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Original Article

MicroRNA-664 functions as an oncogene in cutaneous squamous cell carcinomas (cSCC) via suppressing interferon regulatory factor 2

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ABSTRACT

Background: Aberrant expression of microRNA-664 was involved in tumor growth and metastasis of various cancers. The specific role of miR-664 in cutaneous squamous cell carcinoma (cSCC) is yet to be elucidated.
Objective: The present study aimed to investigate the molecular mechanisms underpinning of cSCC development and provide translational insights for future therapeutics.
Methods: Human cSCC specimens were used to determine the miR-664 by *in situ*hybridization and IRF2 by immunohistochemistry. To study the potential mechanisms in tumorigenesis, three cSCC cell lines including HSC-5, HSC-1 and A431 as well as BALB/C mouse tumor model was utilized.
Results: We found that miR-664 was remarkably high in cSCC patient specimens and cSCC cell lines. Overexpression of miR-664 promotes tumorigenic behaviors such as increased cell proliferation, migration and invasion capacities *in vitro* and enhanced tumorigenicity in xenograft mouse model. Our data further identified IRF2 as a direct downstream target of miR-664. Knockdown of IRF2 reverses pro-tumorigenesis phenotype of miR-664; whereas IRF2 over-expression inhibits miR-664 tumorigenesis in cSCC. Together, it revealed miR-664 functions as an oncogene in cSCC *via* suppression of IRF2.
Conclusion: Our data demonstrates that aberrant expression of miR-664 plays a critical role in carcinogenesis of cSCC. The discovery of novel targets such as miR-664 and IRF2 will facilitate future development of therapeutic interventions.

1. Introduction

Epidermal keratinocyte-derived cutaneous squamous cell carcinoma (cSCC) is the second most common cancer with over one million new cases annually worldwide [1–4]. Chronic exposure to sunlight ultraviolet (UV) radiation is a major risk factor for skin cancer such as cSCC [5,6]. cSCC is also associated with a substantial risk of metastasis, contributing to approximately 20% of skin cancer-related deaths [7,8]. Nonetheless, metastatic or locally advanced cSCC patients are not applicable for surgical or radiotherapy strategies. Chemotherapy combined with interferon α (IFN-α) and cis retinoic acid are active but no favorable survival benefits were observed [9]. Despite that the U. S. Food and Drug Administration (FDA) has recently approved an immune checkpoint blockade drug, Cemiplimab, the clinical efficacy in immunocompromised cSCC patients remains unclear [10]. Furthermore, there is no systemic treatment have been approved for advanced cSCC [11].

Given the very limited targeted therapies for cSCC, the molecular mechanisms underpinning this life-threatening disease are of particular interests. MicroRNAs (miRNAs or miRs) are a family of small non-coding RNAs, which regulate gene expression by binding to the 3′untranslated region (3′UTR) of mRNA [12,13]. Mutation or abnormal expression of miRNAs can function as tumor suppressors or oncogenes in various cancers [14–17]. A number of

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studies reported that ectopic expression of miR-664 was implicated in a variety of malignances including cutaneous malignant melanoma, hepatocellular carcinoma and breast cancer [18–20]. Interestingly, we found that the expression level of miR-664 is remarkably higher in cSCC patient samples as well as cell lines.

Interferon regulatory factor 2 (IRF2) is a member of the interferon regulatory transcription factor family [21,22]. This family plays a critical role in adaptive immunity as well as modulates cellular response in tumorigenesis [23,24]. Upregulation of IRF2 expression level was associated with attenuated proliferative capacity of murine medullo blastoma cells [25]. Silencing of IRF2 reduces p53 expression levels and its related genes expression [26]. The potential interactions between miR-664 and IRF2 are yet to be elucidated. The present study aimed to investigate the molecular mechanisms of miR-664 in tumorigenesis of cSCC, facilitating future development of novel therapeutic targets.

2. Materials and methods

2.1. Cell culture and tumor samples

Cutaneous SCC lines HSC-1 (Dongguang Biojet Biotech. Co., Ltd, Guangzhou, China) and A431, HSC-5 (China Center for Type Culture Collection and Cell Bank of the Chinese Academy of Sciences, Shanghai, China) and human benign epidermal keratinocyte cell line HaCaT (China Center for Type Culture Collection, Wuhan, China) were cultured in Dulbecco's modified Eagle medium (DMEM, Gbico, Suzhou, China) supplemented with 10% fetal bovine serum (FBS, Gbico, Germany) at 37 °C in a humidified 5% CO_2 atmosphere. cSCC samples were obtained from patients diagnosed with cSCC from January 2009 to August 2016 in the departments of dermatology, pathology and oncology at Nanfang Hospital and Zhujiang Hospital, affiliated to Southern Medical University.

2.2. Transfection

HSC-1 and HSC-5 cells were transiently transfected with miR-664 mimic, negative control mimic (NC mimic), miR-664 inhibitor, negative control inhibitor (NC inhibitor), IRF2 siRNA (siIRF2), or negative control siRNA (siNC) (RIBOBIO, Guangzhou, China) using Invitrogen™ Lipofectamine 2000 (Invitrogen, USA), according to the manufacturer's protocol.

2.3. Quantitative reverse transcription PCR (qRT-PCR)

After isolation from culture cells or clinical tissues with Trizol reagent (Ambion, 15596018), according to the manufacturer's instructions. The RNA was quantified by Nanodrop2000. Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems, USA) was used to make cDNA from the total RNA. Human miRNAs and U6 were detected by TaqMan microRNA assay (Applied Biosystems, USA) and TaqMan Fast Advanced Master Mix (Applied Biosystems, USA). The following primer sequences were used for amplifying each genes listed: IRF2 (Forward: ATTTGCCAAGTTGTAGAGG; Reverse: CTATCAGTCGTTTCGCTTT), GAPDH (Forward: GGATATTGTTGCCATCAATGACC; Reverse: AGCCTTCTCCATGGTGGTGAAGA), and mature miR-664 primer (Applied Biosystems, USA). Real-time PCR was performed using the Applied Biosystems 7500 Sequence Detection system.

