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RESEARCH ARTICLE

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microRNA-211 promotes proliferation, migration, and invasion ability of oral squamous cell carcinoma cells via targeting the bridging integrator 1 protein

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Abstract

Oral squamous cell carcinoma (OSCC), the most common pathological type of oral cancer, is still a frequent malignancy with unsatisfactory prognosis. Accumulating studies have proven some microRNAs (miRNAs) can function as oncogenes in OSCC by targeting tumor suppressors. In this study, we first investigated the expression and role of tumor suppressor bridging integrator-1 (BIN1) in OSCC tissues and cells. Our results indicated that BIN1 was low expressed in the OSCC tissues and cell lines (SCC6, SCC9, SCC25, HN4, and HN6) along with miR-211 was highly expressed in OSCC tissues and cell lines, and BIN1 overexpression could evidently inhibit their proliferation, migration, and invasion abilities. Next, we used bioinformation algorithms to predict the potential miRNA targeting BIN1 and chose miR-211 for further study. miR-211, a highly expressed miRNA in OSCC cells, could specifically bind with the 3′-untranslated region (3′-UTR) of BIN1 to trigger its degradation. Addition of miR-211 inhibitor could evidently suppress the malignant behaviors of OSCC cells by upregulating BIN1 expression and inhibit the activation of the EGFR/MAPK pathway. Taken together the findings of the study indicated that miR-211 mediated BIN1 downregulation had crucial significances in OSCC, suggesting the miR-211 might be a novel potential therapeutic target for the OSCC treatment.

KEYWORDS
BIN1, invasion, miR-211, OSCC, proliferation

1 | INTRODUCTION

Oral cancer is the 11th most frequent malignancy worldwide, especially in the developing countries.[1] Oral squamous cell carcinoma (OSCC) is the main pathological type, accounts for about 90% of oral cancer cases. Despite the improvement of treating approaches including surgery, chemotherapy, and radiotherapy, the overall prognosis of patients with OSCC remains unsatisfactory. The silence of some tumor suppressor genes plays pivotal roles in the progression of OSCC.[2-4] Therefore, a more adequate understanding of the mechanisms of aberrant tumor suppressor gene expression might be essential for

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establishing novel treatment strategies for improving the prognosis of patients with OSCC.

Bridging integrator-1 (BIN1) is a nucleocytoplasmic adapter protein which was originally defined as a tumor suppressor due to its possibility of interacting with c-MYC protein to neutralize its tumorigenesis effects.[5] The expression of BIN1 is attenuated or even lost in almost 50% of the tumor cell lines as well as in multiple malignant tumors, such as melanoma, lung, prostate, and breast cancer, while its ectopic expression can suppress malignant behaviors of cancer cells.[6-8] In our previous study, we demonstrated that BIN1 expression was attenuated in the tumor microenvironment of esophageal squamous cell carcinoma (ESCC), and low expression of BIN1 was associated with undesirable clinical parameters and poor postoperative survival.[9] In addition, we observed a low expression of BIN1 in non-small–cell lung cancer (NSCLC), and further found that BIN1 could suppress the activation of the EGFR/MAPK pathway.[10] As far as we know, the expression and functions of BIN1 in OSCC still remain unknown.

The Encyclopedia of DNA Elements (ENCODE) project has confirmed that most of the genome is transcribed as noncoding RNAs including microRNAs (miRNAs).[11] miRNAs are evolutionarily conserved single-stranded RNAs that consist of about 21 to 24 nucleotides. miRNAs can trigger messenger RNA (mRNA) degradation or translation inhibition by combining 3′-untranslated regions (UTRs) of target mRNAs.[12] miR-211-5p (miR-211) is a recognized miRNA participating tumorigenesis, but its function varies in different cancers. For instance, miR-211 could suppress tumor progression in hepatocellular carcinoma (HCC) by targeting SPARC,[13] but it acted as oncogene promoting NSCLC proliferation and invasion by inhibiting MxA.[14] As for OSCC, miR-211 could downregulate the tumor suppressor TCF12, and enhance the proliferation, invasion, and colony formation ability of OSCC cells.[14,15] Taken above, miR-211 could be regarded as an oncogene in OSCC that might trigger the anergy of numerous tumor suppressors.

In this study, we evaluated the expression and biological function of BIN1 in OSCC. Next, for elucidating the mechanism of BIN1 attenuation in OSCC, we identified the correlation between BIN1 and miR-211 expression in OSCC cells lines. The results showed that BIN1 could function as a tumor suppressor in OSCC, and it could be downregulated by miR-211.

2 | MATERIALS AND METHODS

2.1 | Cell lines

The human oral squamous cell carcinoma cell lines (SCC6, SCC9, SCC25, HN4, and HN6) and normal human oral keratinocytes (HOK) were obtained from the Research Center of Hebei Medical University (Shijiazhuang, China). All of the used cell lines were cultured in medium RPMI-1640 (Thermo Fisher Scientific, Waltham, Massachusetts) containing 5% fetal bovine serum (FBS; Solarbio, Beijing, China), 100 U/mL penicillin and 100 μg/mL streptomycin, at 37°C in the humidified atmosphere of the 5% CO_2 incubator.

