Applied Biochemistry and Biotechnology
https://doi.org/10.1007/s12010-024-04897-4

ORIGINAL ARTICLE

LINC00963 Promotes Cisplatin Resistance in Esophageal Squamous Cell Carcinoma by Interacting with miR-10a to Upregulate SKA1 Expression

Dongxin Hu[1] · Anqun Ma[1] · Hongda Lu[1] · Zhen Gao[1] · Yue Yu[1] · Jiaming Fan[1] · Shang Liu[1] · Yancheng Wang[1] · Mingyan Zhang[2]

Accepted: 4 March 2024

Abstract
Long non-coding RNA (lncRNA) is associated with a large number of tumor cellular functions together with chemotherapy resistance in a variety of tumors. LINC00963 was identified to regulate the malignant progression of various cancers. However, whether LINC00963 affects drug resistence in esophageal squamous cell carcinoma (ESCC) and the relevant molecular mechanisms have never been reported. This study aims to investigate the effect of LINC00963 on cisplatin resistance in ESCC. After detecting the level of LINC00963 in human esophageal squamous epithelial cells (HET-1 A), ESCC cells (TE-1) and cisplatin resistant cells of ESCC (TE-1/DDP), TE-1/DDP cell line and nude mouse model that interfered with LINC00963 expression were established. Then, the interaction among LINC00963, miR-10a, and SKA1 was clarified by double luciferase and RNA immunoprecipitation (RIP) assays. Meanwhile, the biological behavior changes of TE-1/DDP cells with miR-10a overexpression or SKA1 silencing were observed by CCK-8, flow cytometry, scratch, Transwell, and colony formation tests. Finally, the biological function of the LINC00963/SKA1 axis was elucidated by rescue experiments. LINC00963 was upregulated in TE-1 and TE-1/DDP cell lines. LINC00963 knockdown inhibited SKA1 expression of both cells and impaired tumorigenicity. Moreover, LINC00963 has a target relationship with miR-10a, and SKA1 is a target gene of miR-10a. MiR-10a overexpression or SKA1 silencing decreased the biological activity of TE-1/DDP cells and the expression of SKA1. Furthermore, SKA1 overexpression reverses the promoting effect of LINC00963 on cisplatin resistance of ESCC. LINC00963 regulates TE-1/DDP cells bioactivity and mediates cisplatin resistance through interacting with miR-10a and upregulating SKA1 expression.

Keywords LINC00963 · miR-10a · SKA1 · Esophageal squamous cell carcinoma · Cisplatin resistance

Extended author information available on the last page of the article

Published online: 20 March 2024

Introduction

Esophagus is a long tubular organ that runs through the neck, thoracic cavity, and abdominal cavity, with the main role of assisting the food mass into the stomach. The cause of esophageal squamous cell carcinoma (ESCC) is usually associated with factors such as inattention to diet, mycotoxin infection, long-term smoking, and drinking [1, 2]. Chemoradiotherapy that preserves the function of esophageal vessels is also a good treatment option [3]. In a previous study, our team found that the current status of ESCC treatment is not promising [4]. Chemotherapy is the most important clinical treatment for intermediate to advanced ESCC, and cisplatin-based multidrug combination therapy is the most common chemotherapy regimen [5]. However, acquired resistance has inevitably become the major obstacle to the clinical management of ESCC and is closely associated with a poorer prognosis [6].

LncRNA is a functional unit that does not encode protein nucleotides [7]. The phosphorylation modification and localization of proteins are methodically carried out under the influence of lncRNA. Multiple studies confirm that lncRNA is associated with a large number of tumor cellular functions and together with chemotherapy resistance in a variety of tumors [8–10]. Several oncogenic lncRNAs, including LINC00022, LINC00680, and LOC146880, and anti-oncogenic lncRNAs, including GATA2-AS1, IRF1-AS, and RPL34-AS1,, were identified in ESCC [11–16]. LINC00963 has an essential role in the malignant progression of many cancers [17–19]. In patients with lung cancer, LINC00963 was negatively associated with prognosis and had the potential to be a therapeutic target [20]. LINC00963 functioned as an oncogene by sponging miR-608 in acute myeloid leukemia [21]. In our research, the level of LINC00963 in human esophageal squamous epithelial cells (HET-1 A), ESCC cells (TE-1), and cisplatin resistant cells of ESCC (TE-1/DDP) were pre-analyzed. The findings showed that LINC00963 was increased in TE-1 and TE-1/DDP cell lines. Our preliminary result and the finding that LINC00963 was associated with cisplatin resistance and oxaliplatin resistance [22], suggesting that LINC00963 may be involved in the resistance of ESCC to cisplatin.

Spindle and kinetochore-associated protein 1 (SKA1) is crucial for stabilizing the microtubule attachment of the mitotic spindle during mitosis [23]. It has been confirmed that SKA1 has oncogene characteristics and is closely associated with patient prognosis such as tumor recurrence and metastasis and shorter survival [24]. Moreover, multiple literatures have demonstrated that SKA1 serves as an oncogene in various cancers, including gastric, lung, hepatocellular, and renal cancers [25–28]. Similarly, our previous study has identified SKA1 positively expressed in ESCC and as a potential therapeutic target for ESCC [27]. In consideration of the importance of SKA1, whether SKA1 was regulated by non-coding RNAs holding the scientists' attention. Several miRNAs and lncRNAs were reported to target SKA1 in cancers. For instance, miR-10a mediated SKA1 to exert antitumor function in hepatocellular carcinoma [29]. SKA1 was targeted by miR-1182, and miR-1182/SKA1 was regulated by LINC00339 in hepatocellular carcinoma [30]. And in renal cell carcinoma patients, miR-455 and miR-10a were identified to regulate SKA1 [23, 28, 31]. Besides, lncRNA ZFAS1 promoted cell biological behavior of clear cell renal cell carcinoma via miR-10a/SKA1 [28]. Of note, our pre-experiments revealed that the level of SKA1 was decreased in the TE-1/DDP cell line that interfered with LINC00963. However, how LINC00963 regulates SKA1 expression, in other words, whether the regulation is working through sponging a miRNA, needs further investigation.

Therefore, the aim of our research was to investigate whether LINC00963 affects ESCC cisplatin resistance by targeting SKA1 and to explore its possible molecular mechanisms.

Materials and Methods

Cell Lines

Human esophageal squamous epithelial cells (HET-1 A) and ESCC cells (TE-1) were purchased from ATCC. The chemotherapy-resistant cell line of ESCC (TE-1/DDP) was constructed with medium-concentration and intermittent cisplatin (DDP, Sigma). Logarithmic growth phase TE-1 cells were inoculated in RPMI1640 medium. After treatment with 2 mmol/L DDP for 48 h, drug-containing medium was abandoned and fresh medium was added for further culture. One to two days later, the floating dead cells were cleaned by replacing the medium and the DDP concentration was increased. The cells were intermittently induced by repeated fluid exchange and passage with gradually increasing DDP concentration until the cells were maintained in the medium containing 35 mmol/L DDP.

