



MiR-525-5p Repressed Metastasis and Anoikis Resistance in Cervical Cancer via Blocking UBE2C/ZEB1/2 Signal Axis

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Abstract

Background Accumulating evidence indicated that miRNAs are important regulators involved in cancer biology.

Aims We aimed to investigate the biological functions and potentially underlying molecular mechanism of miR-525-5p in CC.

Methods RT-PCR and Western blot assay were performed to detect mRNA and protein expression. Cell proliferation, anoikis resistance, and cell invasion were analyzed.

Results We observed that the expression of miR-525-5p was declined in several CC cell lines. Additionally, introduction of miR-525-5p dramatically hampered cell viability, invasiveness, and migration ability through modulating epithelial-to-mesenchymal transition (EMT) marked genes as reflected by the upregulation of E-cadherin, as well as the downregulation of vimentin and N-cadherin. Furthermore, administration of miR-525-5p markedly reduced anchorage-independent growth and anoikis resistance accompanied by a decrease in the expression of anti-apoptotic protein Bcl-2 and an increase in the expression of pro-apoptotic protein Bax, C-caspase 3, and C-PARP1. Most importantly, analysis using publicly available algorithms predicted that UBE2C was a direct and functional target of miR-525-5p. Luciferase assays coupled with RT-PCR and Western blot analysis further verified that miR-525-5p negatively regulated UBE2C expression. Interestingly, miR-525-5p modulated ZEB1/2 expression via targeting UBE2C. Mechanically, administration of UBE2C partially blunted the salutary effects of miR-525-5p on invasive ability, EMT, and anoikis resistance, indicating that miR-525-5p acts as a tumor suppressor in CC largely through repression of UBE2C/ZEB1/2 signaling.

Conclusions Taken together, our data identify a novel signaling axis of miR-525-5p/UBE2C/ZEB1/2 in repressing EMT and anoikis resistance, and likely serve as a potential therapeutic target for CC metastasis and prognosis as well as a therapeutic application.

Keywords MiR-525-5p · Metastasis · Anoikis resistance · UBE2C/ZEB1/2 signal axis · Cervical cancer

Introduction

Cervical cancer (CC) is one of the most common gynecology tumors and the third leading cause of cancer deaths among females in the world [1]. Cancer progression is a multistep complex process affected by multiple procedures including loss of cell adhesion, anchorage-independent cell growth, and hydrolysis of the ECM leading to cell migration and invasion. Tumor invasion and distant metastasis are the main causes for treatment failure and death in cancer

patients, particularly in CC [2]. These processes involved in tumor initiation and progression [3]. Despite the development of advanced therapeutic strategies, there is still no effective and reliable means to prevent or control metastasis. Therefore, it is important to determine how the metastasis process is regulated and to identify potential targets for repressing cancer metastasis.

The fundamental events for cancer progression and metastases include loss of cell adhesion, cell proliferation, anchorage-independent cell growth (evading anoikis), cell migration, and cell invasion [4]. All these events leading to cancer progression happen in a favorable nurturing tumor microenvironment. Anoikis is a specific programmed cell death induced by inappropriate cell–cell and cell–ECM attachment leading to metastases. Anoikis resistance has been demonstrated to facilitate distant metastases of cancers

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[5]. It is the primary condition of invasion and metastasis for the characteristics of anoikis resistance in tumor cells, attributed to changes in cell polarity and the induction of EMT. Multiple lines of evidence revealed that anoikis resistance is involved in CC metastasis, and reducing the anoikis-resistant capacity of CC cells could reverse their invasive properties [4]. Therefore, invasiveness and resistance to anoikis are critical for the systemic spread of cancer cells. Despite the importance of invasiveness and anoikis resistance in the progression of cancer, the molecular mechanisms that underlie this process have yet to be fully identified. Thus, this fact underscores the urgency and importance of understanding the molecular mechanisms of metastasis.

Typically, microRNAs (miRNAs) recognize and bind to partially complementary sites in the 3'-UTR of target mRNAs, consequently leading to mRNA degradation or translational inhibition [6]. Accumulating evidence has implicated that deregulation of miRNAs contributes to the developmental and pathological processes in multiple malignancies [7]. They can function as oncogenes or tumor suppressor genes to regulate various cellular activities including cell proliferation, differentiation, invasion, angiogenesis, and apoptosis via relevant targets or crucial signaling pathways. Growing evidences have shown that some miRNAs are aberrantly expressed and regulate tumor invasion- and metastasis-related processes, such as the EMT or anoikis [8–12]. As one of well-documented miRNAs, miR-525-5p has been confirmed to be a tumor suppressor in various kinds of malignant tumors. However, the regulatory mechanisms of miR-525-5p in the development and progression of CC have not been fully elucidated. In this study, we investigated the functional characterization of miR-525-5p/UBE2C/ZEB1/2 axis in the metastases of CC through modulating EMT and anoikis resistance.

Materials and Methods

Cell Culture

Human CC cell lines HeLa, SiHa, Caski, C4-1, SW756, and C-33A and normal human cervical epithelial cell line Ect1/E6E7 were obtained from ATCC (Manassas, VA, USA). Cells were cultured in ATCC recommended medium containing 10% FBS (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C in 5% CO₂ incubator.