2.2 | Patients and specimens

The specimens of OSCC tissues, normal oral epithelial tissues were collected from 86 patients who underwent OSCC surgery at the Fourth Hospital of Hebei Medical University (Shijiazhuang, China) between March 2013 and April 2014. This study was approved by the Ethics Committee of the Hebei Medical University and all informed consents were signed by patients. The median patient age at the time of surgery was 54 years (range, 29-73 years). None of the patients with OSCC received preoperative radiotherapy, chemotherapy, or immunotherapy. The clinical stage and histological tumor type were determined according to the AJCC Classification of 2018 (eighth edition). Patient's clinical information was collected and stored in a database, which was updated every 3 months by telephone follow-up. Complete follow-up was updated until death or June 2018. The specimens were collected and treated promptly after surgery. Each specimen was sufficient to be cut into two pieces and treated differently for various uses, put in liquid nitrogen for extracting RNA, or fixed in 10% formaldehyde for making paraffin-embedded blocks.

2.3 | RNA isolation and real-time quantitative PCR

To quantify BIN1 mRNA expression in OSCC cell lines, total RNA was extracted from cells with TRIzol reagent (Invitrogen, Carlsbad, California), and complementary DNA (cDNA) was synthesized using a TaqMan MultiScribe Reverse Transcriptase (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed using an ABI Prism 7900-HT Sequence Detection System (96-well, Applied Biosystems). BIN1 Forward: 5′-CAAGTCCCCATCT CAGCCAG-3′, Reverse: 5′-GGATCACCAGCACCACAT CA-3′, GPDH Forward: 5′-ACCACAGTCCATGCCAT CACT-3′, Reverse: 5′-TCCACCACCCTGTTGCTGTA-3′. The relative expression of BIN1 was normalized to GAPDH expression. Different BIN1 mRNA levels of other cell lines were relative to that of HOK cells. Experiments were performed in triplicate.

2.4 | Western blot analysis

HOK, SCC6, SCC9, SCC25, HN4, and HN6 cells were lysed with 250 μL of lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA, pH 8.0, 0.2 mM Na_3VO_4, 0.2 mM phenylmethylsulfonyl fluoride, and 0.5% NP-40). The lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were electrotransferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, Massachusetts). The membranes were incubated in phosphate-buffered saline (PBS) containing 5% bovine serum albumin for 2 hours at room temperature, followed by overnight incubation at 4°C with different dilutions of the primary antibodies, including antibodies to EGFR (1:1000), p-EGFR (1:2000), MEK (1:2000), p-MEK (1:2000), ERK (1:2000), and p-ERK (1:1000). MEK (12671, D2R10), p-MEK (16211, 166F8), ERK (12950, D3I5V), p-ERK (14474, 197G2), EGFR (4405, 15F8), and p-EGFR (2236, 1H12) were all purchased from Cell Signaling Technology, Inc. (CST, California); antibody to total BIN1 (ab185950, EPR13463-25) and GAPDH (ab9485) were purchased from Abcam, Inc. (Cambridge, Massachusetts). The membranes were developed with the Odyssey infrared imaging system according to the manufacturer's instruction. The protein level in each sample were normalized relative to that of GAPDH. Each experiment contained triplicate wells of each sample, and all experiments were repeated at least three times.

2.5 | Immunohistochemical assay

Immunohistochemical (IHC) analysis was performed by the streptavidin-peroxidase (SP) method. 4-μm sections were cut from paraffin-embedded archived tissues, and then dewaxed, rehydrated, and antigen retrieved Tris-EDTA buffer, pH 9.0 in a steamer for 10 minutes. The rabbit polyclonal antibody against human BIN1 (ab185950, EPR13463-25) at a dilution of 1:100 was used for the detection. The staining was evaluated by scanning the section under low magnification (100-fold) and confirmed under high magnification (200-fold). For evaluating the expression of BIN1 in OSCC tissues, the staining was visualized and classified based on the percentage of positive cells and the intensity of staining according to the 0 to 4 semi-quantitative system. The total scores were determined by multiplying the percentage score and intensity score and graded as low for a score of 0 to 4 and high for a score of 5 to 12. Each section was scored independently by two pathologists and a third pathologist determined the final score if there was any inconsistency.

2.6 | Cell transfection

A eukaryotic expression plasmid of the human BIN1 gene was constructed using a pCDH vector (Invitrogen). BIN1 cDNA expression vector was constructed on the basis of the sequence in NCBI (accession number U68485). The empty vector was used as negative control. SCC6 cells were cultured in six-well plates until they reached 80% to 90% confluence, and then transient transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer instructions. At 48 hours after transfection, gene expression was confirmed via immunoblotting analysis and qRT-PCR.

2.7 | CCK-8 assay

The effect of BIN1 on OSCC cell proliferation was determined by a cell-counting kit-8 (CCK-8) assay according to the manufacturer's protocol. Briefly, about 2×10^3 cells were plated into 96-well plates. When cells were well adhered, 10 μL of CCK-8 (Solarbio) was added to each well and incubated for 2 hours in a 37°C environment containing 5% CO_2. The absorbance of each well was detected at a wavelength of 450 nm. Proliferation rates were determined at 0, 24, 48, 72, and 96 hours after the transfection. Experiments were performed in triplicate.

2.8 | Wound-healing experiment

5×10^5 HN4 cells were seeded in 24-well plates. After scraping the cell monolayer with a sterile micropipette tip, the wells were washed with serum-free medium three times. The first image of each scratch was acquired at time zero. After 24 hours, each scratch was examined and captured at the same location and the healed area was measured.

