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RESEARCH PAPER

MMP1 regulated by NEAT1/miR-361-5p axis facilitates the proliferation and migration of cutaneous squamous cell carcinoma via the activation of Wnt pathway

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ABSTRACT

Cutaneous squamous cell carcinoma (CSCC) is one of the most malignant tumors worldwide. It has been validated that matrix metallopeptidase 1 (MMP1) expression was obviously up-regulated in CSCC tissues. However, its specific role in CSCC is still unclear. RT-qPCR analysis and western blot assays were used to measure the mRNA and protein expressions, respectively. MTT and colony formation assays were conducted to assess proliferative ability. Transwell assays were adopted to evaluate migratory and invasive abilities. Flow cytometry and caspase-3/8/9 activity assays were carried out to evaluate cell apoptosis. Relevant mechanism experiments were finally performed to delineate molecular relationship among genes. We found that the expression of MMP1 was up-regulated in CSCC cells, and knockdown of MMP1 suppressed cell proliferation and invasion in CSCC. Subsequently, miR-361-5p was validated to target MMP1. Moreover, miR-361-5p was proved to be sponged by nuclear paraspeckle assembly transcript 1 (NEAT1) in CSCC. We further demonstrated that NEAT1 could activate Wnt pathway to affect cell proliferation and invasion. Finally, miR-361-5p inhibition rescued the suppressing effects of NEAT1 depletion on cell proliferation, invasion as well as Wnt pathway in CSCC. In summary, MMP1 regulated by NEAT1/miR-361-5p axis facilitated CSCC malignant behaviors via Wnt pathway activation.

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MMP1; NEAT1; miR-361-5p; cutaneous squamous cell carcinoma

Introduction

Cutaneous squamous cell carcinoma (CSCC) is one of the most common skin malignant tumors and may lead to approximately 20% of total skin tumor-associated death globally.[1,2] The morbidity of CSCC is rising in the last decade.[3] Also, one of the features of CSCC is its metastasis to other organs. The long-term prognosis for CSCC patients in highly metastatic cases is very poor, with a disease-specific survival at 1 year of 44–56%.[4] Hence, to find out novel therapeutic targets for improving the poor clinical outcome of CSCC is critical.

Messenger RNAs (mRNAs) are capable of encoding proteins which play a vital role in biological activities. In recent years, it has been well established that the mRNA stability and expression level can be regulated by long non-protein coding RNAs (lncRNAs).[5] MRNAs contain partial binding sites, named micro response elements (MRE) that could be recognized and targeted by microRNAs (miRNAs). This target relationship contributes to the significant degradation of mRNA.[6] Matrix metalloproteinases (MMPs) are a type of extracellular matrix remodeling proteinase. MMPs have been revealed to play essential roles in the pathology of cancers.[7] Matrix metalloproteinase-1 (MMP1) was found to promote early perineural invasion of pancreatic cancer cells.[8] Recently, it has been reported that MMP1 has been up-regulated in tissues of patients with CSCC.[9] However, whether the differentially expressed MMP1-induced impacts on the development of CSCC have never been elucidated.

With more than 200 nucleotides (nt) in length, lncRNAs represent a group of RNAs with very limited protein-coding ability or without the potential to encode proteins. Nevertheless, lncRNAs can exert regulatory effects on gene expression, thus affecting carcinogenesis process. Similar to mRNA, lncRNAs harbor partial MRE and can competitively bind to specific miRNA. This competitive endogenous RNA (ceRNA) regulatory mechanism creates connections among lncRNA, miRNA and mRNA and further affects disease pathogenesis.[10] MiR-361-5p is a relatively well-studied tumor-suppressing gene which is usually lowly expressed in malignancies, such as in prostate cancer[11] and lung cancer.[12] Furthermore, the overexpression of miR-361-5p will lead to better prognosis for patients suffering from breast cancer.[13] Previous researches have uncovered that miR-361-5p suppresses glycolytic metabolism, proliferation and invasion of breast cancer via targeting its downstream FGFR1 and MMP1.[14] NEAT1 is an identified carcinogenic gene in various cancers, such as in esophageal cancer and hepatocellular carcinoma.[15] Importantly, the link between NEAT1 and miR-361-5p has been revealed in hemangioma. NEAT1 has been

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elucidated to serve as a ceRNA to increase VEGFA expression via sequestering miR-361-5p in hemangioma.[16]

Here, we testified whether MMP1 elicited biological function in CSCC and further probed into the possible underlying mechanism of MMP1 in the regulation of CSCC.

Materials and methods

Cell culture

Four human CSCC cells (A431, HSC-5, SCC13, SCL-1) and one human normal cutaneous cell (HaCaT), available from ATCC (Manassas, VA), were incubated under the standard condition of 5% CO_2 and 37°C. DMEM medium was commercially acquired from Gibco (Grand Island, NY) for cell culture, with 10% FBS and Pen/Strep antibiotics solution (1%) as supplements.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total cell RNA was acquired using TRIzol method (Invitrogen, Carlsbad, CA), for cDNA synthesis with Reverse Transcription Kit (TaKaRa Bio, Shiga, Japan). The resulting products were treated with SYBR Green PCR Master Mix (Invitrogen) and then calculated with $2^{-\Delta\Delta CT}$ method. GAPDH or U6 was used as the internal control.

Cell transfection

The two specific shRNAs and control-shRNAs were produced by Genepharma Company (Shanghai, China) to silence MMP1 or NEAT1 in A431 and HSC-5 cells applying the transfection kit Lipofectamine 2000 (Invitrogen). The miR-361-5p mimics/inhibitor and NC mimics/inhibitor were procured from Genechem (Shanghai, China) for 48 h of cell transfection. In addition, the mutant sequences of MMP 3′UTR and NEAT1 were introduced by the authors.

MTT assay

A431 and HSC-5 cells were seeded in 96-well plates at 4×10^3 cells/well after transfection. Cell viability was detected after adding 5 mg/ml MTT for 4 h and then treated with 150 μl of DMSO. The absorbance at 490 nm was examined at indicated times using microplate reader.

