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miR-146b Functions as an Oncogene in Oral Squamous Cell Carcinoma by Targeting HBP1

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Abstract

Oral squamous cell carcinoma (OSCC) represents more than 90% of all oral cancer and is the most common oral threat around the world. In this study, we examined the roles of miR-146b in OSCC cells. The miR-146b expression in OSCC tissues and cell lines was evaluated by quantitative real-time PCR (qRT-PCR). MTT assay was used to investigate the impact of miR-146b on the growth of OSCC cells *in vitro*. Transwell assay was utilized to analyze the effect of miR-146b on the migration and invasion of OSCC cells. Target prediction and luciferase assay were employed to demonstrate the interaction between miR-146b and HMG-Box Transcription Factor 1 (HBP1). Western blot was carried out to investigate the protein expressions of HBP1 related genes. miR-146b expression was significantly higher in OSCC tissues and cells compared with paired normal tissues and normal oral keratinocyte cells. Inhibition of miR-146b decreased cell proliferation, migration, and invasion of OSCC cells. Further studies found that HBP1 was a direct target of miR-146b. Co-inhibition of HBP1 reversed the suppressive impact of miR-146b inhibition on OSCC cell proliferation, migration, and invasion. In conclusion-ourresults reveal that miR-146b potentially regulates the proliferation, migration, and invasion of OSCC cells through binding and downregulating HBP1 expression in OSCC cells.

Keywords

OSCC, qRT-PCR, MTT, HBP1, miR-146b

Abbreviations

OSCC, Oral squamous cell carcinoma; qRT-PCR, quantitative real-time PCR; HBP1, HMG-Box Transcription Factor 1; TSCC, tongue squamous cell carcinoma; CDKN1B, cyclin-dependent kinase inhibitor 1B expression; DND1, dead end homolog 1

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Introduction

As the most common oral malignancy, oral squamous cell carcinoma (OSCC) accounts for more than 90% of oral cancer.[1] The incidence of OSCC ranks the eighth among all kinds of tumors in men.[2] For patients with OSCC, surgery is usually performed, and preoperative induction chemotherapy or postoperative adjuvant chemoradiotherapy is applied according to the condition of the lesion. However, there are still many patients who have recurrence symptoms after surgery with a poor survival rate.[3,4] Clinically, the determination of the treatment approach for OSCC and the judgment of prognosis are usually based on tumor size, lymph node size, and metastasis (TNM staging). However, this system does not provide a comprehensive assessment of tumors in individual OSCC patients. For example, there is a significant difference between the biological behavior and clinical efficacy of tumors in the same stage.[5] Based on the pieces of evidence mentioned above, how to achieve early diagnosis and adopt reasonable treatment of OSCC is crucial for improving patient survival rate. Therefore, there is an urgent need for reliable molecular markers to provide early, specific diagnosis and accurately targeted therapy for OSCC patients.

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MicroRNAs (miRNAs) are a family of non-coding single-stranded RNA particles with a length of approximately 20-25 nt, which regulate about 30% of all human genes through complete or incomplete pairing with 3' –UTR (3'-untranslated regions). MicroRNAs (miRNAs) can silence or block the translation of their target genes. Thus, miRNAs function as post-transcriptional regulators.[6] In the process of tumorigenesis and development, miRNAs widely regulate various biological behaviors of tumor cells such as differentiation, proliferation, apoptosis, metabolism, invasiveness, and drug.[7] Meanwhile, studies have shown that miRNA can inhibit oncogenes or promote the expression of tumor suppressor genes to play a role in cancer therapy.[8,9] For example, miRNA-21 with up-regulated expressions may play an oncogene role in various tumors such as gastric cancer, pancreatic cancer, and ovarian cancer.[10,11] miRNA-184 expression is up-regulated in tongue squamous cell carcinoma (TSCC). Meanwhile, miRNA-184 inhibitor could reduce the cell proliferation rate and induces apoptosis of OSCC cells by targeting SOX7.[12] Also, overexpression of miRNA-24 in TSCC tumor samples and cell lines could down-regulate dead end homolog 1(DND1) expression, which inhibits cyclin-dependent kinase inhibitor 1B expression (CDKN1B). Therefore, miRNA-24 can indirectly regulate TSCC cell proliferation and apoptosis.[13] Based on the above research evidence, miRNAs offer the possibility of early diagnosis and adjuvant therapy for OSCC.

Recent studies characterized miR-146b as a tumor suppressor or oncogene in several human cancers, including thyroid cancer, colon cancer, breast cancer, and melanoma.[14-16] Qiu et al found that the upregulation of miR-146b in papillary thyroid carcinoma and miR-146b could promote proliferation and migration in TPC-1 cells.[17] In prostate cancer, miR-146b could inhibit autophagy by targeting PTEN/Akt/mTOR pathway.[18] However, its role in OSCC is still unknown. In the present study, we aimed to decipher the biological function of miR-146b in OSCC.

Method and Materials

Clinical Samples and Cells

All of the OSCC samples and their paired pericarcinomatous tissues were collected in the Department of Pathology, Wuxi stomatology hospital between June, 2018 and August, 2018. All experimental protocols were reviewed and approved by the wuxi stomatology hospital ethics committee (License number: wx-o-2018-7-20). All patients had read and signed the informed consent. The collected tissues were quickly solidified within the fluid nitrogen and stored at -80°C until further study. Tca8113, SCC9, SCC25, CAL27, HN12, HSU3, FADU, and NHOK cells were purchased from ATCC (Virginia, USA). Cells were cultured with RPMI 1640 with 10% (v/v) FBS (Invitrogen, Carlsbad, CA) in a humidified chamber at 5% CO_2, at 37°C.

