Extracellular Vesicles Long Non-Coding RNA AGAP2-AS1 Contributes to Cervical Cancer Cell Proliferation Through Regulating the miR-3064-5p/SIRT1 Axis

Min Li1*, Jing Wang2, Hongli Ma3, Li Gao3, Kuxiang Zhao4 and Tingting Huang3

1 Pathology Department, Jinan Second Maternal and Child Health Care Hospital, Jinan, China, 2 The Second Children & Women’s Healthcare of Jinan City, Jinan, China, 3 Department of Obstetrics, Tai’an City Central Hospital, Tai’an, China, 4 Department of Obstetrics and Gynecology, Pengquan Community Health Service Center, Jinan, China

Cervical cancer is one of the most severe and prevalent female malignancies and a global health issue. The molecular mechanisms underlying cervical cancer development are poorly investigated. As a type of extracellular membrane vesicles, EVs from cancer cells are involved in cancer progression by delivering regulatory factors, such as proteins, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs). In this study, we identified an innovative function of extracellular vesicle (EV) lncRNA AGAP2-AS1 in regulating cervical cancer cell proliferation. The EVs were isolated from the cervical cancer cells and were observed by transmission electron microscopy (TEM) and were confirmed by analyzing exosome markers. The depletion of AGAP2-AS1 by siRNA significantly reduced its expression in the exosomes from cervical cancer and in the cervical cancer treated with AGAP2-AS1-knockdown exosomes. The expression of AGAP2-AS1 was elevated in the clinical cervical cancer tissues compared with the adjacent normal tissues. The depletion of EV AGAP2-AS1 reduced cell viabilities and Edu-positive cervical cancer cells, while it enhanced cervical cancer cell apoptosis. Tumorigenicity analysis in nude mice showed that the silencing of EV AGAP2-AS1 attenuated cervical cancer cell growth in vivo.

Regarding the mechanism, we identified that AGAP2-AS1 increased SIRT1 expression by sponging miR-3064-5p in cervical cancer cells. The overexpression of SIRT1 or the inhibition of miR-3064-5p reversed EV AGAP2-AS1 depletion-inhibited cancer cell proliferation in vitro. Consequently, we concluded that EV lncRNA AGAP2-AS1 contributed to cervical cancer cell proliferation through regulating the miR-3064-5p/SIRT1 axis. The clinical values of EV lncRNA AGAP2-AS1 and miR-3064-5p deserve to be explored in cervical cancer diagnosis and treatments.

Keywords: cervical cancer, proliferation, lncRNA AGAP2-AS1, miR-3064-5p, sirtuin 1 (SIRT1), extracellular vesicles
INTRODUCTION

Cervical cancer ranks the top five of female malignancy globally with a high incidence and represents a primary global health issue (1). It is estimated that there are over half a million new cases and approximately 300,000 dead cases every year (1). The incidence of cervical cancer is closely related with the chronic infection of human papilloma virus (HPV), which leads to almost all cases of cervical cancer. Hence, the prevention of HPV is a primary manner for prevention of cervical cancer, which mostly relies on the HPV vaccines (2). Surgical operation is a standard treatment for patients at early stage (3). However, radiotherapy and/or chemotherapy and anti-angiogenesis therapy were applied to those with advanced cancer stages or metastasis (3, 4). Nevertheless, the developed drug resistance highlights the importance of developing novel therapeutic strategies.

For the past decade, extracellular vesicles (EVs) have been identified as a new manner for the communication between cells (5). Accumulating research studies have indicated that EVs derived from cancer cells played critical role in development and progression of various cancers, due to their capacity of delivering from cancer cells played critical role in development and transcriptional and transcriptional regulation, while the most frequently reported function of lncRNAs is as a sponge of miRNA (8). Among the lncRNAs related to cancer progression, lncRNA AGAP2-AS1 was recently recognized as a prognostic biomarker and oncogenic factor in glioblastoma multiforme (9). Dong et al. also suggested that AGAP2-AS1 mediated the chemoresistance in breast cancer (10), whereas its function during the development of cervical cancer is not clear.