Cell Transfection

The miR-525-5p mimics and negative control (NC) were synthesized and purchased from Genepharma (Shanghai, China). Transient transfection was performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according

to the manufacturer's protocol. After transfection for 48 h, the transfection efficiency was validated by RT-PCR.

RNA Extraction and RT-PCR Assay

Total RNA was isolated from cells using Trizol reagent (Invitrogen) following manufacturer's recommended protocols. RNA concentration was determined using a NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was generated using the PrimeScript RT reagent kit (Takara, Otsu, Japan) in accordance with the manufacturer's instructions. The mRNA levels were determined using SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) on ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). RT-PCR analysis of miR-525-5p was conducted with the TaqMan MicroRNA Assay Kit (Applied Biosystems). β -actin or U6 was used as internal control for the normalization of mRNA or miRNA, respectively.

Western Blot Analysis

Total proteins were extracted using M-PER reagent (Pierce, Rockford, IL). The protein concentration was quantified by the BCA Assay Kit (Pierce, Rockford, IL, USA). Equivalent amounts of proteins were fractionated using SDS-PAGE and then transferred onto PVDF membranes (Millipore, Billerica, MA, USA). After blocking with 5% non-fat dry milk at 37 °C for 1 h, the membranes were incubated with appropriate primary antibodies at 4 °C overnight. Subsequently, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology, CA, USA) for 1 h at room temperature. The bands were visualized with the enhanced chemiluminescence (ECL) system (Perkin Elmer, Waltham, MA, USA) and quantified by the Image J software (NIH, Bethesda, MD, USA). β -actin was used as the endogenous control.

Cell Counting Kit-8 (CCK-8) Assay

CCK-8 assay was conducted to evaluate cell proliferation (Sigma-Aldrich, MO, USA). In brief, the cells were plated onto 96-well plates (1×10^4 cells/well) and subjected to the indicated transfection. After 72 h of incubation, 10 μ L of CCK-8 solution was added into each well and incubated for another 4 h at 37 °C. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Richmond, CA, USA).

Invasion and Migration Assay

Transwell chambers with Matrigel-coated filters (8 μ m pore size, BD Biosciences, San Jose, CA, USA) were used to

assess for cell invasive capacity. Briefly, cells (1×10^5) suspended in serum-free DMEM were plated into the upper chamber, and DMEM supplemented with 10% FBS was placed into the lower chamber. After 24 h of incubation, the cells that remained on the upper chambers were removed, and the cells that invaded to the lower chambers were fixed with 20% methanol followed by staining with 0.1% crystal violet. The number of invaded cells was determined under a microscope (Olympus, Tokyo, Japan) from five randomly selected fields. Cell migration assays were performed using Transwell chambers without Matrigel.

Soft Agar Cloning Assay for Anchorage-Independent Growth

About 1500 cells/well were suspended in pre-warmed culture medium containing 0.25% agarose, and seeded in culture dishes that were pre-coated with 0.5% agarose in culture medium. Cells were incubated for 2–3 weeks at 37 °C under a 5% CO₂ atmosphere. The formed colonies were counted and imaged under a light microscope (Olympus, Tokyo, Japan).

Anoikis Assessment Assay

The cells (1×10^6 /well) were seeded in plates coated with Poly-HEMA (Sigma) to avoid the adhesion of cells. After incubation for 24 h, the cells were harvested and washed with cold PBS and subjected to flow cytometry analysis using an Annexin V/FITC Apoptosis Detection Kit (BD Pharmingen™, Franklin lake, New Jersey, USA) according to the manufacturer's instructions.

Bioinformatics Analysis

Prediction of the interaction between miR-525-5p and UBE2C was performed using TargetScan, miRanda, and PicTar databases.

Luciferase Reporter Assay

The 3'-UTR of UBE2C mRNA containing predicted miR-525-5p binding sites or mutant binding sites was PCR amplified and inserted into pMIR-control vectors. For luciferase reporter assays, the cells were co-transfected with wild-type (WT) or mutated (Mut) versions of reporter plasmids, miR-525-5p mimics, and pRL-TK reporter plasmid using Lipofectamine 3000 reagent. At 48 h after transfection, the luciferase activities were measured with a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's recommendations. Data were normalized to Renilla activity.

Statistical Analysis

All analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism software 7.0 (GraphPad Software Inc., San Diego, CA, USA), and all data were presented as the mean \pm SD. The comparison of difference between two groups was performed using Student's *t* test; comparison among multiple groups was analyzed using one-way ANOVA. $P < 0.05$ was considered to be statistically significant.

Results

MiR-525-5p Was Dysregulated in CC Cells

To explore the functions of miR-525-5p in CC progression, we initially investigated the expression pattern of miR-525-5p in human CC cells. The resultant data showed that miR-525-5p was aberrantly downregulated in several CC cell lines (Fig. 1a). Taken together, these results suggested that miR-525-5p may function as a tumor suppressor in CC. Among these CC cell lines, miR-525-5p displayed the lowest expression level in HeLa cells. Therefore, Huh7 cells were selected for the subsequent experiments. These observations indicated that miR-525-5p plays critical roles in CC progression. To examine this notion, HeLa cells were transfected with miR-525-5p mimics, and transfection efficiency was confirmed by RT-PCR assay (Fig. 1b).