Quantitative Real-Time Polymerase Chain (qRT-PCR) Analysis

Total RNA was extracted with RNApure Tissue&Cell Kit (CWBio, China). The miRNA expression was detected with Bio-Rad IQ5 system. The real-time PCR reaction contained: 10μL GoldStar Probe Mixture (Low ROX) (CWBio, China), 1μL sense primer (10 nM), 1μL anti-sense primer (10 nM), 2μL cDNA template (10 ng), and 6μL H_2O. The program qRT-PCR was set as following: 95°C, 30 seconds, 40 cycles (95°C, 5 seconds, and 60°C, 10 seconds). $2^{-\Delta\Delta}$Ct cycle method was used to calculate the relative expression level of mRNAs. GAPDH was employed as internal control. Primer sequences were as follows: HBP1: F-5'-TCATCACCATTGGAAGGAGGA-3'; R-5'-TTGCACCATCCCAAATCATCA-3'; GAPDH-F: 5'-GGAGCGAGATCCCTCCAAAAT-3'; R-5'-GGCTGTTGTCATACTTCTCATGG-3'. To detect the level of mature miR-146b, miRNA-specific stem-loop RT primers (General Biosystem, ChuZhou, China) were used to amplify miR-146b. The sequence information was listed as following: miR-146b: F-5'-TGACCCATCCTGGGCCTCAA-3; R:5'-CCAGTGGGCAAGATGTGGGCC-3'; U6: F-5-CTCGCTTCGGCAGCACA-3'; R: 5'-AACGCTTCACGAATTTGCGT-3'. The qRT-PCR was performed in a 10 μL of reaction: 2μL RT buffer, 0.5μL GoldStar Probe Mixture (Low ROX) (CWBio, China), 1μL miR-146b RT primer (10 nM), 1μL total RNA, and 5.5 μL of H_2O.

Transfection

The oligonucleotides were transfected into SCC9 and SCC25 cells with Liprofectamine2000 (Thermo Fisher Scientific, USA) at 80–90% confluency according to the manufacturer's instructions. Cells were seeded on six-well plates with a density of 5×10^5 cells per well. DMEM containing 10% FBS without penicillin and streptomycin overnight was used as a culture medium. OPTI-MEM serum-free medium (M5650, Sigma Aldrich) was used in transfection tests. The siRNAs of HBP1 were synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). Two sequences of si-HBP1 information was GAAUACCCAAGAUCAUCU UTT (si-HBP1 -1), UAC CUC AGA CAU ACC AGA ATT (si-HBP1-2), Final concentration of 100 nM siRNA was introduced in this study. Meanwhile, the similar experimental operations were carried out with human miR-146b inhibitor and miR-146b mimic. miR-146b inhibitor and control were obtained from General Biosystem (General Biosystem, ChuZhou, China). The sequence of miR-146b-inhibitor information was 5'- CCGGGCACCAGAACTGAGTCCACAGGGCATTGCTAGAGCTCACAGCCTATGGAATTCTGTTCTCAGTGCCAGG-3'. Beside, miR-146b mimic and control were obtained from General Biosystem (ChuZhou, China). The sequence of miR-146b mimic information was 5'-CCUGGCACUGAGAACUGAAUUCCAUAGGCUGUGAGCUCUAGCAAUGCCCUGUGGACUCAGUUCUGGUGCCCGG-3'. Lipofectamine 2000 (Thermo Fisher Scientific, USA) was used to transfect 200 nM miR-146b inhibitor or

negative control inhibitor (negative control), as well as 200 nM miR-146b mimic and negative control miRNA (negative control) into 5×10^5 SCC9 and SCC25 cells.

MTT Assay

MTT assay was carried out with a commercial kit (C0009, Beyotime) according to the protocol provided by manufacture. In brief, a density of 4×10^3 cells /well was seeded in 96-well plates. After the cells were attached for 4-6 hours, the medium was changed. After incubation at 37°C for 24 h, 48 h, and 72 h, 20μLMTT was added to each well for 4 h. The supernatant was discarded, 150μL DMSO was added to each well to detect the A value.

Dual Fluorescein Reporter Gene Analysis

In different groups, dual Luciferase Reporter Gene Assay Kit (Yeasen, China) was used according to the instruments provided by manufacture. The degree of activation of the reporter gene for different sample purposes was compared based on the ratio obtained.

Migration and Invasion Assay.

After cell digesting, each well of the 6-well plate was covered with a single layer. The small gun head was used for vertical scratching. Photographs were taken under a 72 h microscope and analyzed by Image J software. The relative migration distance of cells = (D treatment group—D control group) /2. Cell invasion was analyzed by using Cell Invasion Assay Kit (ab235885, Abcam) according to standard procedures. 1 x 10^3 cells with different treatments were seeded on the upper wells, which were coated with Matrigel basement extract. 500 μl of serum free media were added at the bottom of each well. After 48 h, the non-invasive cells on the upper surface were removed. Cells go through to the lower surface were fixed. Subsequently, cells were cultured at 37°C in CO_2 incubator for 1 h. Optical density value was measured with fluorimetric analysis (485 excitations, 520 nm emission).