Noteworthy, lncRNAs exhibit their functions through multiple biological regulatory manners, including epigenetic regulation, transcriptional and transcriptional regulation, while the most frequently reported function of lncRNAs is as a sponge of miRNA (8). MiRNAs also belong to the big family of non-coding RNAs, identified by the short and conservative sequence around 20 nucleotides, and were related to cancers due to their function of targeting mRNAs and impeding gene expression (11, 12). MiR-3064-5p was previously reported to function as competing RNA of lnc PXN-AS1 to mediate the suppression of pancreatic cancer development (13). Zhang et al. reported that expression of miR-3064-5p apparently reduced cancer sections of liver cancer patients and negatively correlated with the levels of proangiogenic factors (14). Sirtuin 1 (SIRT1) was previously verified to be overexpressed in cervical cancer, as well as regulating cell proliferation and apoptosis, by multiple studies (15, 16). For instance, SIRT1 mediated the beta2-AR associated chemoresistance of cervical cancer (17). So and colleagues determined that SIRT1 was overexpressed in cervical cancer cells infected with HPV and may function through impeding antiviral immunity (16). In this study, we identified an elevation of EV lncRNA AGAP2-AS1 in cervical cancer and clarified its function to interact with and regulate the miR-3064-5p/SIRT1 axis, which further facilitated the progression of cervical cancer. Our study provided novel evidence for participation of EV lncRNAs in the pathogenesis of cervical cancer and highlighted their role as potential therapeutic targets.

MATERIALS AND METHODS

Collection of Patient Samples

Human cervical cancer cell lines Hela (HPV-18 positive) and C33A (HPV negative) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), cultured in high glucose-DMEM supplied with 10% fetal bovine serum (FBS, Hyclone, USA) and 1% penicillin/streptomycin (SolarBio, China). The cells were set in a 37°C incubator with humidified 5% CO2. All cervical cells used in this work were within 30 passages.

The small interfering RNA targeting AGAP2-AS1 (si-AGAP2-AS1), miR-3064-5p mimics, miR-3064-5p inhibitor, and their corresponding scramble controls (NCs), as well as the overexpressing vector pCMV-SIRT1 were synthesized and purchased from RiboBio (China). Lipofectamine 2000 was adopted to conduct transfection in accordance with the manufacturer’s protocol. Briefly, Hela and C33A were seeded in six-well plates or culturing dishes to form a 70% confluence. The siAGAP2-AS1 (50 nM), miR-3064-5p mimics (50 nM), miR-3064-5p inhibitor (50 nM), pCMV-SIRT1 (0.75 μg), or the NCs were mixed with lipofectamine 2000 in a Opti-MEM for 20 min. The mixture was added to each well, and 48 h later, the cells were collected for the following experiments.

Isolation and Identification of EVs

EVs were isolated from the supernatant of Hela cells after 2 days’ culture in exosome-free medium by using a Total exosome separation kit (Thermo, USA) following the manufacturer’s protocol. The identification of isolated EVs through morphology

| Variables | Number |
|-----------|--------|
| Age       |        |
| <50 years | 18     |
| ≥50 years | 32     |
| Histology |        |
| Squamous cell carcinoma | 44     |
| Adenocarcinoma           | 6      |
| HPV infection           |        |
| Positive               | 36     |
| Negative              | 14     |
| Total                 | 50     |
was realized by transmission electron microscopy (TEM) (Philips, Holland). In brief, one drop of isolated sample was put on cleaned copper grid covered with carbon (200 mesh) for TEM. The grids were washed by Milli-Q water and subsequently stained by uranyl acetate for 1 min. After that, the grids were air-dried and observed by TEM. The size distribution of EVs was detected by using Nanoparticle Tracking Analysis (NTA) (Malvern, Germany). The biomarkers of EVs, including CD63, CD9, CD54, Annexin, and negative biomarker Calnexin were measured by western blotting. For in vitro study, the cells were cultured in exosome-free medium, and 5 µg/ml of extracted EVs was added with RNase A (1 µg/ml) every 48 h.

**Exosome Internalization**

EVs were labeled with PKH67 (Sigma) following the manufacturer’s description. Cervical cancer cells were seeded in 24-well plates, followed by incubation with EVs (5 µg/100 µl PBS) for 48 h. Rhodamine phalloidin (Sigma) was adopted to label actin fibers. For each sample, five random fields were photographed by fluorescence microscope (Leica, Germany).