Introduction of MiR-525-5p Impaired Invasion and Migration of CC Cells

To dissect the impact of miR-525-5p on the biologic activity of CC cells, CCK-8 assay was performed to assess cell proliferation. It was observed that introduction of miR-525-5p obviously impeded cell proliferation capacity (Fig. 2a). Cellular invasion and migration is critical for CC metastasis. We then evaluated the effect of miR-525-5p on the invasion and migration of HeLa cells. Matrigel invasion chamber assay was conducted to examine the invasive potential of CC cells. As illustrated in Fig. 2b, forced miR-525-5p expression greatly impaired the cell invasive capability. Meanwhile, addition of miR-525-5p evidently lessened the number of migration, as determined by Transwell assay, suggesting that miR-525-5p restrained cell migration ability (Fig. 2c). These observations point to the crucial role of miR-525-5p in repressing CC cell invasion and migration potential. Matrix metalloproteinases (MMPs) have also been regarded as major causes of tumor migration and invasion. Results depicted in Fig. 2d, e showed that administration of miR-525-5p resulted in the decreased production of MMP-2 and MMP-9, which participate in the metastasis of CC. EMT

Fig. 1 MiR-525-5p is down-regulated in CC cells. **a** The expression levels of miR-525-5p were quantified by RT-PCR assay. * $P < 0.05$ versus Ect1/E6E7. **b** RT-PCR was carried out to evaluate the transfection efficiency of miR-525-5p mimics. * $P < 0.05$ versus control/NC

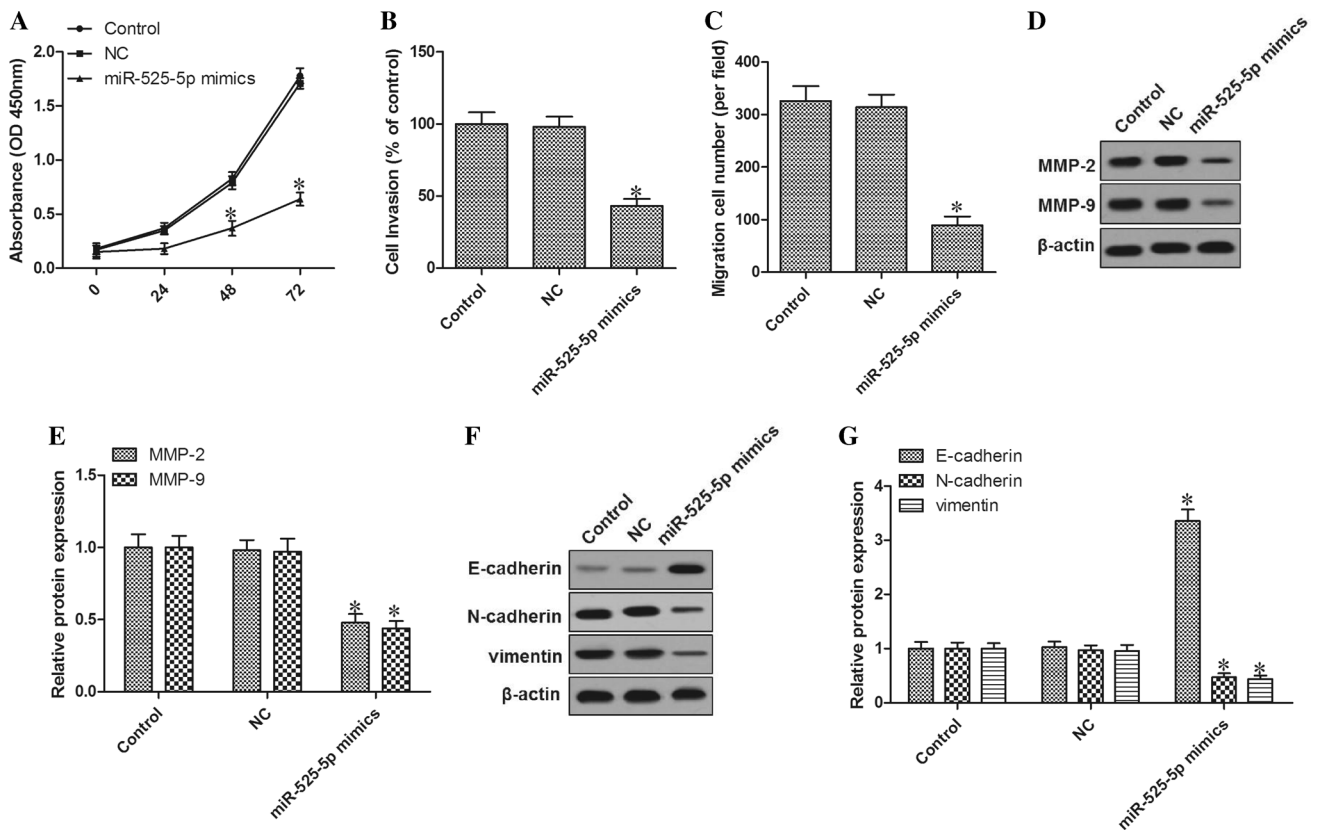
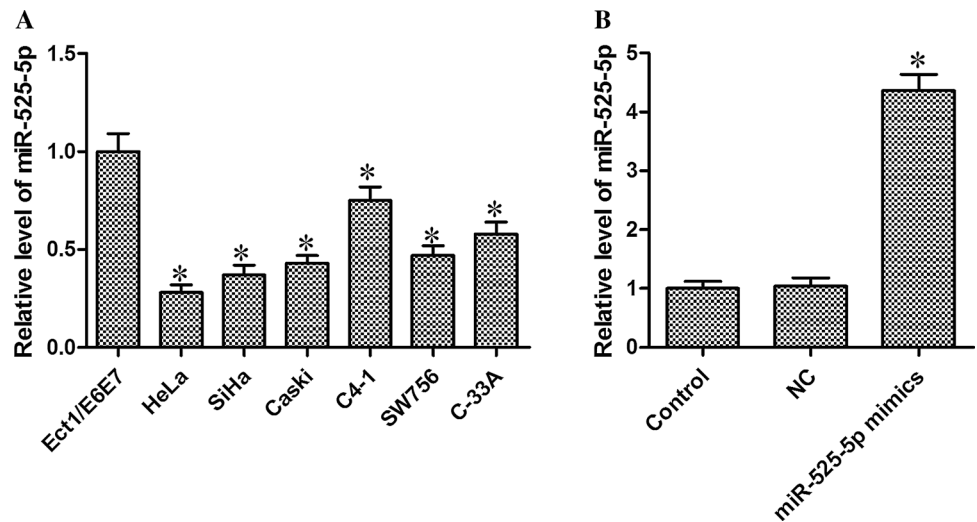


