSP70) did not have any apparent effects on luciferase activity.

To further validate that HSP70 was a direct target of let-7c, we analyzed the expression of HSP70 in heat-denatured dermal fibroblasts transfected with let-7c mimics or inhibitor. As shown in Figs. 3C and 3D, we found that the expression of HSP70 decreased significantly in those cells transfected with let-7c mimics compared with cells transfected with the mimics control, both at the mRNA and protein levels. When protein expression was quantified, an approximately 67% reduction in HSP70 was found in the cells transfected with let-7c mimics relative to mimics control group (Fig. 3E), whereas the let-7c inhibitor exerted the opposite effects. These results indicate that HSP70 is a direct target gene of let-7c.

Let-7c is involved in regulating Bax and Bcl-2 expression

As described above, the observations demonstrated that HSP70 was a direct target of let-7c; therefore, we reasoned that the expression of downstream genes of HSP70, namely Bax and Bcl-2 (Stankiewicz et al., 2005; Zyllicz et al., 2001), may be affected by let-7c transfection. To verify this hypothesis, we analyzed the effect of let-7c mimics and inhibitor on Bax and Bcl-2 mRNA and protein levels by qRT-PCR and Western blotting, respectively. The results demonstrated that, compared with the control group, knockdown of let-7c decreased the expression of Bax (Figs. 4A-4C), while it increased the expression of Bcl-2 (Figs. 4D-4F). In contrast, let-7c overexpression exerted the opposite effects (Figs. 4A-4F).

Inhibition of HSP70 abrogates the effects of let-7c

To further assess the contribution of HSP70 to the biological effects of let-7c, the expression of HSP70 was silenced via co-transfection with let-7c inhibitor and the HSP70-specific siRNA harboring no specific binding sites with let-7c. Western blotting

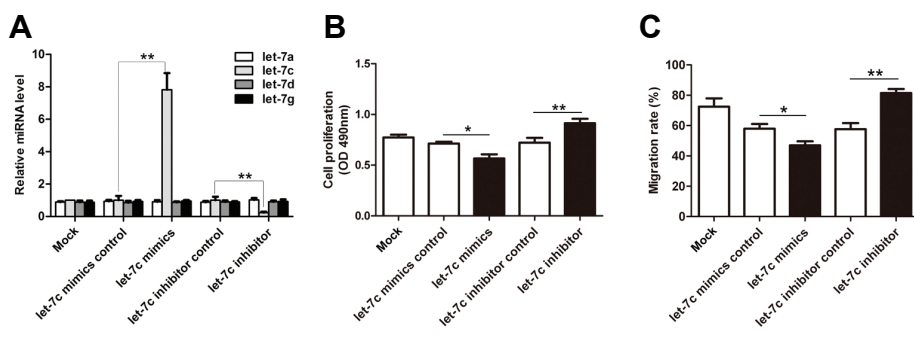


Fig. 2. Effect of let-7c on proliferation and migration in human dermal fibroblasts. (A) qRT-PCR analysis of let-7a, let-7c, let-7d, and let-7g expression in the dermal fibroblasts cells transfected with let-7c mimics or inhibitor for 48 h. Effect of let-7c mimics or inhibitor on fibroblasts proliferation (B) and migration (C) using MTT analysis or transwell migration assay 48 h post heat damage. * $p < 0.05$, ** $p < 0.01$ vs. mock or let-7c mimics (inhibitor) control.