Colony formation

Clonogenic cells of A431 and HSC-5 were cultured at 500 cells/well in the 6-well plates for the 14-day culture process. After treatment with 0.5% crystal violet, colonies were counted manually.

Transwell assay

Cells (1×10^5) were transfected into the top of Transwell chamber containing polycarbonate filters (8 μm pore; Corning Incorporated, Corning, NY), which was coated with Matrigel (BD Biosciences, Franklin Lakes, NJ) or not for analyzing cell invasion or migration. The lower chamber was supplied with complete medium. After 24 h, cells on the bottom were fixed in 4% paraformaldehyde for crystal violet staining and then counted under microscope.

Flow cytometry apoptosis analysis

Cells were cultured in PBS washing buffer and suspended in the 100 mL of binding buffer with 5 mL of Annexin V-FITC and 5 mL of propidium iodide (PI). After 15 min, stained cells were detected with flow cytometry (BD Biosciences, CA, USA).

Caspase-3/8/9 activity detection

Based on the standard methods, the activity of caspase-3/8/9 was examined using the caspase-3/8/9 activity kits (Beyotime, Shanghai, China) in A431 and HSC-5 cells. The activities were detected by microplate reader at 405 nm.

RNA immunoprecipitation (RIP) assay

After being washed in PBS, cell lysates were acquired by incubation in RIP lysis buffer and centrifugation and then cultured with the antibody-beads mixture overnight at 4°C. Antibodies specific to human AGO2 and normal control IgG were procured from Abcam (Cambridge, MA). QRT-PCR was conducted for analyzing the precipitated RNAs.

Luciferase reporter assay

The fragments of MMP1 3′UTR or NEAT1 covering the wild-type or mutant miR-361-5p binding sites were used to construct the luciferase reporter plasmids using luciferase reporter pmirGLO vector (Promega, Madison, WI). Following the co-transfection with miR-361-5p mimics or NC mimics in A431 and HSC-5 cells for 48 h, luciferase assay was carried out by Luciferase Reporter Assay System (Promega).

Subcellular fractionation

The nuclear or cytoplasmic RNA fraction was isolated from A431 and HSC-5 cells using PARIS™ Kit method (Invitrogen). After RNA purification, qRT-PCR was conducted to analyze the NEAT1 expression in both fractions.

Fluorescence in situ hybridization (FISH) assay

Based on the established method, RNA FISH assay was conducted in hybridization buffer using the specifically designed NEAT1-FISH probe (Ribobio). Then, cells were washed for Hoechst staining, finally observed with microscope.

Statistical analyses

Graphpad Prism 6 software was employed for statistical analysis in all bio-triplicates. The measurement data were represented as the mean ± standard deviation (SD). Comparisons