2.9 | Transwell migration and invasion assay

Tumor cell migration assay was performed in a 24-well transwell chamber (Corning, New York), which contained an 8-μm pore size polycarbonate membrane filter and was precoated with 100 μg Matrigel for invasion assay (Becton-Dickinson, Bedford, Massachusetts). Briefly, the cells were seeded in the upper chambers and incubated in 500 μL RPMI 1640 medium without FBS, while 500 μL medium with 10% FBS was placed in the lower chambers. The plates were incubated for 24 hours in a 5% CO_2 humidified incubator at 37°C. Cells on the upper side of the filters were removed by cotton-tipped swabs. Next, the cells on the lower side were fixed in 4% formaldehyde and stained with 1% crystal violet in PBS for 5 minutes at room temperature. The cells on the lower side of the filters were defined as migration cells and counted at × 200 magnification in 5 random fields of each filter.

2.10 | Flow cytometry (FCM) assay

Apoptosis rate was assayed with FCM using a FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, San Jose, California) according to manufacturer's protocol. Briefly, the SCC6 or SCC9 cells were harvested using trypsin without EDTA. After washed two times with pre-cooling PBS, about 5×10^5 cells were resuspended in 100 μL 1 × binding buffer,

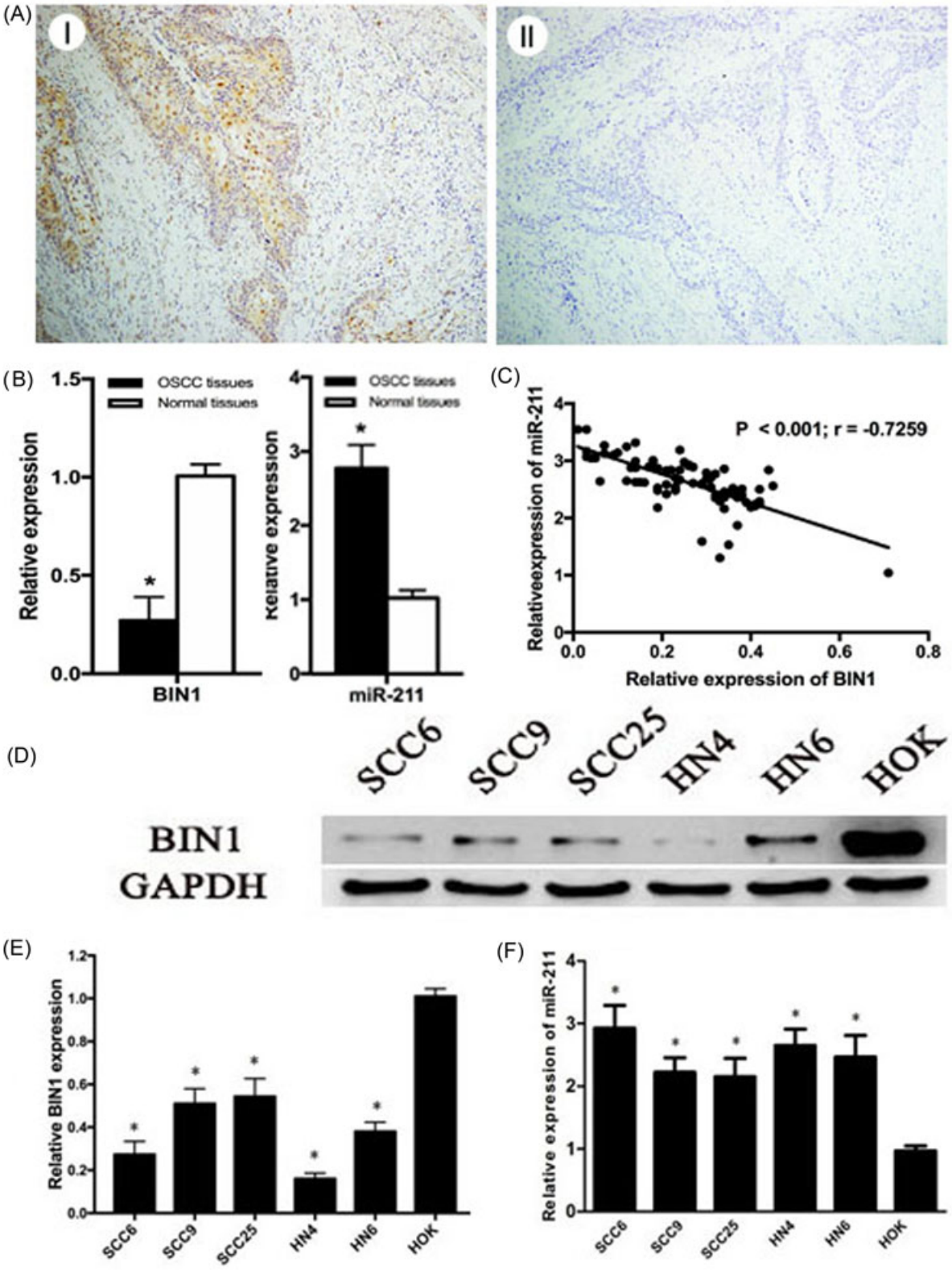

FIGURE 1 Continued.

then 5 μL propidium iodide (PI) and 5 μL FITC Annexin V were added. After incubated for 10 minutes at room temperature in the dark, 400 μL 1 × binding buffer was added to each tube and stained cells were analyzed by a FACS Calibur Flow Cytometer (BD Biosciences).