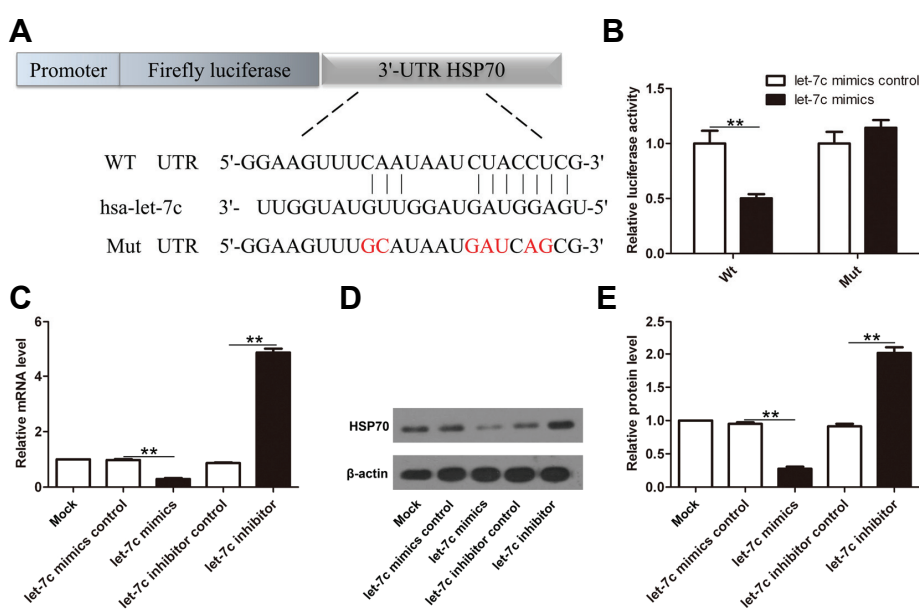


Fig. 3. HSP70 is a direct target gene of let-7c. (A) Schematic representation of luciferase constructs used for luciferase reporter assays. (B) Relative luciferase activity of reporter gene expression in HEK-293 cells transfected with let-7c mimics normalized to the average luciferase activity value of HEK-293 cells transfected with a let-7c mimics control. (C) Quantification of relative levels of HSP70 mRNA by qRT-PCR analysis. (D) Western blotting analysis of HSP70 protein levels in different groups. (E) Quantification analysis of relative protein levels of HSP70 using Image-Pro Plus 6.0 software and normalized to β -actin. ** $p < 0.01$ vs. mock or let-7c mimics (inhibitor) control.

results demonstrated that the effects of the let-7c inhibitor on the expression of HSP70, Bax and Bcl-2 were apparently blocked by the siHSP70 (Figs. 5A-5I). Moreover, the migration (Fig. 6A) and proliferation (Fig. 6B) increased by the let-7c inhibitor were significantly abrogated by siHSP70. These results indicated that let-7c played a role in heat-denatured dermal fibroblasts through regulating HSP70 expression.

DISCUSSION

In this study, we characterized let-7c as a negative regulator of proliferation and migration in heat-denatured dermal fibroblasts. First, we identified dynamic expression of let-7c during recovery from wound healing in heat-denatured dermal fibroblasts. The down-regulation of let-7c was found to be associated with a high degree of proliferation and migration. Further investigation of the mechanism identified HSP70 as a direct target of let-7c and inhibition of let-7c promoted HSP70 expression. Accordingly, inhibition of HSP70 expression partially reversed the stimulatory effects of the let-7c inhibitor on the proliferation and migration of heat-denatured dermal fibroblasts. In summary, our study indicates that let-7c negatively regulates the proliferation and migration of heat-denatured dermal fibroblasts by down-

regulating HSP70.

Several miRNAs and cell factors that participate in cellular stress responses have been identified, but the exact mechanism by which these miRNAs and components regulate cellular stress responses is not yet clear. Let-7c miRNA is highly conserved across animal species (Pasquinelli et al., 2000). Earlier research on let-7c has mainly concentrated on its roles and functions in cancer. It has been reported that let-7c inhibits human non-small-cell lung cancer metastasis by regulating ITGB3 and MAP4K3 (Zhao et al., 2014), whereas in breast cancer, let-7c inhibits cell proliferation by Myb (Bjorner et al., 2014). Recent genetic screening identified that the let-7 miRNA family was significantly down-regulated in numerous skin-related diseases, including melanomas (Schultz et al., 2008) and systemic sclerosis (Koba et al., 2013). Moreover, down-regulation of let-7c was observed in denatured dermis (Liang et al., 2012). Meanwhile, recent studies have indicated that let-7c is involved in the TGF signal in angiogenesis including a suggestion that an increase in let-7c levels reverses this process (Chen et al., 2012). Despite these important biological functions, little is known regarding the role of let-7c in wound healing.

After undergoing heat denaturation, fibroblasts are activated and become major cells at the wound site, which can secrete