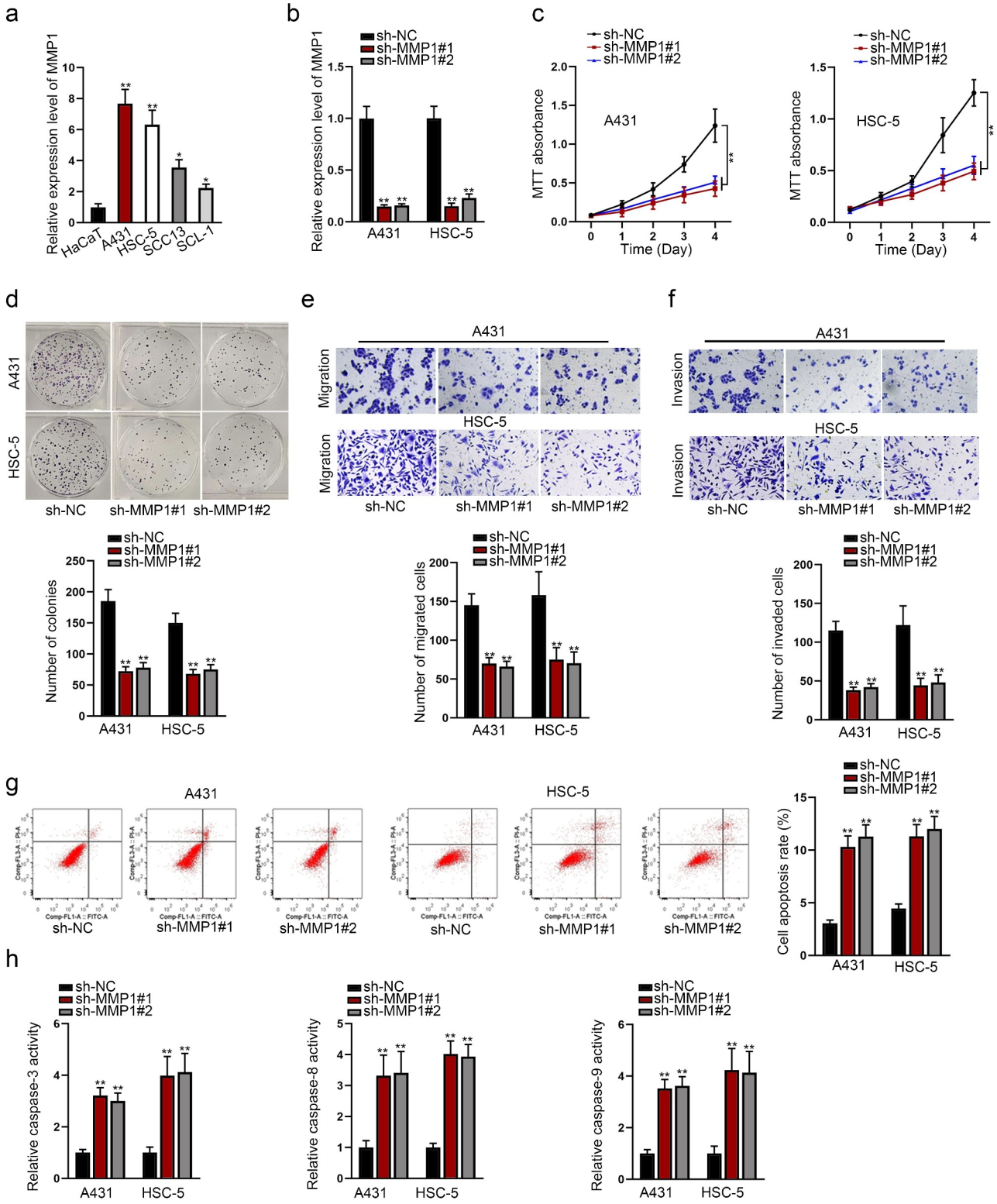

Figure 1. MMP1 promotes cell growth, migration, and invasion of CSCC. (a). Expression of MMP1 in CSCC cell lines and normal one was evaluated by qRT-PCR assay. (b). The interfering efficiency of sh-MMP1 was detected and validated by qRT-PCR in A431 and HSC-5 cells. (c,d). The proliferation ability of A431 and HSC-5 cells was assessed by MTT and colony formation assays. (e,f). The migration and invasion capacities were measured by Transwell assays in MMP1-silenced A431 and HSC-5 cells. (g,h). CSCC cells apoptosis was explored by flow cytometry and caspase 3/8/9 activity test. *P < .05; **P < .01.

of groups were examined by Student's t test or one-way ANOVA, with $p < .05$ seen as significant.

Results

MMP1 promotes cell growth, migration and invasion of CSCC

For exploring the potential biological role of MMP1 in CSCC, the expression level of MMP1 was evaluated in CSCC cells (A431, HSC-5, SCC13, SCL-1) and human normal cutaneous cell (HaCaT). Compared with HaCaT cell, MMP1 mRNA level was found distinctively up-regulated in CSCC cells, particularly in A431 and HSC-5 cells (Figure 1(a)). Hence, they were selected for following assays. Then, MMP1 was stably silenced in A431 and HSC-5 cells for the purpose of loss-of-function assays (Figure 1(b)). After that, MTT assay was conducted to assess CSCC cell vitality. It showed that MMP1 silencing evidently weakened the viability of CSCC cells (Figure 1(c)). Consistently, colony formation assay further proved the inhibitory effect of MMP1 silencing on the proliferation of selected CSCC cells (Figure 1(d)). Besides, Transwell migration and invasion assays indicated that MMP1 silencing dampened the migratory and invasive capacities of A431 and HSC-5 cells (Figure 1(e,f)). Additionally, flow cytometry assay manifested that MMP1 silencing markedly promoted cell apoptosis of A431 and HSC-5 (Figure 1(g)). Caspase-3/8/9 activity test revealed that MMP1 silencing remarkably enhanced activities of caspase-3, caspase-8, and caspase-9 (Figure 1(h)). What's more, pcDNA3.1 vectors were constructed to detect the overexpression efficiency of MMP1 (Fig. S1A). After that, MTT, colony formation and Transwell assays were conducted to verify the effect of MMP1 overexpression on the malignant behaviors of CSCC cells (Fig. S1B-S1E). As shown by the results, after the transfection of pcDNA3.1/MMP1, a totally different effect was achieved on the malignant behaviors of CSCC cells compared with those transfected with sh-MMP1#1/2. Aside from that, to exclude the possibility that the changes in the malignant behaviors of CSCC cells were caused by transfection toxicity, relevant rescue assays were conducted by us. First of all, we compared the expression of MMP1 in CSCC cells transfected with sh-MMP1#1 and sh-MMP1#1 + pcDNA3.1/MMP1 (Figure. S2A). As shown by the figure, the expression of the former one was close to that of cells transfected with sh-NC. Next, MTT and colony formation assays were adopted, the result of which showed that the proliferative ability of CSCC cells transfected with sh-MMP1#1 could be partially reversed by the transfection of pcDNA3.1/MMP1 (Figure. S2B-S2C). What's more, the same results could be achieved in Transwell, flow cytometry assays and caspase-3/8/9 activity test (Figure S2D-S2G). Therefore, the malignant behavior changes were caused by the silencing and overexpression of MMP1 rather than transfection toxicity. Overall, these results demonstrated that the silencing of MMP1 could suppress cell growth and migration in CSCC.

MiR-361-5p is the molecular upstream gene of MMP1

Increasing protein-coding RNAs have been discovered to be mediated by miRNA post-transcriptionally. To probe into the mechanism of MMP1, we firstly found the potential upstream target miRNA of MMP1. Utilizing miRmap and miRanda databases, we obtained eight potential target miRNAs of MMP1 (Figure 2(a)). We examined the expression pattern of these miRNAs in CSCC cells and miR-361-5p was found in CSCC cells with markedly low expression (Figure 2(b)). The potential wild and mutant sequences of miR-361-5p in the 3′UTR sequence of MMP1 were shown in Figure 2(c). RIP assay manifested a significant enrichment of miR-361-5p and MMP1 in the AGO2-bound complexes (Figure 2(d)). We ensured that miR-361-5p mimics could effectively increase the level of miR-361-5p in selected CSCC cells (Figure 2(e)). Luciferase reporter assay data showed that miR-361-5p mimics obviously suppressed the luciferase activity of MMP1-WT (Figure 2(f)). Furthermore, MMP1 mRNA level was down-regulated in miR-361-5p-overepxressed A431 and HSC-5 cells (Figure 2(g)). We then confirmed the interfering effect of miR-361-5p inhibitor by qRT-PCR (Figure 2(h)). MMP1 mRNA level was up-regulated after the transfection of miR-361-5p inhibitor (Figure 2(i)). To conclude, MMP1 could bind to miR-361-5p and was regulated by miR-361-5p in CSCC negatively.

MiR-361-5p inhibits the malignant behaviors of CSCC

MiR-361-5p has been found to exert an inhibitory role in the development of an array of malignancies. However, its functional role in CSCC remains unclear. First of all, we detected the protein level and activity of MMP1 in CSCC cells after the transfection of miR-361-5p mimics (Fig. S3A) after the reference of two papers.[17,18] Functional experiments indicated that miR-361-5p inhibited CSCC cells proliferative (Figure 3(a,b)), migratory (Figure 3(c)), and invasive (Figure 3(d)) abilities. Meanwhile, miR-361-5p strengthened the apoptotic capacity of CSCC cells (Figure 3(e,f)). To further verify our thought, the protein level and activity of MMP1 in CSCC cells after the transfection of miR-361-5p inhibitor were detected (Figure S3B). After that, functional experiments were carried out in cells transfected with miR-361-5p inhibitor. As shown by the results, it was indicated that the transfection of miR-361-5p inhibitor enhanced the proliferative (Fig. S3C-S3D), migratory (Fig. S3E), and invasive (Fig. S3F) abilities of CSCC cells. Therefore, we could conclude that miR-361-5p could inhibit the malignant behaviors of CSCC.