Fig. 2 Effects of miR-525-5p on proliferation, invasion, and EMT in CC cells. **a** CCK-8 assay was used to determine the proliferation ability of CC cells. **b** Matrigel invasion assay was applied to examine the invasion ability of CC cells. **c** Cell migration was detected by the Transwell chamber assay. **d, e** The levels of metastasis-related pro-

tein MMP-2 and MMP-9 were evaluated by Western blot analysis. **f, g** Western blot assay was applied to evaluate the protein expression of EMT markers E-cadherin, vimentin, and N-cadherin. * $P < 0.05$ versus control/NC

processes are correlated with cell invasive capability. To elucidate the molecular basis whereby miR-525-5p repressed the malignant behaviors of CC cells, we explored the expression levels of EMT-related proteins. As presented in Fig. 2f,

g, introduction of miR-525-5p evidently enhanced the expression of epithelial marker E-cadherin as well as dramatically diminished the expression of mesenchymal marker N-cadherin and vimentin, indicating that miR-525-5p was

capable to repress efficient EMT signaling. Consequently, the above data implied anti-survival, tumor-suppressive, and anti-metastatic functions of miR-525-5p in HeLa cells.

Enforced Expression of miR-525-5p Repressed the Anchorage-Independent Growth and Anoikis Resistance of CC Cells

Because anoikis resistance has been considered as a key step in the progression of tumor metastasis [13, 14], enhancing anoikis resistance is a key mechanism underlying integrin-mediated metastatic dissemination. We further investigated the effect of miR-525-5p on the anchorage-independent growth in HeLa cells assessed by a soft agar assay. As depicted in Fig. 3a, forced expression of miR-525-5p strikingly attenuated the number of colonies in CC cells. Subsequently, anoikis assay analysis revealed that overexpression of miR-525-5p depressed the anoikis resistance of CC cells (Fig. 3b), implying that miR-525-5p restrained CC cells

with resistance to anoikis. Moreover, higher expression of miR-525-5p resulted in the enhanced expression of Bax, the main pro-apoptotic proteins in anoikis, and the decreased expression of Bcl-2, the main anti-apoptotic proteins in anoikis (Fig. 3c, d). In the meanwhile, the results of Western blot revealed that expressions of apoptosis-related factors, cleaved caspase 3 (C-caspase 3) and cleaved-PARP1 (C-PARP1), were enhanced drastically (Fig. 3e, f). Together with the data we present here demonstrated that miR-525-5p obstructed anchorage-independent growth events and anoikis resistance of CC cells.

UBE2C Was a Direct Target for MiR-525-5p

As is well known, miRNAs exert their function through suppressing the expression of their target genes, we therefore aimed to identify direct target of miR-525-5p that is essential for clarifying of the molecular mechanisms. In order to achieve this aim, several available prediction