2.4. Construction of recombinant expression vectors

Validation of miRNA targets was performed by cloning partial IRF2 3′UTRs containing the sequence recognized by the miR-664 core seed region. The oligos carrying native and mutated miR-664 binding sites with SpeI and SacI (NEB, USA) restriction sites for cloning were annealed and cloned into the pMIR-reporter vector (Promega, Beijing, China).

2.5. Dual luciferase reporter assay

For dual luciferase experiments, HEK293T cells were grown in 12-well plates and transfection, including pMIR-reporter constructs and Renilla luciferase reporter vector (Promega, Beijing, China), and either 100 nM miR-664 or control mimic (RIBOBIO, Guangzhou, China), were performed when confluence reaches 80% using Lipofectine 2000 (Invitrogen, USA) according to the manufacture' manual. Cells were incubated for 48 h after transfection. Cell lysates were harvested and luciferase activities were assayed using Dual-Luciferase Reporter Assay Kit (TransGen Biotech, Beijing, China). Correction for differences in transfection efficiency was performed by normalizing firefly luciferase activity to total Renilla luciferase.

2.6. Cell proliferation analysis

Cell proliferation was measured with the CCK-8 assay kit (Dojindo, Tokyo, Japan). Cells were plated into a 96-well microplate (Corning Incorporated, New York, USA) and incubated at 37 °C in 5% CO_2 five hours after transfection. Each data point represents the measurement of three replicates. After 24, 48 and 72 h of culture, 10 μl of CCK-8 solution was added to each well with 100 μl of serum-free medium for 120 min incubation. Finally, the absorbance was measured at 450 nm as described according to the manufacturer instructions.

2.7. Colony formation assays

For the colony forming assay, transfected cells were trypsinized and seeded on 6-well plates (1000 cells per well) and cultured for 7–14 days. Colonies were collected after being washed twice with PBS and fixed in 3.7% paraformaldehyde for 2 min followed by 100% methanol 20 min. Finally, the cells were stained with 0.1% crystal violet for 15 min. Visible colonies were photographed and counted.

2.8. Transwell assay

Migration of cSCC cells *in vitro* were assayed using a Transwell chamber (Millicell, Germany) with a polycarbonic membrane (8 μm pore size). After 24 h of transfection, 1.0×10^5 cells added to the upper chamber with 200 μl of serum-free medium, and 750 μl of medium with 10% FBS was added to the lower chamber. The cells were cultured for 16–24 h at 37 °C. The chambers were washed with PBS twice and fixed with 3.7% formaldehyde. Cells were permeabilized using 100% methanol at room temperature, stained with 0.1 crystal violet, and observed using a microscope after the cells remained in the wells being scraped off with cotton swabs.

2.9. Matrigel invasiveness assay

For the assessment of invasive ability, Matrigel invasion chambers (Corning, USA) were used to culture transfected cSCC cells, 2.0×10^5 cells were seeded into the upper chambers. Other treatments were performed as in the migration assay.

2.10. Western blot analysis

The transfected cSCC cells were lysed with cold RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China)

and quantified by Bradford assay. Then the lysis buffer was mixed with the loading buffer (Beyotime Institute of Biotechnology, Haimen, China) and boiled at 100 °C for 10 min. Equal amounts of protein from each sample were subjected to SDS-PAGE electrophoresis and transferred to a polyvinylidene fluoride membrane (Millipore Corporation, Billerica, MA, USA). The membrane was then soaked in tris-buffered saline with Tween-20 (TBST, 150 mM NaCl, 20 mM Tris-HCl pH 8.0, 0.05% Tween-20) containing 5% skim milk powder for 1 h at room temperature, followed by gentle shaking and subsequent incubation with specific antibody against IRF2 (1:1000, Proteintech, Wuhan, China), GAPDH (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. Afterwards, the membrane was washed and incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000, Invitrogen, USA) for 1 h at room temperature. Protein bands were detected using a chemiluminescent HRP substrate (Millipore Corporation, USA).

2.11. microRNA-FISH

The formalin-fixed paraffin-embedded cSCC sections were used for miRNA hybridization. Specimen was deparaffinized and hydrated, followed by treatment with proteinase K and refixation in 4% paraformaldehyde. After washing with phosphate-buffered saline and air-drying, the sections were hybridized with the fluorescein isothiocyanate-labeled LNA-miR-664 probe. Nuclei were routinely stained using DAPI.

2.12. Immunohistochemistry

Five-micron-thick sections of formalin-fixed and paraffin-embedded TMA were deparaffinized and rehydrated, followed by high-temperature antigen retrieval *via* microwave in 0.1 M citrate solution (pH 6.0) for 15 min. After blocked in 5% normal goat serum at room temperature for 30 min, the sections were incubated with the mouse anti-interferon regulatory factor 2 (IRF2) antibody (1:500, Proteintech, Wuhan, China) at 4 °C overnight, then incubated with biotinylated secondary antibody at room temperature for 30 min, and finally immunostained by the avidin-biotin complex technique using 3, 3′-diaminobenzidine. Hematoxylin was used as a counterstain.

2.13. DNA constructs

The IRF2 expressing construct was purchased from Transheep (http://www.transheep.com) with a signed material transfer agreement.