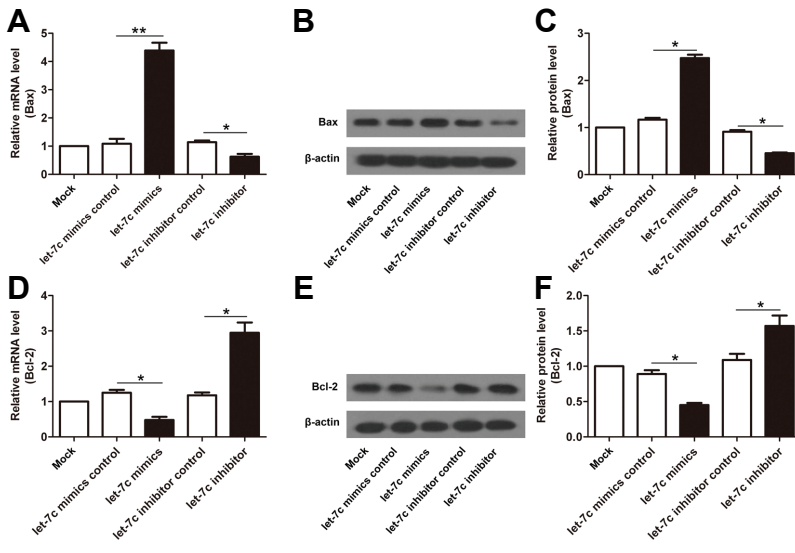


Fig. 4. Effect of let-7c on the expression of Bax and Bcl-2. qRT-PCR analysis of Bax (A) and Bcl-2 (D) mRNA expression in dermal fibroblasts cells transfected with let-7c mimics or inhibitor. Western blotting assay of Bax (B) and Bcl-2 (E) protein expression in the different treated groups. Quantitative analysis of relative protein levels of total Bax (C) and Bcl-2 (F) using Image-Pro Plus 6.0 software and normalized to β -actin. After transfection for 48 h, cells were harvested for analysis. * $p < 0.05$, ** $p < 0.01$ vs. mock or let-7c mimics (inhibitor) control.

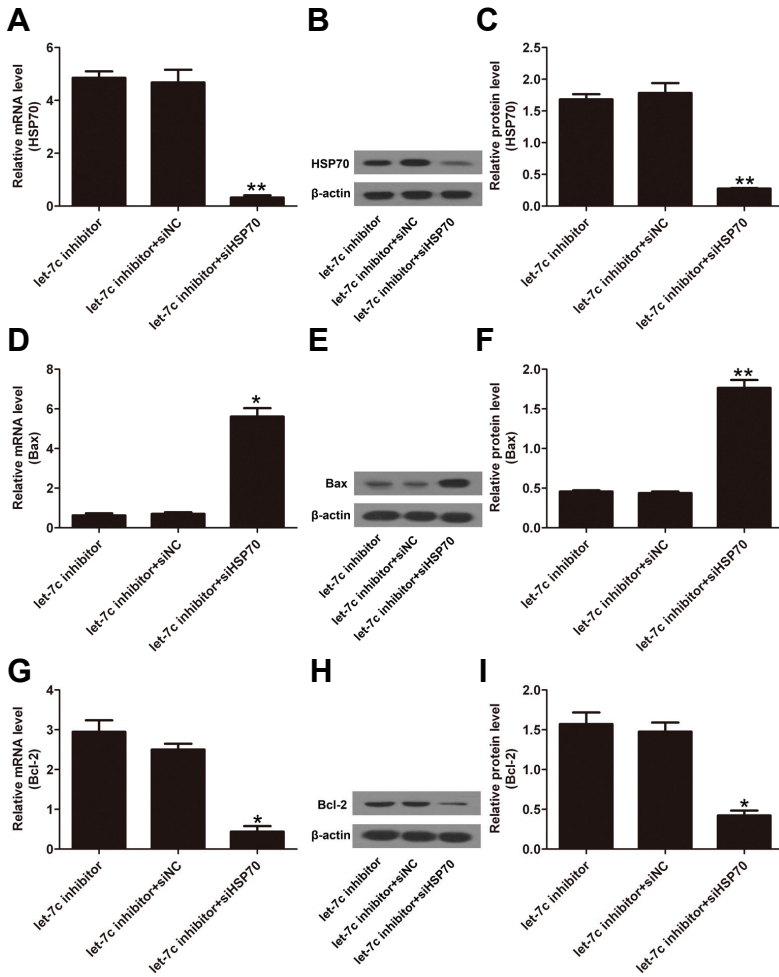


Fig. 5. Inhibition of HSP70 abrogates the effects of let-7c on Bax and Bcl-2 expression. qRT-PCR analysis of HSP70 (A), Bax (D) and Bcl-2 (G) mRNA levels in dermal fibroblasts cells. Western blotting assay of HSP70 (B), Bax (E) and Bcl-2 (H) protein levels in the different treated groups. Quantitative analysis of the relative protein levels of total HSP70 (C), Bax (F) and Bcl-2 (I) using Image-Pro Plus 6.0 software and normalized to β -actin. * $p < 0.05$, ** $p < 0.01$ vs. let-7c inhibitor or siNC.