**Fluorescence In Situ Hybridization Analysis**

Localization of lncAGAP2-AS1 was determined by FISH assay. The FISH probe against lncAGAP2-AS1 was obtained from Gene Pharma (China). Cervical cells were fixed in 4% PFA, washed with PBS, treated with pepsin, dehydrated with ethanol, followed by incubation with FISH probe in the hybridization buffer. After that, the cells were washed and observed with Prolong Gold Antifade Reagent with DAPI. The staining was visualized under fluorescence microscope (Leica, Germany).

**Western Blotting**

Total protein was extracted from EVs, cervical cancer cells after indicated transfection, or tumor samples by using a RIPA lysis buffer (SolarBio, China) containing protease inhibitors (Sigma, USA). The total protein level was detected by using BCA kit (Beyotime). Cells were collected, washed with PBS, and stained with pepsin, dehydrated with ethanol, followed by incubation with FISH probe in the hybridization buffer. After that, the cells were washed and observed with Prolong Gold Antifade Reagent with DAPI. The staining was visualized under fluorescence microscope (Leica, Germany).

**Apoptosis**

The apoptosis of transfected Hela and C33A cells was determined by using an Annexin V-FITC/PI dual staining kit (Beyotime). Cells were collected, washed with PBS, and stained in FITC-conjugated AV and PI solution for 15 min in dark. After staining, the cells were immediately analyzed in a C6 flow cytometer (BD Biosciences, USA). The data were analyzed by FlowJo software, and the cells in LR and UR were defined as early and late phase apoptotic cells.

**RNA Immunoprecipitation**

The RIP assay was performed by using a Magna RIP kit (Millipore, USA) according to the manufacturer’s description. Briefly, the cells were lysed, and the supernatant was incubated with Dynabeads coated with Ago2 antibody or IgG antibody conjugated dynabeads (Invitrogen) at 4°C for 10 h. Subsequently, the beads were washed and incubated with proteinase K. The RNA was extracted and examined by qPCR.

**Pull-Down Assay**

Biotin-labeled wild-type miR-3064-5p (Bio-miR-3064-5p-WT), mutated miR-3064-5p (Bio-miR-3064-5p-MUT), and negative control (Bio-NC) were synthesized by GenePharma. Hela and C33A cells were transfected with these probes for 48 h, harvested, and lysed. The cell extracts were then incubated with magnetic
beads (Sigma) at 4°C for 3 h, followed by washing with PBS and qPCR detection of AGAP2-AS1.

Xenograft Tumor Model
To determine in vivo tumor growth, Hela cells (1 × 10⁶) were subcutaneously injected into flanks of 6-week aged female SCID/ Nude mice (n = 5) at a total number of 1 × 10⁶ cells. When the tumor size reached 100 mm³, EVs (15 µg in 20 µl PBS) were injected into the tumor sites every three days. Tumor size and body weight of mice were measured as indicated. The tumor volume was calculated with following formula: ½ × length × width². At the end time point, the mice were sacrificed, and the tumors were isolated for following experiments. All animal experimental protocols were approved by the Ethics Committee of our hospital.

Dual Luciferase Reporter Assay
The interactions between miR-3064-5p with IncRNA AGAP2-AS1 and SIRT1 were predicted by online website ENCORI and confirmed by the dual luciferase reporter assay. The sequences of IncAGAP2-AS1 and the 3'UTR of SIRT1 were inserted into the pmirGLO plasmid (Promega, USA) to generate AGAP2-AS1-WT and SIRT1-WT, separately. Similarly, the site-specific mutation was performed to generate the AGAP2-AS1-Mut and SIRT1-Mut. Hela and C33A cells were transfected with the WT or Mut together with miR-3064-5p mimics or NC for 48 h. The pRLTK vector (Promega) was also co-transfected as the internal control. The values of luciferase activity were measured by a luciferase detection kit (Promega).

Statistics
Data in our study are presented as mean ± standard deviation of three independent experiments and analyzed by GraphPad software. The statistical differences were defined by p values lower than 0.05, evaluated by a Student’s t-test or one-way ANOVA analysis. The correlation among AGAP2-AS1, miR-3064-5p, and SIRT1 in cervical tumors was conducted by Pearson analysis.