To exclude the possibility that all other readouts probably stem from the cell apoptosis, we designed a series of experiments. First we detected the efficiency of anti-miR-361-5p (Fig. S4A-B). Then the cell viability, proliferation, migration and invasion abilities were tested by RT-qPCR respectively when miR-361-5p mimics were transfected into the cells (Fig. S4C-F). We found that all these abilities weakened. However, after co-transfected with anti-miR-361-5p, the above abilities recovered. Next, cell apoptosis was measured by flow cytometry and caspase-3/8/9 activity (Fig. S4G-H). Also, the effects caused by miR-361-5p overexpression were rescued by co-transfection

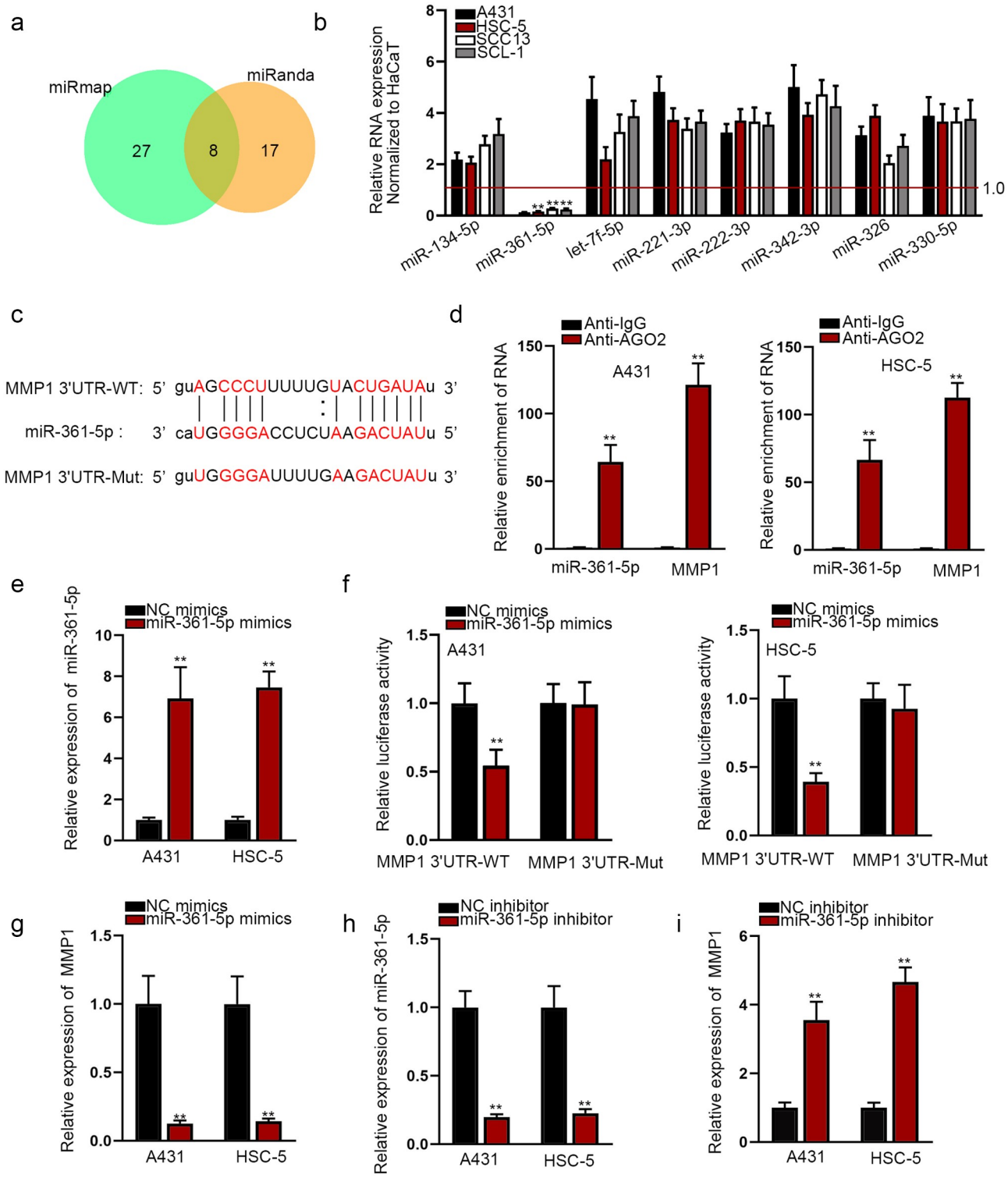

Figure 2. MiR-361-5p is the molecular upstream gene of MMP1. (a). StarBase screened potential miRNAs that may bind to MMP1. (b). The expression patterns of candidate miRNAs were detected in CSCC cells and normal one. (c). Putative and mutant binding site between miR-361-5p and MMP1. (d). RIP assays showed significant enrichment of miR-361-5p and MMP1 in response to antibody targeting AGO2. (e). QRT-PCR showed that the expression of miR-361-5p was increased by miR-361-5p mimics. (f). Luciferase reporter assays found that miR-361-5p overexpression attenuated the luciferase activity of MMP1-WT. (g). MMP1 mRNA level was measured after transfecting miR-361-5p mimics in selected CSCC cells. (h). QRT-PCR showed that the expression of miR-361-5p declined in response to miR-361-5p inhibitor. (i). MMP1 mRNA level was measured after transfecting miR-361-5p inhibitor in selected CSCC cells. **P < .01.