Western Blot Analysis

Total cellular protein was isolated with 1% PMSF (Cell Signaling Technology, USA) and RIPA lysis buffer (Thermo Fisher Scientific, USA). After boiled with SDS-PAGE sample buffer for 5 min, the samples were performed for sodium dodecyl lsulfate–polyacrylamide gel electrophoresis. Then the proteins were transferred onto a polyvinylidene difluoride membrane (Millipore, USA). After being blocked for 1 h at room temperature, the membrane was incubated with rabbit polyclonal anti-mouse HBP1 antibody (1:1000) (ab83402, abcam), E-cadherin (1:800) (MAB7481, R&D system), β-catenin (1:500) (#9562, Cell Singling technology), Vimentin (1:500) (MAB2105, R&D system), MMP-2(1:500) (AF1488, R&D system), MMP-9(1:500) (AF909, R&D system), and GAPDH (1:1500) (MAB5718, R&D system) overnight. Primary antibody-treated samples were incubated with horseradish peroxidase-labeled secondary antibody (1:200) for 1 h at room temperature. The results were finally detected by ECL Chemiluminescence Kit (Thermo Fisher Scientific, USA). The bands were obtained by GeneGnome 5 (Synoptics Ltd., UK).

Statistical Analysis

SPSS21.0 software was used in this study. The test values in experimental results were expressed as mean ± s. The mean comparison between the two groups was carried out with t-test method. $P < 0.05$ was considered statistically significant.

Results

miR-146b Expression Is Significantly Up-Regulated in OSCC Tissues and Cell Lines

Firstly, we analyzed miR-146b expression in 60 pericarcinomatous tissues and their paired OSCC tissues. The results suggested that miR-146b expression in OSCC tissues was significantly higher than that in the paired pericarcinomatous tissues ($P < 0.0001$) (Figure 1A). The clinical information of all patients was summarized in Table 1. 60 patients with OSCC were divided into miR-146b low expression group ($n = 30$) and miR-146b high expression group ($n = 30$) according to the median expression value of miR-146b in the OSCC tissue. The chi-square test was used to calculate the relationship between miR-146b expression and clinicopathological data of OSCC. The results suggested that miR-146b expression was significantly associated with tumor size, differentiation, TNM stage, and lymph node metastasis ($p < 0.001$). Meanwhile, patient age, gender, and location of the tumor were not associated with miR-146b expression. Furthermore, we studied miR-146b expression in 7 OSCC cell lines, including Tca8113, SCC9, SCC25, CAL27, HN12, HSU3, FADU, and normal oral keratinocyte cell line NHOK. The results suggested that miR-146b expressions in OSCC cell lines were significantly higher than those in NHOK cells ($P < 0.01$) (Figure 1B). The highest miR-146b expressions was detected in SCC25 and SCC9 cells. Therefore, both cells were selected to carry out further study.

Silencing of miR-146b Inhibits OSCC Cell Proliferation, Migration and Invasion

To further probe the functional role of miR-146b in OSCC cells, we knocked down miR-146b expressions in SCC25 and SCC9 cells. qRT-PCR analysis suggested that the successful knockdown of miR-146b could be observed in SCC25 and SCC9 cells ($P < 0.001$) (Figure 2A). Next, we studied cell proliferation in control and miR-146b-silenced SCC25 and SCC9 cells at different time points. Figure 2B indicated that knockdown miR-146b expression significantly decreased cell proliferation at 48 h, 72 h, and 96 h ($P < 0.05$). Also, cell migration analysis suggested that miR-146b knockdown significantly reduced the migration of SCC25 and SCC9 cells ($P < 0.01$) (Figure 2C). In addition, cell invasion analysis suggested that knockdown of miR-146b also significantly reduced cell invasion ($P < 0.01$) (Figure 2D). In summary, miR-146b

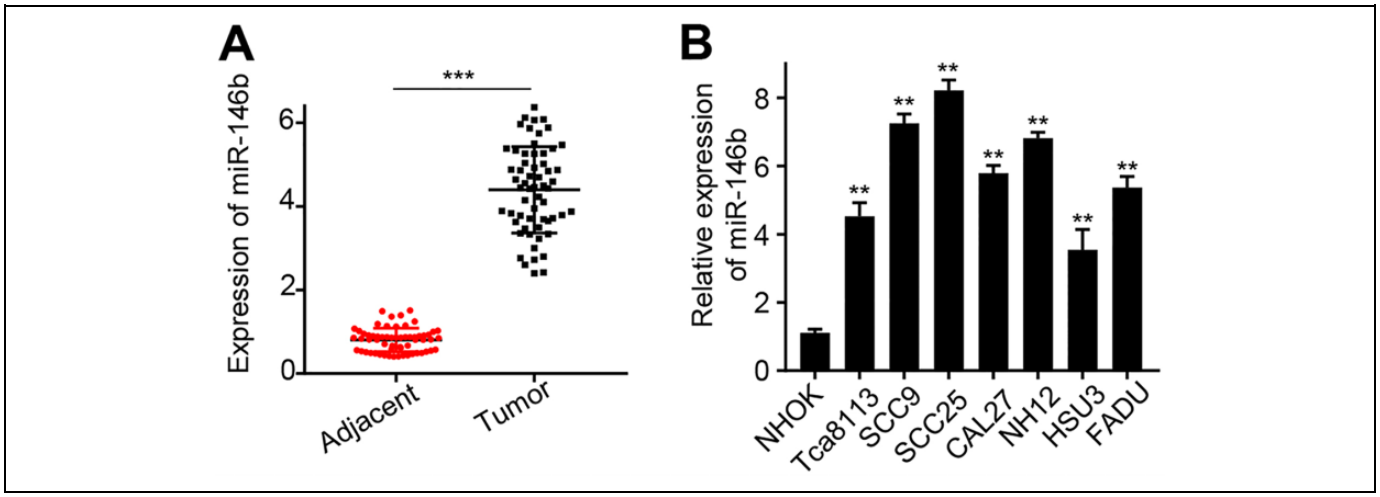

Figure 1. miR-146b expression analysis in clinical tissues and cell lines A) miR-146b expression analysis of 60 OSCC and paired paracancerous tissues with qRT-PCR; B) qRT-PCR analysis of miR-146b expression in Tca8113, SCC9, SCC25, CAL27, HN12, HSU3, FADU, and NHOK cells. **P < 0.01, ***P < 0.001.