collagen fibers and several growth factors (Martin, 1997). In this study, we examined the role of let-7c in heat-denatured dermal fibroblasts. The results demonstrated a down-regulation of let-

7c expression in the heat-denatured dermal fibroblasts, consistent with the miRNA array results described earlier (Liang et al., 2012). Moreover, the expression of let-7c was shown to be

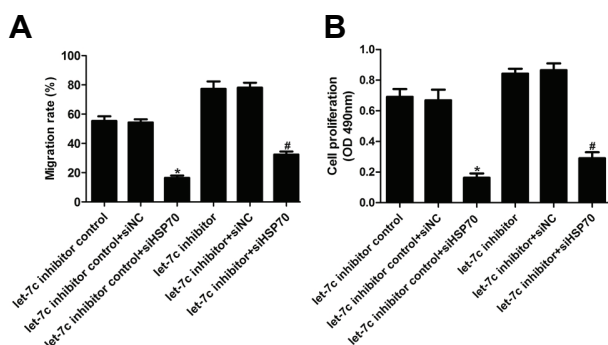


Fig. 6. Knockdown of HSP70 blocks the effect of the let-7c inhibitor on the proliferation and migration of heat-denatured fibroblasts. Cell migration (A) and proliferation (B) were analyzed by transwell migration assay and MTT assay, respectively. * $p < 0.05$ vs. let-7c inhibitor control or let-7c inhibitor control + siNC; # $p < 0.05$ vs. let-7c inhibitor or let-7c inhibitor + siNC.

time-dependent during the recovery of heat-denatured dermis. Furthermore, inhibition of let-7c accelerated cell proliferation and migration, whereas overexpression of let-7c exerted the opposite effects. These data suggested that let-7c may play an important role in regulating dermal fibroblasts during wound healing.

As we know, miRNAs function by targeting the 3'-UTR of the target genes. RAS, CDC25A and MYC have been identified as the let-7c targets in various human cancers (Johnson et al., 2005; Nadiminty et al., 2012; Zhu et al., 2015). In this study, we illustrate for the first time that HSP70 is a direct target gene of let-7c as the mediator of fibroblasts proliferation and migration. Among the more than 100 putative targets of let-7c known from bioinformatic analysis, we noticed that HSP70 was the special heat stimuli stress-associated gene that was predicted by miRanda and TargetScan (data not shown). Furthermore, in a number of cell lines, heat and other physiological and environmental stimuli such as hypoxia, hyperoxia and surgical stress have been suggested to induce the transcriptional up-regulation of HSPs (Ciocca and Calderwood, 2005). In cancer cells, HSP70 is expressed at unusually high levels, leading to an antiapoptotic effect (Wang et al., 2014). HSP70 is also shown to be up-regulated in the keloid fibroblasts (Shin et al., 2015). Our results indicate that HSP70 is a direct target gene of let-7c. Accordingly, inhibition of let-7c promotes the expression of HSP70 in heat-denatured fibroblasts. HSP70 is believed to be induced transcriptionally upon heat shock. However, upon heat shock, we identified an approximately 5-fold and 2-fold increase in HSP70 at the mRNA and protein levels, respectively in the let-7c inhibitor-transfected fibroblasts compared with the untransfected controls, suggesting an important role of let-7c in regulating HSP70 upon heat shock. Furthermore, the knockdown of HSP70 expression significantly attenuated and reversed the enhanced proliferation and migration induced by the let-7c inhibitor, in agreement with earlier research showing that HSP70 promotes cell proliferation and viability in cancer cells (Singh and Suri, 2014).

To summarize, we have identified let-7c as a novel suppressor of cell proliferation and migration in heat-denatured dermal fibroblasts. The inhibition of let-7c expression promotes HSP70 expression which contributes to increasing cell proliferation and migration. Overall, this study indicates that let-7c could be a

promising therapeutic target for the treatment of burn diseases, although further investigation is needed to reveal the underlying mechanism.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

