

# CircRNA\_0016418 expedites the progression of human skin melanoma via miR-625/YY1 axis

Y. ZOU<sup>1</sup>, S.-S. WANG<sup>1</sup>, J. WANG<sup>1</sup>, H.-L. SU<sup>1</sup>, J.-H. XU<sup>1,2</sup>

<sup>1</sup>Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China

<sup>2</sup>Shanghai Institute of Dermatology, Shanghai, China

**Abstract. – OBJECTIVE:** Some circular RNAs (circRNAs) have been testified to play crucial roles in the regulation of skin melanoma, including circRNA\_0016418 (circ0016418). However, the regulatory mechanism of circ0016418 in skin melanoma is undiscovered.

**MATERIALS AND METHODS:** The RNA expression was examined through quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and associated-proteins levels were measured via Western blot. Cell counting kit-8 (CKK-8) assay was used for detecting cell proliferation. Transwell assay was conducted to assess the abilities of migration and invasion. The target relation was analyzed by Dual-Luciferase reporter assay.

**RESULTS:** The levels of circ0016418 and Yin Yang 1 (YY1) were up-regulated in skin melanoma tissues and cells. Knockdown of both circ0016418 and YY1 had suppressive effects on proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) of skin melanoma cells. YY1 overexpression reverted the inhibitory effects on skin melanoma cells caused by circ0016418 knockdown. Circ0016418 negatively modulated microRNA-625 (miR-625) expression and miR-625 directly targeted YY1. Circ0016418 functioned as a competitive endogenous RNA (ceRNA) of miR-625 to regulate YY1 expression.

**CONCLUSIONS:** Circ0016418 regulated proliferation, migration, invasion, and EMT of skin melanoma cells through miR-625/YY1 axis. Circ0016418 might be a useful indicator of the therapeutic strategies of skin melanoma.

*Key Words:*

CircRNA, Circ0016418, Skin melanoma, MiR-625, YY1.

## Introduction

Skin melanoma is a kind of skin cancers recognized as a common malignancy along with squamous cell carcinoma, basal cell carcinoma, and so

on<sup>1</sup>. Actually, skin melanoma accounts for only a small percentage of skin cancers, but it is responsible for the great mass of skin cancers deaths<sup>2</sup>. Due to the complexity of risk factors, including melanocytic nevi, the exposure of ultraviolet (UV), familiar history, and genetic susceptibility<sup>3</sup>, searching more biomarkers is pivotal for the treatment and prognosis of skin melanoma.

Circular RNAs (circRNAs) are highly stable and relatively resistant to degradation due to the covalent closed-loop structures with the deficiency of accessible tail ends<sup>4,5</sup>. Authors<sup>6-9</sup> suggested that circRNAs exerted the fundamental function in the regulation and tumorigenesis of human cancers, as transcriptional/post-transcriptional regulators. Recently, circRNAs were reported to generate a significant effect on the progression of skin cancers, such as circRNA006612, circRNA0025039, and circRNA005537<sup>10</sup>. CircRNA\_0016418 (circ0016418) is derived from the Vasohibin2 (VASH2) gene and located on-chr1 (213134507-213147412). Nevertheless, there is little research on the regulatory mechanism of circ0016418 in skin melanoma.

As acknowledged tumor regulators, microRNAs (miRNAs) combined with the 3'-untranslated regions (3'-UTRs) of targets to degrade mRNA and reduce translation<sup>11</sup>. MiRNAs were showed to be dysregulated and efficient for the therapy and evolution of skin melanoma<sup>12-14</sup>. For example, miR-195 restrained cell proliferation in skin melanoma cells by regulating prohibitin 1 (PHB1)<sup>15</sup>; miR-138 had a reduction of cell growth and metastasis in skin melanoma cells via negatively targeting the hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ )<sup>16</sup>. However, the regulatory mechanism of microRNA-625 (miR-625) is not fully understood.

Yin Yang 1 (YY1) is a zinc finger protein of human glioma-associated oncogene (GLI)-Kruppel family<sup>17</sup> and exerts enormous impacts on the

regulation of tumor growth and development at the transcriptional level<sup>18,19</sup>. Liu et al<sup>20</sup> showed that YY1 inhibited the proliferation and migration in pancreatic ductal adenocarcinoma by modulating some signal pathways. Su et al<sup>21</sup> declared that YY1 promoted proliferation and migration of nasopharyngeal carcinoma (NPC) cells. YY1 has been reported to be a carcinogen in skin melanoma<sup>22</sup>, but further researches about the concrete mechanism are needed.

In our present report, both the effects of circ0016418 on cellular behaviors of skin melanoma cells and the regulatory mechanism were explored. This study emphasizes the modulatory mechanism of circ0016418 in skin melanoma and aims to exploit a novel biomarker and provide the basis for the skin melanoma progression.

## Patients and Methods

### Tissues Collection and Cell Culture

30 pairs of skin melanoma tissues and relative adjacent tissues were collected from patients who were subjected to the melanoma resection (no treatment for skin melanoma before surgery) at Huashan Hospital, Fudan University (Shanghai, China). All tissues were immediately stored at  $-80^{\circ}\text{C}$ . Informed consent was signed by all participants. Our research was authorized by the Ethics Committee of Huashan Hospital, Fudan University (Shanghai, China).

Human normal keratinocyte cell line HaCaT was purchased from Cell Lines Service GmbH (CLS GmbH, Eppelheim, Germany) and skin melanoma cell lines (SKMEL1 and SKMEL5) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). These cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) replenished with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and antibiotics (100 U/mL penicillin and 100  $\mu\text{g}/\text{mL}$  streptomycin; Gibco, Rockville, MD, USA) in a  $37^{\circ}\text{C}$  incubator with 5%  $\text{CO}_2$ .

### Cell Transfection

Small interfering RNA (siRNA) against circ0016418 (si-circ0016418#1, si-circ0016418#2 and si-circ0016418#3), siRNA targeting YY1 (si-YY1#1, si-YY1#2 and si-YY1#3), pcDNA-YY1 overexpression vector (YY1), miR-625 mimic (miR-625), miR-625 inhibitor (anti-miR-625), and corresponding negative controls (si-NC, vec-

tor, miR-NC, anti-NC) were purchased from GENEWIZ (Suzhou, China). Lipofectamine 3000 kit (Invitrogen, Carlsbad, CA, USA) was used for cell transfection. When inoculated cells reached 80% of the monolayer coverage, vectors/oligonucleotides-lipid complexes were added to cells following the operating manual. Then, cells were harvested at the specific time according to the requirements of assays.

### Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The extraction of total RNA was implemented utilizing TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Following the reference manuals, reverse transcription experiment and PCR reaction were administrated by PrimeScript<sup>TM</sup> RT Master Mix and TB Green<sup>®</sup> Premix Ex Taq<sup>TM</sup> II Kit (TaKaRa, Dalian, China), respectively. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and small nuclear RNA U6 were used for normalizing the data. The primers were synthesized from GENEWIZ and listed as follows: circ0016418 (Forward: 5'-CTCCGACCCAAGTGAGAAGC-3', Reverse: 5'-CAGCCTGTAGTTTGGGACC-3'); VASH2 (Forward: 5'-ACGTCTCAAAGATGCTGAGG-3', Reverse: 5'-CTCTCCGACCCAAGTGAGAA-3'); miR-625 (Forward: 5'-CCAGGGGAAAGTTCTATAGTCC-3', Reverse: 5'-CAGTGCGTGTCGTGGAGT-3'); YY1 (Forward: 5'-TAACTTTGCTTGCGGTAGATT-3', Reverse: 5'-CTACAACTGAGCACCACCTTTCT-3'); GAPDH (Forward: 5'-GGGAACTGTGCGGTGAT-3', Reverse: 5'-GAGTGGGTGTCGCTGTTGA-3'); U6 (Forward: 5'-GC-GCGTCGTGAAGCGTTC-3', Reverse: 5'-GTGCAGGGTCCGAGGT-3'). The analysis of relative expression levels was performed through the  $2^{-\Delta\Delta\text{Ct}}$  method.