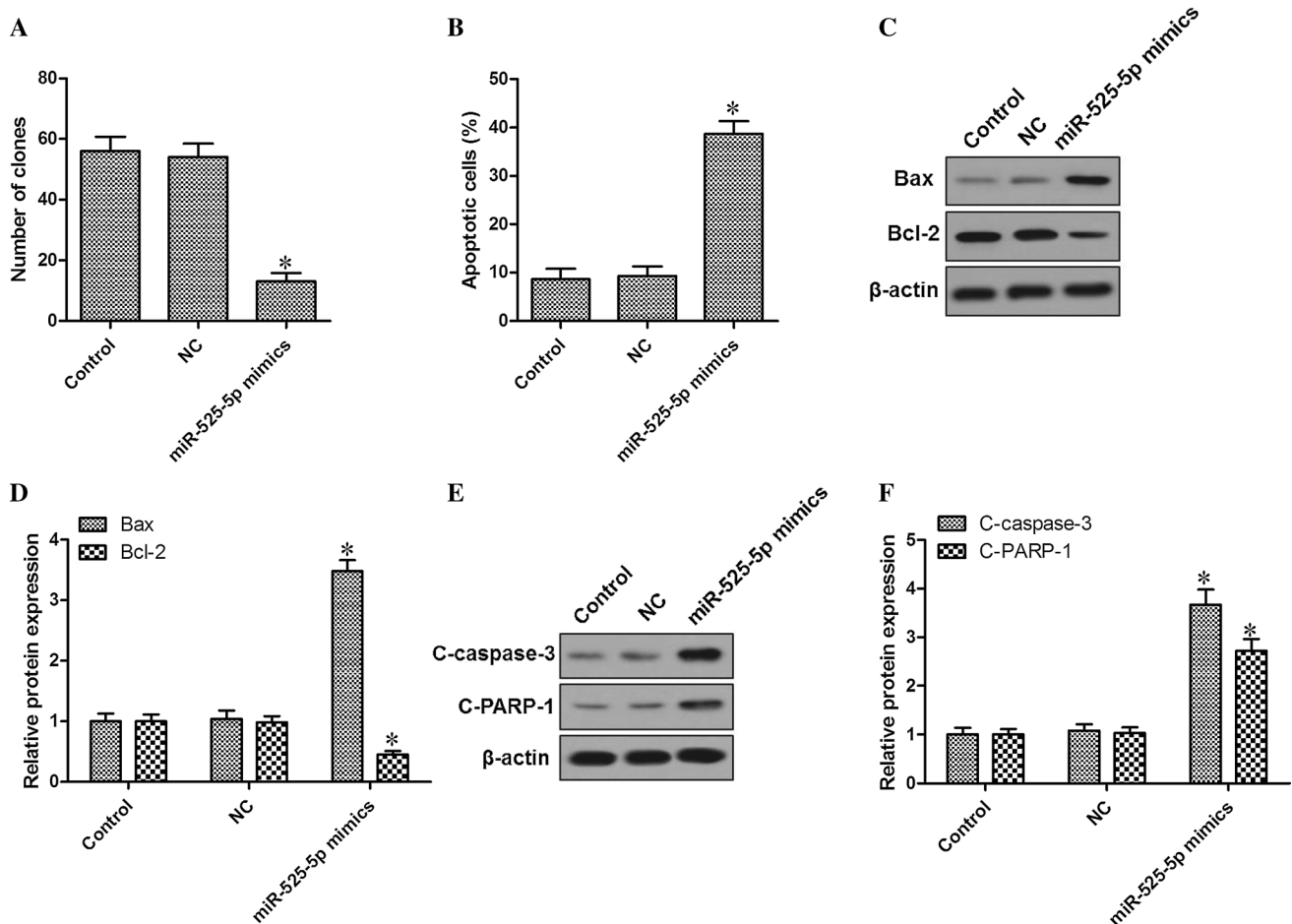


Fig. 3 Effects of miR-525-5p on anchorage-independent growth and anoikis resistance. **a** The cellular anchorage-independent growth was estimated by soft agar cloning assay. **b** Anoikis of HeLa cells that were seeded into poly-HEMA pre-coated plates was detected

by Annexin V/PI staining. **c, d** Western blot was performed to determine the expression of apoptosis-associated Bax and Bcl-2. **e, f** The expression of cleavage of caspase 3 and PARP 1 was assessed by using Western blot assay. * $P < 0.05$ versus control/NC

algorithms TargetScan, miRanda, and PicTar were adopted to search for potential targets of miR-525-5p. Bioinformatics instruments showed that the 3'-UTR of UBE2C included the targeting site of miR-525-5p (Fig. 4a), implying that UBE2C was a putative target of miR-525-5p. Dual-luciferase reporter assay revealed that ectopic expression of miR-525-5p restrained the luciferase activity of the WT 3'-UTR of UBE2C, but that of the Mut 3'-UTR of UBE2C was not affected, which confirmed that UBE2C was a direct target of miR-525-5p (Fig. 4b). We also defined the relationship between miR-525-5p and UBE2C. As presented in Fig. 4c, the delivery of miR-525-5p mimics distinctly downregulated the mRNA expression of UBE2C. Meanwhile, the similar results were obtained by using Western blot analysis of UBE2C protein (Fig. 4d, e). On the basis of these findings, we implicated that miR-525-5p affected UBE2C expression by directly binding to its 3'-UTR region.

Restoration of UBE2C Reversed the Protective Effects of MiR-525-5p in CC Cells

We further investigated whether UBE2C is indeed involved in miR-525-5p-mediated tumor suppression in CC. As can be seen, Western blot results showed that UBE2C overexpression largely abrogated the ability of miR-525-5p to ameliorate UBE2C expression (Fig. 5a, b). It is worth noting that UBE2C overexpression impaired the inhibitory effects of miR-525-5p on the expression of ZEB1 and ZEB2 (Fig. 5a, b). In Fig. 5c, it was manifested that restoration of UBE2C expression obviously impeded the benefit effect of miR-525-5p on the invasive capacity. Besides, administration of UBE2C remarkably reversed the suppressive effect of miR-525-5p on EMT process (Fig. 5d, e). Taken together, these observations demonstrated that downregulated expression of UBE2C expression was responsible for the protective effect of miR-525-5p on the invasive capacity and EMT of CC cells. We further monitored whether UBE2C was involved in anoikis resistance mediated by miR-525-5p, and the data