REFERENCES

- Barrientos, S., Stojadinovic, O., Golinko, M.S., Brem, H., and Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. *Wound Repair Regen.* 16, 585-601.
- Bartel, D.P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281-297.
- Beere, H.M. (2004). "The stress of dying": the role of heat shock proteins in the regulation of apoptosis. *J. Cell Sci.* 117, 2641-2651.
- Bjorner, S., Fitzpatrick, P.A., Li, Y., Allred, C., Howell, A., Ringberg, A., Olsson, H., Miller, C.J., Axelson, H., and Landberg, G. (2014). Epithelial and stromal microRNA signatures of columnar cell hyperplasia linking Let-7c to precancerous and cancerous breast cancer cell proliferation. *PLoS One* 9, e105099.
- Calin, G.A., Sevignani, C., Dumitru, C.D., Hyslop, T., Noch, E., Yendamuri, S., Shimizu, M., Rattan, S., Bullrich, F., Negrini, M., et al. (2004). Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl. Acad. Sci. USA* 101, 2999-3004.
- Chen, P.Y., Qin, L., Barnes, C., Charisse, K., Yi, T., Zhang, X., Ali, R., Medina, P.P., Yu, J., Slack, F.J., et al. (2012). FGF regulates TGF-beta signaling and endothelial-to-mesenchymal transition via control of let-7 miRNA expression. *Cell Rep.* 2, 1684-1696.
- Ciocca, D.R., and Calderwood, S.K. (2005). Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress Chaperones* 10, 86.
- Clayton, A., Turkes, A., Navabi, H., Mason, M.D., and Tabi, Z. (2005). Induction of heat shock proteins in B-cell exosomes. *J. Cell Sci.* 118, 3631-3638.
- De-quan, L., Xiao-yuan, H., and Xing-hua, Y. (2008). Morphology of human skin fibroblasts after heat injury *in vitro*. *J. Clin. Rehabilitative Tissue Engineering Res.* 12, 2056.
- Felicetti, F., Errico, M.C., Bottero, L., Segnalini, P., Stoppacciaro, A., Biffoni, M., Felli, N., Mattia, G., Petrin, M., Colombo, M.P., et al. (2008). The promyelocytic leukemia zinc finger-microRNA-221/222 pathway controls melanoma progression through multiple oncogenic mechanisms. *Cancer Res.* 68, 2745-2754.
- Hartl, F.U. (1996). Molecular chaperones in cellular protein folding. *Nature* 381, 571-579.
- Huang, X., Yang, X., Lei, S., Xiao, M., Zhang, M., and Zeng, J. (2001). With the preservation of denatured dermis and autologous skin grafting to repair of deeply burned hands. *Zhonghua Shao Shang Za Zhi* 17, 60-61.
- Jego, G., Hazoume, A., Seigneuric, R., and Garrido, C. (2013). Targeting heat shock proteins in cancer. *Cancer Lett.* 332, 275-285.
- Johnson, S.M., Grosshans, H., Shingara, J., Byrom, M., Jarvis, R., Cheng, A., Labourier, E., Reinert, K.L., Brown, D., and Slack, F.J. (2005). RAS is regulated by the let-7 microRNA family. *Cell* 120, 635-647.
- Johnson, C.D., Esquela-Kerscher, A., Stefani, G., Byrom, M., Kelnar, K., Ovcharenko, D., Wilson, M., Wang, X., Shelton, J., and Shingara, J. (2007). The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Res.* 67, 7713-7722.
- Kariya, A., Furusawa, Y., Yunoki, T., Kondo, T., and Tabuchi, Y. (2014). A microRNA-27a mimic sensitizes human oral squamous cell carcinoma HSC-4 cells to hyperthermia through downregulation of Hsp110 and Hsp90. *Int. J. Mol. Med.* 34, 334-340.
- Koba, S., Jinnin, M., Inoue, K., Nakayama, W., Honda, N., Makino, K., Kajihara, I., Makino, T., Fukushima, S., and Ihn, H. (2013). Expression analysis of multiple microRNAs in each patient with scleroderma. *Exp. Dermatol.* 22, 489-491.
- Li, D., Wang, A., Liu, X., Meisgen, F., Grunler, J., Botusan, I.R., Narayanan, S., Erikci, E., Li, X., Blomqvist, L., et al. (2015).