### Ribonuclease R (RNase R) Treatment

For the sake of analyzing the stability of circ0016418, 2  $\mu\text{g}$  extracted RNA was incubated using 3U/ $\mu\text{g}$  RNase R (Epicentre Technologies; Madison, WI, USA) for 30 min under the temperature of  $37^{\circ}\text{C}$ . After RNase R was treated, the relative RNA levels of circ0016418 and VASH2 were examined through qRT-PCR.

### Western Blot

After total proteins were extracted by cell lysis buffer (Sigma-Aldrich, St. Louis, MO, USA), Western blot was administrated first through sodium dodecyl sulfate-polyacrylamide gel

electrophoresis (SDS-PAGE). After that, the polyvinylidene difluoride (PVDF) membranes (Sigma-Aldrich, St. Louis, MO, USA) were utilized to transfer proteins, following blocked by 5% skim milk (Sigma-Aldrich; St. Louis, MO, USA) for more than 3 h. After that, under the temperature of 4°C, the membranes were incubated with primary antibodies anti-YY1 (Abcam, CA, UK, ab109237, 1:1000), anti-E-cadherin (Abcam, ab40772, 1:1000), anti-N-cadherin (Abcam, ab76011, 1:1000), anti-Vimentin (Abcam, ab92547, 1:1000) or anti-GAPDH (Abcam, ab181602, 1:3000) overnight. After incubation with primary antibodies, secondary antibody (Abcam, ab205718, 1:5000) was incubated for about 45 min at room temperature. Eventually, the bands of proteins were emerged by means of enhanced chemiluminescence (ECL) reagent (Sigma-Aldrich; St. Louis, MO, USA).

#### **Cell Counting Kit-8 (CCK-8)**

SKMEL1 and SKMEL5 cells were inoculated into a 96-well plate to perform cell transfection. At 0 h, 24 h, 48 h, and 72 h, cell incubation of 10  $\mu$ L CCK-8 (Sigma-Aldrich; St. Louis, MO, USA) lasted for another 2 h. Then, the optical density (OD) value at the wavelength of 450 nm could be detected with the help of the microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

#### **Transwell Migration and Invasion Assays**

In the migration assay, cells were first seeded into the upper chamber of transwell 24-well chamber (Corning, Corning, NY, USA). Next, the lower chamber was added with DMEM containing 10% FBS in moderation. 24 h later, cells were fastened by 4% paraformaldehyde and stained by crystal violet (Sangon Biotech, Shanghai, China). Finally, migrated cells were recognized by a microscope. Unlike the migration assay, the upper chamber must be packaged using Matrigel (Corning Life Sciences, Tewksbury, MA, USA) before cell seeding. Then, invaded cells can be observed.

#### **Dual-Luciferase Reporter Assay**

Circular RNA Interactome and starBase were used for the prediction of circRNA-miRNA and miRNA-gene, respectively. The sequences of wide-type (WT) circ0016418 and 3'-UTR of WT-YY1 were severally cloned into pGL3 basic Luciferase vector (Promega, Madison, WI, USA) to construct WT-circ0016418 and YY1 3'-UTR-WT, along with their mutant-type (MT) controls (MUT-circ0016418 and YY1 3'-UTR-MUT). Sub-

sequently, four Luciferase reporter plasmids were transfected into SKMEL1 and SKMEL5 cells with miR-625 or miR-NC, respectively. Then, Dual-Luciferase reporter system (Promega, Madison, WI, USA) was used for the measurement of relative Luciferase activity from lysed cells, in light of the user's guideline.

#### **Statistical Analysis**

Data were revealed as the mean  $\pm$  standard deviation (SD) with repetition at least three times. Biological analysis and data handling were carried out by SPSS 19.0 (IBM, Armonk, NY, USA) and GraphPad Prism 7 (San Diego, CA, USA). Linear relationship was analyzed with Spearman's correlation coefficient. Differences analysis was executed through Student's *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett's test. The difference was significant when  $p < 0.05$ .