Cell Transfection and Groups

LINC00963 interference vector (sh-LINC00963) and control (sh-NC) were used to perform the role of LINC00963. miR-10a mimic and mimic-NC or si-SKA1 and si-NC were used to perform the effects of miR-10a or SKA1 on TE-1/DDP cells. The rescue experiments were further performed using co-transfection with sh-LINC00963 and pcDNA3.1-SKA1 in TE-1/DDP cells. All cell transfection procedures were performed with the Lipofectamine™ 3000 transfection (Thermo Fisher Scientific, USA).

Tumor Formation Experiment

Experiments of 4–5 weeks BALB/c nude mice were been reviewed by the Animal Protection and Use Committee of our hospital. Mice were randomly divided into TE-1 + sh-NC, TE-1 + sh-LINC00963, TE-1/DDP + sh-NC, and TE-1/DDP + sh-LINC00963 groups (5 per group). 2×10^6 cell suspension was inoculated into the right axillary subcutis, and the tumor volume was detected for 4 weeks. Finally, the mice were anesthetized and sacrificed for tumor weighed.

QRT-PCR

Total RNA was extracted through TRIZOL Reagent (Kangwei Century Biotechnology Co. LTD, Beijing, China) and reversed transcription into cDNA. Real-time PCR was performed by SYBR Green PCR Master Mix (Accurate Biotechnology Co. LTD., Changsha, China). The mRNA level of LINC00963, miR-10a, and SKA1 were tested via $2^{-\Delta\Delta CT}$ with actin or U6 as normalizing control. The primer sequences were as follows: LINC00963: Forward, 5'-GGTAAATCGAGGCCCAGAGAT-3', Reverse: 5'-ACGTGGATGACAGCG TGTGA-3'; miR-10a, : Forward, 5'-GGATACCCTGTAGATCCGAA-3', Reverse, 5'-CAG TGCGTGTCGTGGAGT-3'; SKA1: Forward, 5'-CCTGAACCCGTAAAGAAGCCT-3',

Reverse, 5'-TCATGTACGAAGGAACACCATTG-3'; U6: Forward, 5'-GCTCGCTTC GGCAGCACA-3', Reverse, 5'-GAACGCTTCACGAATTTGCGTG-3'.

CCK-8

Cells digested with trypsin and adjusted for concentration were inoculated at 1×10^4/well in 96-well plates. After incubation for corresponding time (24 h, 48 h, 72, and 96 h), 10 μL CCK-8 (Service Biotechnology Co. LTD., Wuhan, China) was added to each well. Then, incubate for another 4 h and measure the absorbance at 460 nm.

Colony Formation

After digested and resuspended to suspension, 1000 cells/well were cultured until clones were visible to the naked eye. Then, the clones were fixed, stained, and recorded through a digital camera.

Flow Cytometry

After centrifugated and resuspended, 1×10^5 cells suspension was pipetted and incubated with Annexin V-PE/7-AAD. After staining incubation and buffer addition, cell apoptosis was tested using a flow cytometry (Agilent Technologies, CA, USA).

Cells with approximately 80% confluency were trypsin digested, resuspended, centrifuged, and supernatant discarded, and the cell precipitate was washed. Then, cells were fixed in pre-chilled 75% ethanol, centrifuged again to remove the fixative, and washed the precipitate. Finally, the cells were incubated with 1 ml of D-Hanks containing RNase (10 mg/mL) and PI (2 mg/mL), and cell cycle was tested through a flow cytometry (Agilent Technologies, CA, USA).

Scratch test

After the cultured cell ($\sim 1.5 \times 10^5$/well) layers reached confluence, the wounded layers are scored and washed to remove cell debris. Then, low concentration serum medium of 0.5% FBS was added and incubated at 37 °C. The cell migration rate was calculated by fluorescence microscope at 24 and 48 h.

Transwell Assay

Serum-free cell suspensions were prepared and adjusted to 2×10^5/mL. Cell suspension was added to the upper cavity which was coated with matrigel, and 10% FBS was added to the lower cavity. Twenty-four hours later, the invasion cells were fixed, stained, and observed through a light microscope at x100 magnification.

Western Blot

Total proteins extracted from the cells were quantified by BCA protein assay kit (Beyotime, China). After resolved with SDS-PAGE and transferred to PVDF, proteins were sealed

with 5% skim milk and immunodetected with antibodies against SKA1 (1:1000, Abcam, Cambridge, UK) and GAPDH (1:5000, Proteintech, California, USA). GAPDH was used as an internal control. After incubated with peroxidase conjugate antibody containing horseradish and chemiluminescence, protein members were analyzed through Image Lab Software (Bio-Rad, Hercules, USA).

Luciferase Reporter Test

TargetScan7 was used to predict the binding sites. Then, wild-type and mutant 3'UTRs of LINC00963 with miR-10a or miR-10a with SKA1 were obtained by pGL3/Luciferase vector. After cloned downstream into luciferase gene, the assay was performed using the dual luciferase reporting system (Promega, Madison, USA).

RNA Immunoprecipitation (RIP)

RIP assay was tested by EZMagna RIP kit (Millipore Corporation, Burlington, USA). Cell extracts were collected with RIP lysis buffer and incubated with magnetic beads conjugated with anti-argonaute 2 (Ago2) or control anti-IgG antibody. The coprecipitated RNA was purified and analyzed.

Statistical Methods

GraphPad software was applied for data processing and expressed as mean ± SD. Data were studied by *t*-test between two groups and LSD test following ANOVA between multiple groups. *P* less than 0.05 was statistically considered significant.

Results

Silencing LINC00963 Inhibits Cisplatin Resistance in ESCC

The level of LINC00963 in different cells was studied by qRT-PCR, and the content of LINC00963 in TE-1 cells was increased than that in HET-1A cells ($P < 0.05$, Fig. 1A). LINC00963 level was the highest in TE-1/DDP cells ($P < 0.05$, Fig. 1A). Next, LINC00963 was knocked down in TE-1 and TE-1/DDP cell lines. Silencing LINC00963 was found to cause a dramatical decrease of SKA1 in TE-1 and TE-1/DDP cells than that in the sh-NC group ($P < 0.05$, respectively, Fig. 1B). Meanwhile, SKA1 level was slightly lower in TE-1/DDP + sh-LINC00963 cells than that in TE-1 + sh-LINC00963 cells (Fig. 1B). To estimate the role of LINC00963 in vivo, BALB/c nude mice xenograft model was established. As Fig. 1C showed, overtly reduction in tumor growth was found in TE-1 + sh-LINC00963 and TE-1/DDP + sh-LINC00963 groups compared to the sh-NC groups ($P < 0.05$, respectively). And a slight decrease was found in TE-1/DDP + sh-LINC00963 group compared to TE-1 + sh-LINC00963 group (Fig. 1C). The findings suggested that silencing LINC00963 inhibited SKA1 expression and impaired tumorigenicity in ESCC cells, especially in chemoresistent ESCC cells.