2.14. Tumor xenograft mouse model

Male athymic nude mice (BALB/C-nu/nu, 4–5 weeks old) were subcutaneously injected with 1×10^6 or 3×10^6 cells of HSC-5 transfected with miR-664 mimic/negative control mimic (NC) or miR-664 inhibitor/negative control mimic (NC) into the left and right flanks of nude mice. The experiments were performed "blind" with respect to the different treatments. Tumor were measured

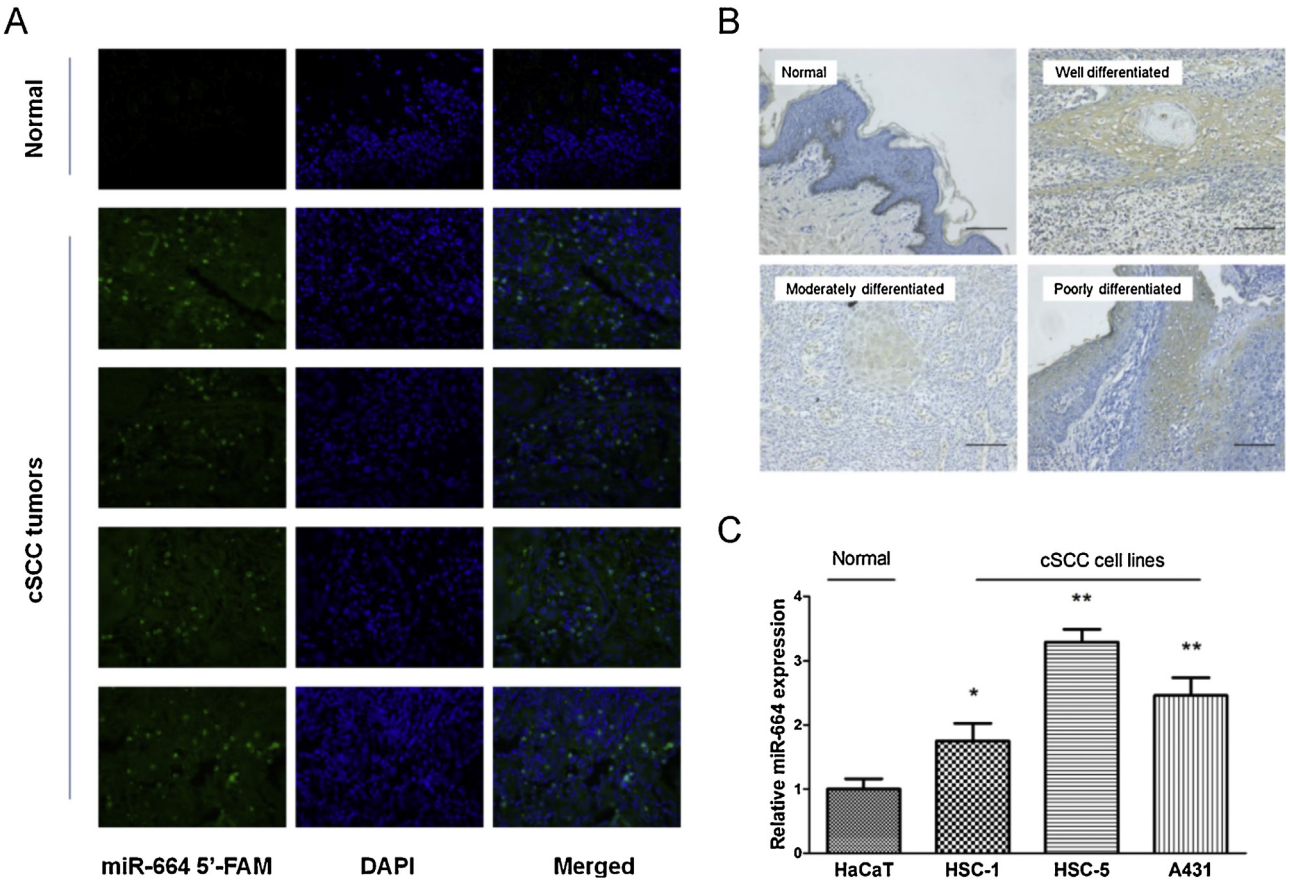

Fig. 1. miR-664 is upregulated in cSCC tumor tissues and cell lines. (A and B) miR-664 FISH (400×) and *In situ* hybridization (200×) results reveal a highly positive rate of miR-664 on paraffin sections of cSCC tumors. (C) High transcriptional level of miR-664 was found in human cSCC cell lines but not human benign epidermal keratinocytes by qRT-PCR. The low expression level of IRF2 in cSCC cell lines and tumors. Scale bar: 100 μm. (*P < 0.05, **P < 0.01, ***P < 0.001).