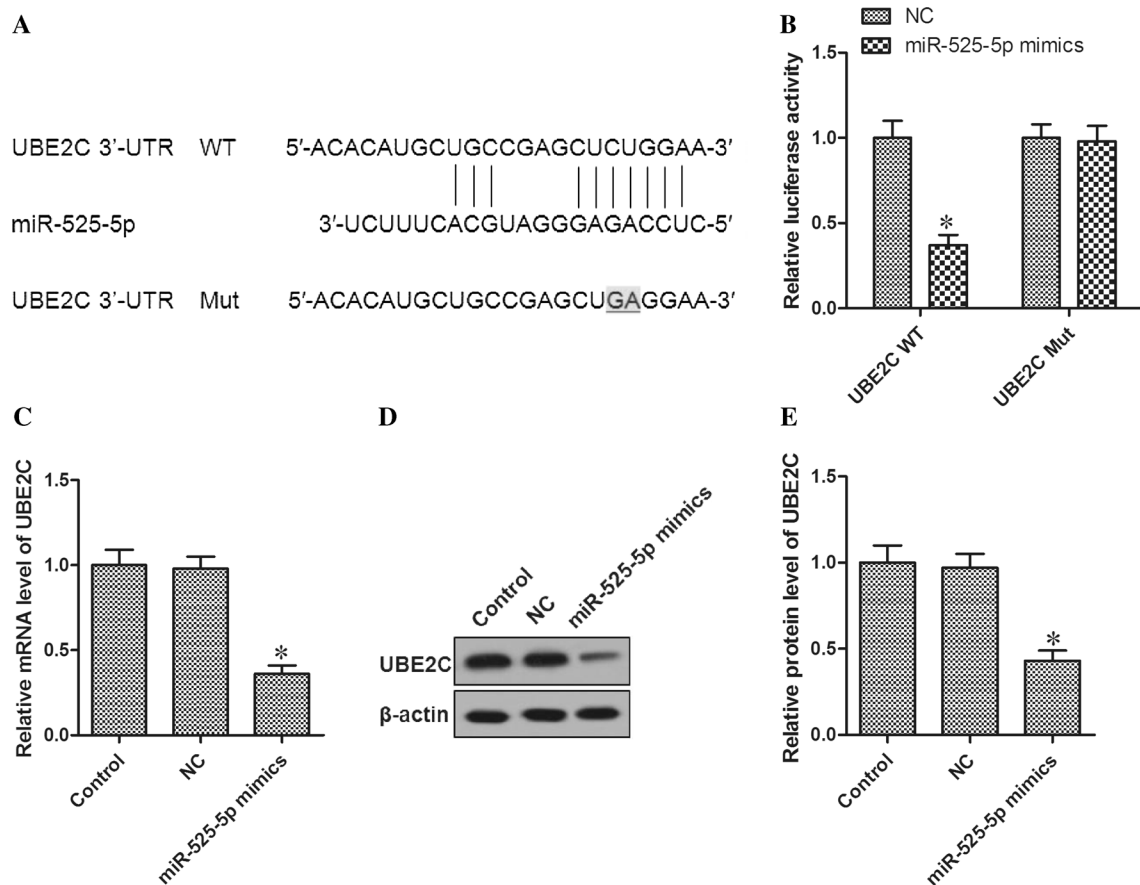


Fig. 4 MiR-525-5p directly targets UBE2C and decreases its expression. **a** Schematic of in silico analysis of predicted binding sites on miR-525-5p binding to the 3'-UTR region of UBE2C mRNA. **b** Dual-luciferase reporter assay was applied to evaluated relative luciferase

activities. **c** The mRNA expression of UBE2C was detected using RT-PCR assay. **d, e** Western blot analysis was conducted to assess UBE2C protein expression. * $P < 0.05$ versus control/NC