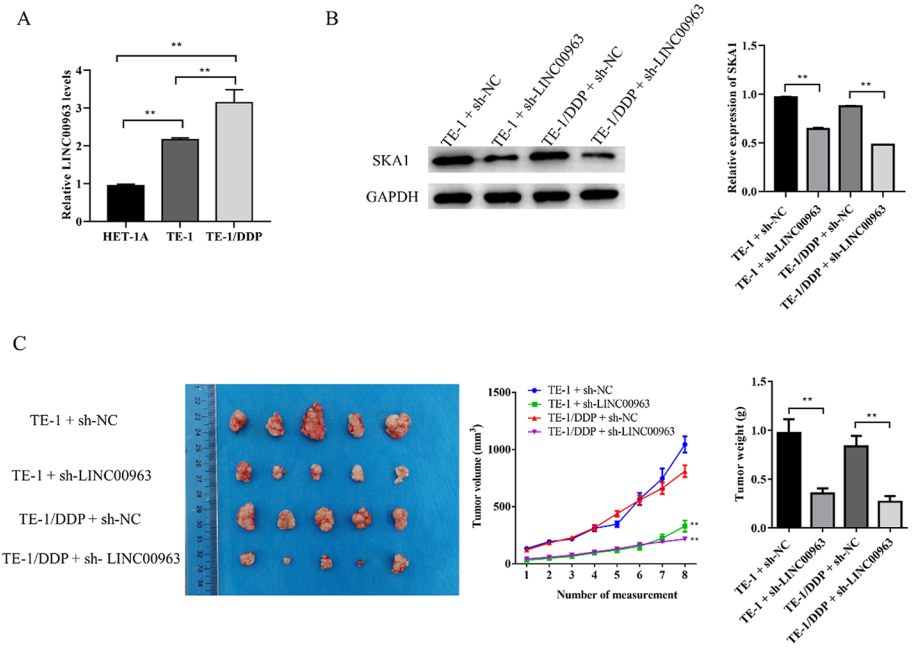

Fig. 1 Silencing LINC00963 inhibits cisplatin resistance in ESCC. **A** QRT-PCR was used to detect the mRNA expression of LINC00963 in HET-1 A, TE-1, and TE-1/DDP cells. **B** Western blot was used to detect the protein expression of SKA1 in TE-1 and TE-1/DDP cells. **C** Tumor volume and tumor weight. $^{**}p < 0.01$ vs. sh-NC group

The Relationship Between LINC00963, miR-10a, and SKA1

To explore the mechanism of LINC00963, the downstream target was identified. The luciferase activity of miR-10a was found to be reduced in WT 3'UTR, but not in Mut 3'UTR ($P < 0.05$, Fig. 2A). Meanwhile, LINC00963 and miR-10a were enriched preferentially in miRNPs containing Ago2 compared to anti-IgG immunoprecipitation (Fig. 2B). These results indicated the direct interaction between LINC00963 and miR-10a. Moreover, the role of miR-10a in SKA1 expression was tested. In Fig. 2C and D, miR-10a mimic obviously alleviated the mRNA and protein expression of SKA1 ($P < 0.01$). Moreover, miR-10a mimic enhanced the level of miR-10a (Fig. 2D). Those findings suggested that miR-10a is a target gene of LINC00963 and regulates SKA1.

LINC00963 Regulates Cisplatin Resistance in ESCC Through Targeting miR-10a

Next, miR-10a was overexpressed in TE-1/DDP cells to assess the role of miR-10a in cell biological behaviors. MiR-10a mimic transfection significantly suppressed the growth of TE-1/DDP cells (Fig. 3A and B, $P < 0.05$). Similarly, the abilities of migration and invasion of TE-1/DDP cells were notably reduced after miR-10a overexpression (Fig. 3C and D, $P < 0.05$). In addition, the apoptosis rate was notably increased in miR-10a mimic group than that in mimic-NC group (Fig. 3E, $P < 0.05$). Moreover, miR-10a overexpression

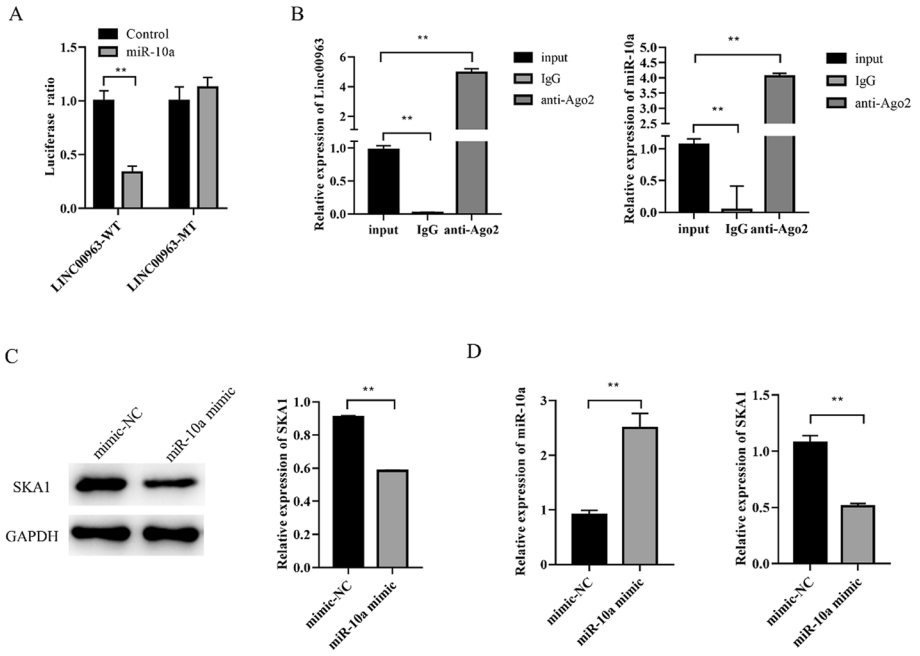

Fig. 2 miR-10a is a target gene of LINC00963 and regulates SKA1. **A** Double luciferase assay. **B** RIP assay. **C** Western blot assay. **D** QRT-PCR assay. $*p < 0.05$, $**p < 0.01$ vs. mimic-NC group

caused cells to arrest in G1 phase (Fig. 3E and F). All those findings indicated that LINC00963 regulates cisplatin resistance in ESCC by targeting miR-10a.