Table 1. Clinical Information of All Patients Recruited in This Study.

Clinical data	High, no.cases	Low, no.cases	χ^2	P value
Age				
≥60	13	15	0.268	0.605
<60	17	15		
Gender				
Male	19	16	0.617	0.432
Female	11	14		
Differentiation				
High	15	4	14.507	0.001
Moderate	9	6		
Low	6	20		
Clinical stage				
I-II	5	12	4.022	0.045
III-IV	25	18		
Tumer diameter				
≥2cm	24	12	10.000	0.002
<2cm	6	18		
Location				
Tongue	4	5	0.913	0.979
Gingiva	7	5		
Bucca mucosa	6	9		
Floor of the mouth	5	5		
Others	7	7		
Lymph node metastasis				
Yes	20	7	11.380	0.001
No	10	23		

knockdown treatment in SCC25 and SCC9 cells could inhibit OSCC cell proliferation, migration, and invasion.

miR-146b Targets HBP1 in OSCC Cells

We then studied the regulatory mechanisms of miR-146b in OSCC. Target prediction revealed that HBP1 was the potential target of miR-146b (Figure 3A). Therefore, we carried out luciferase reporter gene assay to verify the targeted relationship between HBP1 and miR-146b. Figure 3A showed that miR-146b mimic significantly inhibited luciferase activity in SCC25 and SCC9 cells (P < 0.05). However, no difference in luciferase activity was observed in SCC25 and SCC9 cells when we mutated the HBP1 binding site for miR-146b. This result indicated that HBP1 was a direct target of miR-146b. Furthermore, qRT-PCR result suggested that miR-146b knockdown significantly promoted HBP1 expression (P < 0.01) (Figure 3B). The similar results could be obtained by performing western blot analysis (Figure 3C). We also investigated HBP1 expressions in 60 pericarcinomatous tissues and the paired OSCC tissues. The result suggested that HBP1 expression was significantly downregulated in OSCC tissues compared with that in pericarcinomatous tissues (P < 0.01) (Figure 3D). Based on spearman correlation coefficient test, we found that HBP1 expression was negatively correlated with miR-146b expression in OSCC tissues (R = 0.4447) (Figure 3E). In summary, miR-146b, which directly interacts with HBP1, reduces HBP1 expression in OSCC cells.

Knockdown of HBP1 Rescues the Decreased Malignant Phenotype Caused by miR-146b Silencing

Next, we further investigated the functional relationship between HBP1 and miR-146b. To this end, we inhibited HBP1 expression in miR-146b-silenced cells. Cell proliferation analysis was carried out in three different groups at 0 h, 24 h, 48 h, 72 h, and 96 h. The results revealed that the proliferation of inh-miR-146b OSCC cells was lower than of inh-NC OSCC cells at 96 h (P < 0.01) (Figure 4A). However, cell proliferation was partially rescued by HBP1 co-inhibition (P < 0.01) (Figure 4A). Moreover, migration and invasion analysis indicated that inh-miR-146b treatment significantly reduced cell migration and invasive abilities (P < 0.01), and HBP1 co-inhibition reversed the effects of inh-miR-146b (P < 0.01) (Figure 4B, C). In addition, we have employed western blot analysis to study the HBP1 related protein expressions, including HBP1, E-cadherin, β-catenin, Vimentin, MMP-2, MMP-9, and