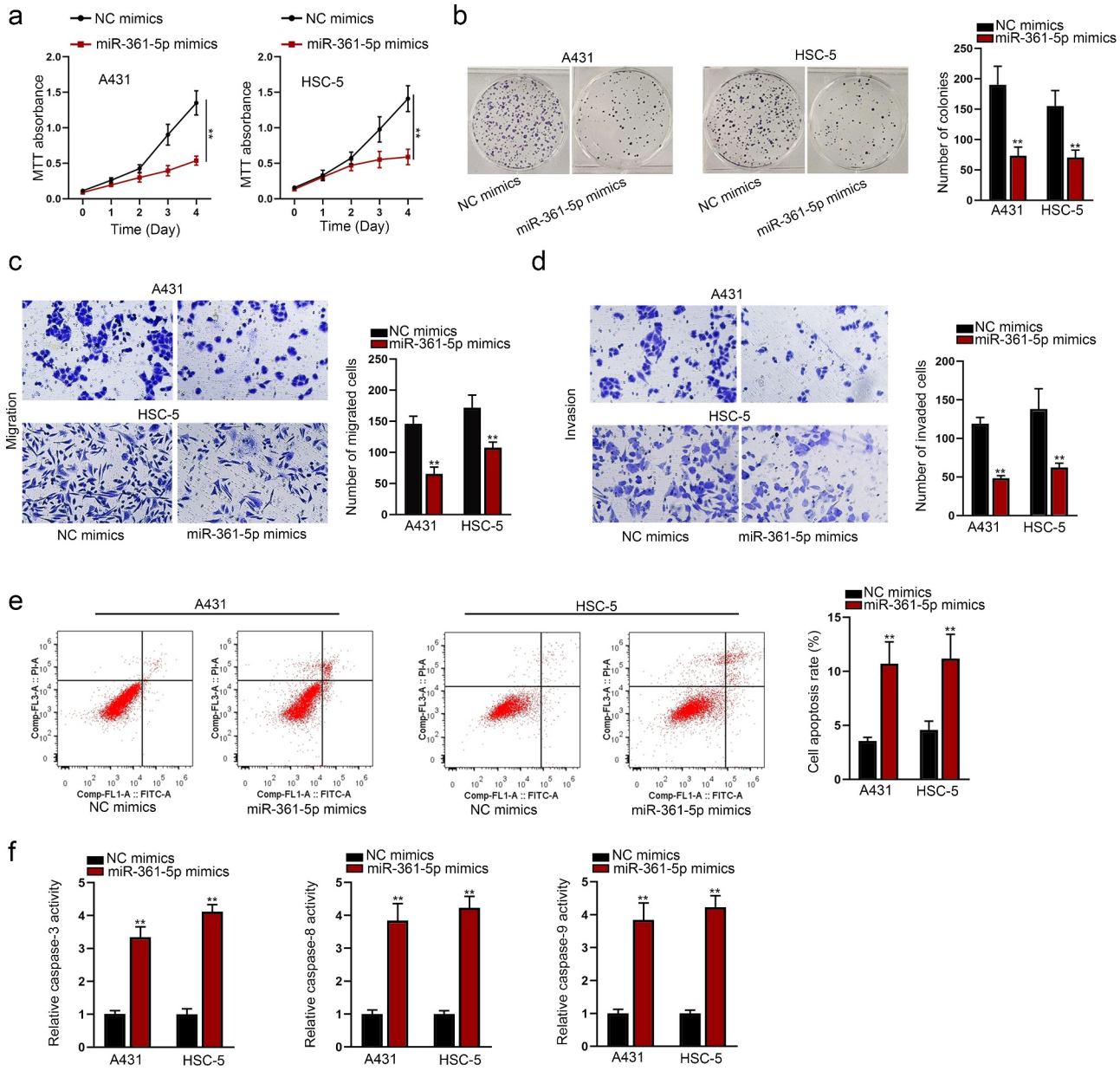

Figure 3. The function of miR-361-5p in CSCC was explored. (a,b). The proliferation ability was assessed in A431 and HSC-5 after overexpressing miR-361-5p. (c,d). The migration and invasion capacities were testified by transwell assays in miR-361-5p-overexpressed A431 and HSC-5 cells. (e,f). Apoptosis was explored by flow cytometry and caspase 3/8/9 activity test in miR-361-5p-overexpressed A431 and HSC-5 cells. **P < .01.

with anti-miR-361-5p. In conclusion, all the phenotypes were caused by miR-361-5p rather than cell apoptosis.

NEAT1 interacts with miR-361-5p

Since miRNAs have been increasingly reported to be regulated by lncRNAs, we detected the potential lncRNAs that might bind to miR-361-5p. With the help of starBase (http://starbase.sysu.edu.cn/), eight lncRNAs that might share binding sites with miR-361-5p were sifted out. The expression of NEAT1 was found to be obviously high in CSCC cells (Figure 4(a)). To determine the potential regulatory role of NEAT1, we firstly studied its distribution in A431 and HSC-5 cells. Subcellular fractionation and FISH assays manifested that NEAT1 was located in both cytoplasm and nucleus of A431 and HSC-5 cells, but predominantly in cytoplasm (Figure 4(b,c), Fig. S5A). RIP assay revealed a marked enrichment of NEAT1, miR-361-5p and MMP1 by antibody directly targeting AGO2 (Figure 4(d)). We found that NEAT1 had binding sites with miR-361-5p (Figure 4(e)). Besides, miR-361-5p mimics significantly decreased the luciferase activity of NEAT1-WT in A431 and HSC-5 cells (Figure 4(f)). To determine the effects of NEAT1 on miR-361-5p-regulated MMP1, we silenced NEAT1 by constructing sh-NEAT1 (Figure 4(g)). The protein level and activity of MMP1 in CSCC cells after the transfection with sh-NEAT1#1 or the co-transfection with sh-NEAT1#1 and miR-361-5p inhibitor were detected (Figure S5B). MMP1 mRNA was down-regulated in