miR-10a Regulates Cisplatin Resistance in ESCC by Targeting SKA1

Further, the relationship between miR-10a and SKA1 was identified. Figure 4A showed that the luciferase activity of SKA1 in WT 3'UTR was decreased, but that of Mut 3'UTR was not ($P < 0.05$), suggesting the direct interaction between SKA1 and miR-10a. Then, si-SKA1 and si-NC were used to test the role of SKA1 on TE-1/DDP cells. Figure 4B showed the successful depletion of SKA1 in TE-1/DDP cells. With the decrease of SKA1, the proliferation and clony formation of TE-1/DDP cells were significantly suppressed ($P < 0.05$, respectively, Fig. 4C and D). Besides, the cell movement was markedly suppressed in SKA1 silencing group ($P < 0.05$, respectively, Fig. 4E and F). Moreover, SKA1 downregulation induced cells to arrest in G1 phase and cell apoptosis (Fig. 4G and H). Those findings suggested that miR-10a regulated cisplatin resistance in ESCC via targeting SKA1.

LINC00963 Promotes Cisplatin Resistance in ESCC by Interacting with miR-10a to Upregulate SKA1

To further clarify the functions of LINC0096, miR-10a, and SKA1 in cisplatin resistance in ESCC, the SKA1 in TE-1/DDP cells was overexpression on the basis of LINC00963 silencing. Compared with control group, growth and movement of TE-1/DDP cells was markedly reduced in sh-LINC00963 group (Fig. 5A–D, $P < 0.05$, respectively).

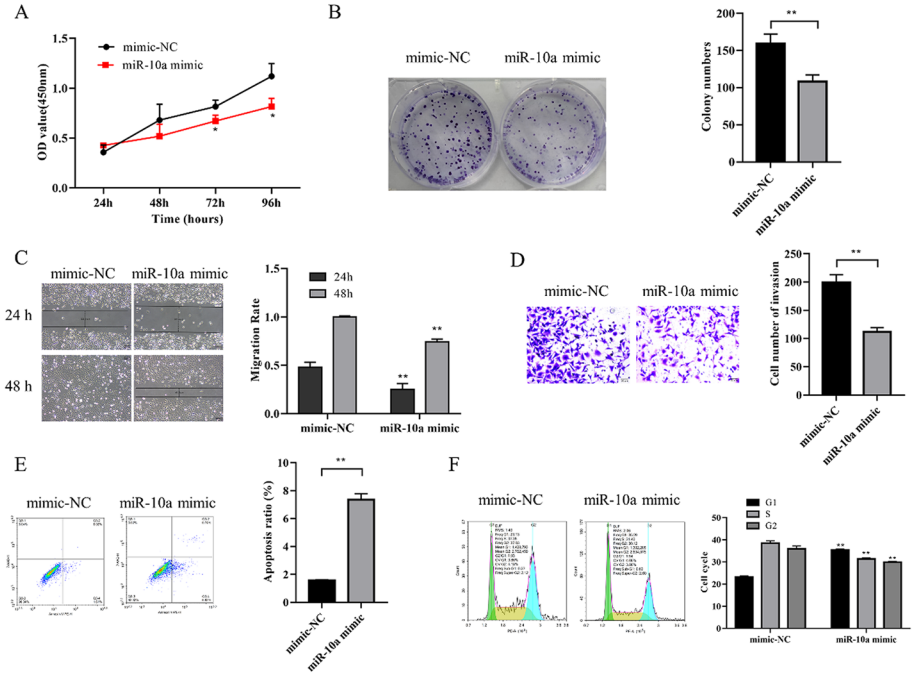

Fig. 3 LINC00963 regulates cisplatin resistance in ESCC through targeting miR-10a. **A** CCK-8 assay. **B** Colony formation assay. **C** Scratch assay. **D** Transwell assay. **E** and **F** Flow cytometry assay. $*p<0.05$, $**p<0.01$ vs. mimic-NC group

However, after cotransfected with sh-LINC00963 and SKA1, growth and movement of TE-1/DDP cells were markably enhanced compared to the sh-LINC00963 + OE-NC group (Fig. 5A–D, $P<0.05$, respectively). Moreover, the ratios of apoptosis and cells arrested in G1 phase were significantly enhanced in sh-LINC00963 group compared to control group (Fig. 5E and F, $P<0.05$, respectively). After SKA1 overexpression, the ratios were obviously reduced compared to the sh-LINC00963 + OE-NC group (Fig. 5E and F, $P<0.05$, respectively). These results displayed that overexpression of SKA1 could reverse the effect of LINC00963 knockdown on biological behaviors of TE-1/DDP cells. Taken together, these results found that LINC00963 regulates TE-1/DDP cells bioactivity and mediates cisplatin resistance by interacting with miR-10a to upregulate SKA1 expression.

Discussion

As a member of non-coding RNAs, LINC00963 is a key component of the transcriptome [22]. Mass of studies have demonstrated that LINC00963 exerts a facilitating role in the malignant progression of many cancers, including promoting cancer cell growth, movement, and suppress apoptosis [17–19]. In patients with hepatocellular carcinoma or cervical cancer, LINC00963 upregulation was correlated with dismal prognosis and clinical features [22, 32]. Besides, a meta-analysis study reported that LINC00963 could serve as a biomarker of prognosis and therapeutical target in pan-cancer [33].