RESULTS
Cervical Cancer Transferees AGAP2-AS1 by EVs
Firstly, the EVs were isolated from the Hela and C33A cells and were observed by TEM (Figure 1A). Meanwhile, we also confirmed the overexpression of exosome markers, such as CD63, CD9, CD54, and Annexin, and negative expression of intracellular biomarker Calnexin in the EVs from Hela and C33A cells (Figure 1B and Figure S1A). Besides, NTA analysis demonstrated that the size of particle from extracts mainly distributed around 100 nm (Figure 1C). Through FISH assay with specific probe for IncAGAP2-AS2, we determined that IncAGAP2-AS2 mainly localized in the nuclei section of cervical cells (Figure S1B). Next, the expression of AGAP2-AS1 was measured in cell culture medium of Hela and C33A cells treated with RNase A or co-treated with RNase A and Triton X-100. We observe the stable AGAP2-AS1 expression under the treatment of RNase A, while was remarkably decreased under the simultaneous co-treatment of RNase A with Triton X-100 (Figure 1D), which manifested that the EVs were broken by Triton X-100, and the IncRNAs inside the EVs were degraded by RNase. Hela and C33A cells were transfected with PKH67 labeled-EVs, and the green fluorescence inside Rhodamine-stained cytoskeleton indicated the internalization of EVs by cervical cells (Figure 1E). Meanwhile, the depletion of AGAP2-AS1 by siRNA significantly reduced its expression in the EVs from Hela and C33A cells (Figure 1F). In addition, the Hela and C33A cells were treated with EVs from Hela and C33A cells transfected with AGAP2-AS1 siRNA, and we observed a significantly down-regulated AGAP2-AS1 expression under the treatment (Figure 1G). As shown in Figure 1H, treatment with EVs extracted from the siRNA control-transfected group also notably increased the expression of AGAP2-AS1 in Hela and C33A cells, compared with treatment with PBS.

EV AGAP2-AS1 Contributes to Cervical Cancer Cell Proliferation In Vitro and In Vivo
Then, we determined the correlation of EV AGAP2-AS1 with cervical cancer. Our data showed that the expression of AGAP2-AS1 was elevated in the EVs derived from clinical cervical cancer tissues (n = 50) relative to the adjacent normal tissues (n = 50) (Figure 2A). Next, the Hela and C33A cells were treated with EVs from Hela and C33A cells transfected with AGAP2-AS1 siRNA, SIRT1 siRNA control, or the non-transfected cells, and the silencing of AGAP2-AS1 repressed cell viabilities of Hela and C33A cells (Figures S1C, D). The efficacy of transfection was manifested by notably decreased levels of AGAP2-AS1 in cell extraction and EVs collected from Hela and C33A cells (Figures S1E, F). The depletion of EV AGAP2-AS1 reduced the Edu-positive Hela and C33A cells (Figures 2D, E). Hela and C33A cell apoptosis was enhanced by the knockdown of AGAP2-AS1 (Figures 2F, G). The effect of EV AGAP2-AS1 on cervical cancer cell growth was also analyzed in vivo in the nude mice. Tumorigenicity analysis showed that the depletion of EV AGAP2-AS1 significantly inhibited tumor volume and tumor weight in the nude mice (Figures 2H, I). Moreover, the AGAP2-AS1 levels were decreased in tumors treated with siAGAP2-AS1-depleted EVs (Figure 2J).

AGAP2-AS1 Interacts With miR-3064-5p
Regarding the mechanism, we identified a binding site between AGAP2-AS1 and miR-3064-5p (Figure 3A). The treatment of miR-3064-5p mimic was able to significantly enhance the miR-3064-5p expression (Figure 3B) and repress the luciferase activity of AGAP2-AS1 in the Hela and C33A cells (Figure 3C). Meanwhile, the silencing of AGAP2-AS1 by siRNA could increase miR-3064-5p expression in the Hela and
C33A cells (Figure 3D). To further identify the direct interaction between AGAP2-AS1 and miR-3064-5p, we also conducted RIP and RNA pulldown experiments. As shown in Figure 3E, the enrichment of AGAP2-AS1 and miR-3064-5p by anti-Ago2 was notably higher than that in anti-IgG group, which suggested that miR-3064-5p could directly interact with AGAP2-AS1. The results from RNA pulldown demonstrated that the wild type miR-3064-5p rather than the mutated miR-3064-5p could effectively pull down AGAP2-AS1 (Figure 3F).