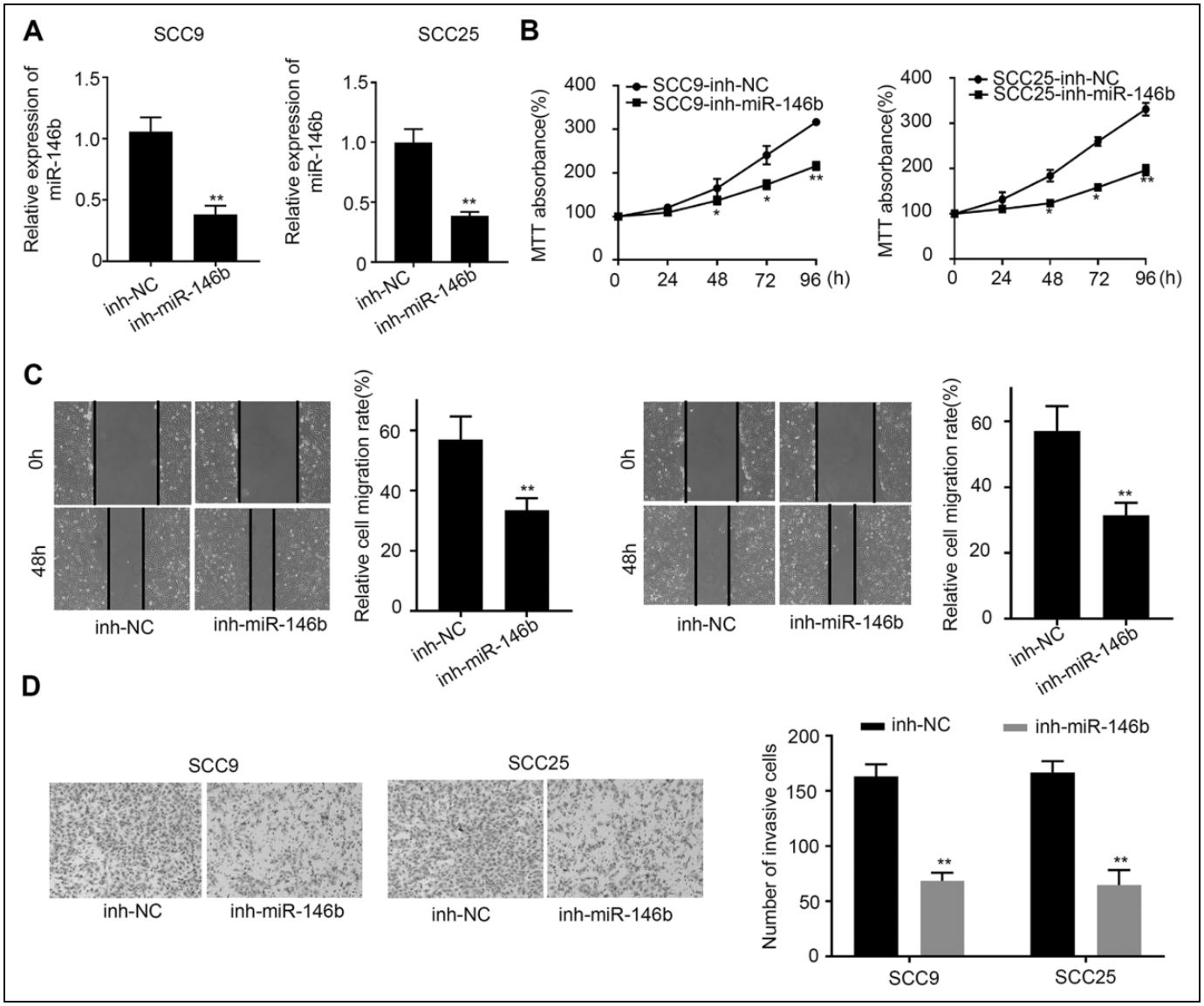

Figure 2. Cell proliferation, migration and invasion analysis of miR-146b knockdown treated SCC9 and SCC25 cells A) qRT-PCR analysis of miR-146b expressions in miR-146b knockdown treated SCC9 and SCC25 cells. B) Cell proliferation analysis of miR-146b knockdown treated SCC9 and SCC25 cells at 0 h, 24 h, 48, 72 h, 96 h. C) Cell migration analysis of miR-146b knockdown treated SCC9 and SCC25 cells. D) Invasion analysis of miR-146b knockdown treated SCC9 and SCC25 cells. *P < 0.05, **P < 0.01.

GAPDH, in SCC25 and SCC9 cells. The results suggested that the protein expressions of β-catenin, Vimentin, MMP-2, MMP-9 in inh-miR-146b OSCC cells was lower than those in inh-NC OSCC cells at 96 h, whereas E-cadherin upregulated by inh-miR-146b (P < 0.01) (Figure 4D). However, their expressions could be partially rescued by HBP1 inhibition (P < 0.01). In summary, knockdown of HBP1 effectively rescues the decreased malignant phenotype caused by miR-146b silencing.

Overexpression of HBP1 Rescues the Increased Malignant Phenotype Caused by miR-146b Mimic

To further clarify the relationship between miR-146b and HBP1, HBP1 overexpression was used in cells transfected with miR-146b mimic. Cell proliferation analysis revealed that the proliferation of miR-146b-mimic OSCC cells was higher than mimic-NC OSCC cells (P < 0.01) (Figure 5A). However, this promotion of proliferation was partially rescued by HBP1 overexpression (P < 0.01) (Figure 5A). Moreover, migration and invasion analysis indicated that miR-146b mimic treatment significantly increaed cell migration and invasive abilities (P < 0.01), and HBP1 co-transfection reversed the effects of miR-146b (P < 0.01) (Figure 5B, C). In addition to, we have employed western blot analysis to study the HBP1 related protein expressions, including E-cadherin, β-catenin, Vimentin, MMP-2, MMP-9, and GAPDH, in SCC25 and SCC9 cells. The results suggested that the protein expressions of β-catenin, Vimentin, MMP-2, MMP-9 in miR-146b-mimic OSCC cells