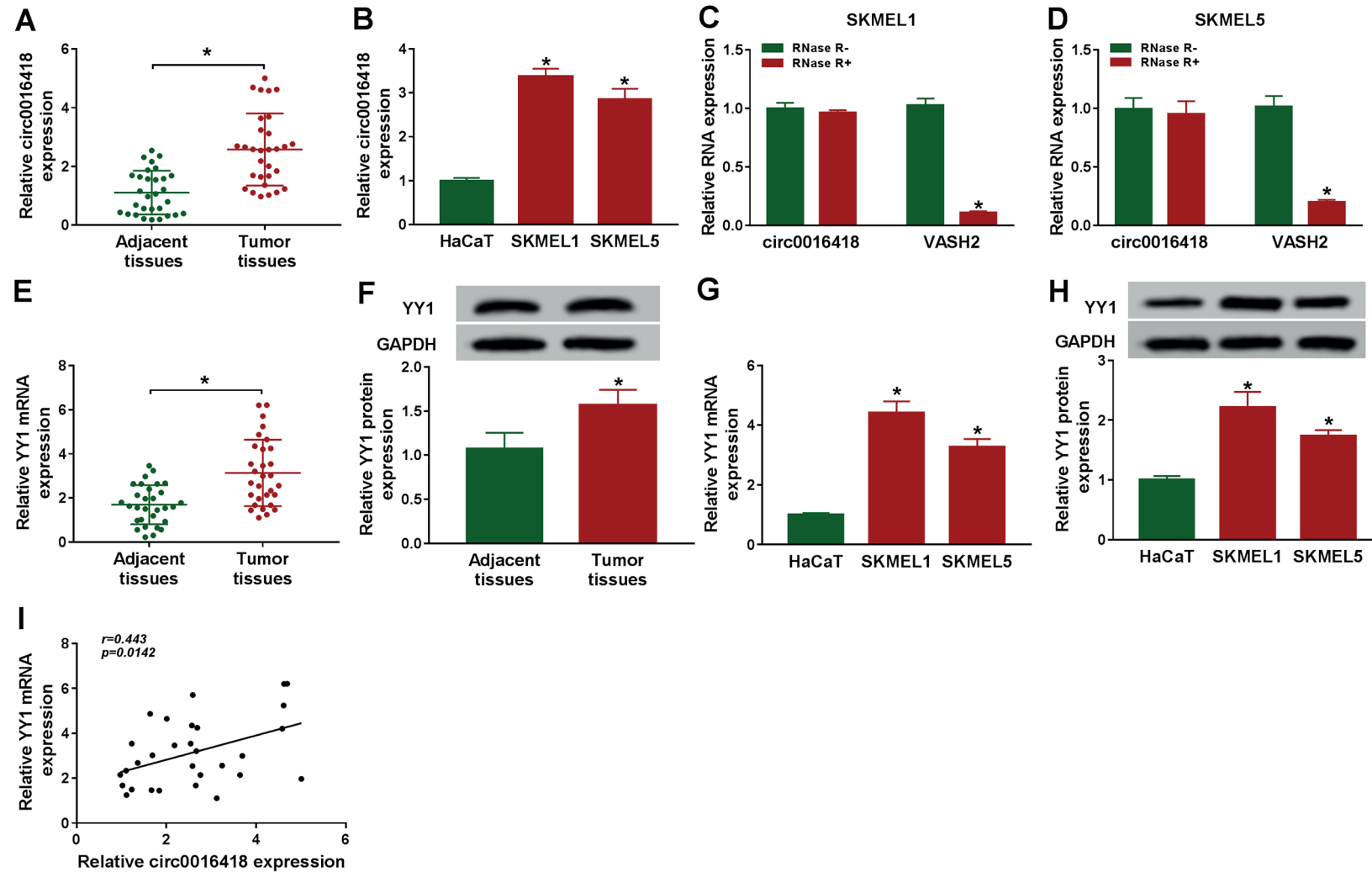
## **Results**

#### ***Circ0016418 and YY1 Were Up-Regulated in Skin Melanoma Tissues and Cells***

The expression of circ0016418 in skin melanoma was first measured. Through the analysis of qRT-PCR, an apparent increase of circ0016418 level was observed in skin melanoma tissues (Figure 1A) and cells (SKMEL1 and SKMEL5; Figure 1B) by comparison with normal adjacent tissues and HaCaT cell line. The resistance of circ0016418 to RNase R was much higher than the original gene VASH2 in SKMEL1 (Figure 1C) and SKMEL5 (Figure 1D) cells, insinuating that circ0016418 was more stable. Besides, the mRNA and protein levels of YY1 were both distinctly up-regulated in skin melanoma tissues contrasted to adjacent tissues (Figure 1E and F). Likewise, there was a significant elevation in YY1 mRNA (Figure 1G) and protein (Figure 1H) levels in SKMEL1 and SKMEL5 cells. Due to the identical trend of high expression, we analyzed the linear relation between circ0016418 and YY1 in skin melanoma tissues, and a significant positive correlation was observed ( $r=0.443$ ,  $p=0.0142$ ; Figure 1I). The up-regulation of circ0016418 and YY1 might be related to the regulation of skin melanoma.

#### ***Knockdown of Circ0016418 Reduced Proliferation, Migration, Invasion, and Epithelial-Mesenchymal Transition (EMT) in Skin Melanoma Cells***

The role of circ0016418 in skin melanoma was first explored. In SKMEL1 and SK-



**Figure 1.** Circ0016418 and YY1 were up-regulated in skin melanoma tissues and cells. **A-B**, QRT-PCR was used to determine the circ0016418 expression in skin melanoma tissues (**A**) and cells (**B**). **C-D**, Circ0016418 and VASH2 levels were measured by qRT-PCR in SKMEL1 and SKMEL5 cells treated with RNase R. **E-H**, MRNA and protein levels of YY1 were detected by qRT-PCR and Western blot in skin melanoma tissues (**E** and **F**) and cells (**G** and **H**). **I**, Linear correlation between circ0016418 and YY1 was analyzed with Spearman's correlation coefficient. \* $p < 0.05$ .

MEL5 cells, the expression of circ0016418 was strikingly interfered by si-circ0016418#1, si-circ0016418#2, and si-circ0016418#3 (Figure 2A and B). Si-circ0016418#2 with the highest interference efficiency was selected to perform the subsequent assays. After transfection with si-circ0016418#2, cell proliferation was overtly suppressed in both SKMEL1 (Figure 2C) and SKMEL5 (Figure 2D) cells compared with si-NC group. Similarly, the numbers of migrated cells (Figure 2E) and invaded cells (Figure 2F) in si-circ0016418#2 group were fewer than those in si-NC group. In addition, the protein level of E-cadherin (EMT inhibited marker) was notably increased while the levels of N-cadherin and Vimentin (EMT promoted markers) were both descended in SKMEL1 (Figure 2G) and SKMEL5 (Figure 2H) cells transfected with si-circ0016418#2. The above data demonstrated that cell proliferation, migration, invasion, and EMT were all restrained in skin melanoma cells after circ0016418 was knocked down.

#### ***Inhibition of YY1 Generated Suppressive Effects on Proliferation, Migration, Invasion and EMT in Skin Melanoma Cells***

YY1 was knocked down to investigate the function in skin melanoma. QRT-PCR and Western blot showed that the knockdown efficiencies of si-YY1#1, si-YY1#2, and si-YY1#3 were all prominent, and si-YY1#1 was the greatest of both mRNA (Figure 3A and B) and protein (Figure 3C and D) levels. Thus, SKMEL1 and SKMEL5 cells were transfected with si-YY1#1 or si-NC. CCK-8 revealed that the OD value was lower in si-YY1#1 group than that in si-NC group (Figure 3E and F). The transwell assay indicated that cell migration (Figure 3G) and invasion (Figure 3H) were both refrained by transfection of si-YY1#1. Also, down-regulation of YY1 significantly enhanced the protein level of E-cadherin but reduced the levels of N-cadherin and Vimentin in SKMEL1 (Figure 3I) and SKMEL5 (Figure 3J) cells. In short, inhibition of YY1 had inhibitory effects on proliferation, migration, invasion, and EMT of skin melanoma cells.