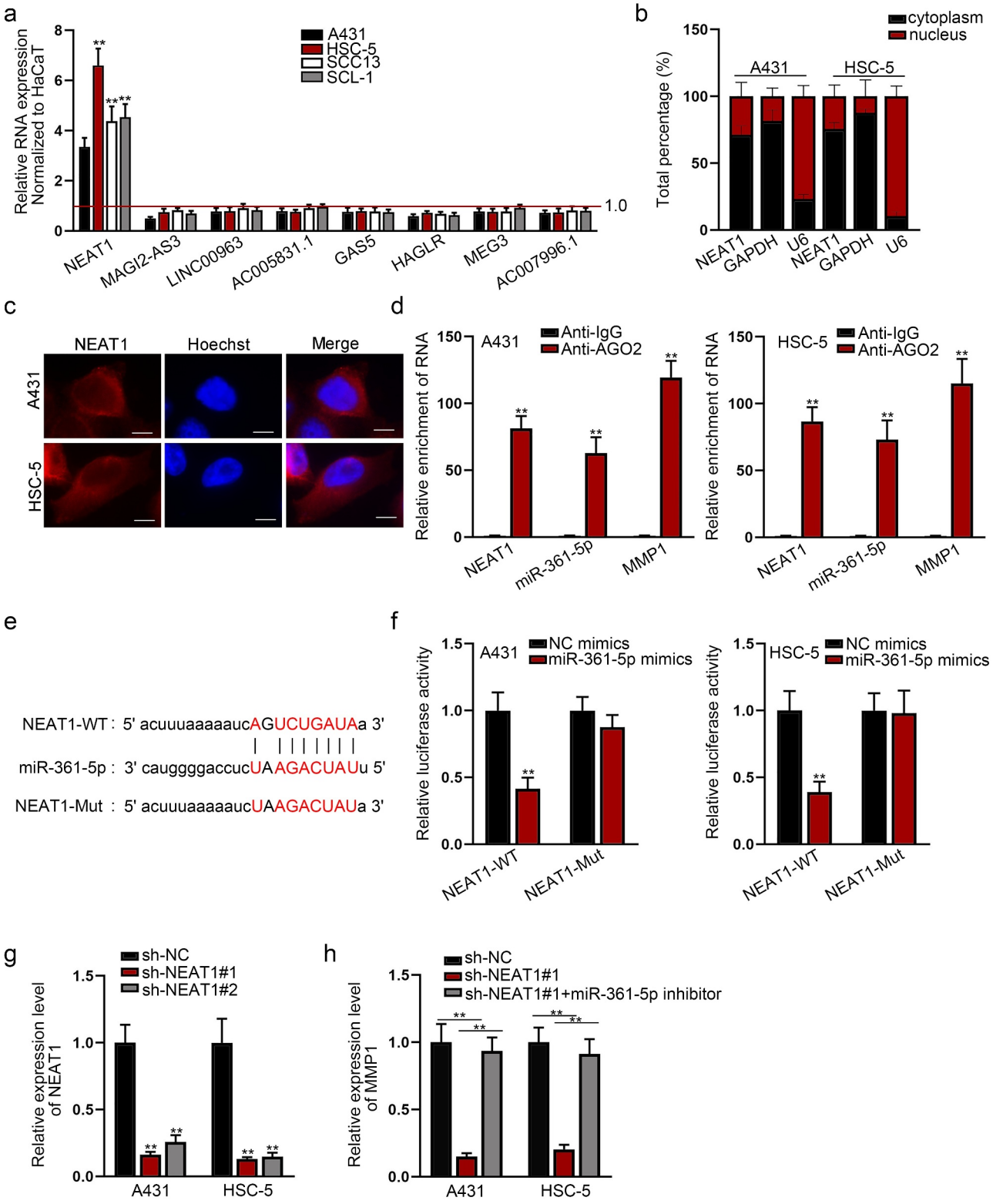

Figure 4. NEAT1 interacts with miR-361-5p. (a). The expression levels of potential upstream lncRNA that may interact with miR-361-5p were detected in CSCC cells and normal one. (b,c). Subcellular fractionation and FISH assays were utilized to understand the location of NEAT1. (d). RIP assays indicated the co-existence of NEAT1, miR-361-5p and MMP1 in anti-AGO2 group. (e). The wild and mutant binding sites with miR-361-5p in the sequence of NEAT1. (f). Luciferase reporter assays indicated the binding of miR-361-5p with the wild type of NEAT1. (g). The interfering efficiency of sh-NEAT1 was validated in selected CSCC cells. (h). MMP1 mRNA was evaluated after co-transfecting miR-361-5p inhibitor into NEAT1-silenced A431 and HSC-5 cells. **P < .01.