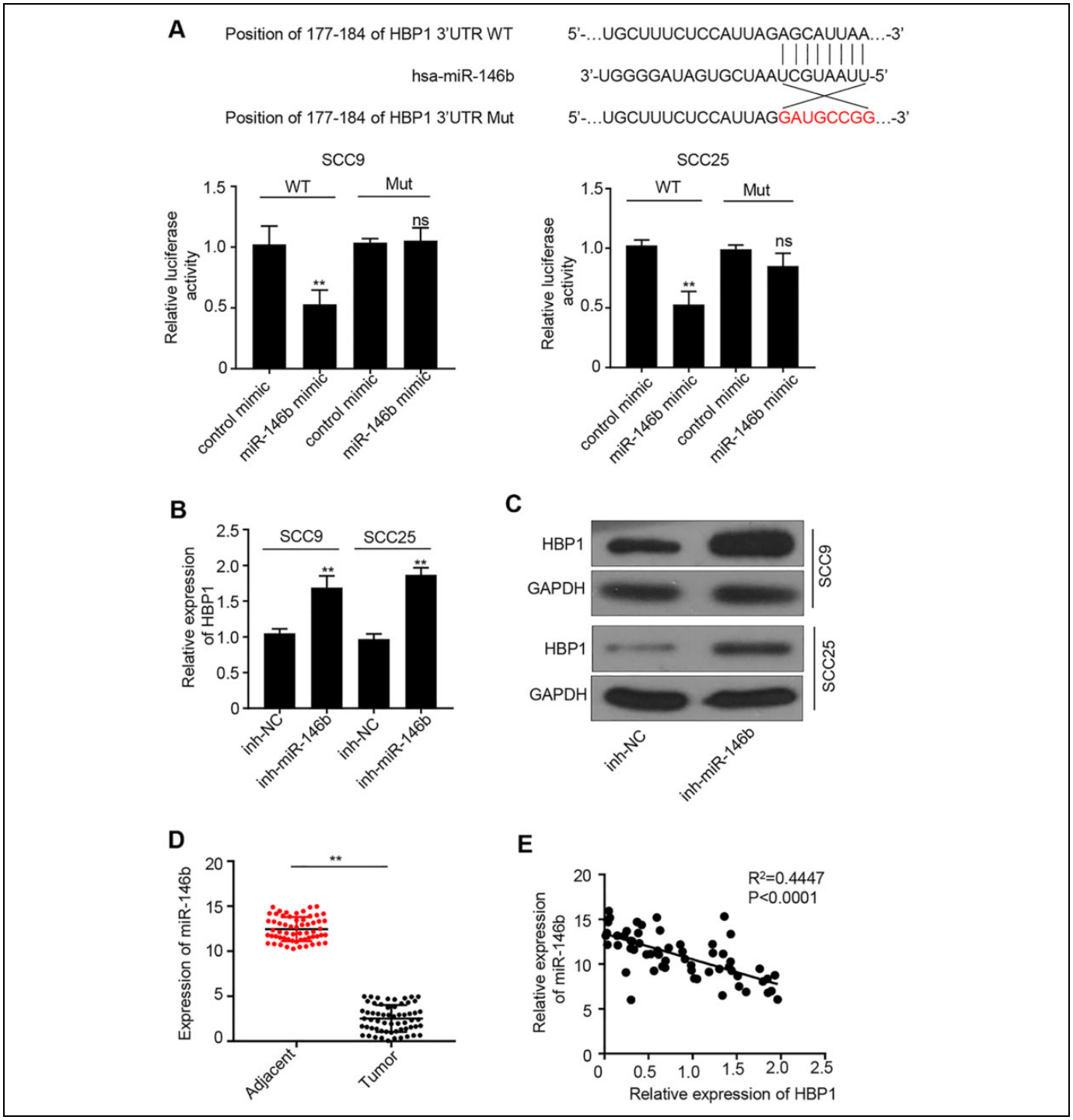

Figure 3. miR-146b directly interacted with HBP1 gene A) Target prediction of miR-146b with miRanda (http://www.microrna.org/). Meanwhile, verification of the luciferase reporter gene (miR-146b) assay had been performed in SCC9 and SCC25 cells with HBP1 gene mutation. B) qRT-PCR analysis of HBP1 expressions in cells with different treatments. C) western blot analysis of HBP1 protein expressions in cells with different treatments. D) qRT-PCR analysis of miR-146b expression in 60 OSCC and paired paracancerous tissues. G) Spearman correlation coefficient analysis of the correlation between miR-146b and HBP1 expression in OSCC tissues. **P < 0.01.

was higher than those in mimic-NC OSCC cells, whereas E-cadherin downregulated by miR-146b mimic ($P < 0.01$) (Figure 5D). However, their expressions could be partially rescued by HBP1 overexpression ($P < 0.01$). Consequently, upregulation of HBP1 effectively rescues the increased malignant phenotype caused by miR-146b.