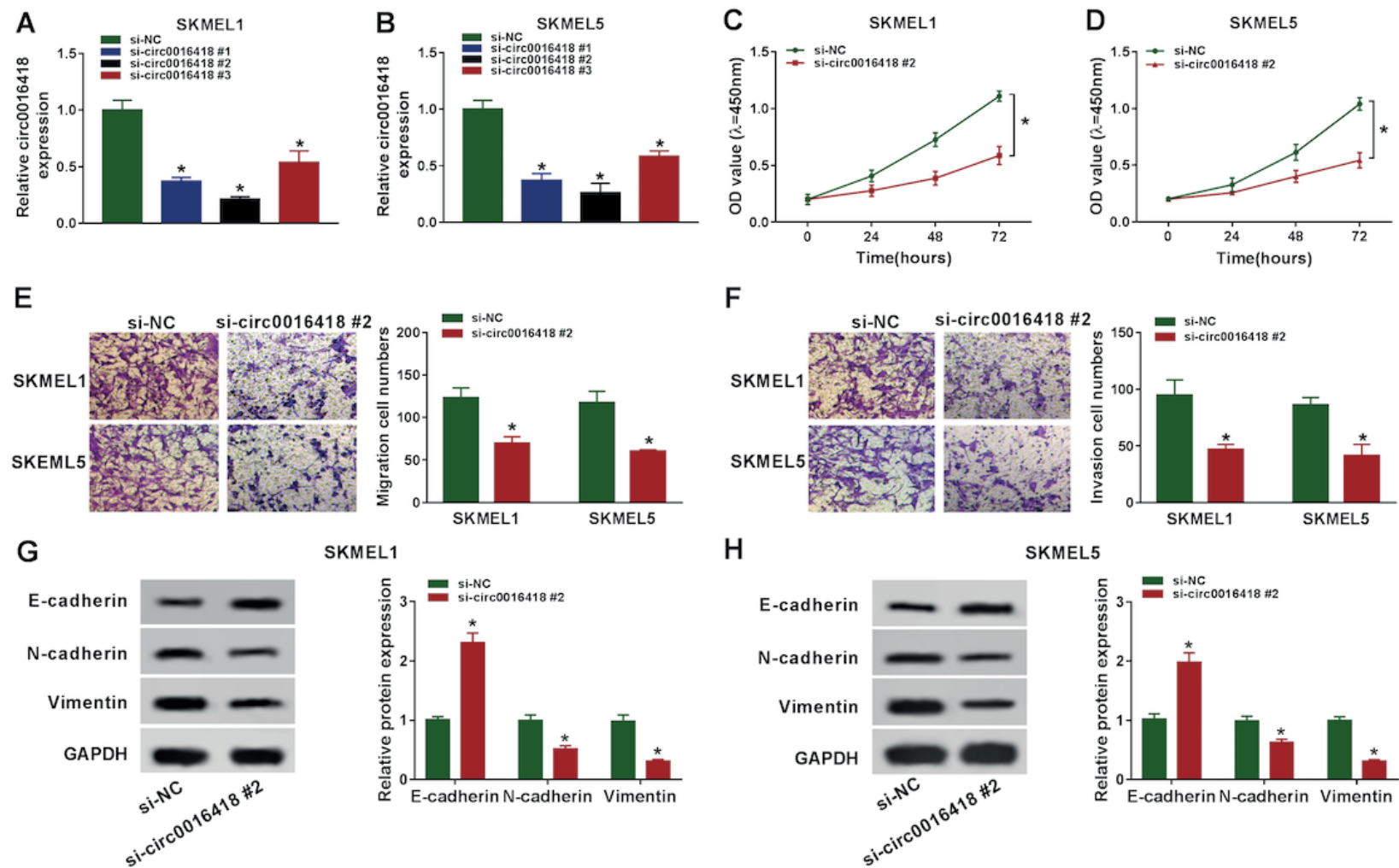
#### ***Overexpression of YY1 Reversed the Si-Circ0016418-Induced Effects on Skin Melanoma Cells***

Si-circ0016418#2, si-circ0016418#2+YY1 or matched controls were transfected into SKMEL1 and SKMEL5 cells for exploring the interaction between circ0016418 and YY1 in cellular

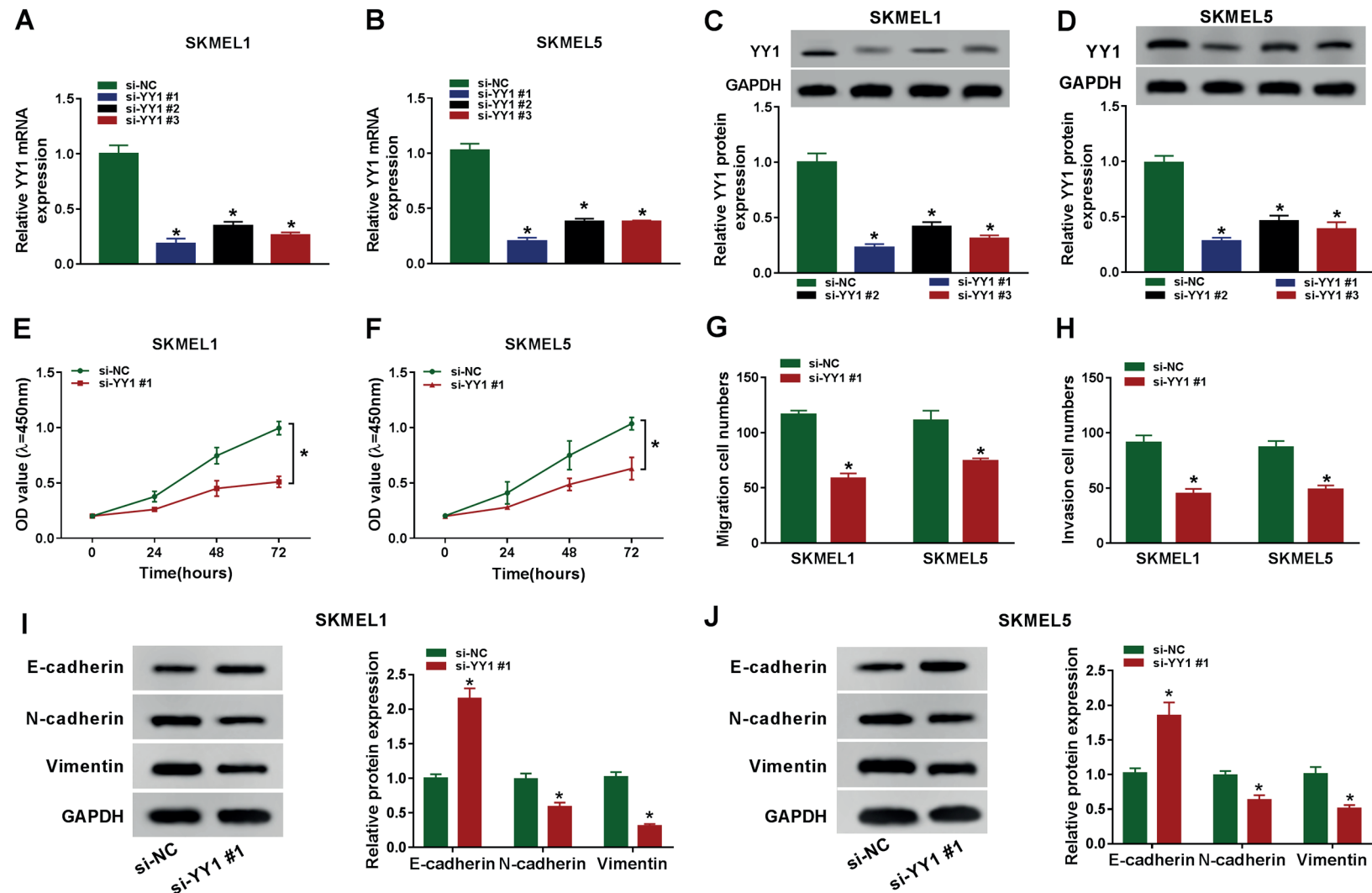
processes. After the analysis of qRT-PCR and Western blot, si-circ0016418#2 transfection markedly decreased the YY1 mRNA (Figure 4A) and protein (Figure 4B) levels, but the decrease was alleviated by YY1 transfection. Of note, the repression of cell proliferation caused by si-circ0016418#2 was abated by the up-regulation of YY1 (Figure 4C and D). Similarly, the transfection of YY1 signally receded the si-circ0016418-induced suppressant effects on migration (Figure 4E) and invasion (Figure 4F). Besides, there was a notable mitigation of si-circ0016418#2-induced E-cadherin, N-cadherin, and Vimentin expression after YY1 expression was elevated in SKMEL1 and SKMEL5 cells (Figure 4G and H). Taken together, the si-circ0016418-induced repressive effects on proliferation, migration, invasion, and EMT of skin melanoma cells were all ameliorated by YY1 overexpression.