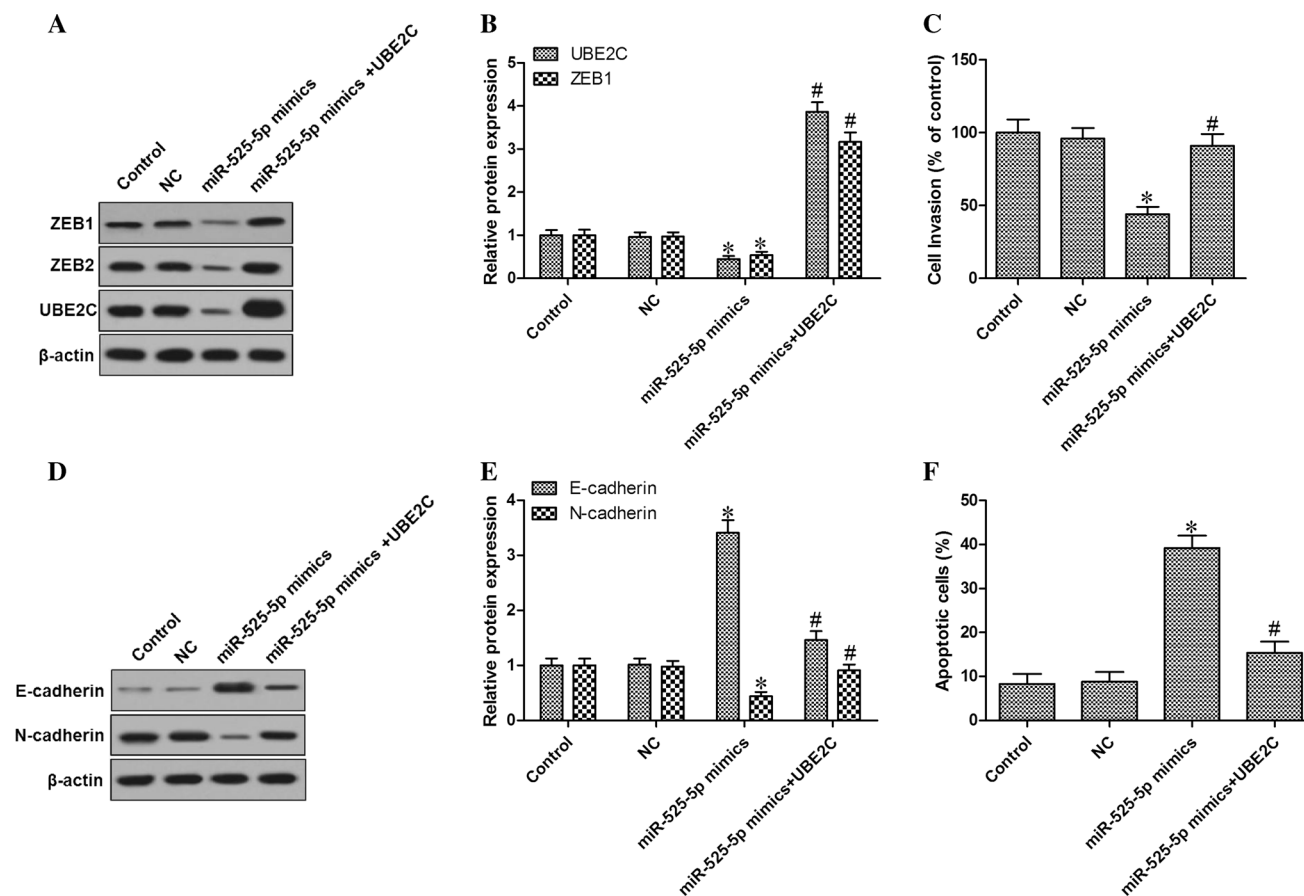


Fig. 5 UBE2C/ZEB1/2 axis contributes to the inhibitory effects of miR-525-5p on metastatic capability and anoikis resistance. **a** Western blot was applied to evaluate the protein expression of UBE2C and ZEB1/2. Quantification of the bands is shown on the **b**. **c** Matrigel

invasion assay was employed to estimate cell invasion. **d**, **e** The protein expression of E-cadherin and N-cadherin was determined using Western blot. **f** Anoikis was assessed by Annexin V/PI staining. * $P < 0.05$ versus control/NC; # $P < 0.05$ versus miR-525-5p mimics

in Fig. 5f implied that re-expression of UBE2C effectively counteracted the promotion of anoikis induced by miR-525-5p, indicating that miR-525-5p as a potential regulator of UBE2C that repressed the anoikis resistance. These data provided sufficient evidence that miR-525-5p exerted a protective effect by blocking UBE2C/ZEB1/2 signal axis.

Discussion

MiRNAs have a critical role in tumorigenesis and metastasis, which are major obstacles of cancer therapy. Accumulating evidence suggests that miR-525-5p has tumor-suppressive functions. However, the exact role of miR-525-5p in CC has not been fully understood to date. In the current study, we attempted to explore the expression and function of miR-525-5p in CC, and we observed that the expression level of miR-525-5p was downregulated in multiple CC cells, suggesting that miR-525-5p may contribute to the malignant progression of CC. This study was designed to explore the

anti-tumor activity of miR-525-5p, and we performed the CCK-8 and Transwell invasion assay to realize its biological functions. As a result, we found that overexpression of miR-525-5p depressed cell proliferation, invasion, and migration capability of CC cells, implying that miR-525-5p functions as an important tumor suppressor with both anti-proliferation and anti-invasive abilities in CC tumorigenesis. There are a number of studies demonstrating the key involvement of MMPs in tumor invasion and metastases [15]. Simultaneously, the addition of miR-525-5p dramatically reduced the production of MMP-2 and MMP-9 to inhibit the degradation of extra-cellular matrix, thus preventing invasive ability of CC cells.

EMT is a common mechanism for promoting tumor cell metastasis [16]. EMT stimulated invasion and migration of tumor cells exerts a powerful effect on the process of occurrence, development, invasion, and metastasis of CC [2, 17]. Thus, we examined the protein expression of several EMT-related marker genes including E-cadherin, N-cadherin, and vimentin, and the results demonstrated that forced

expression of miR-525-5p markedly augmented the expression of E-cadherin, and distinctly attenuated the expression of N-cadherin, and vimentin, indicating that miR-525-5p modulated EMT processes by regulating EMT-related genes. Overall, these preceding observations strongly indicated that miR-525-5p displayed suppressive effects on EMT processes to inhibiting metastasis of CC. Collectively, these researches illuminated that miR-525-5p displays anti-proliferation and anti-invasive properties, suggesting therapeutic potential of miR-525-5p in CC.