2.11 | Dual-luciferase reporter assay

Plasmids containing the firefly luciferase reporter were constructed with BIN1 3′-UTR-wild type (BIN1 3′-UTR-WT) and BIN1 3′-UTR-mutation (BIN1 3′-UTR-M). 5×10^4 cells were seeded in 24-well plates and allowed to settle overnight. On the next day, the cells were transfected with recombinant plasmids or an empty plasmid encoding the firefly luciferase reporter with Lipofectamine 2000. Renilla luciferase reporter pRL-CMV (Promega, Madison, Wisconsin) was cotransfected into cells as a normalizing transfection control. After 48 hours, the reporter luciferase activity was measured with the dual-luciferase reporter assay system (Promega) according to the manufacturer's instructions. All transfection assays were carried out in triplicate.

2.12 | miRNA inhibition

The miR-211 and miR-NC inhibitor were purchased from Thermo Fisher Scientific. For inhibiting miR-211, the OSCC cells were transfected with inhibitor at a final concentration of 25 nmol/L, and miR-NC inhibitor was used as a control. The cells were plated in 6-well plates for 24 hours before miRNA inhibitor transfection. The transfections were performed with Lipofectamine 2000 according to the manufacturer's instruction. The qRT-PCR analysis was performed to confirm the transfection efficiency.

2.13 | Statistical analysis

Statistical analysis was performed with SPSS statistics software, version 24.0 (SPSS, Chicago, Illinois). Data were presented as mean ± SD. A P value of less than 0.05 was considered statistically significant, and all P values were two-tailed. Data were obtained from at least three independent experiments with a similar pattern.

3 | RESULTS

3.1 | The expression status of BIN1 and miR-211 in OSCC tissues and cell lines

First, the IHC assay was used to determine BIN1 and miR-211 expression in carcinoma tissues of 86 patients with OSCC . The IHC results indicated that BIN1 staining in cell nucleus was rare in cancer cells (Figure 1A; $P < 0.001$). Then, we examined *BIN1* and *miR-211* expression status at the gene level with qRT-PCR and found that the relative optical density of *BIN1* and *miR-211* in tumor tissue was 0.26 ± 0.12 and 2.62 ± 0.43, respectively, when compared with matched normal tissues (Figure 1B). In addition, *BIN1* mRNA expression was negatively correlated with *miR-211* in OSCC tissues ($r = -0.7259$, $P < 0.001$; Figure 1C; $P < 0.001$).

To verify the expression status of BIN1 and miR-211 in OSCC cell lines, we evaluated BIN1 and miR-211 expression levels in normal human oral keratinocytes (HOK) cells and OSCC cell lines (SCC6, SCC9, SCC25, HN4, and HN6). The results demonstrated that the protein expressions of BIN1 were low in all of the OSCC cell lines, compared with that of HOK cells (Figure 1D; all $P < 0.01$). Then, the mRNA expression of BIN1 and miR-211 was detected with qRT-PCR, and the results showed that the BIN1 mRNA was low in the OSCC cell lines, compared with that of HOK cells, which was consistent with the corresponding protein expressions (Figure 1E; all $P < 0.01$). However, expressions of miR-211 were high in SCC6, SCC9, SCC25, HN4, and HN6 cells, which was almost the opposite of BIN1 expression levels (Figure 1F; $P < 0.05$). Among these OSCC cell lines, the HN4 and SCC6 cells had the lowest expression of BIN1 and highest expression of miR-211, thus it was chosen for following experiments.

3.2 | BIN1 overexpression could suppress the proliferation, migration, and invasion ability of HN4 and SCC6 cells

For revealing the biological role of BIN1 in OSCC, we performed plasmid transfection to overexpress BIN1 in HN4 and SCC6 cells. First, we used the CCK-8 method to evaluate the effect of BIN1 on the proliferation ability of

FIGURE 1 Expression of BIN1 and miR-211 in OSCC tissues and cell lines. A, Protein expression of BIN1 was low in OSCC tissues used by immunohistochemistry (SP × 100). I: Representative positive staining of BIN1 in OSS tissue; II: Representative negative staining of BIN1 in OSCC tissue. B, The gene expression of *BIN1* and *miR-211* in OSCC tissues and matched normal tissues. C, The correlation between *BIN1* and *miR-211* in OSCC tissues. D, BIN1 was attenuated in OSCC cell lines (SCC6, SCC9, SCC25, HN4, and HN6) compared with normal human oral keratinocytes (HOKs) used by Western blot analysis assay. and F, mRNA expression status of *BIN1 and miR-211* in OSCC cell lines and HOK cells examined by qRT-PCR. Significant P values marked by the asterisk: * $P < 0.05$ and ** $P < 0.001$. BIN1, bridging integrator-1; miR, microRNA; mRNA, messenger RNA; qRT-PCR, quantitative real-time polymerase chain reaction; OSCC, oral squamous cell carcinoma

HN4 and SCC6 cells. The results showed that BIN1 overexpression significantly decreased the proliferation rate of OSCC cells (Figures 2A and 3A). Next, we assessed the effect of BIN1 overexpression on the migration and invasion ability with wound healing and transwell experiment. As Figures 2B and 3B show, BIN1 overexpression led to an evident inhibition on migration ability of HN4 and SCC6 cells, compared with the corresponding control cells (50.67 ± 3.51 μm vs 82.67 ± 9.07 μm, $P = 0.005$). Similarly, BIN1 overexpression also inhibited the invasion ability of HN4 and SCC6 cells, compared with the corresponding control cells (165.33 ± 11.50 cells/field vs 73.00 ± 7.21 cells/field, $P < 0.001$; Figures 2C and 3C). Meanwhile, we detected the apoptosis rate by FCM to realize the effect of BIN1 on cell apoptosis. The results demonstrated that BIN1 overexpression significantly increased the apoptosis rate of HN4 and SCC6 cells ($10.57 \pm 0.96\%$ vs $21.27 \pm 2.26\%$, $P = 0.002$; Figures 2D and 3D). Taken above, BIN1 overexpression could inhibit proliferation, migration, and invasion ability while increase apoptosis of HN4 and SCC6 cells, which indicated that BIN1 played important roles in the malignant behaviors of HN4 and SCC6 cells.