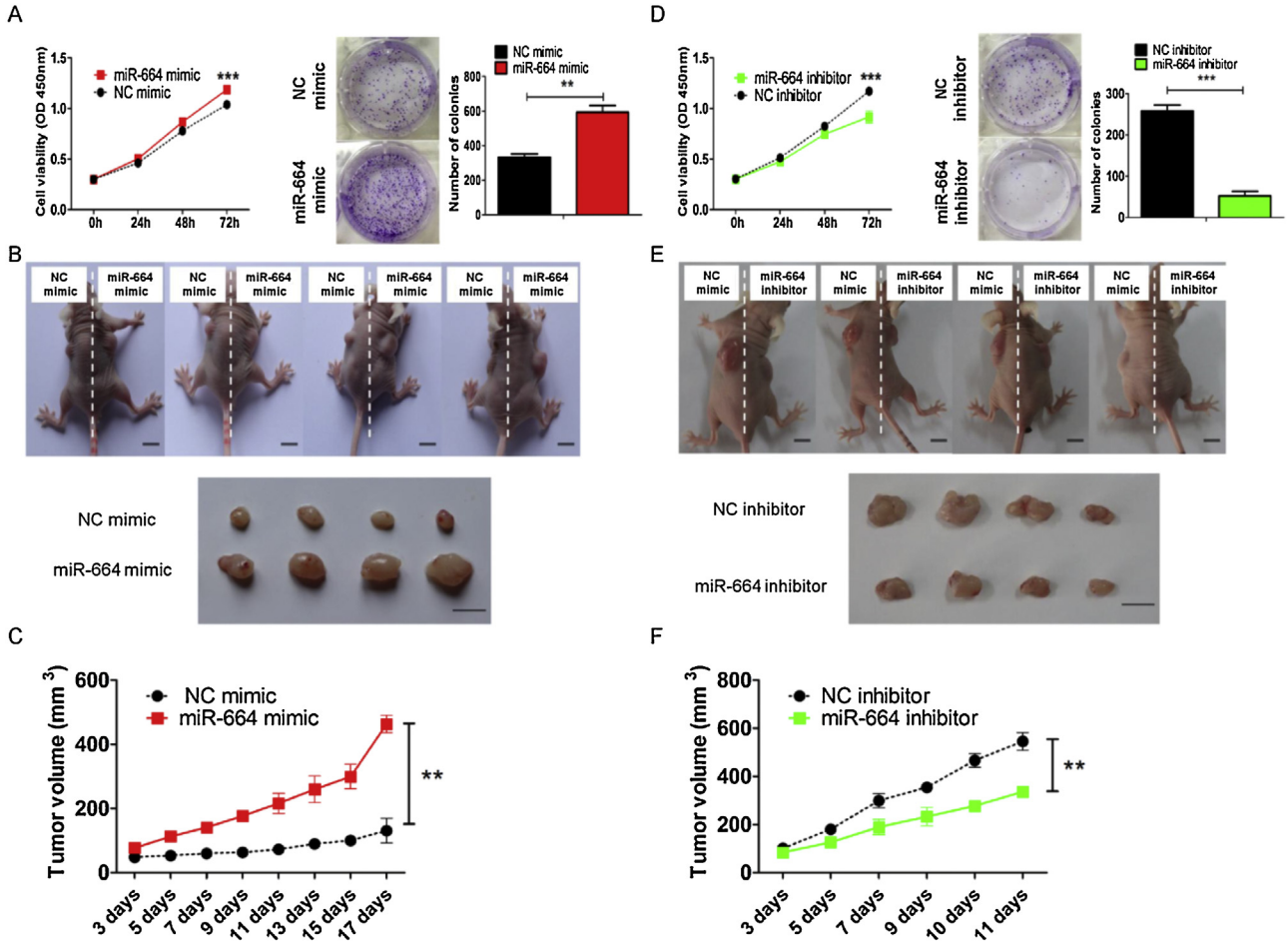

Fig. 2. miR-664 promotes tumorigenicity of cSCC. (A) Increased cell viability and proliferation were found in miR-664 over-expression (miR-664 mimic) *versus* NC transfected HSC-5 cells. (B) Representative images of tumor growth in nude mice injected with HSC-5 cells were transfected with NC or miR-664 mimic. (C) The dynamics of tumor growth in NC mimic and miR-664 mimic HSC-5 injected mouse model. (D) Reduced cell viability and proliferation were found in miR-664 inhibition *versus* non-inhibition control. (E) Representative images of tumor growth in nude mice injected with miR-664 inhibited *versus* non-inhibited HSC-5 cells. (F) The dynamics of tumor growth in miR-664 inhibited or non-inhibited HSC-5 injected mouse model. (Scale bar =1 cm) (*P < 0.05, **P < 0.01, ***P < 0.001).

weekly by calipers, and tumor volumes were calculated as formula: volume = length × width^2/2.

2.15. Statistical analysis

Data were presented as mean ± standard deviation (SD). Statistical tests were performed for independent-samples with an unpaired *t*-test or one-way ANOVA tests (SPSS version 20.0, SPSS Inc.) and graph presentations were completed using GraphPad Prism 5 Software. All statistical tests incorporated two-tailed tests and homogeneity of variance tests, and were considered to reflect significant differences if *P < 0.05, **P < 0.01, or ***P < 0.001. Details of statistical analyses including sample numbers (n) are included in the respective figure legends.

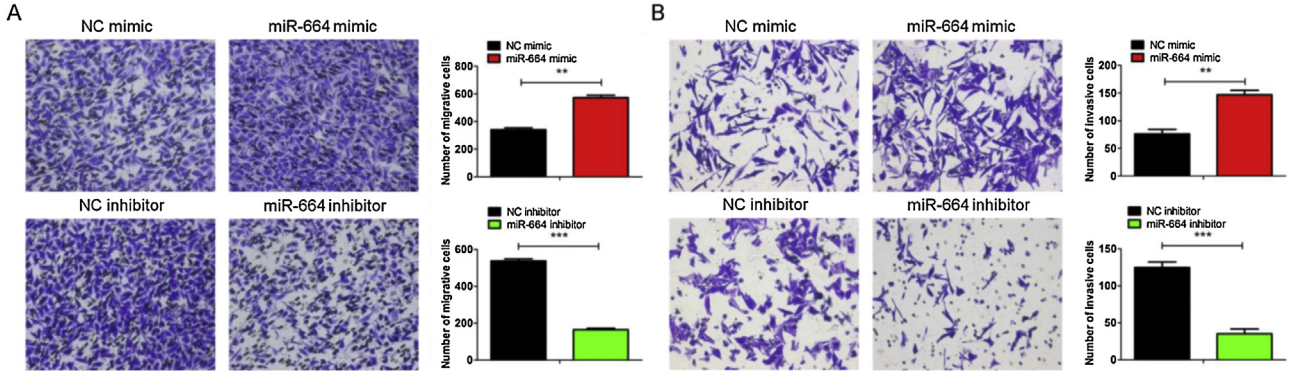

Fig. 3. miR-664 promotes the migration and invasion ability of cSCC cell lines. (A) Increased migration capacity was found in miR-664 overexpression HSC-5 cells; the attenuated migration capacity was observed in miR-664 inhibited HSC-5 cells. (B) Enhanced invasiveness was found in miR-664 overexpression HSC-5 cells; the reduced invasion capacity was observed in miR-664 inhibited HSC-5 cells. (*P < 0.05, **P < 0.01,***P < 0.001).