#### ***Circ0016418 Negatively Modulated MiR-625 Expression and MiR-625 Directly Targeted YY1***

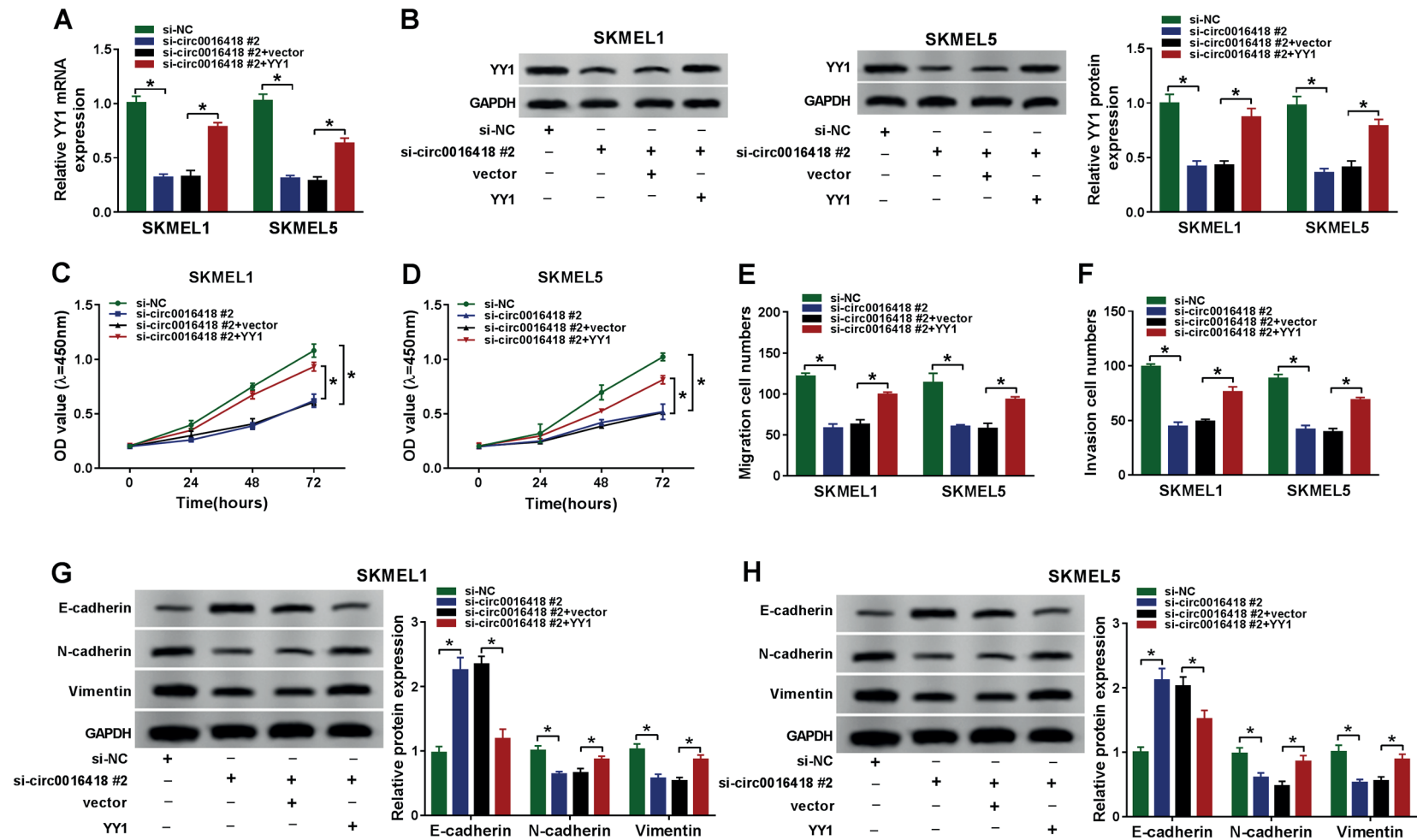
After the prediction of circular RNA Interactome and starBase, we found that WT-circ0016418 and YY1 3'UTR-WT both contained the binding sites of miR-625, as opposed to their mutant types (Figure 5A). Afterward, the target relation was analyzed by Dual-Luciferase reporter assay, in which the Luciferase activities of WT-circ0016418 (Figure 5B and C) and YY1 3'UTR-WT (Figure 5D and E) groups in SKMEL1 and SKMEL5 cells transfected with miR-625 were definitely declined, whereas the decline was blocked by MUT-circ0016418 and YY1 3'UTR-MUT. After that, qRT-PCR indicated that miR-625 expression was boosted by the knockdown of circ0016418 (Figure 5F). Overexpression effect of miR-625 was evident (Figure 5G) and miR-625 significantly reduced the YY1 mRNA (Figure 5H) and protein (Figure 5I) levels. In addition, the level of miR-625 was clearly down-regulated in skin melanoma tissues (Figure 5J) and cells (SKMEL1 and SKMEL5; Figure 5K) in comparison to normal adjacent tissues and HaCaT cells. In skin melanoma tissues, there was a conspicuous negative linear relation between circ0016418 and miR-625 expression ( $r=-0.4958$ ,  $p=0.0053$ ; Figure 5L), and between the levels of miR-625 and YY1 ( $r=-0.529$ ,  $p=0.0027$ ; Figure 5M). Thus, circ0016418 negatively regulated the miR-625 level and YY1 was a target of miR-625.