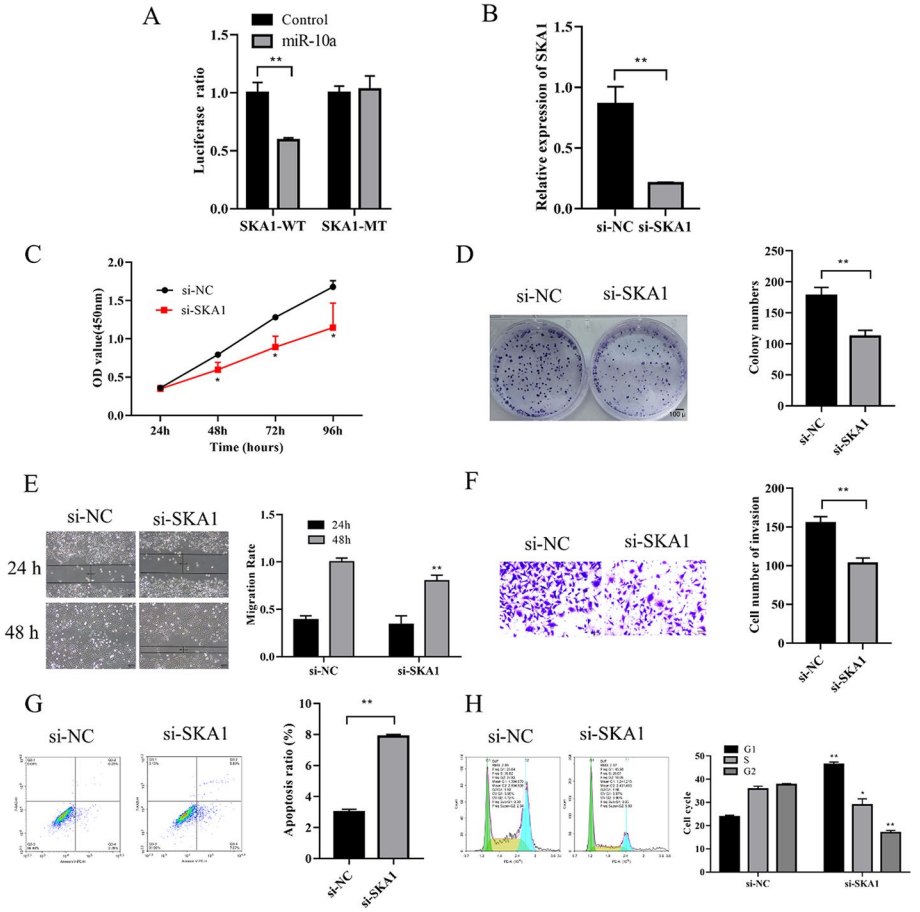

Fig. 4 miR-10a regulates cisplatin resistance in ESCC through targeting SKA1. **A** Double luciferase assay. **B** QRT-PCR assay. **C** CCK-8 assay. **D** Colony formation assay. **E** Scratch assay. **F** Transwell assay. **G** and **H** Flow cytometry assay. $*p < 0.05$, $**p < 0.01$ vs. si-NC group

LINC00963 could combined with EZH2, a histone methyltransferase, to hinder p21 expression, so as to promote glioma progression [34]. Moreover, LINC00963 overexpression could enhance drug resistance in gastric cancer and radioresistance in breast cancer [35, 36]. Besides, lncRNAs can combine with miRNAs and participate in cell cycle and cell death regulation in many malignancies, thereby affecting malignant biological behaviors [37]. Literatures have demonstrated that LINC00963 can serve as a miRNA sponge for at least 9 miRNAs, including miR-608, miR-625, and miR-506 [21, 38, 39]. And LINC00963 was reported to suppress colorectal cancer progression partly by sponging miR-10a [40]. In line with these aforementioned studies, in the present work, we found that LINC00963 expression was increased in TE-1/DDP cells and depletion of LINC00963 could significantly inhibit tumor growth in xenograft model. In addition, luciferase reporter and RIP assays identified a novel direct target, miR-10a, for LINC00963. These findings implied that silencing LINC00963 could inhibited cisplatin resistance in ESCC via miR-10a.

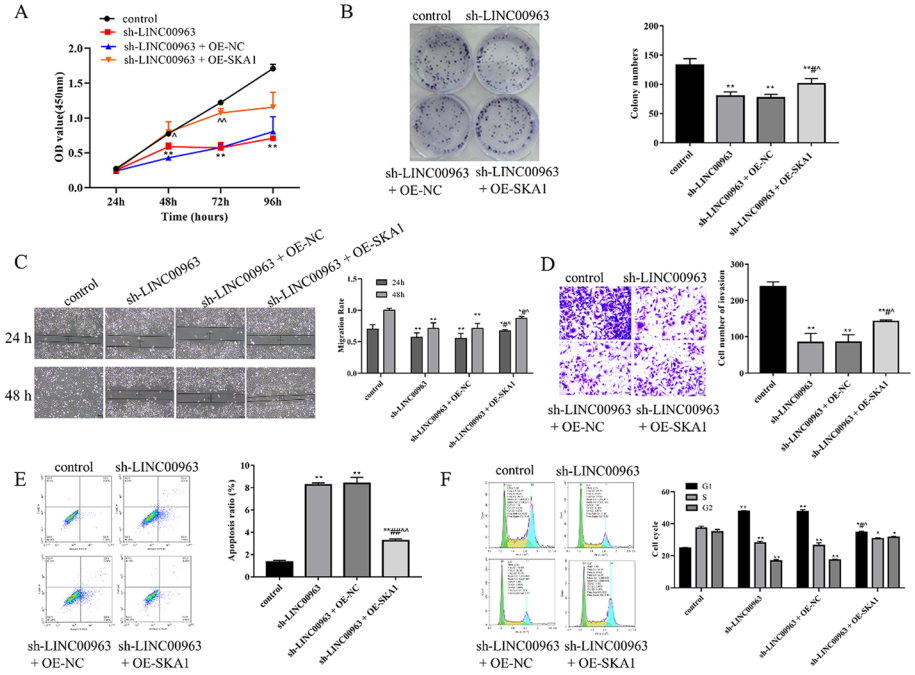

Fig. 5 LINC00963 promotes cisplatin resistance in ESCC through interacting with miR-10a to upregulate SKA1 expression. **A** CCK-8 assay. **B** Colony formation assay. **C** Scratch assay. **D** Transwell assay. **E** and **F** Flow cytometry assay. $^{*}p<0.05$, $^{**}p<0.01$ vs. control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ vs. sh-LINC00963 group; $^{\wedge}p<0.05$, $^{\wedge\wedge}p<0.01$ vs. sh-LINC00963 + OE-NC group

Accumulating evidence has reported the critical role of miRNA-10a in multiple cancers. miR-10a could exert positive or negative function in different cancers. For example, miR-10a was found to be upregulated and promoted metastasis and cell growth in non-small cell lung cancer [41]. Wang et al. revealed that miR-10a could contribute to epithelial-mesenchymal transition and maintaining stem cell stemness in pancreatic cancer [42]. And there are other studies about the positive function in cancer development. However, various literatures have also demonstrated the opposite role of miR-10a. Liu et al. reported that miR-10a-5p was downregulated and hindered the aggressive behaviors of ovarian cancer cells [43]. miR-10a may act as a tumor suppressor in colorectal and prostate cancers [44, 45]. In addition, Liu et al. revealed that miR-10a inhibited malignant behaviors and metastasis of ESCC via Tiam1 in vitro and in vivo [46]. These investigations suggest that miR-10a exerts diverse roles in different cancers. In the present study, miR-10a upregulation obviously suppressed the malignant phenotype of TE-1/DDP cells. Because LINC00963 could not only regulate SKA1 expression but also bind to miR-10a, the relationship between miR-10a and SKA1 was further explored. We found that miR-10a directly targeted SKA1. Silencing SKA1 suppressed the malignant phenotype of TE-1/DDP cells. Obviously, these findings elucidated that LINC00963 exerted its function via miR-10a/SKA1. Further, the following rescue experiments were conducted to validate the point. TE-1/DDP cells were co-transfected with sh-LINC00963 and pcDNA3.1-SKA1. The results showed that overexpression of SKA1 could reverse the effect of LINC00963 knockdown on biological behaviors of

TE-1/DDP cells. These findings suggested that SKA1 overexpression was essential for the cisplatin resistance-promoting effect of LINC00963 in ESCC.