3. Results

3.1. miR-664 is upregulated in cSCC

To investigate the role of miR-664 in skin carcinogenesis, the translational level of miR-664 were evaluated in patient samples by *in situ* hybridization. Interestingly, miR-664 was highly upregulated in tumor regions compared to normal skin tissue, independent of the degrees of pathological differentiation (Fig. 1A, B). We next to determine the transcriptional level of miR-664 in three cSCC cell lines, A431, HSC-1 and HSC-5 cells *in vitro*. In contrast to the human benign epidermal keratinocyte cell line HaCaT, qRT-PCR results demonstrated an increased transcriptional level of miR-664 in three cSCC cell lines (Fig. 1C). Together, our results indicated that the upregulation of miR-664 may be associated with cSCC tumorigenesis.

3.2. miR-664 promotes proliferation and tumorigenicity of cSCC

To understand the functional role of miR-664 in cSCC development, HSC-5 cell line was transfected with miR-664 mimic oligonucleotide. The cell viability and proliferation capacity between miR-664 over-expression *versus* vector control were assessed by the Cell Counting Kit-8 (CCK-8) and colony formation assays, respectively. Both cell viability and proliferation ability were significantly increased in miR-664 over-expression (miR-664 mimic) group ($P < 0.001$ and $P < 0.01$, respectively) (Fig. 2A). We next to ask whether miR-664 promotes cSCC development *in vivo*, HSC-5 xenograft tumor model was established in nude mice. In accordance with *in vitro* findings, miR-664 over-expression (miR-664 mimic) group clearly promoted tumor growth compared with vector control tumors (Fig. 2B). The dynamics of tumor size changes between miR-664 over-expression and vector control groups further corroborated this finding ($P < 0.01$) (Fig. 2C).

Loss-of-function experiments were also performed to confirm the role of miR-664 in cSCC development. After inhibiting of miR-664 in HSC-5 cells, both cell viability and proliferation capacity were dramatically attenuated when comparing with non-inhibition control (NC inhibitor) ($P < 0.001$ and $P < 0.001$, respectively) (Fig. 2D). The miR-664 inhibition group also suppressed tumor growth *in vivo* (Fig. 2E, F). In parallel, the gain-/loss- of functions phenotype of miR-664 was also evidenced in another cSCC cell line HSC-1 *in vitro* (Figure S1).

3.3. miR-664 enhances migration and invasion capacities of cSCC

We next thought to study whether miR-664 would affect the migration and invasion properties in cSCC cells. According to the migration assays, over-expression of miR-664 (miR-664 mimic) was capable of facilitating HSC-5 cells migration ($P <$ 0.01). In