3.3 | miR-211 directly targeted the 3′-UTR of BIN1 in HN4 and SCC6 cells

Aiming at revealing the miRNA regulating the expression of BIN1, we first used the Targetscan (http://www.targetscan.org/vert_71/) and miRanda (http://www.microrna.org) algorithms to predict the potential miRNAs. miR-211 was predicted to be able to regulate the expression of BIN1. As Figure 4A shows, there was one hypothetic miR-211 binding site in the BIN1 3′-UTR. Thus, we performed reporter assays with a luciferase plasmid harboring the 3′-UTR sequence of BIN1 containing the predicted site for binding miR-211. Furthermore, we built mutant reporter vectors containing a mutation in the miR-211 binding site (BIN1 3′-UTR-M). These plasmids were transfected into HN4 and SCC6 cells with miR-211 inhibitor (Figure 4B). The results showed that miR-211 inhibitor increased the luciferase activity in HN4 and SCC6 cells which were transfected with BIN1 3′-UTR-WT, but not in those with BIN1 3′-UTR-M (Figure 4C-F). This result suggested that miR-211 specifically targeted the binding site in the BIN1 3′-UTR. Meanwhile, BIN1 protein expression was increased in the presence of miR-211 inhibitor in HN4 and

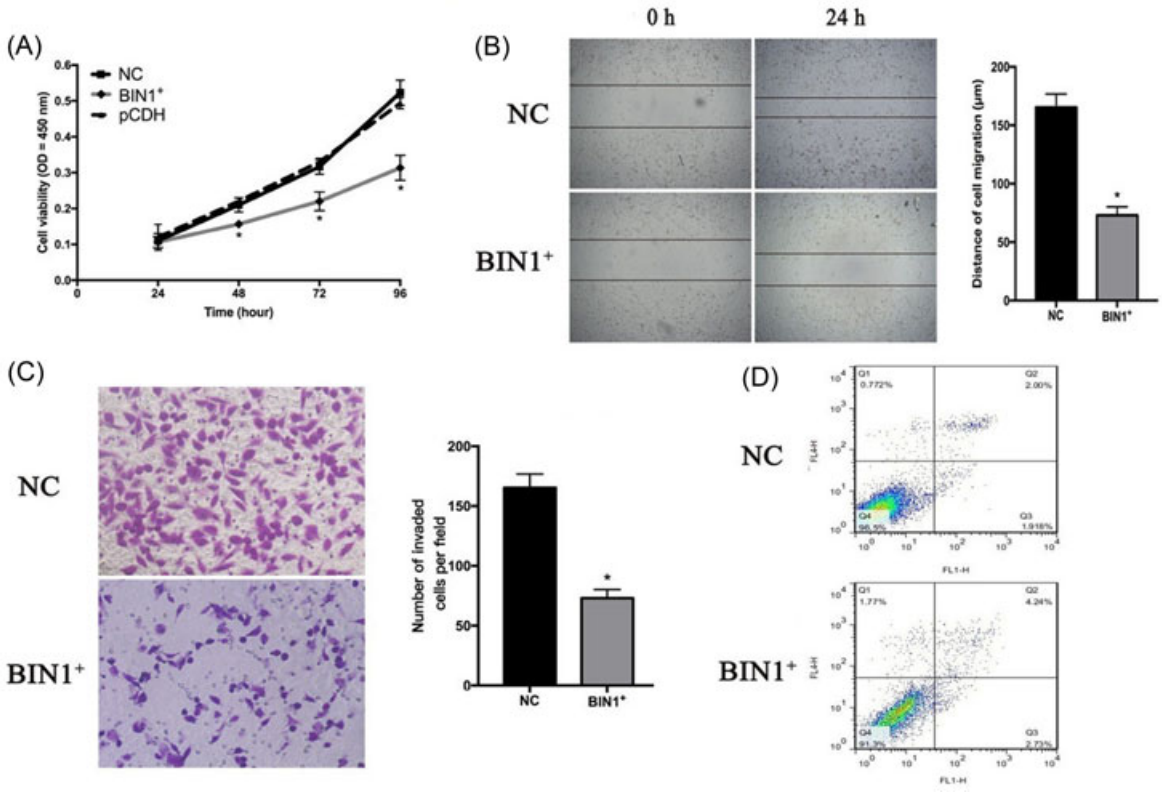

FIGURE 2 Effect of BIN1 overexpression on malignant behaviors of HN4 cells. A, Effect of BIN1 overexpression on the proliferation ability inhibition of HN4 cells, analyzed with CCK-8 assay. B, Effect of BIN1 overexpression on the migration ability suppression of HN4 cells, assayed with wound healing experiment. C, Effect of BIN1 overexpression on the invasion ability of HN4 cells, assayed with transwell experiment. D,Effect of BIN1 overexpression on the apoptosis ability of HN4 cells. Significant P values marked by asterisk: $^{*}P < 0.05$ and $^{**}P < 0.001$. BIN1, bridging integrator-1