- MicroRNA-132 enhances transition from inflammation to proliferation during wound healing. *J. Clin. Invest.* 125, 3008-3026.
- Liang, P., Lv, C., Jiang, B., Long, X., Zhang, P., Zhang, M., Xie, T., and Huang, X. (2012). MicroRNA profiling in denatured dermis of deep burn patients. *Burns* 38, 534-540.
- Martin, P. (1997). Wound healing—aiming for perfect skin regeneration. *Science* 276, 75-81.
- Motoyama, K., Inoue, H., Nakamura, Y., Uetake, H., Sugihara, K., and Mori, M. (2008). Clinical significance of high mobility group A2 in human gastric cancer and its relationship to let-7 microRNA family. *Clin. Cancer Res.* 14, 2334-2340.
- Nadiminty, N., Tummala, R., Lou, W., Zhu, Y., Zhang, J., Chen, X., eVere White, R.W., Kung, H.J., Evans, C.P., and Gao, A.C. (2012). MicroRNA let-7c suppresses androgen receptor expression and activity via regulation of Myc expression in prostate cancer cells. *J. Biol. Chem.* 287, 1527-1537.
- Nardai, G., Vegh, E.M., Prohaszka, Z., and Csermely, P. (2006). Chaperone-related immune dysfunction: an emergent property of distorted chaperone networks. *Trends Immunol.* 27, 74-79.
- Pasquinelli, A.E., Reinhart, B.J., Slack, F., Martindale, M.Q., Kuroda, M.I., Maller, B., Hayward, D.C., Ball, E.E., Degnan, B., Muller, P., et al. (2000). Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 408, 86-89.
- Reinhart, B.J., Slack, F.J., Basson, M., Pasquinelli, A.E., Bettinger, J.C., Rougvie, A.E., Horvitz, H.R., and Ruvkun, G. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* 403, 901-906.
- Reinke, J.M., and Sorg, H. (2012). Wound repair and regeneration. *Eur. Surg. Res.* 49, 35-43.
- Schmitt, E., Parcellier, A., Gurbuxani, S., Cande, C., Hammann, A., Morales, M.C., Hunt, C.R., Dix, D.J., Kroemer, R.T., Giordanetto, F., et al. (2003). Chemosensitization by a non-apoptogenic heat shock protein 70-binding apoptosis-inducing factor mutant. *Cancer Res.* 63, 8233-8240.
- Schultz, J., Lorenz, P., Gross, G., Ibrahim, S., and Kunz, M. (2008). MicroRNA let-7b targets important cell cycle molecules in malignant melanoma cells and interferes with anchorage-independent growth. *Cell Res.* 18, 549-557.
- Shin, J.U., Lee, W.J., Tran, T.N., Jung, I., and Lee, J.H. (2015). Hsp70 knockdown by siRNA decreased collagen production in keloid fibroblasts. *Yonsei Med. J.* 56, 1619-1626.
- Singh, S., and Suri, A. (2014). Targeting the testis-specific heat-shock protein 70-2 (HSP70-2) reduces cellular growth, migration, and invasion in renal cell carcinoma cells. *Tumour Biol.* 35, 12695-12706.
- Stankiewicz, A.R., Lachapelle, G., Foo, C.P., Radicioni, S.M., and Mosser, D.D. (2005). Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation. *J. Biol. Chem.* 280, 38729-38739.
- Wang, X., Chen, M., Zhou, J., and Zhang, X. (2014). HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy (Review). *Int. J. Oncol.* 45, 18-30.
- Yi, R., O'Carroll, D., Pasolli, H.A., Zhang, Z., Dietrich, F.S., Tarakhovskiy, A., and Fuchs, E. (2006). Morphogenesis in skin is governed by discrete sets of differentially expressed microRNAs. *Nat. Genet.* 38, 356-362.
- Yu, F., Yao, H., Zhu, P., Zhang, X., Pan, Q., Gong, C., Huang, Y., Hu, X., Su, F., and Lieberman, J. (2007). let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell* 131, 1109-1123.
- Zhao, B., Han, H., Chen, J., Zhang, Z., Li, S., Fang, F., Zheng, Q., Ma, Y., Zhang, J., Wu, N., and Yang, Y. (2014). MicroRNA let-7c inhibits migration and invasion of human non-small cell lung cancer by targeting ITGB3 and MAP4K3. *Cancer Lett.* 342, 43-51.
- Zhu, X., Wu, L., Yao, J., Jiang, H., Wang, Q., Yang, Z. and Wu, F. (2015). MicroRNA let-7c inhibits cell proliferation and induces cell cycle arrest by targeting CDC25A in human hepatocellular carcinoma. *PLoS One* 10, e0124266.
- Zylicz, M., King, F.W., and Wawrzynow, A. (2001). Hsp70 interactions with the p53 tumour suppressor protein. *EMBO J.* 20, 4634-4638.