However, there are also some limitations in our work. Only one ESCC cell line (TE-1) was used in this study. Besides, a study of Feng et al. manifested that miR-10a-5p was also sponged by lncRNA FAM83H-AS1 in ESCC [47], suggesting the complexity of lncRNA-miR-10a regulatory network. Therefore, further investigations are needed to unveil the crosstalk of various lncRNAs regulating miR-10a in ESCC.

Conclusion

In sum, this research found that the knockdown of LINC00963 could inhibit the tumorigenicity of TE-1 and TE-1/DDP cells and the possible mechanism is related to inhibit the expression of SKA1 through interacting with miR-10a. These data may have strong implications for understanding the process of cisplatin resistance in ESCC and may help to explain the mechanism of LINC00963 in ESCC. Our work provides a novel idea for therapeutic strategy for chemoresistent ESCC.

Author contribution Dongxin Hu, Anqun Ma,Hongda Lu, Zhen Gao, Yue Yu, Shang Liu, Yancheng Wang and Jiaming Fan contributed to the material preparation, data collection and analyses; Dongxin Hu and Mingyan Zhang contributed to the first draft; All authors contributed to the study conception and design, read and approved the final manuscript.

Funding This work is supported by the Natural Science Foundation of Shandong Province, No. ZR2020QH194.

Data Availability Not applicable.

Declarations

Ethical Approval This study was approved by Shandong Provincial Hospital Affiliated to Shandong University Laboratory Animal Ethics Committee (No. 2019-020).

Consent to Participate All authors have their consent to participate.

Consent for Publication All authors have their consent to publish their work.

Competing Interests The authors declare no competing interests.

References

1. Bollschweiler, E., Plum, P., & Monig, S. P. (2017). Current and future treatment options for esophageal cancer in the elderly. *Expert Opinion on Pharmacotherapy*, *18*(10), 1001–1010. https://doi.org/10.1080/14656566.2017.1334764
2. Lam, A. K. (2020). Introduction: Esophageal squamous cell carcinoma-current status and future advances. *Methods in Molecular Biology*, *2129*, 1–6. https://doi.org/10.1007/978-1-0716-0377-2_1
3. Domper Arnal, M. J., Ferrandez Arenas, A., & Lanas Arbeloa, A. (2015). Esophageal cancer: Risk factors, screening and endoscopic treatment in western and eastern countries. *World Journal of Gastroenterology*, *21*(26), 7933–7943. https://doi.org/10.3748/wjg.v21.i26.7933

4. Hu, D., Li, Z., Li, X., & Fu, H. (2019). SKA1 overexpression is associated with the prognosis of esophageal squamous cell carcinoma and regulates cell proliferation and migration. *International Journal of Molecular Medicine*, *44*(5), 1971–1978. https://doi.org/10.3892/ijmm.2019.4343
5. Akiyama, Y., Iwaya, T., Endo, F., Chiba, T., Takahara, T., Otsuka, K., Nitta, H., Koeda, K., Mizuno, M., Kimura, Y., & Sasaki, A. (2018). Investigation of operative outcomes of thoracoscopic esophagectomy after triplet chemotherapy with docetaxel, cisplatin, and 5-fluorouracil for advanced esophageal squamous cell carcinoma. *Surgical Endoscopy*, *32*(1), 391–399. https://doi.org/10.1007/s00464-017-5688-5
6. Hirata, H., Niida, A., Kakiuchi, N., Uchi, R., Sugimachi, K., Masuda, T., Saito, T., Kageyama, S. I., Motomura, Y., Ito, S., Yoshitake, T., Tsurumaru, D., Nishimuta, Y., Yokoyama, A., Hasegawa, T., Chiba, K., Shiraishi, Y., Du, J., Miura, F., Morita, M., Toh, Y., Hirakawa, M., Shioyama, Y., Ito, T., Akimoto, T., Miyano, S., Shibata, T., Mori, M., Suzuki, Y., Ogawa, S., & Ishigami, K., Mimori, K. (2021). The evolving genomic Landscape of esophageal squamous cell Carcinoma under Chemoradiotherapy. *Cancer Research*, *81*(19), 4926–4938. https://doi.org/10.1158/0008-5472.CAN-21-0653
7. Bridges, M. C., Daulagala, A. C., & Kourtidis, A. (2021). LNCcation: lncRNA localization and function. *Journal of Cell Biology*, *220*(2). https://doi.org/10.1083/jcb.202009045
8. Evans, J. R., Feng, FY&, & Chinnaiyan, A. M. (2016). The bright side of dark matter: lncRNAs in cancer. *J Clin Invest*, *126*(8), 2775–2782. https://doi.org/10.1172/JCI84421
9. Meng, Q., Li, Z., & Pan, J. (2020). Long noncoding RNA DUXAP8 regulates proliferation and apoptosis of ovarian cancer cells via targeting miR-590-5p. *Human Cell*, *33*(4), 1240–1251. https://doi.org/10.1007/s13577-020-00398-8
10. Luo, Y., Zheng, S., Wu, Q., Wu, J., Zhou, R., Wang, C., Wu, Z., Rong, X., Huang, N., Sun, L., Bin, J., Liao, Y., & Shi, M. (2021). Long noncoding RNA (lncRNA) EIF3J-DT induces chemoresistance of gastric cancer via autophagy activation. *Autophagy*, *17*(12), 4083–4101. https://doi.org/10.1080/15548627.2021.1901204
11. Xue, S. T., Zheng, B., Cao, S. Q., Ding, J. C., Hu, G. S., Liu, W., & Chen, C. (2022). Long non-coding RNA LINC00680 functions as a ceRNA to promote esophageal squamous cell carcinoma progression through the miR-423-5p/PAK6 axis. *Molecular Cancer*, *21*(1), 69. https://doi.org/10.1186/s12943-022-01539-3
12. Cui, Y., Zhang, C., Ma, S., Li, Z., Wang, W., Li, Y., Ma, Y., Fang, J., Wang, Y., Cao, W., & Guan, F. (2021). RNA m6A demethylase FTO-mediated epigenetic up-regulation of LINC00022 promotes tumorigenesis in esophageal squamous cell carcinoma. *Journal of Experimental & Clinical Cancer Research : Cr*, *40*(1), 294. https://doi.org/10.1186/s13046-021-02096-1
13. Tang, J., Xu, H., Liu, Q., Zheng, J., Pan, C., Li, Z., Wen, W., Wang, J., Zhu, Q., & Wang, Z. (2021). LncRNA LOC146880 promotes esophageal squamous cell carcinoma progression via miR-328-5p/FSCN1/MAPK axis. *Aging (Albany NY)*, *13*(10), 14198–14218. https://doi.org/10.18632/aging.203037
14. Niu, Y., Guo, Y., Li, Y., Shen, S., Liang, J., & Guo, W. (2022). LncRNA GATA2-AS1 suppresses esophageal squamous cell carcinoma progression via the mir-940/PTPN12 axis. *Experimental Cell Research*, *416*(2), 113130. https://doi.org/10.1016/j.yexcr.2022.113130
15. Huang, J., Li, J., Li, Y., Lu, Z., Che, Y., Mao, S., Lei, Y., Zang, R., Zheng, S., Liu, C., Wang, X., Li, N., Sun, N., & He, J. (2019). Interferon-inducible lncRNA IRF1-AS represses esophageal squamous cell carcinoma by promoting interferon response. *Cancer Lett, 459*, 86–99. https://doi.org/10.1016/j.canlet.2019.05.03
16. Zhang, H., Pan, E., Zhang, Y., Zhao, C., Liu, Q., & Pu, Y. (2022). LncRNA RPL34-AS1 suppresses the proliferation, migration and invasion of esophageal squamous cell carcinoma via targeting miR-575/ACAA2 axis. *Bmc Cancer*, *22*(1), 1017. https://doi.org/10.1186/s12885-022-10104-6
17. Yu, T., Zhao, Y., Hu, Z., Li, J., Chu, D., Zhang, J., Li, Z., Chen, B., Zhang, X., Pan, H., Li, S., Lin, H., Liu, L., Yan, M., & He, X. (2017). MetaLnc9 facilitates Lung Cancer Metastasis via a PGK1-Activated AKT/mTOR pathway. *Cancer Research*, *77*(21), 5782–5794. https://doi.org/10.1158/0008-5472.CAN-17-0671
18. Wu, J. H., Tian, X. Y., An, Q. M., Guan, X. Y., & Hao, C. Y. (2018). LINC00963 promotes hepatocellular carcinoma progression by activating PI3K/AKT pathway. *European Review for Medical and Pharmacological Sciences*, *22*(6), 1645–1652. https://doi.org/10.26355/eurrev_201803_14574
19. Jiao, H., Jiang, S., Wang, H., & Li, Y. (2018). Upregulation of LINC00963 facilitates melanoma progression through miR-608/NACC1 pathway and predicts poor prognosis. *Biochemical and Biophysical Research Communications*, *504*(1), 34–39. https://doi.org/10.1016/j.bbrc.2018.08.115
20. Hu, R., Xu, B., Ma, J., Li, L., Zhang, L., Wang, L., Zhu, J., Guo, T., Zhang, H., & Wang, S. (2023). LINC00963 promotes the malignancy and metastasis of lung adenocarcinoma by stabilizing Zeb1