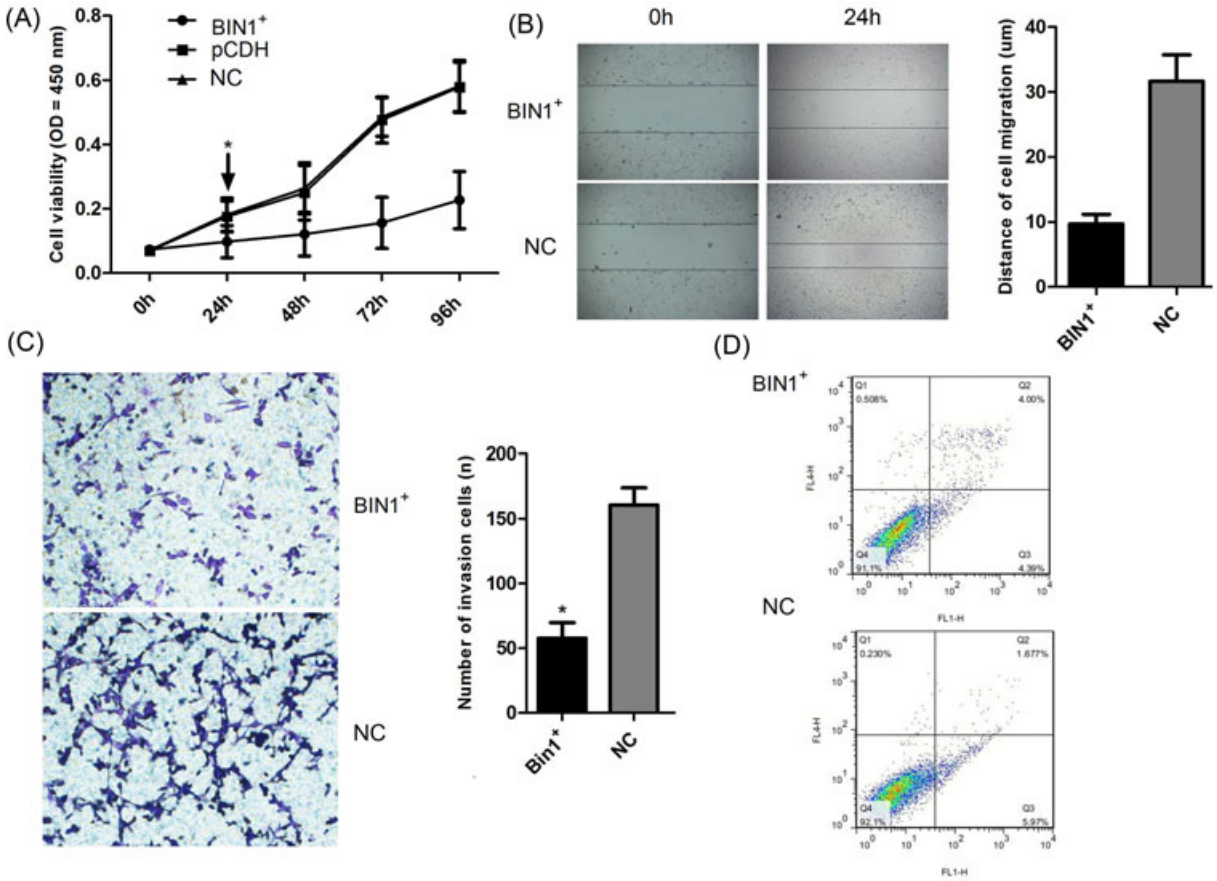

FIGURE 3 Effect of BIN1 overexpression on malignant behaviors of SCC6 cells. A, Effect of BIN1 overexpression on the proliferation ability inhibition of SCC6 cells analyzed with the CCK-8 assay. B, Effect of BIN1 overexpression on the migration ability suppression of SCC6 cells assayed with wound healing experiment. C, Effect of BIN1 overexpression on the invasion ability of SCC6 cells assayed with transwell experiment. D, Effect of BIN1 overexpression on the apoptosis ability of SCC6 cells. Significant *P* values marked by the asterisk: $*P < 0.05$ and $**P < 0.001$. BIN1, bridging integrator-1; NC, negative control

SCC6 cells, which was consistent with the result of the luciferase reporter assay (Figure 4G and 4H). These results demonstrated that miR-211 could downregulate BIN1 expression through binding its 3′-UTR.

3.4 | miR-211 inhibition could suppress the malignant behaviors of OSCC cells by deactivating the EGFR/MAPK pathway

Considering that miR-211 could bind the 3′-UTR of BIN1 to inhibit its expression, we next evaluated the effect of miR-211 on the malignant behaviors of OSCC cells. Thus we performed wound healing and transwell experiments. As Figure 5A and 5B showed, the invasion and migration ability of HN4 cells could be significantly suppressed by a miR-211 inhibitor. However, as for the HN4 cells which were treated with siR-BIN1, the miR-211 inhibitor did not suppress their invasion and migration ability. Since our previous study showed that, in NSCLC cells, BIN1 could inhibit the activation of EGFR/MAPK pathway which was also an important signaling pathway in OSCC cells,[16] we then detected its expression and phosphorylation level. As Figure 5C showed, miR-211 inhibitor evidently suppressed the activation of EGFR/MAPK pathway-related proteins including EGFR, MEK, and ERK, suggesting that miR-211 played important roles in the activation of EGFR/MAPK pathway. Furthermore, we observed the changes in EGFR, MEK, and ERK in BIN1 overexpression cells to examine the correlation between miR-211, BIN1, and EGFR/MAPK pathway. The results showed that BIN1 overexpression could inactivate EGFR/MAPK pathway, indicating that miR-211 promoted proliferation, migration, and invasion ability of OSCC cells via targeting BIN1 and its downstream EGFR/MAPK pathway (Figure 5D).