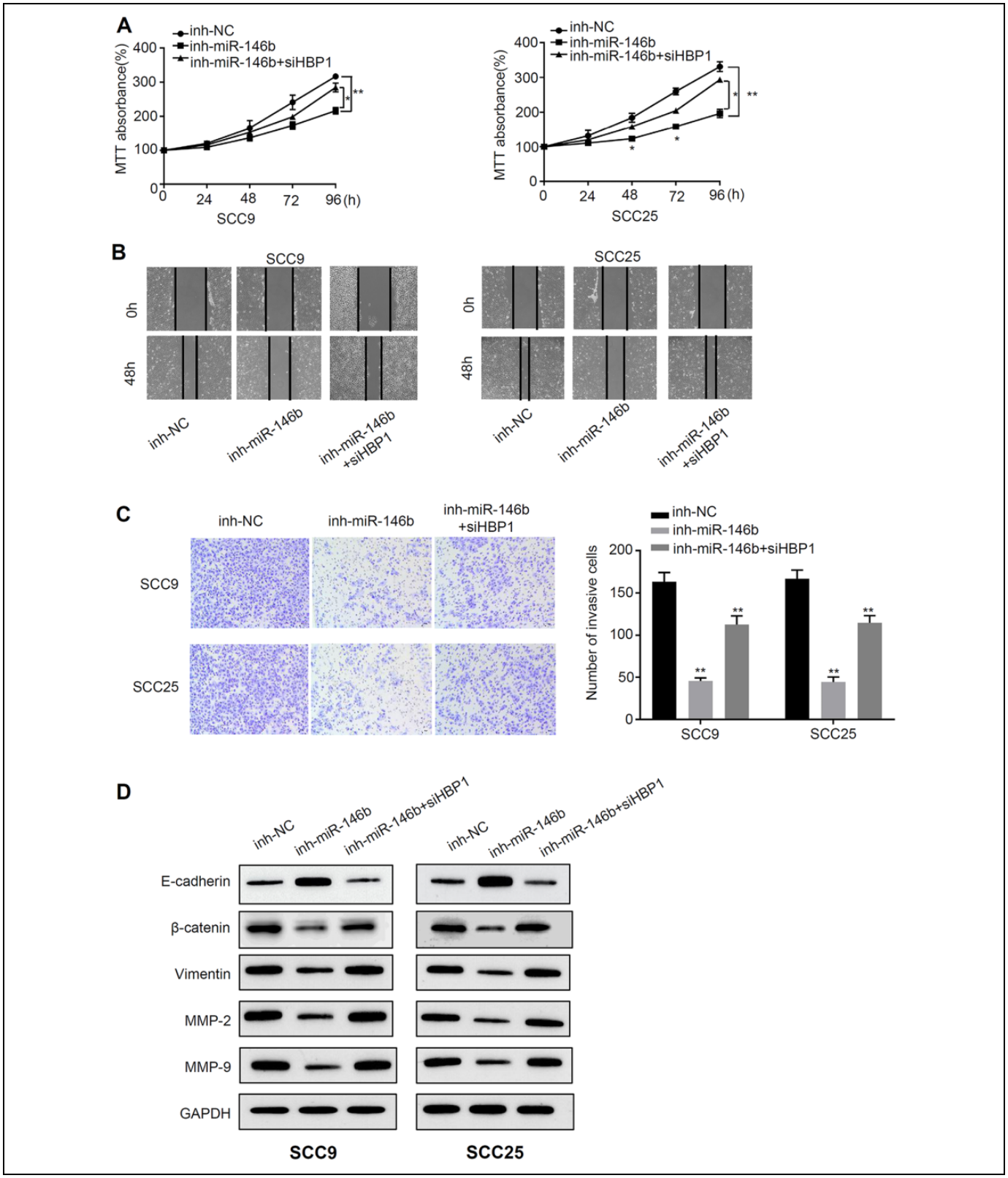

Figure 4. Knockdown of HBP1 effectively rescues the decreased malignant phenotype caused by miR-146b silencing A) MTT assay was used to detect the light absorption at 570 nm at 0 h, 24 h, 48 h, 72 h and 96 h in different groups of SCC9 and SCC25 cells (inh-NC, inh-miR-146b, inh-miR-146b+si-HBP1). B) Scratch test to detect migration ability in different groups of SCC9 and SCC25 cells (inh-NC, inh-miR-146b, inh-miR-146b+si-HBP1). C) Transwell assay to detect the invasive ability of different groups of SCC9 and SCC25 cells (inh-NC, inh-miR-146b, inh-miR-146b+si-HBP1). D) Western blot analysis of HBP1 related protein expression. *P < 0.05, **P < 0.01.