and exosomes-induced M2 macrophage polarization. *Molecular Medicine*, *29*(1), 1. https://doi.org/10.1186/s10020-022-00598-y
21. Zuo, W., Zhou, K., Deng, M., Lin, Q., Yin, Q., Zhang, C., Zhou, J., & Song, Y. (2020). LINC00963 facilitates acute myeloid leukemia development by modulating miR-608/MMP-15. *Aging (Albany NY)*, *12*(19), 18970–18981. https://doi.org/10.18632/aging.103252
22. Xie, Z., Zhong, C., Shen, J., Jia, Y., & Duan, S. (2022). LINC00963: A potential cancer diagnostic and therapeutic target. *Biomedicine & Pharmacotherapy*, *150*, 113019. https://doi.org/10.1016/j.biopha.2022.113019
23. Arai, T., Okato, A., Kojima, S., Idichi, T., Koshizuka, K., Kurozumi, A., Kato, M., Yamazaki, K., Ishida, Y., Naya, Y., Ichikawa, T., & Seki, N. (2017). Regulation of spindle and kinetochore-associated protein 1 by antitumor miR-10a-5p in renal cell carcinoma. *Cancer Science*, *108*(10), 2088–2101. https://doi.org/10.1111/cas.13331
24. Song, G. Q., He, T. L., Ji, K. J., Duan, Y. M., Zhang, J. W., & Hu, G. Q. (2022). SKA1/2/3 is a biomarker of poor prognosis in human hepatocellular carcinoma. *Frontiers in Oncology*, *12*, 1038925. https://doi.org/10.3389/fonc.2022.1038925
25. Sun, W., Yao, L., Jiang, B., & Guo, L. (2014). Spindle and kinetochore-associated protein 1 is overexpressed in gastric cancer and modulates cell growth. *Molecular and Cellular Biochemistry*, *391*(1–2), 167–174. https://doi.org/10.1007/s11010-014-1999-1
26. Chen, C., Guo, Q., Song, Y., & Xu, G. (2020). SKA1/2/3 serves as a biomarker for poor prognosis in human lung adenocarcinoma. *Transl Lung Cancer Res*, *9*(2), 218–231. https://doi.org/10.21037/tlcr.2020.01.20
27. Chen, Y., Zhao, J., Jiao, Z., Wang, W., Wang, D., Yu, X., Shi, Z., Ge, N., Pan, Q., Xia, J., Niu, W., Zhao, R., Zhang, X., & Du, W. (2018). SKA1 overexpression is associated with poor prognosis in hepatocellular carcinoma. *Bmc Cancer*, *18*(1), 1240. https://doi.org/10.1186/s12885-018-5119-6
28. Dong, D., Mu, Z., Wei, N., Sun, M., Wang, W., Xin, N., & Shao, Y. (2019). Long non-coding RNA ZFAS1 promotes proliferation and metastasis of clear cell renal cell carcinoma via targeting miR-10a/SKA1 pathway. *Biomedicine & Pharmacotherapy*, *111*, 917–925. https://doi.org/10.1016/j.biopha.2018.12.143
29. Shen, D., Zhao, H. Y., Gu, A. D., Wu, Y. W., Weng, Y. H., Li, S. J., Song, J. Y., Gu, X. F., Qiu, J., & Zhao, W. (2021). miRNA-10a-5p inhibits cell metastasis in hepatocellular carcinoma via targeting SKA1. *Kaohsiung Journal of Medical Sciences*, *37*(9), 784–794. https://doi.org/10.1002/kjm2.12392
30. Xiao, J., & Yu, H. (2019). LINC00339 promotes growth and invasiveness of hepatocellular carcinoma by the miR-1182/SKA1 pathway. *Onco Targets Ther*, *12*, 4481–4488. https://doi.org/10.2147/OTT.S207397
31. Yamada, Y., Arai, T., Kojima, S., Sugawara, S., Kato, M., Okato, A., Yamazaki, K., Naya, Y., Ichikawa, T., & Seki, N. (2018). Anti-tumor roles of both strands of the miR-455 duplex: Their targets SKA1 and SKA3 are involved in the pathogenesis of renal cell carcinoma. *Oncotarget*, *9*(42), 26638–26658. https://doi.org/10.18632/oncotarget.25410
32. Chang, A., & Wang, P. (2022). LINC00963 may be Associated with a poor prognosis in patients with cervical Cancer. *Medical Science Monitor*, *28*, e935070. https://doi.org/10.12659/MSM.935070
33. He, J., Wang, Z., Wang, Y., Liu, F., Fu, L., Jiang, X., Wang, P., Chen, H., Hu, M., & Cai, H. (2023). A systematic review and meta-analysis of long noncoding RNA 00963 expression and prognosis and clinicopathological characteristic in human cancers. *Pathology, Research and Practice*, *242*, 154291. https://doi.org/10.1016/j.prp.2022.154291
34. Gai, Q., & Guo, W. (2021). EZH2-mediated long-chain non-coding RNA LINC00963 promotes proliferation and invasion of glioma cells through inhibiting p21 expression. *J BUON, 26*(2):380–387.
35. Zhang, N., Zeng, X., Sun, C., Guo, H., Wang, T., Wei, L., Zhang, Y., & Zhao, J. (2019). LncRNA LINC00963 promotes tumorigenesis and radioresistance in breast Cancer by sponging mir-324-3p and inducing ACK1 expression. *Mol Ther Nucleic Acids*, *18*, 871–881. https://doi.org/10.1016/j.omtn.2019.09.033
36. Hou, M., & Li, C. (2021). LINC00963/miR-4458 regulates the effect of oxaliplatin in gastric cancer by mediating autophagic flux through targeting of ATG16L1. *Scientific Reports*, *11*(1), 20951. https://doi.org/10.1038/s41598-021-98728-9
37. Wu, X., Sui, Z., Zhang, H., & Wang, Y. (2020). Integrated Analysis of lncRNA-Mediated ceRNA Network in Lung Adenocarcinoma. *Frontiers in Oncology*, *10*, 554759. https://doi.org/10.3389/fonc.2020.554759
38. Wu, Z., Wang, W., Wang, Y., Wang, X., Sun, S., Yao, Y., & Zhang, Y. (2020). Long noncoding RNA LINC00963 promotes breast cancer progression by functioning as a molecular sponge for microRNA-625 and thereby upregulating HMGA1. *Cell Cycle*, *19*(5), 610–624. https://doi.org/10.1080/15384101.2020.1728024