**SIRT1 Is a Target of miR-3064-5p**

Next, we identified the binding region between SIRT1 and miR-3064-5p (Figure 4A). The treatment of miR-3064-5p mimic was able to suppress the luciferase activity of SIRT1 mRNA 3’UTR (Figure 4B) and inhibit the mRNA expression of SIRT1 in the Hela and C33A cells (Figure 4C). Moreover, western blot analysis showed that the knockdown of AGAP2-AS1 remarkably repressed SIRT1 expression, while the inhibition of miR-3064-5p could rescue the expression of SIRT1 in the Hela and C33A cells (Figure 4D). Besides, Pearson correlation analysis manifested the negative correlation between miR-3064-5p with AGAP2-AS1 and SIRT1, and the positive correlation between AGAP2-AS1 and SIRT1 (Figures S1G–I).

**MiR-3064-5p/SIRT1 Axis Is Involved in EV AGAP2-AS1-Induced Cervical Cancer Cell Proliferation In Vitro**

Next, we were concerned whether miR-3064-5p/SIRT1 axis was involved in EV AGAP2-AS1-induced cervical cancer cell proliferation. To this end, the Hela and C33A cells were treated with EVs from Hela and C33A cells transfected with AGAP2-AS1 siRNA or PBS. The expression of SIRT1 was measured by qPCR.
SIRT1 overexpression vectors. Our data showed that the depletion of EV AGAP2-AS1 attenuated the numbers of Edu-positive Hela and C33A cells, while the overexpression of SIRT1 or the inhibition of miR-3064-5p rescued the results (Figures 5A, B). Conversely, the knockdown of EV AGAP2-AS1 enhanced Hela and C33A cell apoptosis, while the overexpression of SIRT1 or the inhibition of miR-3064-5p blocked the enhancement in the cells (Figures 5C, D). Besides, the miR-3064-5p level was decreased, and SIRT1 level was elevated in recipient cells after exosome treatment (Figures S1J, K). These results suggested that AGAP2-AS1 is transferred to cells by EVs and functions through miR-3064-5p and SIRT1 axis.

**DISCUSSION**

EVs derived from cancer cells, such as exosomes and microvesicles, are involved in the modulation of cancer progression by delivering regulatory factors, such as proteins, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs). In this study, we identified an innovative function of EV lncRNA AGAP2-AS1 in regulating cervical cancer cell proliferation. It has been reported that SP1-enhanced lncRNA AGAP2-AS1 contributes to chemoresistance by epigenetically regulating MyD88 in breast cancer (10). AGAP2-AS1 serves as a competitive endogenous RNA of miR-16-5p to upregulate...
ANXA11 expression and enhances hepatocellular carcinoma metastasis and proliferation (18). AGAP2-AS1 functions as prognostic biomarker and oncogenic lncRNA in glioma (9). Our data showed that AGAP2-AS1 could be detected in the EVs from cervical cancer cells. And the expression of AGAP2-AS1 was enhanced in the plasma EVs from clinical cervical cancer tissues relative to the adjacent normal tissues. These data imply the potential association of AGAP2-AS1 with cervical cancer development. Meanwhile, EV lncRNAs are well reported in the regulation of the progression of cervical cancer. It has been
FIGURE 4 | SIRT1 is a target of miR-3064-5p. (A) The binding site between SIRT1 and miR-3064-5p was analyzed in ENCORI database. (B, C) The HeLa and C33A cells were treated with miR-3064-5p mimic for 48 h. The expression of SIRT1 was detected by qPCR, and the luciferase activity was measured by dual luciferase reporter assays (C). (D) The expression of SIRT1 was detected by western blot analysis in the HeLa and C33A cells transfected with AGAP2-AS1 siRNA or co-treated with AGAP2-AS1 siRNA and miR-3064-5p inhibitor. mean ± SD, **P < 0.01 vs Control.