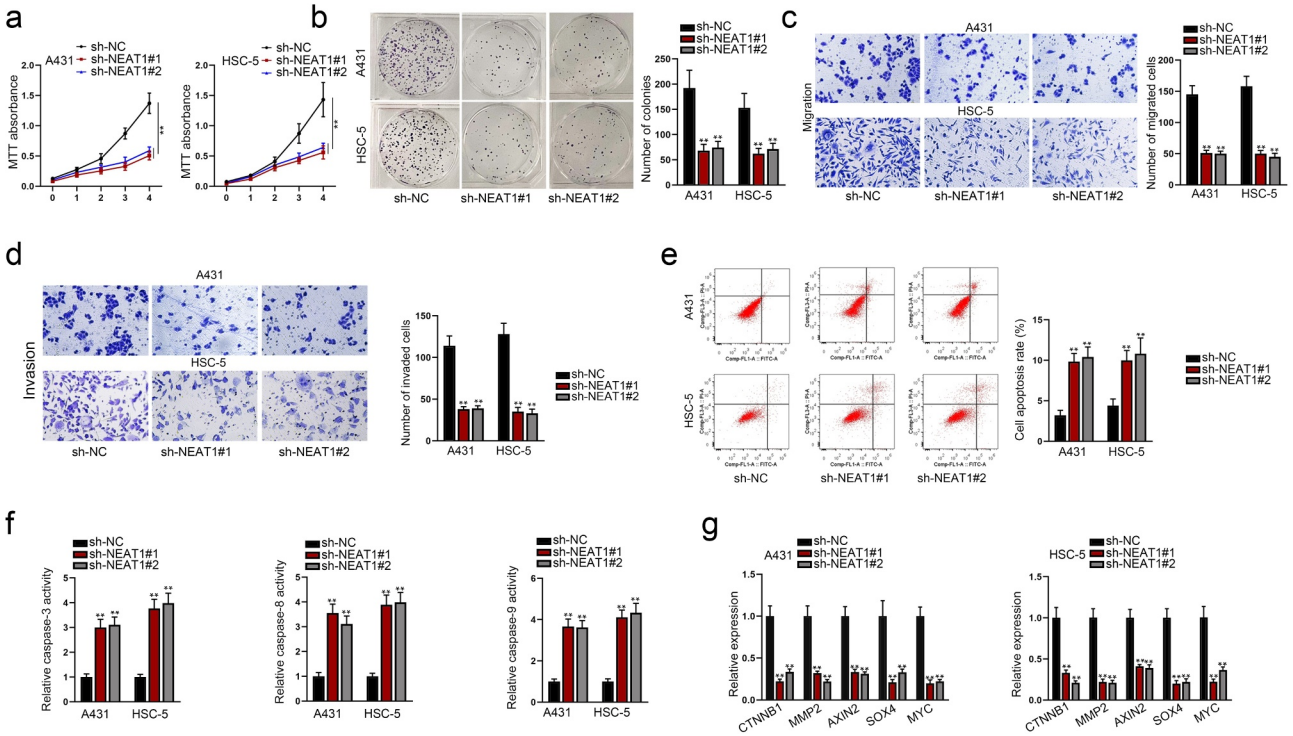

Figure 5. NEAT1 knockdown suppresses cell growth and migration via activation of Wnt pathway in CSCC. The effects of silencing NEAT1 on CSCC cells proliferation (a, b), migration (c), invasion (d), and apoptosis (e,f) were explored. G. QRT-PCR was conducted to evaluate the mRNA expression level of genes associated with Wnt pathway. **P < .01.

NEAT1-depleted A431 and HSC-5 cells, but restored by co-transfection of miR-361-5p inhibitor (Figure 4(h)).

NEAT1 knockdown suppresses cell growth and migration via activation of Wnt pathway in CSCC

To determine whether NEAT1 influences the development of CSCC, we performed loss-of-function assays in selected CSCC cells. As expected, we found that NEAT1 knockdown suppressed cell proliferation (Figure 5(a,b)), migration (Figure 5(c)), and invasion (Figure 5(d)). Also, NEAT1 silencing would lead to an increase in the number of apoptotic cells in selected CSCC cells (Figure 5(e,f)). Since NEAT1 has been reported to activate Wnt pathway via increasing β-catenin nuclear transport and down-regulated ICAT, GSK3B, and Axin2 expression in the progression of glioblastoma,[19] we aimed at verifying whether NEAT1 exerted carcinogenic role in CSCC via the activation of Wnt pathway. QRT-PCR was conducted to evaluate the mRNA expression level of genes associated with Wnt pathway, including CTNNB1, MMP2, AXIN2, SOX4, and MYC. It showed that NEAT1 silencing greatly down-regulated these mRNA expressions in CSCC cells (Figure 5(g)). In conclusion, NEAT1 silencing suppressed cell growth and migration via activating Wnt pathway in CSCC.

MiR-361-5p inhibitor rescues the oncogenic function of NEAT1 silencing on CSCC

To further elucidate whether the biological function of MMP1 could be indirectly regulated by NEAT1 through miR-361-5p, rescue experiments were then conducted. MTT analysis and colony formation assay showed that the cell viability or proliferation was impaired by NEAT1 knockdown, but partially restored by down-regulating miR-361-5p (Figure 6(a,b)). Additionally, the migration and invasion abilities were weakened by NEAT1 knockdown, while partially restored by down-regulating miR-361-5p (Figure 6(c,d)). Flow cytometry analysis and caspase 3/8/9 activity test indicated that cells apoptosis was promoted by NEAT1 knockdown, yet hampered by down-regulating miR-361-5p (Figure 6(e,f)). Moreover, we measured the expression level alternation of Wnt pathway-relevant genes. We found that the expression levels of CTNNB1, MMP2, AXIN2, SOX4, and MYC were down-regulated by NEAT1 knockdown; however, this interfering outcome was reversed by co-transfecting miR-361-5p inhibitor (Figure 6(g)). According to the result, it was suggested that NEAT1 regulated Wnt pathway via sponging miR-361-5p. Overall, above data manifested that miR-361-5p inhibitor rescued the oncogenic function of NEAT1 in CSCC, which was closely related with the restoration of Wnt pathway activity.