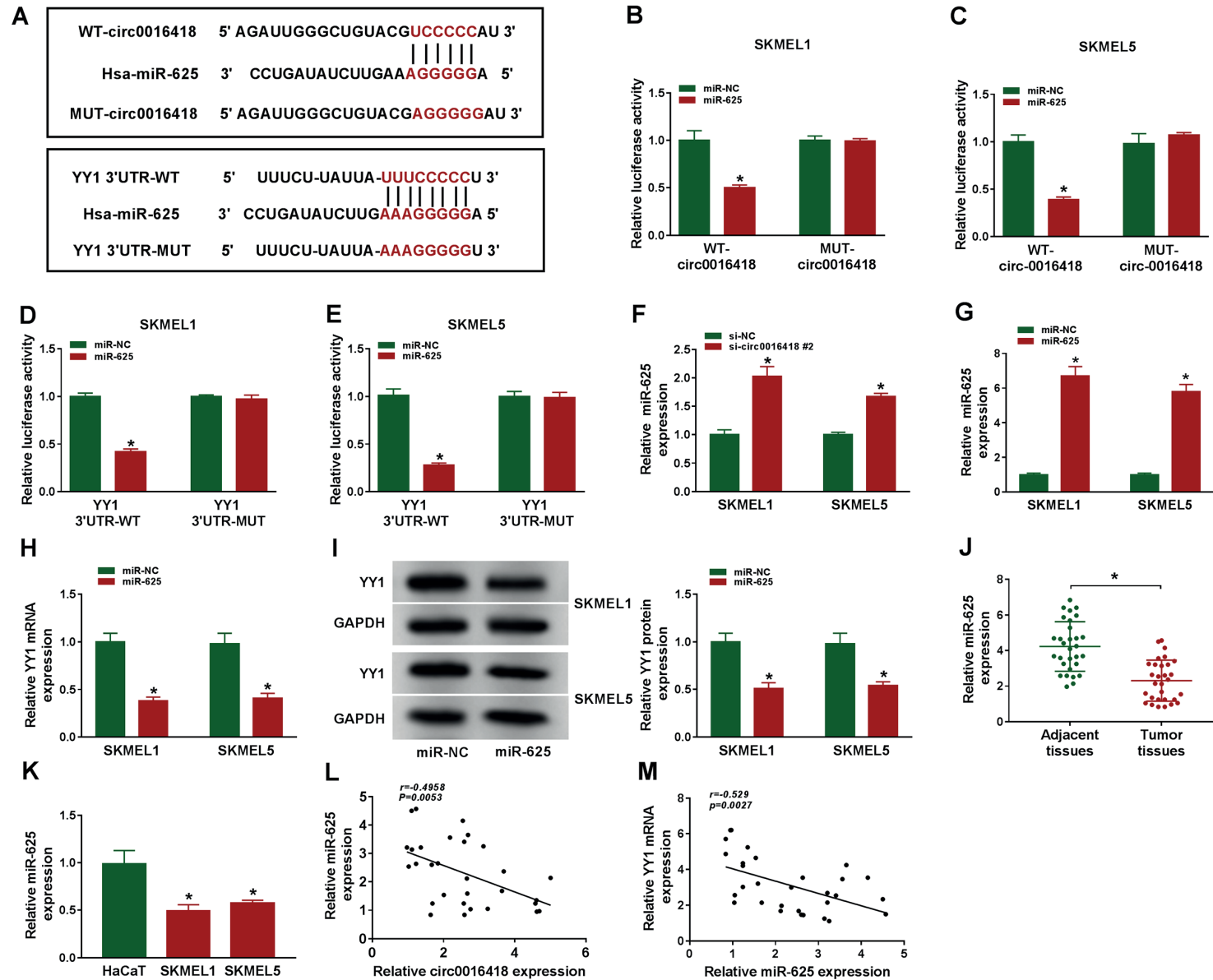
**Figure 2.** Knockdown of circ0016418 reduced proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) in skin melanoma cells. **A, B**, QRT-PCR was used for evaluating the interference efficiencies of si-circ0016418#1, si-circ0016418#2, and si-circ0016418#3 in SKMEL1 and SKMEL5 cells. **C, D**, CCK-8 was used to detect cell proliferation in SKMEL1 and SKMEL5 cells transfected with si-circ0016418#2 or si-NC. **E, F**, Transwell assay was administrated to measure cell migration (**E**) and invasion (**F**) (magnification: 300 $\times$ ). **G, H**, Western blot was utilized for examining the levels of EMT-associated proteins. \* $p < 0.05$ .



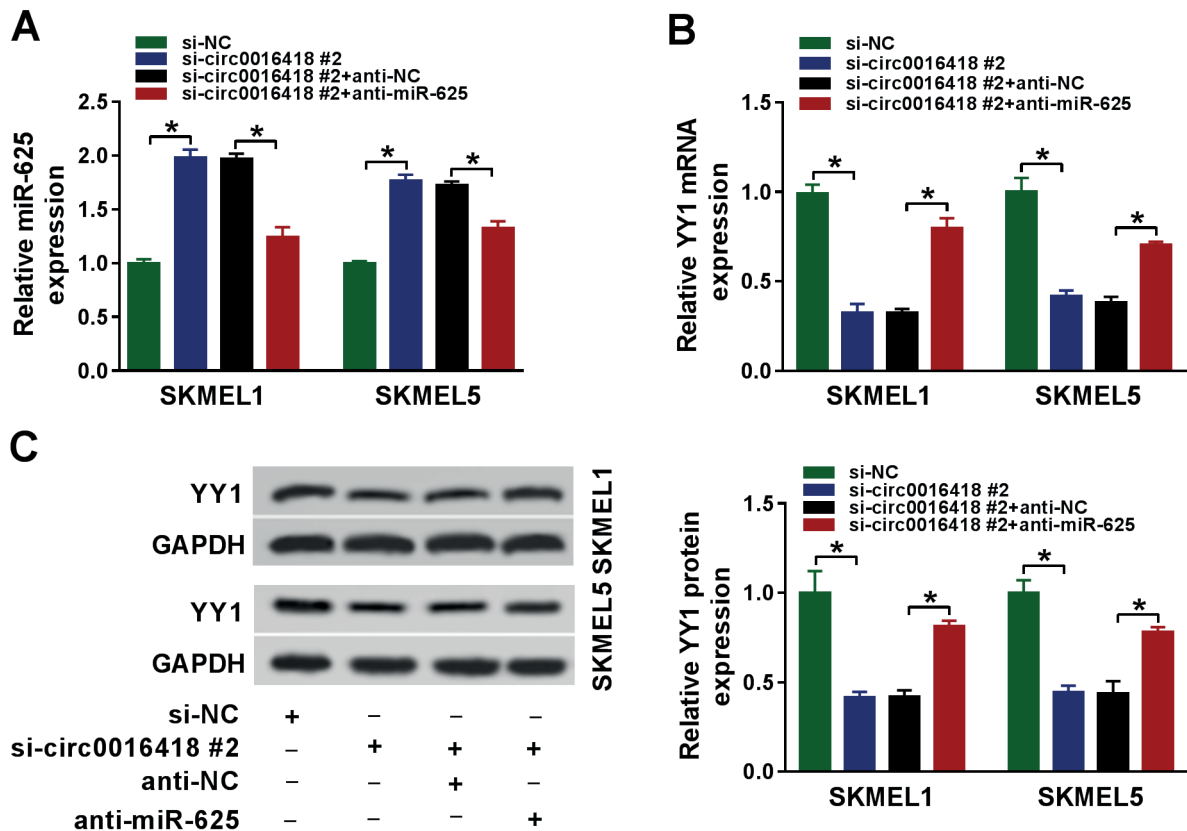
**Figure 3.** Inhibition of YY1 generated suppressive effects on proliferation, migration, invasion, and EMT in skin melanoma cells. **A-D**, Knockdown efficiencies of si-YY1#1, si-YY1#2, and si-YY1#3 were assessed by qRT-PCR (**A** and **B**) and Western blot (**C** and **D**) in SKMEL1 and SKMEL5 cells. **E, F**, Cell proliferation was examined by CCK-8 after transfection with si-YY1#1 or si-NC. **G, H**, Cell migration and invasion were determined by transwell assay. **I, J**, EMT-associated proteins levels were measured by Western blot. \* $p < 0.05$ .



**Figure 4.** Overexpression of YY1 reversed the si-circ0016418-induced effects on skin melanoma cells. **A, B**, YY1 mRNA and protein levels were measured by qRT-PCR and Western blot in SKMEL1 and SKMEL5 cells transfected with si-circ0016418#2, si-circ0016418#2+YY1 or relative controls. **C, D**, Detection of cell proliferation was implemented by CCK-8. **E, F**, Examination of cell migration (**E**) and invasion (**F**) was carried out by transwell assay. **G, H**, Analysis of EMT-associated proteins levels was conducted by Western blot. \* $p < 0.05$ .



**Figure 5.** Circ0016418 negatively modulated miR-625 expression and miR-625 directly targeted YY1. **A**, Circular RNA Interactome and starBase were used for predicting the targets of circ0016418 and miR-625, respectively. **B-E**, Dual-Luciferase reporter assay was conducted to verify the relationship between circ-0016418 and miR-625 (**B** and **C**) or miR-625 and YY1 (**D** and **E**) in SKMEL1 and SKMEL5 cells. **F**, Level of miR-625 was determined by qRT-PCR after transfection with si-circ0016418#2 or si-NC. **G**, QRT-PCR was applied to detect the overexpression effect of miR-625. **H**, **I**, YY1 mRNA and protein levels were measured by qRT-PCR and Western blot after transfection with miR-625 or miR-NC. **J**, **K**, QRT-PCR was utilized for examining the level of miR-625 in skin melanoma tissues (**J**) and cells (**K**). **L**, **M**, Spearman's correlation coefficient was used to analyze the relation between circ0016418 and miR-625 or miR-625 and YY1 in skin melanoma tissues. \* $p < 0.05$ .