FIGURE 5 | MiR-3064-5p/SIRT1 axis is involved in EV AGAP2-AS1-induced cervical cancer cell proliferation in vitro. (A–D) The HeLa and C33A cells were treated with EVs from HeLa and C33A cells transfected with AGAP2-AS1 siRNA or co-treated with the EVs and miR-3064-5p inhibitor or SIRT1 overexpression vectors. (A, B) The cell proliferation was measured by Edu assays. (C, D) The cell apoptosis was evaluated by flow cytometry analysis. mean ± SD, **P < 0.01.
reported that EV lncRNA HNF1A-AS1 regulates cisplatin resistance by targeting microRNA-34b/TUFT1 in cervical cancer (19). Serum EV lncRNA DLX6-AS1 functions as a prognostic biomarker of cervical cancer (20). EV lncRNA LINC01305 contributes to cervical cancer progression by regulating KHSRP (21). Here, we found that EV AGAP2-AS1 contributes to cervical cancer cell proliferation in vitro and in vivo. It suggests that cervical cancer-derived EVs are involved in promoting cancer progression by delivering lncRNA AGAP2-AS1, and it indicated a new function of AGAP2-AS1 in cervical cancer development, presenting the new investigation evidence of the function of EV lncRNAs and AGAP2-AS1 in cervical cancer. However, we just evaluated the function of EV AGAP2-AS1 under the experimental conditions in limited cell lines, and the broad applicability and clinical significance in cervical cancer need to be investigated in further studies.

MiRNAs are one of the most common downstream factors of EV lncRNAs, and miRNAs broadly participate in the cervical cancer development. MicroRNA-449a is reduced in cervical cancer and represses invasion, migration, and proliferation of cervical cancer cells (22). MiR-155-5p reduces the expression of PDK1 to promote autophagy of cervical cancer cell by mTOR signaling (22). MiR-3064-5p targeted by lncRNA MALAT1 inhibits angiogenesis by targeting the FOXA1/CD24 signaling in hepatocellular carcinoma (14). EV circular RNA circPRRX1 contributes to doxorubicin resistance by modulating miR-3064-5p/PTPN14 axis in gastric cancer (23). Meanwhile, β2-AR stimulation enhances chemoresistance of cervical cancer cells by regulating p53 acetylation via Sirt1 (17). LncRNA TUG1 contributes to cervical cancer development by regulating miR-138-5p/SIRT1 axis (24). MiR-29a serves as a tumor inhibitor by targeting the miR-3064-5p/PTPN14 axis (24). MiR-29a serves as a tumor inhibitor by regulating the miR-3064-5p/SIRT1 axis. The clinical values of EV lncRNA AGAP2-AS1 and miR-3064-5p deserve to be explored in cervical cancer diagnosis and treatments.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Jinan Second Maternal and Child Health Care Hospital. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Jinan Second Maternal and Child Health Care Hospital.

**AUTHOR CONTRIBUTIONS**

ML, JW and HM designed the experiments and prepared the manuscript. LG performed the experiments. KZ and TH wrote the paper. All authors contributed to the article and approved the submitted version.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.684477/full#supplementary-material

**Supplementary Figure 1 | (A) The expression of CD63, CD9, CD54, Annexin V and Calnexin was detected by western blot analysis in the cell lysates and EVs from Hela and C33A cells. (B) FISH assay for localization of AGAP2-AS1. Green, AGAP2-AS1 probe; Blue, DAPI. (C, D) MTT detection of the viability of Hela (C) and C33A (D) cells treated with AGAP2-AS1 siRNA. (E, F) qPCR experiment of AGAP2-AS1 in cell extraction and EVs. (G–I) Pearson correlation analysis among miR-3064-5p, AGAP2-AS1, and SIRT1 level in Hela and C33A cells after exosome treatment was detected by qPCR assay, mean ± SD, **P < 0.01.