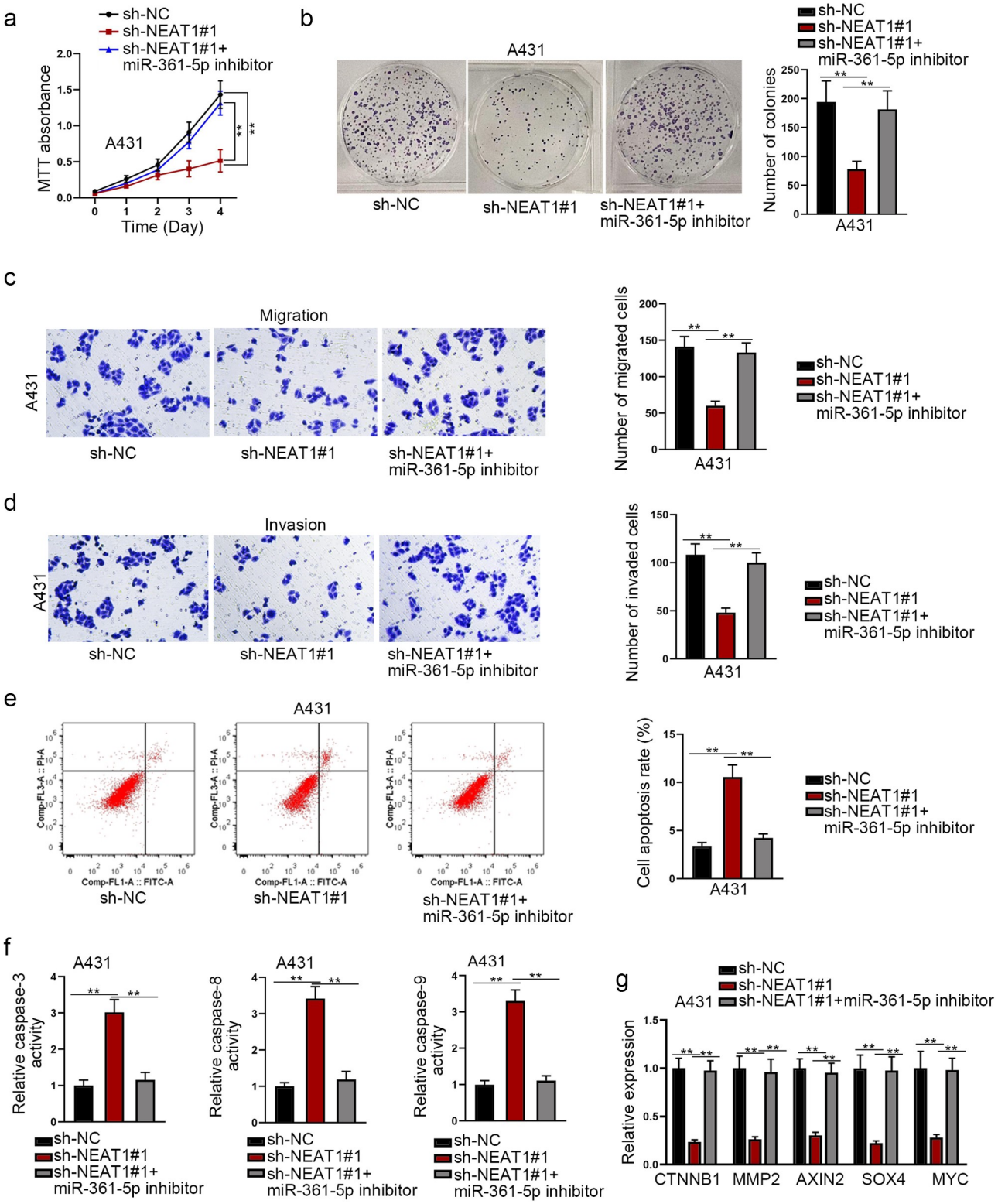

Figure 6. MiR-361-5p inhibitor rescues the oncogenic function of NEAT1 in CSCC. (a,b). The rescuing effects of miR-361-5p inhibitor on sh-NEAT1 induced vitality and proliferation alternation were determined by MTT and colony formation, severally. (c,d). The rescuing effects of miR-361-5p inhibitor on sh-NEAT1 induced migration and invasion. (e,f). The rescuing effects of miR-361-5p inhibitor on sh-NEAT1 mediated apoptosis. (g). QRT-PCR analysis was conducted to evaluate the mRNA expression variation of genes associated with Wnt pathway after co-transecting miR-361-5p inhibitor into NEAT1-depleted cells. **P < .01.

Discussion

Extensive reports have revealed the critical role of MMP1 in the metastasis of cancers. MMP1 expression level was reported to be significantly elevated in penile carcinoma tissues and served as a predictive biomarker for lymph node metastasis in patients with penile carcinoma.[20] Besides, previous investigation demonstrated that MMP1 overexpression advanced non-small cell lung cancer (NSCLC) progression and may serve as a useful biomarker for NSCLC patients.[21] According to our study, we detected an up-regulation of MMP1 in CSCC cell lines and found out that the knockdown of MMP1 hampered cell proliferative, migratory and invasive abilities, while facilitated the apoptotic capacity of CSCC cells.

MiRNAs could restrain the production of proteins via impacting the stability of their target mRNA or translational repression. The expression of MMP1 could be regulated by miRNAs in a variety of diseases. MiR-623 directly repressed MMP1 and relieved MMP1-induced migration, invasion, and metastasis in pancreatic cancer.[22] In this study, we explored the potential regulatory mechanism for the aberrant overexpression of MMP1. Also, miR-361-5p was verified to be an upstream molecular gene of MMP1 through bioinformatics analysis. MiR-361-5p expression was uncovered to be inversely correlated with vascular endothelial growth factor A (VEGFA) in CSCC.[23] Here, we found the expression of miR-361-5p in CSCC cell lines was low, which was contrary to the expression pattern of MMP1. MiR-361-5p was proved to inhibit the malignant behaviors of CSCC *in vitro*.

Since miRNAs exert negative impacts on the expression of target mRNA, we deduced that there might be another mechanism of molecular mediation on miR-361-5p. MiR-361-5p was discovered to be epigenetically sponged by many lncRNAs to affect target gene function in the carcinogenesis process. For instance, its repression on target mRNA FOXM1 was offset by the sponge of SBF2-AS1 in cervical cancer.[24] Herein, mechanism exploration revealed that the up-regulated NEAT1 was a molecular sponge for miR-361-5p in CSCC and it exhibited oncogenic properties in CSCC. This finding accorded with its carcinogenic role in diverse cancers, including myeloma,[25] hepatocellular carcinom[26] and thyroid carcinoma[27]. Moreover, the pro-tumor role of NEAT1 in CSCC was identical to its promoting impacts on other type of skin cancer, such as melanoma cancer.[28] Furthermore, inhibition of miR-361-5p countervailed the biological effects induced by silencing NEAT1. NEAT1 was validated to activate Wnt pathway in nasopharyngeal carcinoma[29] and non-small cell lung cancer.[30] Here, silencing NEAT1 inhibited the expression level of Wnt pathway related genes, such as CTNNB1, MMP2, AXIN2, SOX4 and MYC in CSCC, but this effect was reversed by down-regulating miR-361-5p. This phenomenon implied that the NEAT1/miR-361-5p/MMP1 axis contributed to CSCC development via the activation of Wnt pathway.

In a word, our study firstly uncovered that MMP1 regulated by NEAT1/miR-361-5p axis facilitates cutaneous squamous cell carcinoma cell proliferation and migration via the activation of Wnt pathway. This observation might shed new light on novel therapeutic target for CSCC clinical application. However, our study lacked the specific illustration of how lncRNA NEAT1 affect the activity of Wnt pathway, which needs in-depth study in the future.

Acknowledgments

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Disclosure statement

The authors declared no relevant conflicts of interests.

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