39. Ye, F., Xu, R., Ge, Y., Zheng, Y., Liu, X., & Deng, P. (2020). LINC00963 confers oncogenic properties in Glioma by regulating the miR-506/BCAT1 Axis. *Cancer Manag Res*, *12*, 2339–2351. https://doi.org/10.2147/CMAR.S246332
40. Ghaemi, Z., & Mowla, S. J. (2023). Novel splice variants of LINC00963 suppress colorectal cancer cell proliferation via miR-10a/miR-143/miR-217/miR-512-mediated regulation of PI3K/AKT and Wnt/beta-catenin signaling pathways. *Biochim Biophys Acta Gene Regul Mech*, *1866*(2), 194921. https://doi.org/10.1016/j.bbagrm.2023.194921
41. Yu, T., Liu, L., Li, J., Yan, M., Lin, H., Liu, Y., Chu, D., Tu, H., & Gu, A. (2015). MiRNA-10a is upregulated in NSCLC and may promote cancer by targeting PTEN. *Oncotarget*, *6*(30), 30239–30250. https://doi.org/10.18632/oncotarget.4972
42. Wang, C., & Yin, W. (2020). MicroRNA-10a promotes epithelial-to-mesenchymal transition and stemness maintenance of pancreatic cancer stem cells via upregulating the Hippo signaling pathway through WWC2 inhibition. *Journal of Cellular Biochemistry*, *121*(11), 4505–4521. https://doi.org/10.1002/jcb.29716
43. Liu, L. J., Sun, X. Y., & Yang, C. X. (2021). MiR-10a-5p restrains the aggressive phenotypes of ovarian cancer cells by inhibiting HOXA1. *Kaohsiung Journal of Medical Sciences*, *37*(4), 276–285. https://doi.org/10.1002/kjm2.12335
44. Liu, Y., Zhang, Y., Wu, H., Li, Y., Zhang, Y., Liu, M., & Li, X. (2017). miR-10a suppresses colorectal cancer metastasis by modulating the epithelial-to-mesenchymal transition and anoikis. *Cell Death and Disease*, *8*(4), e2739. https://doi.org/10.1038/cddis.2017.61
45. Mu, H., Xiang, L., Li, S., Rao, D., & Wang, S. (2019). MiR-10a functions as a tumor suppressor in prostate cancer via targeting KDM4A. *Journal of Cellular Biochemistry*, *120*(4), 4987–4997. https://doi.org/10.1002/jcb.27774
46. Liu, Y., Wang, X., Jiang, X., Yan, P., Zhan, L., Zhu, H., Wang, T., & Wen, J. (2019). Tumor-suppressive microRNA-10a inhibits cell proliferation and metastasis by targeting Tiam1 in esophageal squamous cell carcinoma. *Journal of Cellular Biochemistry*, *120*(5), 7845–7857. https://doi.org/10.1002/jcb.28059
47. Zhu, H., & Du, F. (2021). Restoration of circPSMC3 sensitizes gefitinib-resistant esophageal squamous cell carcinoma cells to gefitinib by regulating miR-10a-5p/PTEN axis. *Cell Biology International*, *45*(1), 107–116. https://doi.org/10.1002/cbin.11473

Authors and Affiliations

Dongxin Hu[1] · Anqun Ma[1] · Hongda Lu[1] · Zhen Gao[1] · Yue Yu[1] · Jiaming Fan[1] · Shang Liu[1] · Yancheng Wang[1] · Mingyan Zhang[2]

✉ Mingyan Zhang
zhangmingyan@sdfmu.edu.cn

[1] Department of Thoracic Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250021, China

[2] Department of Gastroenterology and Hepatology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324, Jing Wu Road, Huaiyin District, Jinan, Shandong 250021, China