**REFERENCES**

1. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical Cancer. *Lancet* (2019) 393:169–82. doi: 10.1016/S0140-6736(18)32470-X
2. Bhatla N, Singhal S. Primary HPV Screening for Cervical Cancer. *Best Pract Res Clin Obstet Gynaecol* (2020) 65:98–108. doi: 10.1016/j.bpobgnyn.2020.02.008
3. Vu M, Yu J, Awolude OA, Chuang L. Cervical Cancer Worldwide. *Curr Probl Cancer* (2018) 42:457–65. doi: 10.1016/j.crpc.2018.06.003
4. The L. Eliminating Cervical Cancer. *Lancet* (2020) 395:312. doi: 10.1016/S0140-6736(20)30247-6
5. van den Boorn JG, Dasler J, Coch C, Schlee M, Hartmann G. Exosomes as Nuclear Acid Nanocarriers. *Adv Drug Deliv Rev* (2013) 65:331–5. doi: 10.1016/j.addr.2012.06.011
6. Zhang X, Yuan X, Shi H, Wu L, Qian H, Xu W. Exosomes in Cancer: Small Particle, Big Player. *J Hematol Oncol* (2015) 8:83. doi: 10.1186/s13045-015-0181-x
7. Yang G, Lu X, Yuan L. LncRNA: A Link Between RNA and Cancer. *Biochim Biophys Acta* (2014) 1839:1097–109. doi: 10.1016/j.bbagrm.2014.08.012
8. Kopp F, Mendell JT. Functional Classification and Experimental Dissection of Long Noncoding Rnas. *Cell* (2018) 172:393–407. doi: 10.1016/j.cell.2018.01.011
9. Tian Y, Zheng Y, Dong X. Agap2-AS1 Serves as an Oncogenic lncRNA and Prognostic Biomarker in Glioblastoma Multiforme. *J Cell Biochem* (2019) 120:9056–62. doi: 10.1002/jcb.28180
18. Liu Z, Wang Y, Wang L, Yao B, Sun L, Liu R, et al. Long non-Coding RNA

17. Chen H, Zhang W, Cheng X, Guo L, Xie S, Ma Y, et al. Beta2-AR Activation Induces

16. So D, Shin HW, Kim J, Lee M, Myeong J, Chun YS, et al. Cervical Cancer is

20. Ding XZ, Zhang SQ, Deng XL, Qiang JH. Serum Exosomal Lncrna DLX6-AS1 Is a Promising Biomarker for Prognosis Prediction of Cervical Cancer.

19. Luo X, Wei J, Yang FL, Pang XX, Shi F, Wei YX, et al. Exosomal Lncrna HNF1A-AS1 Affects Cisplatin Resistance in Cervical Cancer Cells Through Regulating microRNA-34b/TUFT1 Axis. Cancer Cell Int (2019) 19:323. doi: 10.1186/s12935-019-1042-4

22. Wang L, Zhao Y, Xiong W, Ye W, Zhao W, Hua Y. Microrna-449a Is Downregulated in Cervical Cancer and Inhibits Proliferation, Migration, and Invasion. Oncol Res Treat (2019) 42:564–71. doi: 10.1159/000502122

21. Huang X, Liu X, Du B, Liu X, Xue M, Yan Q, et al. Lncrna LINCO1305 Promotes Cervical Cancer Progression Through KHSRP and Exosome-Mediated Transfer. Aging (Albany NY) (2021) 13:19230-42. doi: 10.18632/aging.202565

23. Wang S, Ping M, Song B, Guo Y, Li Y, Jia J. Exosomal CircPpR1 Enhances Doxorubicin Resistance in Gastric Cancer by Regulating MiR-3064-5p/PTPN14 Signaling. Yonsei Med J (2020) 61:750–61. doi: 10.3349/ymj.2020.61.9.750

24. Zhu J, Shi H, Liu H, Wang X, Li F. Long non-Coding RNA TUG1 Promotes Cervical Cancer Progression by Regulating the miR-138-5p-SIRT1 Axis. Oncotarget (2017) 8:65253–64. doi: 10.18632/oncotarget.18224

25. Nan P, Niu Y, Wang X, Li Q. MiR-29a Function as Tumor Suppressor in Cervical Cancer by Targeting SIRT1 and Predict Patient Prognosis. Oncol Targets Ther (2019) 12:6917–25. doi: 10.2147/OTT.S218043

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Li, Wang, Ma, Guo, Zhao and Huang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.