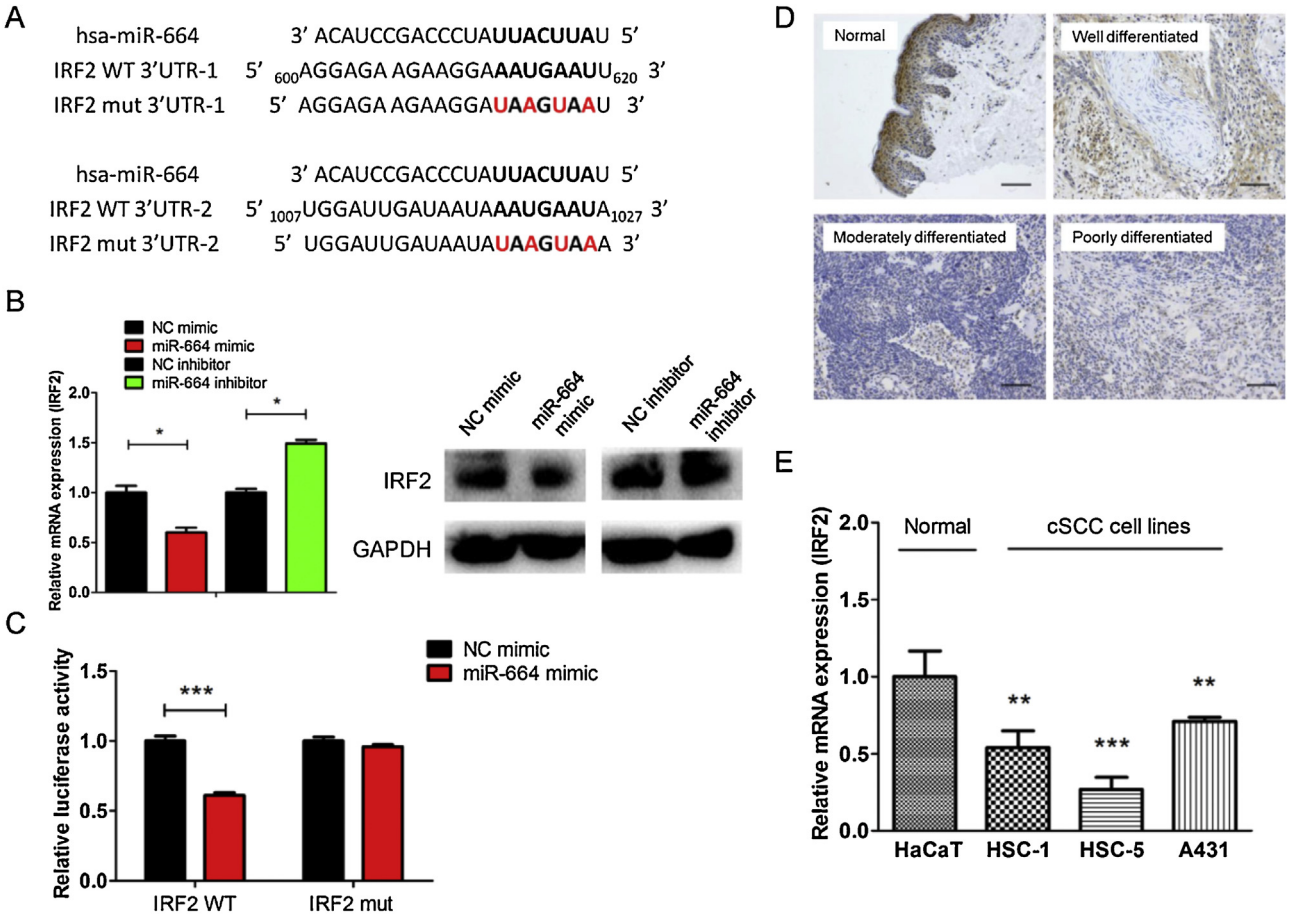

Fig. 4. IRF2 is a direct target of miR-664 and it's downregulated in cSCC tumor tissues and cell lines. (A) The constructed wild genotype (WT)/mutant genotype (mut) of the miR-664-binding site in IRF2 3′UTR. (B) Real time PCR and western blotting analysis showed that both IRF2 protein and IRF2 mRNA expressions were significantly decreased in miR-664 mimic transfected cSCC cells; whereas the inhibition of miR-664 was able to increased IRF2 expression. (C) Luciferase activity of miR-664 mimic was significantly inhibited by co-transfecting with wild 3′UTR construct, compared to mutant construct groups. (D) IHC results revealed low expression level of IRF2 in cSCC specimens in comparison to normal skin tissues. (E) The transcriptional levels of IRF2 in cSCC cells lines were much lower than HaCaT keratinocytes. (200×) Scale bar: 100 μm. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

contrast, the migration ability was largely abrogated after inhibition of miR-664 ($P < 0.001$) (Fig. 3A). Matrigel invasion assay was performed for the assessment of invasive ability in HSC-5 cells. A remarkably increased invasive ability was observed in miR-664 over-expression cells however attenuated sharply after the inhibition of miR-664 ($P < 0.01$ and $P < 0.001$, respectively) (Fig. 3B). The similar findings were also identified in HSC-1 cells (Figure S2).

3.4. IRF2 is a direct target of miR-664 by binding to 3′ UTR region

To investigate the downstream target of miR-664a-3p, TargetScan 7.1 and miRDB were utilized to select IRF2 as a potential target of miR-664. The 3′UTR of IRF2 mRNA contains two presumptive miR-664 binding site, one is in the position 613–619 of 3′UTR IRF2, and the other is 1007–1013 (Fig. 4A). To verify whether IRF2 is a direct target of miR-664, miR-664 mimic/NC mimic or miR-664 inhibitor/NC inhibitor was transfected into cSCC cells. Western blots and qRT-PCR analysis showed that both IRF2 protein and IRF2 mRNA expressions were significantly decreased in miR-664 mimic transfected cSCC cells; whereas the inhibition of miR-664 was able to upregulate IRF2 protein expression (Fig. 4B). These results indicated that miR-664 suppressed IRF2 expression post-transcriptionally. To further validate whether miR-664 mediated-IRF2 downregulation was through the 3′UTR IRF2, we constructed luciferase reporter vectors containing either the wild-type of IRF2 3′UTR sequences or the mutant forms of seeding sites. The luciferase activity of the wild-type IRF2 3′UTR was declined to 61.2% after miR-664 overexpression. In contrast, no significant reduction of luciferase activity in mutant genotype IRF2 3′UTR was observed in 293 T cells (95.9% fluorescence signals) (Fig. 4C). Collectively, our results indicated that miR-664 down-regulated IRF2 expression through directly binding to its 3′UTR.