4 | DISCUSSION

OSCC is one of the most common tumors worldwide with rapidly increasing incidence and mortality, and its prognosis is still unsatisfactory.[1,17] However, the

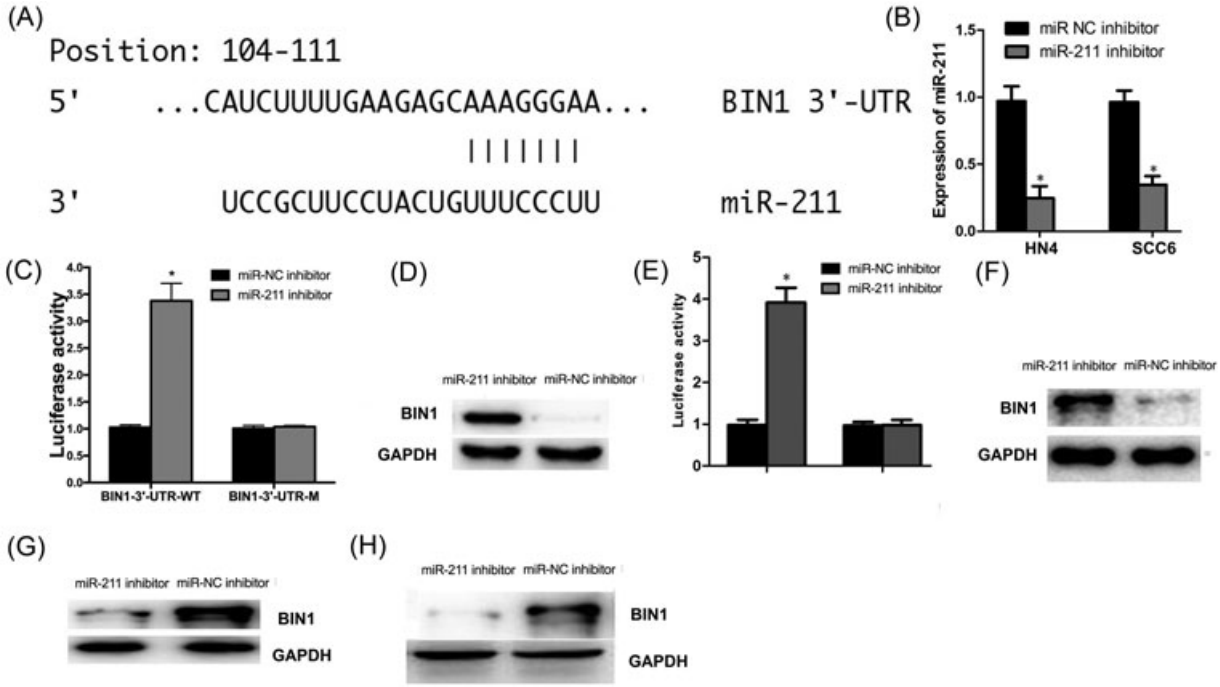

FIGURE 4 Interaction between miR-211 and BIN1 in OSCC HN4 and SCC6 cells. A, The predicted site in BIN1 3′-UTR for binding miR-211. B-F, Luciferase reporter assay of HN4 and SCC6 cells were transfected with the BIN1 3′-UTR-WT or BIN1 3′-UTR-M in the binding sites. Differences were observed when miR-211 inhibitor was added, and miR-NC inhibitor was used as the control. G and H, miR-211 inhibitor could reverse the BIN1 protein expression assayed by Western blot analysis experiment in HN4 and SCC6 cells. Significant *P* values marked by the asterisk: $*P < 0.05$ and $**P < 0.001$. BIN1, bridging integrator-1; miR, microRNA; OSCC, oral squamous cell carcinoma; 3′-UTR, 3′-untranslated region; WT, wild type