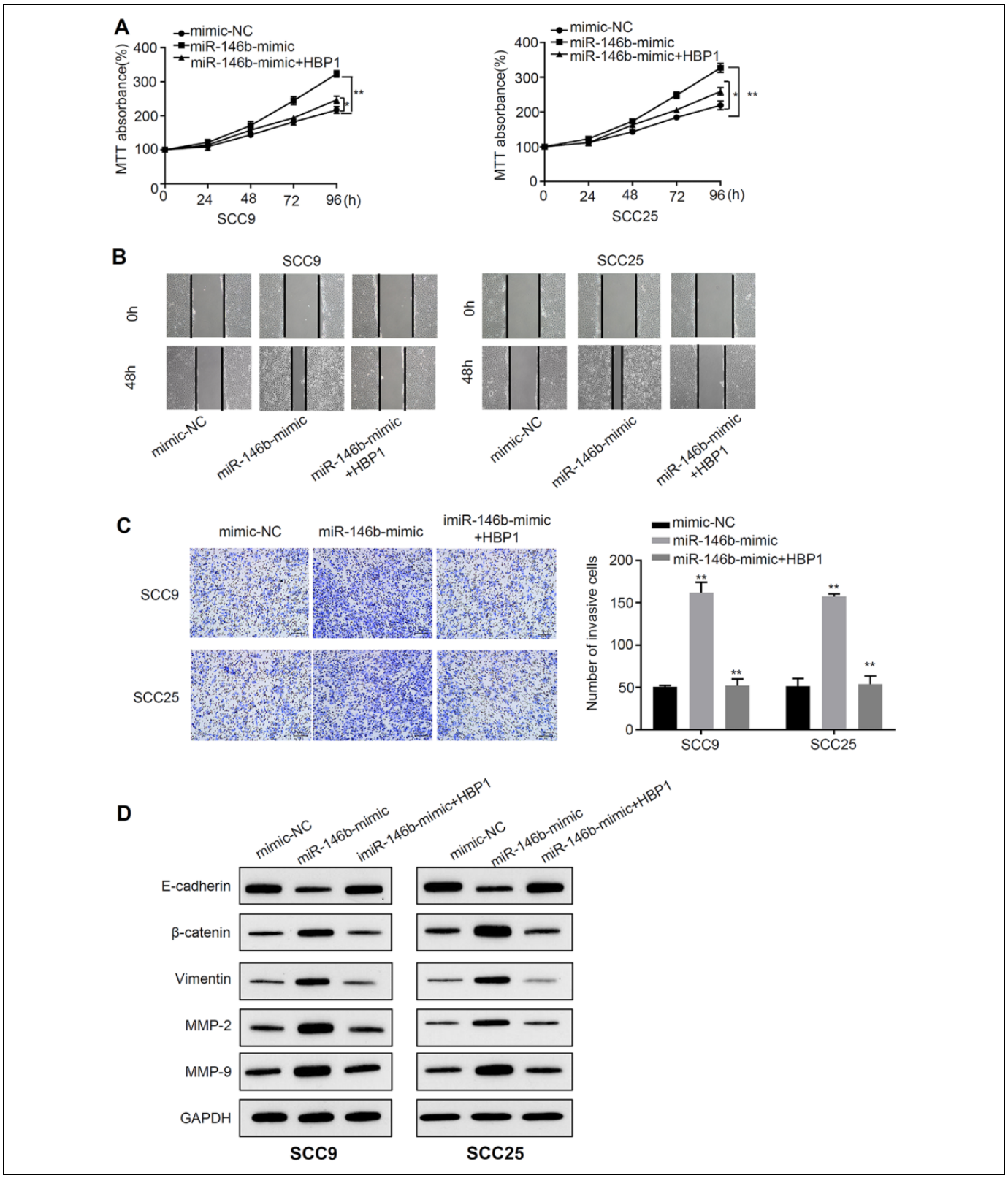

Figure 5. Overexpression of HBP1 rescues the increased malignant phenotype caused by miR-146b mimic A) MTT assay was used to detect the light absorption at 570 nm at 0 h, 24 h, 48 h, 72 h and 96 h in different groups of SCC9 and SCC25 cells (mimic-NC, mimic-miR-146b, mimic-miR-146b+ HBP1). B) Scratch test to detect migration ability in different groups of SCC9 and SCC25 cells (mimic-NC, mimic-miR-146b, mimic-miR-146b+ HBP1). C) Transwell assay to detect the invasive ability of different groups of SCC9 and SCC25 cells (mimic-NC, mimic-miR-146b, mimic-miR-146b+ HBP1). D) Western blot analysis of HBP1 related protein expression. *P < 0.05, **P < 0.01.

Discussion

In humans, miR-146b is localized on chromosome 10 q24.3. miR-146b and mi R-146a belong to the miR-146 family. There is only one base difference between the mature miR-146a and miR-146b.[19] In non-small cell lung cancer, low levels of miR-146b can shorten patient survival. Overexpression of miR-146b can inhibit the proliferation, colony formation, migration and invasion of lung cancer cells.[20] In gallbladder cancer, down-regulated miR-146b expression is associated with tumor grade, liver metastasis, and degree of differentiation.[21] Previous studies have suggested that PTEN-deficient thymocytes can induce the formation of CD4+ T lymphoma. Overexpression of miR-146b can inhibit malignant transformation of thymocytes.[22] In thymocytes, miR-146b directly reduces the expression of the TRAF6 gene in TCR signaling. Menawhile, the down-regulated expression of TRAF6 protein inhibits the expression of oncogene downstream of the NF-k B signaling pathway, thereby inhibiting TCR-mediated cell proliferation.[23] However, miR-146b inhibits PTEN expression in thyroid cancer, thereby highly activating PI3K/AKT signaling pathway and promoting tumor cell proliferation.[24] In follicular thyroid cancer, miR-146b acts on ST8SIA4 to promote cell proliferation, migration and invasion.[25] Moreover, overexpressed miR-146b promotes cell proliferation, migration and glycolysis in colon cancer.[26] In addition, miR-146b is highly expressed in triple negative breast cancer. This molecular can directly regulate BRCA1 expression, resulting in increased cell proliferation and reduced homologous recombination rate.[27] The above evidences suggested that miR-146b played an important role in multiple cancers.

Many studies have shown miRNA have important impact on the progression and development in OSCC. Yang et al used gene overexpression technique and detected that miR-381-3p suppresses the proliferation of OSCC cells by directly targeting FGFR2.[28] In a study about cancer-associated fibroblast-derived exosomal, researchers found that miR-382-5p promotes the migration and invasion in OSCC.[29] Hyein et al detected that miR-197 suppresses the expression of PD-L1 and then facilitates the tumor immunologic escape in OSCC.[30] Moreover, researchers have proven that miR-504 inhibits cell proliferation, migration and invasion by targeting CDK6 in OSCC.[31] However, there are still poor evidences about the functions of miR-146b in OSCC. In this study, we have identified firstly that miR-146b is up-regulated expressed in OSCC. Meanwhile, this molecular could change tumor cell function through HBP1 gene *in vitro*. Therefore, miR-146b is closely related to the carcinogenesis of OSCC and may be a potential biomarker and therapeutic target for OSCC.

Recent studies have shown that HBP1 has a negative regulatory effect on the Wnt-β-cateninsignaling pathway, which can inhibit tumor growth.[32] In addition, studies have shown that HBP1 mutations have been detected in invasive breast cancer, which further confirms that HBP1 has inhibitory effect on the formation of tumors.[33] Li et al have found that the the expression of Vimentin protein in osteosarcoma negatively correlate with HBP1 expression, whereas there is a positively link between E-cadherin and HBP1 protein.[34] Moreover, the MMP2 and MMP9 are key protein in the process of tumor metastasis.[35] In breast cancer, the overexpression of MMP2 and MMP9 could promote the migration and invasion.[36] In this study, we demonstrated that down-regulation of miR-146b inhibited the growth, migration, and invasion of OSCC cell. Furthermore, HBP1 was the a direct target of miR-146b. The proliferation, migration, and invasion of OSCC cell could be affected through regulating miR-146b/HBP1. Our study first revealed the possible link between miR-146b and HBP1 in OSCC cells.

In conclusion, the present study indicated that miR-146b knockdown inhibited cell proliferation, migration, and invasion in OSCC cells by regulating HBP1. Our results could provide detailed information for further studies in OSCC.

Authors' Note

Kui Li, Zheng Zhou, and Ju Li are co-first author.

Authors' Contributions

RX conceived and designed the experiments, KL and ZZ analyzed and interpreted the results of the experiments, JL performed the experiments

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Approval and Consent to Participate

All experiments in this study were approved by the Animal Care and Use Committee of WuXi Stomatology Hospital and performed in accordance with the Guide for the Care and Use of Laboratory Animals.

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