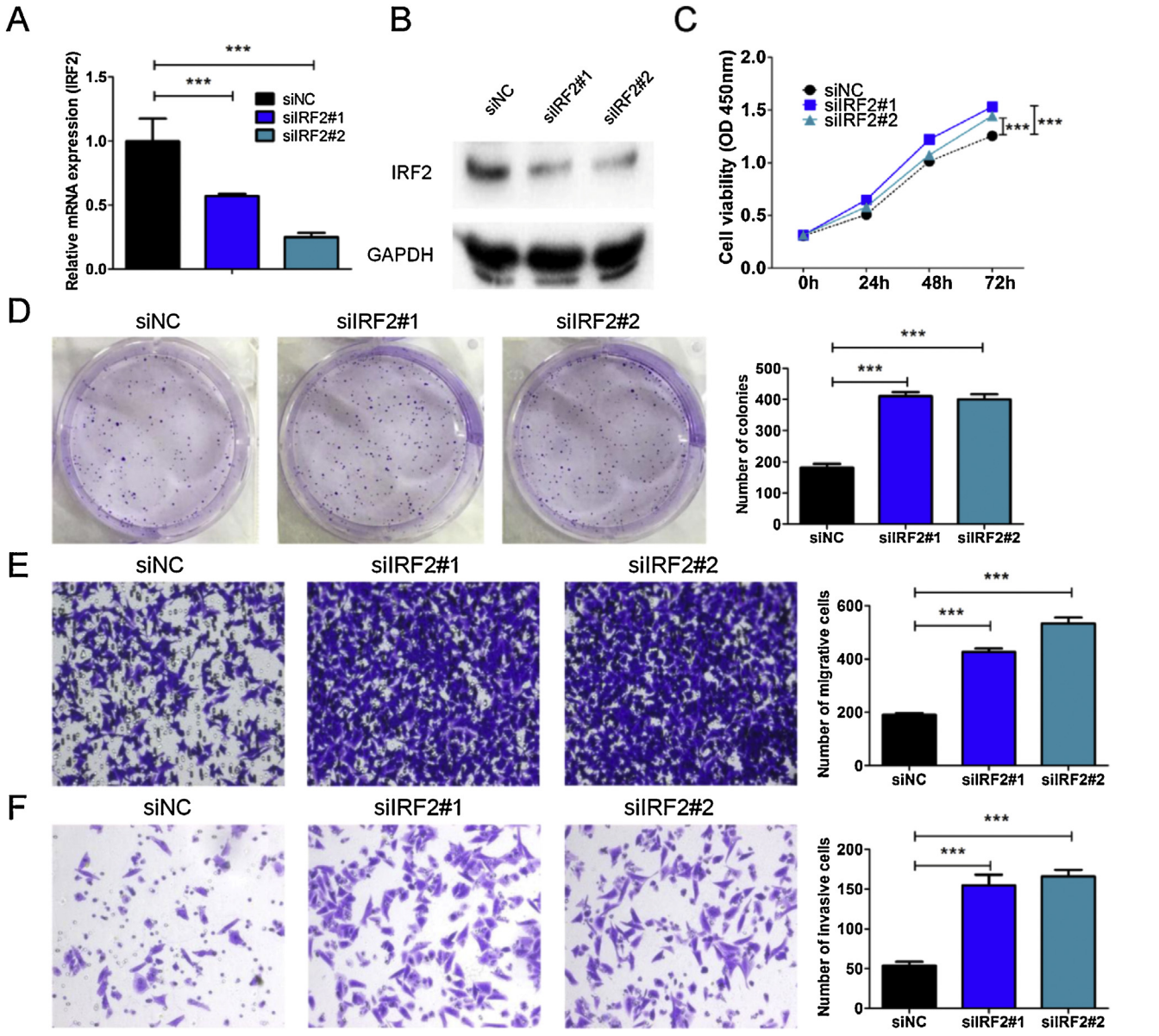

Fig. 5. IRF2 knockdown promotes the proliferation, migration and invasion abilities of cSCC cells. The translational (A) and protein level (B) of IRF2 in siNC- and siIRF2- treated HSC-5 cells. (C) Increased cell viability after IRF2 knockdown by CCK-8 assay. Significantly enhanced proliferation (D), migration (E) and invasion (F) capacities after IRF2 knockdown in HSC-5 cells. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

3.5. IRF2 is downregulated in cSCC

To understand the possible role of IRF2 in cSCC tumorigenesis, we firstly examined the transcriptional and protein levels of IRF2 in cSCC cell lines, *i.e.*, A431, HSC-1 and HSC-5. Unlike to miR-664, mRNA levels of IRF2 in cSCC cell lines were found to be significantly lower than HaCaT keratinocytes (Fig. 4E). IRF2 expression level was also studied in paraffin-embedded sections of human cSCC and normal skin specimens. The immunohistochemistry (IHC) results also indicated a low level of IRF2 in tumor tissues whereas it was high in normal tissue (Fig. 4D). That is to say, both cSCC tissues and cell lines were associated with low IRF2 expression levels.

3.6. IRF2 knockdown reverses miR-664 tumorigenesis in cSCC

To study the functional roles of IRF2 in cSCC development, IRF2 was knocked down by transfecting siRNA oligos into HSC-5 cells. The reduction of IRF2 mRNA and protein levels were achieved by two different siIRF2-treated cells with respect to control (siNC) transfection (Fig. 5A,B). As a result, the cell viability was increased after IRF2 knock down (both $P < 0.001$) (Fig. 5C). Likewise, the proliferation capacity was also increased in IRF2 knock-down cells (both $P < 0.001$) (Fig. 5D). Furthermore, IRF2 knock-down cells significantly enhance migration and invasion abilities compared to siNC (all $P < 0.001$) (Fig. 5E,F). Taken together, IRF2 knock-down positively regulated tumorigenicity of miR-664 in cSCC.

3.7. IRF2 over-expression inhibits miR-664 tumorigenesis in cSCC

Gain-of-function experiments were performed by transfecting IRF2 expression vector (pCMV-IRF2) or negative control (pCMV) into HSC-5 cells. First of all, the over-expression status of IFR2 was validated at transcriptional and protein levels (Fig. 6A,B). Consequently, IRF2 over-expression was found to