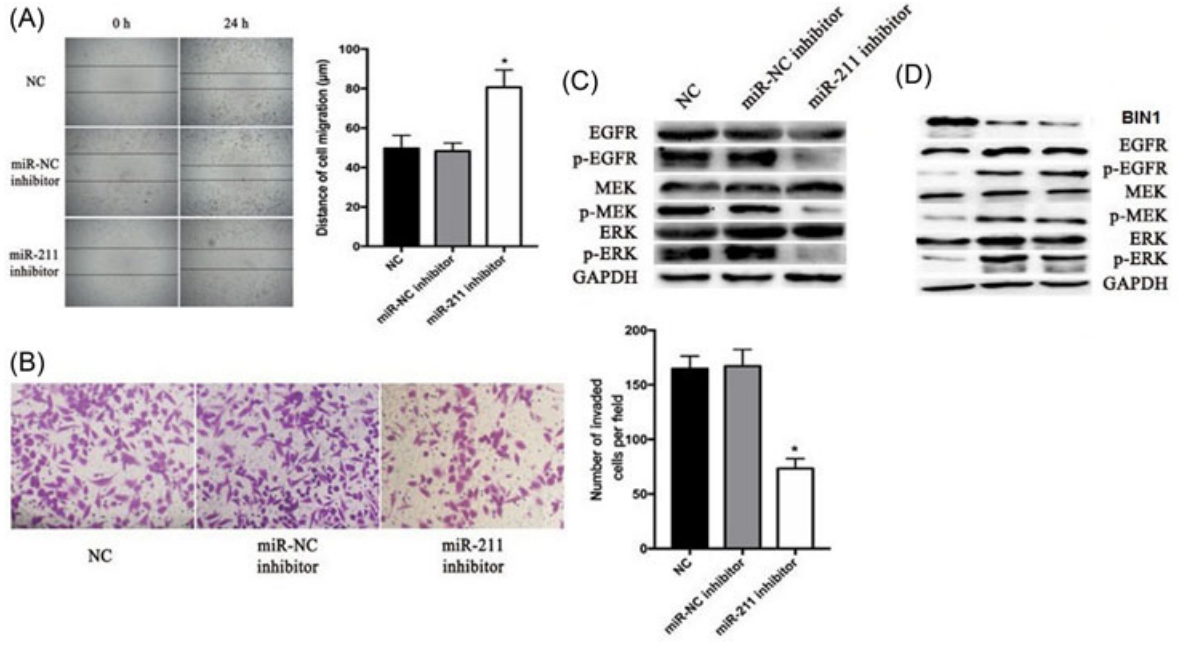

FIGURE 5 The effects of miR-211 inhibitor on OSCC cells. A, Effect of miR-211 inhibitor on the inhibition of migration ability of HN4 cells assayed with the wound healing experiment. B, Effect of miR-211 inhibitor on the suppression of invasion ability of HN4 cells assayed with transwell experiment. C, Effects of miR-211 inhibitor on the activation of EGFR/MAPK pathway analyzed with Western blotting experiment. D, Effect of BIN1 overexpression on EGFR/MAPK pathway OSCC cells. Significant *P* values marked by the asterisk: $*P < 0.05$ and $**P < 0.001$. BIN1, bridging integrator-1; miR, microRNA; NC, negative control; OSCC, oral squamous cell carcinoma

regulating network of OSCC-related proteins and genes remains poorly understood, which limits the improvement of clinical treatment. The deactivation of tumor-suppressing proteins is a frequent cancer hallmark benefiting the progression of OSCC. Thus, a more adequate comprehension of the proteins involved in the malignant behaviors might contribute to the improvement in treating OSCC.

BIN1 is a conserved member of the BAR (Bin-Amphiphysin-Rvs) family of adapter proteins participating in diverse cellular processes including endocytosis, programmed cell death, DNA repair, stress responses, and transcriptional control.[18-20] Numerous studies confirmed that reduction of BIN1 could be frequently observed in multiple cancers, and BIN1 attenuation played important roles in driving tumor progression.[21-23] Ghaneie et al demonstrated that loss of BIN1 might contribute to breast cancer progression by eliminating a mechanism that restrained the ability of activated c-Myc to drive cell division inappropriately.[24] Elliott et al proposed that reduction of BIN1 might facilitate malignancy by inhibiting cancer cell death related to oncogene activation.[25] In the current study, we first investigated the expression and functions in OSCC cells. Our results confirmed that BIN1 was low expressed in OSCC cell lines, compared with the normal human oral keratinocytes HOK cells. For elucidating the function of BIN1 in OSCC, we performed BIN1 overexpression in the HN4 cells which had the lowest BIN1 expression. After BIN1 overexpression, the proliferation, migration, and invasion abilities of HN4 cells significantly decreased. This result indicated that BIN1 could also act as a tumor suppressor in OSCC cells.

Then, we attempted to explain the attenuated expression of BIN1 in OSCC cells. Emerging studies have proven that some miRNAs can undertake in the pathogenesis of tumors by affecting cellular processes including cell proliferation, apoptosis, invasion, and metastasis.[26] For example, miR-101-3p could inhibit the proliferation and clonogenic growth of neuroblastoma by targeting MYCN, and suppress cell growth and tumorigenesis of gastric cancer by targeting SOCS2.[27] On the other hand, miRNA may also lead to a cancer promotion by targeting tumor suppressors.[28] As for OSCC, miRNAs also play crucial roles in mediating the malignant behaviors. miR-133a-3p can inhibit OSCC proliferation and invasion by targeting COL1A1.[29] After predicting the potential miRNA targeting BIN1 with bioinformation algorithms, we chose miR-211 as the research objective. Our results indicated that miR-211 was highly expressed in the OSCC cells, compared with the normal HOK cells. And further study confirmed that miR-211 could lead to a downregulation of BIN1 by interacting with its 3′-UTR to promote the migration and invasion of OSCC cells. Thus, we have demonstrated that miR-211 functioned as an oncogene in OSCC, consistent with the results of Chen et al.[14] Aiming at revealing correlated mechanisms, we detected the effect of miR-211/BIN1 on the activation of EGFR/MAPK pathway, which was proved to be inhibited by BIN1 in NSCLC cells.[16] The results suggested that miR-211 played important roles in regulating the activation of the EGFR/MAPK pathway.

In conclusion, we identified the low expression status and tumor-suppressing function of BIN1 in OSCC. Overexpression of BIN1 could significantly inhibit the proliferation, migration, and invasion ability of OSCC cells. Furthermore, the reduction of BIN1 was positively correlated with the existence of miR-211 in OSCC cells. Addition of miR-211 inhibitor could evidently suppress the malignant behaviors of OSCC cells by inhibiting the activation of the EGFR/MAPK pathway. Taken together, our findings demonstrated that miR-211 mediated BIN1 downregulation had crucial significances in OSCC and the treatment targeting miR-211 might have potentials in improving the treatment efficacies.

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CONFLICTS OF INTEREST

The authors declare that there are no competing financial interests.

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