**Figure 6.** Down-regulation of circ0016418 restrained YY1 expression by promoting miR-625. **A-B**, QRT-PCR was performed to examine the levels of miR-625 (**A**) and YY1 (**B**) in SKMEL1 and SKMEL5 cells transfected with si-circ0016418#2, si-circ0016418#2+anti-miR-625, and matched controls. **C**, Protein level of YY1 was detected by Western blot. \* $p < 0.05$ .

### Down-Regulation of Circ0016418 Restrained YY1 Expression by Promoting MiR-625

To research the regulatory mechanism between circ0016418 and YY1, SKMEL1 and SKMEL5 cells were transfected with si-circ0016418#2, si-circ0016418#2+anti-miR-625, and relative controls. As Figure 6A depicted, anti-miR-625 transfection apparently weakened the promotion of miR-625 expression caused by si-circ0016418#2. Furthermore, the mRNA level of YY1 was markedly decreased by the knockdown of circ0016418, but miR-625 inhibitor reversely elevated the YY1 level (Figure 6B). The suppression of miR-625 also relieved the si-circ0016418#2-induced reduction of YY1 protein level (Figure 6C). These results attested that circ0016418 down-regulation inhibited the level of YY1 via up-regulating miR-625, hinting that circ0016418 acted as a competitive endogenous RNA (ceRNA) of miR-625 to modulate the expression of YY1.

### Discussion

To obstruct the death and recurrence of skin melanoma, it is indispensable to search novel biomarkers for promoting the therapy and prognosis. During the current research, we found circ0016418 was overexpressed in skin melanoma, and that there were inhibitory effects on proliferation, migration, invasion, and EMT in skin melanoma cells after circ0016418 was knocked down. Furthermore, circ0016418 exerted its function via regulating miR-625/YY1 axis, suggesting circ0016418 could be a valid indicator for the treatment of skin melanoma as a novel oncogenic role.

Generated by back-splicing or gene rearrangement, circRNAs are considered as better therapeutic targets and regulatory non-coding RNAs (ncRNAs) with sequence stability in comparison to linear ncRNAs<sup>5,6</sup>. In recent years, circRNAs were presented to be aberrantly expressed and regulate the different types of melanomas. Yang

et al<sup>23</sup> unraveled that some circRNAs were dysregulated and might be promising candidates for the researches of mechanism in uveal melanoma, including circ0047924, circ0128533, circ0032148, and so on. Ju et al<sup>24</sup> showed that circ0005320, circ0067531, and circ0008042 had high expression, but circ0000869 and circ0000853 were down-regulated in metastatic oral mucosal melanoma. For skin melanoma, circ0084043 was showed to be overtly up-regulated and promote cell proliferation, migration, and invasion<sup>25</sup>. In addition, circ0016418 was proved to have an increase of expression in skin melanoma, and cell proliferation and invasion were reduced with the silence of circ0016418<sup>26</sup>. However, the regulatory mechanism of circ0016418 is entirely ill-defined. This study was conducted to address this matter with great research significance. At first, circ0016418 was up-regulated in skin melanoma and more stable than its original gene VASH2. Then, knockdown of circ0016418 had a distinct suppression of proliferation, migration, invasion. Moreover, EMT occurring in the embryonic development is considered as the symbol of canceration, and the inhibition of E-cadherin, as well as the increase of N-cadherin and Vimentin, is shown in the EMT process<sup>27</sup>. In this report, E-cadherin expression was elevated while N-cadherin and Vimentin were down-regulated after circ0016418 was knocked down, implying the repression of EMT. Further, circ0016418 played an oncogenic role in skin melanoma, consistent with the findings of Wang et al<sup>26</sup>.

YY1, a ubiquitous transcription factor, was reported to modulate the cellular phenotype of skin melanoma cells<sup>28</sup>. Zhao et al<sup>29</sup> elucidated that YY1 expression was enhanced in skin melanoma and the down-regulation of YY1 restrained cell proliferation and migration. In correspondence with their report, YY1 was found to be highly expressed in skin melanoma tissues and cells compared to that in normal tissues and cells. They showed repressive impacts of not only proliferation and migration but also invasion and EMT process. Since the identical role of circ0016418 and YY1 in skin melanoma and the positive correlation between circ0016418 and YY1 in tissues, we speculated that certain regulatory mechanism existed between circ0016418 and YY1. Further experiments indicated that when YY1 was overexpressed, the inhibition of proliferation, migration, invasion, and EMT induced by knockdown of circ0016418 was abated, suggesting that knockdown of circ0016418

reduced oncogenesis of skin melanoma by decreasing YY1 level.

CircRNAs, as competing endogenous RNAs (ceRNAs), acted as native miRNA sponges to decrease the combination between miRNAs and their target genes, resulting in the alteration of gene level<sup>30-32</sup>. For instance, Wang et al<sup>33</sup> asserted that circular RNA protein arginine methyltransferase 5 (circ-PRMT5) enhanced the proliferation of non-small cell lung cancer cells via promoting zeste homologue 2 (EZH2) as miR-377/382/498 sponges. Circ0084043 expedited Snail expression by sponging miR-153-3p in skin melanoma cells<sup>25</sup>. Hence, there might be a miRNA connection between circ0016418 and YY1 to form the circRNA-miRNA-gene regulatory network in skin melanoma. As expected, circular RNA Interactome and starBase singled out miR-625 that contained the binding sites of WT-circ0016418 and YY1 3'UTR-WT. Then, further assays demonstrated that circ0016418 negatively targeted miR-625 in skin melanoma cells, and miR-625 targeted YY1. Next, we investigated the level of miR-625 and found there was a reduction of miR-625 both in skin melanoma tissues and cells, in line with the previous report<sup>34</sup>. Furthermore, miR-625 inhibitor relieved the effect on YY1 expression caused by a decrease of circ0016418, manifesting that circ0016418 knockdown reduced the level of YY1 via motivating miR-625 in skin melanoma cells. It was indicated that circ0016418 modulated the expression of YY1 in skin melanoma cells, as a ceRNA for miR-625.

## Conclusions

The knockdown of circ0016418 suppressed proliferation, migration, invasion, and EMT in skin melanoma cells by inhibiting YY1 via accelerating the expression of miR-625. This is the first study on the modulatory mechanism of circ0016418 in skin melanoma, and the circ0016418/miR-625/YY1 axis was also discovered for the first time. Meaningfully, circ0016418 could involve in the advancement of therapeutic methods of skin melanoma, as a promising index. It is worth expecting that circ0016418 can work well in the clinical research of the treatment for skin melanoma patients.

## Conflict of Interests

The Authors declare that they have no conflict of interests.