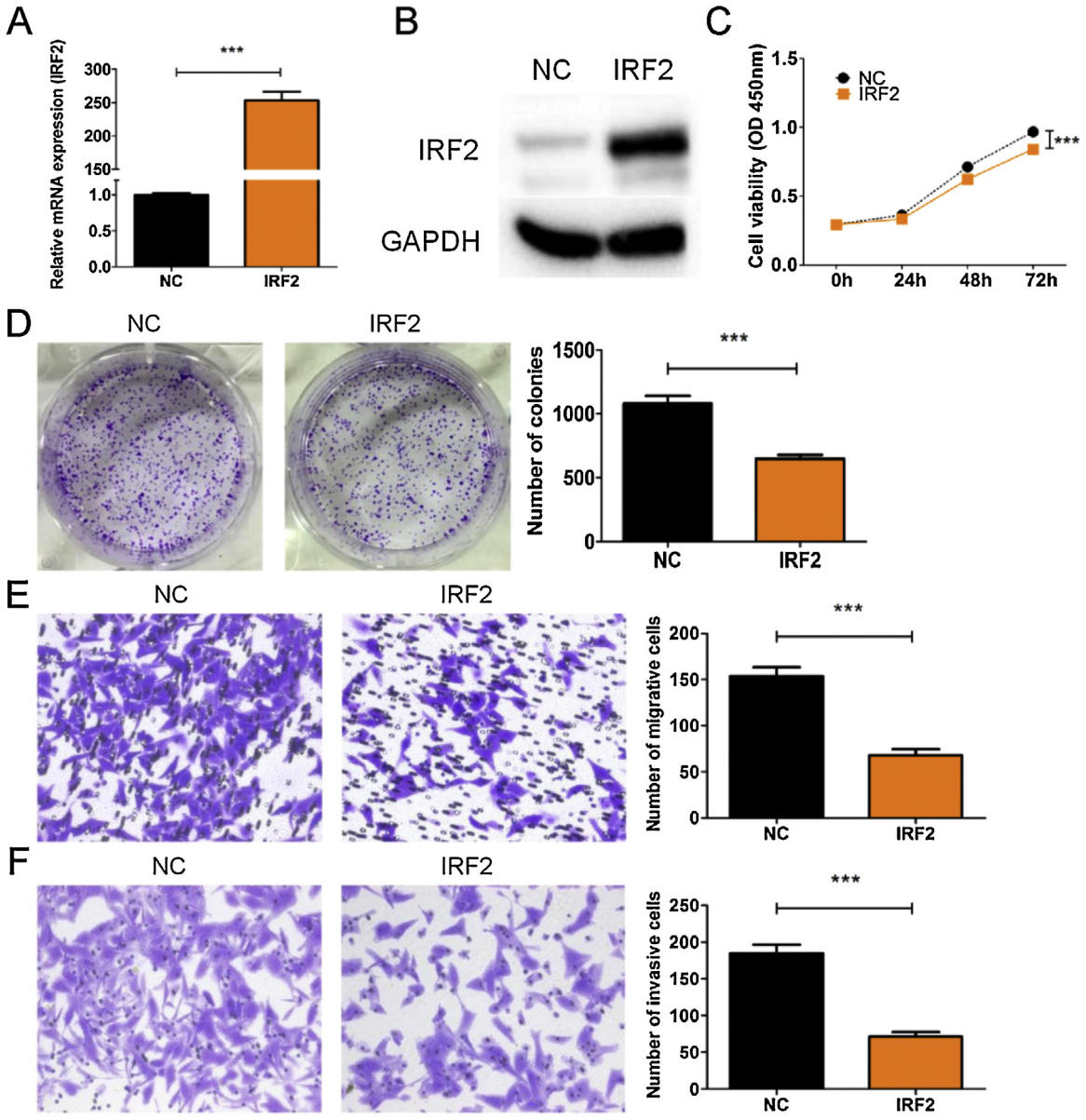

Fig. 6. Overexpression of IRF2 restrains the proliferation, migration and invasion abilities of cSCC cells. The validation of translational (A) and protein (B) levels of IRF2 after IRF2 expression vector (pCMV-IRF2) and negative control (pCMV) were transfected into HSC-5 cells. IRF2 overexpression was found to suppress significantly the cell viability (C), proliferation (D), migration (E) and invasion (F) capacities in HSC-5 cells. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

restore the inhibitory phenotype and restrain cell proliferation, migration, and invasiveness (all $P < 0.001$) (Fig. 6C-F). The IRF2 knock-down and over-expression results were further validated in HSC-cells with identical findings (Figure S3,S4). Together, over-expression of IRF2 dramatically reversed the pro-tumorigenesis effects of miR-664 in cSCC.

4. Discussion

Locally advanced or metastatic cSCC is clinically difficult worldwide [27]. During recent decades, miRNAs have been identified to be a major transcriptional regulator in cell differentiation and carcinogenesis [28,29]. Some miRNAs function as suppressor genes, while others as oncogenes [30]. The conception of miRNAs provides new insights into the diseases, which eventually facilitates mechanistic studies as well as the development of novel therapeutic approaches. Our previous studies revealed that miR-664 could be a tumor suppressor gene in cutaneous malignant melanoma [18]. The pro-carcinogenic role of miR-664 was reported in osteosarcoma [31] as well as lung cancer [32].

The present study shown that miR-664 was upregulated in cSCC tissues and cell lines. It was also found that miR-664 promoted cell proliferation, migration and invasion *in vitro* and facilitated tumor growth *in vivo*. The mechanism of the oncogenic role of miR-664 might function *via* downregulating IRF2 by targeting its 3′-UTR. Those findings suggested that miR-664 played an important role in promoting carcinogenesis and progression of cSCC.

It was reported that IRF2 functions as a tumor suppressor in lung cancer and gastric cancer [30,33], or an oncogene in testicular embryonal carcinoma cells [34]. IRF2 is considered as one of the most desirable targets for cancer therapies [35,36]. Nevertheless, the function and regulatory mechanisms of IRF2 in cSCC remains unclear. Our data revealed that IRF2 was able to suppress cell proliferation, migration and invasion capacity of cSCC. However, further mechanistic studies on the inhibitory functions of IRF2 as well as its associated signaling cascades are warrant.

5. Conclusions

Our study demonstrated that miR-664 was significantly upregulated in cSCC tumor tissues and cell lines. Overexpression of miR-664 promoted tumorigenic malignant transformation such as increased cell proliferation, migration and invasion in cSCC cells and enhanced tumorigenicity in athymic nude mice. According to dual luciferase reporter assay, IRF2 was demonstrated as a direct downstream target of miR-664. Interestingly, IRF2 expression level inversely correlated with the miR-664. Our data implicated IRF2 as an anti-carcinogenic role and was suppressed by miR-664 in cSCC development. Those findings outlined miR-664 functions as an oncogene in cSCC *via* suppression of IRF2. Our data enabled the discovery of novel targets for treatment of cSCC with translational potentials.

Authors' contributions

Z.D. and L.C. designed the experiments. C.Zhou and X.L. wrote the manuscript. X.L. and C.Zhang. performed experiments and animal work. X.L., X.X., Z.Z. and C.Zhou assisted in acquisition, analysis and interpretation of data for the work. M.Z. revised critically the manuscript for important intellectual content. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.05.004.

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