After encountering loss of adhesion, transformed cells evade the anchorage-independent programmed cell death, also known as anoikis. Anoikis is a physiological barrier to metastases, and the ability of cells to obtain anoikis-like apoptotic cell death resistance is a prerequisite for tumor proliferation, metastasis, and chemotherapy resistance [18]. Anoikis plays a critical role in several biological processes, especially during the process of metastasis. Anoikis resistance of tumor cells is crucial for metastasis to occur, since many disseminated tumor cells fail to develop metastasis due to anoikis. The augmented anoikis resistance of tumor cells is in favor of not only the invasion of tumor cells into the target organ but also the development of metastatic lesions. During this phase, the cancer phenotype that causes cell survival in detachment conditions, drug resistance, and EMT is altered. Inhibition of anoikis resistance can potentially be the molecular target in cancer therapy [19]. Anoikis resistance is an important protective process for anchorage-independent growth [20]. Bcl-2 is recognized as a marker of the intrinsic pathway of anoikis [11]. It is recognized that anoikis resistance facilitates metastasis via triggering EMT [4]. The established anoikis resistant displayed more aggressive malignant behaviors, including rapid proliferation, upregulated anti-apoptotic protein levels, and EMT phenotype. In the present study, ectopic expression of miR-525-5p prevented the advent of anoikis resistance and anchorage-independent growth events, which was confirmed by reduced anti-apoptotic Bcl-2 protein, concomitant with elevated levels of pro-apoptotic protein Bax, cleavage caspase 3, and PARP1 protein. These strong evidences demonstrated that miR-525-5p decelerated the anoikis resistance of CC cells, thus repressing tumor metastasis. Collectively, our results shed lights on the roles and mechanisms of miR-525-5p in CC metastases, which were involved in modulation of anoikis resistance and anchorage-independent growth, and highlight miR-525-5p as a therapeutic target in CC.

Ubiquitin-conjugating enzyme E2C (UBE2C; also known as UbcH10) functions as a potential oncogene which involved in tumorigenesis or progression of the tumor [21]. Mounting evidences indicated that UBE2C expression is elevated in CC patients and human cervical cell lines [22–24]. There is now convincing evidence that UBE2C plays a crucial role in cancer progression, invasion, and metastasis

through the induction of EMT and the regulation of angiogenic responses. A previous study showed that dominant negative-UBE2C represses cell proliferation and decreases in resistance to radiation [24]. ZEB1 (zinc finger E-box binding homeobox 1) and ZEB2 are two members of the ZEB family of transcription factors characterized by the presence of two zinc finger clusters and a centrally located homeodomain. Aberrant expression of ZEB1/2 has been observed in cervical cancer [25, 26]. Accumulating evidence suggests that ZEB1/2 can promote invasion and EMT in CC cells [25, 27, 28]. It has been demonstrated that UBE2C directly binds to the 5'-UTR of ZEB1/2, and UBE2C upregulates the expression of ZEB1/2 [29]. In addition, UBE2C promotes EMT in lung cancer cells by directly targeting the 5'-UTR of the transcript encoding ZEB1/2 [29]. A recent study also reported that UBE2C/ZEB1/2-ABCG2/ERCC1 reverses cisplatin resistance by downregulating antidrug genes and reducing EMT in cisplatin resistant NSCLC cells [30]. To gain insight into the detailed molecular mechanisms underlying the regulation of miR-525-5p on CC progression, bioinformatics analysis, luciferase reporter analysis, RT-PCR, and Western blot analysis were applied to discern and examine the relationship between miR-525-5p and its potential target UBE2C. UBE2C was identified as a potential miR-525-5p targeted transcript by multiple miR-target prediction tools. Consistently, RT-PCR and Western blot revealed that miR-525-5p negatively regulated the expression of UBE2C. According to these results, UBE2C was verified to be the target gene of miR-525-5p. It is interesting to notice that miR-525-5p downregulated expression levels of ZEB1/2. To investigate the biological importance of UBE2C as a target of miR-525-5p, we performed a rescue experiment. As we expected, overexpression of UBE2C largely reversed the inhibitory effect of miR-525-5p on UBE2C expression, accompanied by a striking enhancement in expression of ZEB1/2, suggesting that ZEB1/2 involved in miR-525-5p/UBE2C axis mediated CC progression. Furthermore, Transwell assay verified that overexpression of UBE2C abrogated the regulatory effect of miR-525-5p on invasive capability of CC cells, indicated that the protective effect of miR-525-5p on cell invasion might be dependent on UBE2C. Concurrently, the inhibitory effect of miR-525-5p on EMT was reversed by UBE2C overexpression. Furthermore, administration of UBE2C abolished the suppressive effect of miR-525-5p on anoikis resistance and revealed that resistance to anoikis regulated by miR-525-5p attributed to UBE2C. After a series of functional restoration assays, we concluded that the suppressive function of miR-525-5p on CC progression was partially attributed to the repression of UBE2C expression. Based upon the results observed in the present study, we deduced that miR-525-5p inhibited adhesion to initiate the EMT but promoted anoikis to suppress metastasis by blocking UBE2C/ZEB1/2 axis signaling.

Overall, as a summary of our findings in this study, our data revealed that miR-525-5p restrained EMT, anchorage-independent growth and anoikis resistance, and eventually inhibiting tumor metastasis, at least partially by inactivating the UBE2C/ZEB1/2 axis signaling. These data collectively suggest that the miR-525-5p/UBE2C/ZEB1/2 axis may be a potential novel diagnostic and therapeutic targets in treatment of advanced CC, although further study of the in vivo effect is needed.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest for this work.

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