## References

- 1) GRAY-SCHOPFER V, WELLBROCK C, MARAIS R. Melanoma biology and new targeted therapy. *Nature* 2007; 445: 851-857.
- 2) CORRIE P, HATEGAN M, FIFE K, PARKINSON C. Management of melanoma. *Br Med Bull* 2014; 111: 149-162.
- 3) RASTRELLI M, TROPEA S, ROSSI CR, ALAIBAC M. Melanoma: epidemiology, risk factors, pathogenesis, diagnosis and classification. *In Vivo* 2014; 28: 1005-1011.
- 4) WILUSZ JE, SHARP PA. Molecular biology. A circuitous route to noncoding RNA. *Science* 2013; 340: 440-441.
- 5) MEMCZAK S, JENS M, ELEFSINIOTI A, TORTI F, KRUEGER J, RYBAK A, MAIER L, MACKOWIAK SD, GREGERSEN LH, MUNSCHAUER M, LOEWER A, ZIEBOLD U, LANDTHALER M, KOCKS C, LE NOBLE F, RAJEWSKY N. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 2013; 495: 333-338.
- 6) SALZMAN J, GAWAD C, WANG PL, LACAYO N, BROWN PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 2012; 7: e30733.
- 7) BOLISETTY MT, GRAVELEY BR. Circuitous route to transcription regulation. *Mol Cell* 2013; 51: 705-706.
- 8) SALZMAN J. Circular RNA expression: its potential regulation and function. *Trends Genet* 2016; 32: 309-316.
- 9) ZHAO ZJ, SHEN J. Circular RNA participates in the carcinogenesis and the malignant behavior of cancer. *RNA Biol* 2017; 14: 514-521.
- 10) ABI A, FARAHANI N, MOLAVI G, GHEIBI HAYAT SM. Circular RNAs: epigenetic regulators in cancerous and noncancerous skin diseases. *Cancer Gene Ther* 2019 Sep 3. doi: 10.1038/s41417-019-0130-x. [Epub ahead of print]
- 11) CHO WC. OncomiRs: the discovery and progress of microRNAs in cancers. *Mol Cancer* 2007; 6: 60.
- 12) LATCHANA N, GANJU A, HOWARD JH, CARSON WE 3RD. MicroRNA dysregulation in melanoma. *Surg Oncol* 2016; 25: 184-189.
- 13) VARAMO C, OCCELLI M, VIVENZA D, MERLANO M, LO NIGRO C. MicroRNAs role as potential biomarkers and key regulators in melanoma. *Genes Chromosomes Cancer* 2017; 56: 3-10.
- 14) ROSS CL, KAUSHIK S, VALDES-RODRIGUEZ R, ANVEKAR R. MicroRNAs in cutaneous melanoma: role as diagnostic and prognostic biomarkers. *J Cell Physiol* 2018; 233: 5133-5141.
- 15) CIRILO PDR, DE SOUSA ANDRADE LN, CORREA BRS, QIAO M, FURUYA TK, CHAMMAS R, PENALVA LOF. MicroRNA-195 acts as an anti-proliferative miRNA in human melanoma cells by targeting Prohibitin 1. *BMC Cancer* 2017; 17: 750.
- 16) QIU H, CHEN F, CHEN M. MicroRNA-138 negatively regulates the hypoxia-inducible factor 1 $\alpha$  to suppress melanoma growth and metastasis. *Biol Open* 2019; 8: pii: bio042937.
- 17) SHI Y, SETO E, CHANG LS, SHENK T. Transcriptional repression by YY1, a human GLI-Kruppel-related protein, and relief of repression by adenovirus E1A protein. *Cell* 1991; 67: 377-388.
- 18) GORDON S, AKOPYAN G, GARBAN H, BONAVIDA B. Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. *Oncogene* 2006; 25: 1125-1142.
- 19) KHACHIGIAN LM. The Yin and Yang of YY1 in tumor growth and suppression. *Int J Cancer* 2018; 143: 460-465.
- 20) LIU D, ZHANG J, WU Y, SHI G, YUAN H, LU Z, ZHU Q, WU P, LU C, GUO F, CHEN J, JIANG K, MIAO Y. YY1 suppresses proliferation and migration of pancreatic ductal adenocarcinoma by regulating the CDKN3/MdM2/P53/P21 signaling pathway. *Int J Cancer* 2018; 142: 1392-1404.
- 21) SU H, LIU L, ZHANG Y, WANG J, ZHAO Y. Long non-coding RNA NPCCAT1 promotes nasopharyngeal carcinoma progression via upregulating YY1. *Biochimie* 2019; 157: 184-194.
- 22) ZHAN S, WANG T, GE W, LI J. Multiple roles of Ring 1 and YY1 binding protein in physiology and disease. *J Cell Mol Med* 2018; 22: 2046-2054.
- 23) YANG X, LI Y, LIU Y, XU X, WANG Y, YAN Y, ZHOU W, YANG J, WEI W. Novel circular RNA expression profile of uveal melanoma revealed by microarray. *Chin J Cancer Res* 2018; 30: 656-668.
- 24) JU H, ZHANG L, MAO L, LIU S, XIA W, HU J, RUAN M, REN G. Altered expression pattern of circular RNAs in metastatic oral mucosal melanoma. *Am J Cancer Res* 2018; 8: 1788-1800.
- 25) LUAN W, SHI Y, ZHOU Z, XIA Y, WANG J. CircRNA\_0084043 promote malignant melanoma progression via miR-153-3p/Snail axis. *Biochem Biophys Res Commun* 2018; 502: 22-29.
- 26) WANG Q, CHEN J, WANG A, SUN L, QIAN L, ZHOU X, LIU Y, TANG S, CHEN X, CHENG Y, CAO K, ZHOU J. Differentially expressed circRNAs in melanocytes and melanoma cells and their effect on cell proliferation and invasion. *Oncol Rep* 2018; 39: 1813-1824.
- 27) KLETUKHINA S, NEUSTROEVA O, JAMES V, RIZVANOV A, GOMZIKOVA M. Role of mesenchymal stem cell-derived extracellular vesicles in epithelial-mesenchymal transition. *Int J Mol Sci* 2019; 20. pii: E4813.
- 28) SEBERG HE, VAN OTTERLOO E, CORNELL RA. Beyond MITF: multiple transcription factors directly regulate the cellular phenotype in melanocytes and melanoma. *Pigment Cell Melanoma Res* 2017; 30: 454-466.
- 29) ZHAO G, LI Q, WANG A, JIAO J. YY1 regulates melanoma tumorigenesis through a miR-9 ~ RYBP axis. *J Exp Clin Cancer Res* 2015; 34: 66.
- 30) TAY Y, RINN J, PANDOLFI PP. The multilayered complexity of ceRNA crosstalk and competition. *Nature* 2014; 505: 344-352.
- 31) KULCHESKI FR, CHRISTOFF AP, MARGIS R. Circular RNAs are miRNA sponges and can be used as a new class of biomarker. *J Biotechnol* 2016; 238: 42-51.

- 32) PANDA AC. Circular RNAs act as miRNA sponges. *Adv Exp Med Biol* 2018; 1087: 67-79.
- 33) WANG Y, LI Y, HE H, WANG F. Circular RNA circ-PRMT5 facilitates non-small cell lung cancer proliferation through upregulating EZH2 via sponging miR-377/382/498. *Gene* 2019; 720: 144099.
- 34) ZHANG H, FENG C, ZHANG M, ZENG A, SI L, YU N, BAI M. MiR-625-5p/PKM2 negatively regulates melanoma glycolysis state. *J Cell Biochem* 2019; 120: 2964-2972.