High Expression of Circular RNA–Mitochondrial tRNA Translation Optimization 1 Assists the Diagnosis of High-Risk Human Papillomavirus Infection in Cervical Cancer

Xiyun Cheng, BS, Changmei Shen, BS, and Zhenrong Liao, BS

**Objective:** Persistence of high-risk human papillomavirus (HR-HPV) infection is a paramount determinant in cervical cancer (CC) development. Circular RNAs have the potential to be promising biomarkers for various cancers. This study explored circular RNA–mitochondrial tRNA translation optimization 1 (circMTO1) expression in the serum of CC patients and its clinical value in diagnosing CC and predicting HR-HPV infection.

**Materials and Methods:** In total, 125 CC patients (including 78 cases with HR-HPV) were enrolled, with another 76 healthy people as controls. Serum circMTO1 and miR-199a expressions were detected by reverse transcription–quantitative polymerase chain reaction, and the diagnostic efficacy of circMTO1 for CC and HR-HPV infection was analyzed by the receiver operating characteristic curve. According to the median of serum circMTO1 expression, CC patients were assigned into circMTO1 low/high expression groups to analyze the correlation between circMTO1 and clinical parameters using the Fisher and χ² tests. Independent association of circMTO1 with HR-HPV infection in CC was evaluated via logistics multivariate regression analysis. Targeted relationship between miR-199a and circMTO1 was predicted by Starbase Web site and validated via dual-luciferase assay, with their correlation further assessed by Pearson analysis.

**Results:** Serum circMTO1 was increased in CC patients and prominently elevated in HR-HPV–positive CC patients, with a level greater than 1.485 assisting CC diagnosis and a level greater than 2.480 assisting HR-HPV–positive diagnosis. The circMTO1 was interrelated to clinical stage, tumor differentiation, lymph node metastasis, invasion depth, and independently linked with HR-HPV infection in CC. Serum miR-199a was downregulated in HR-HPV–positive CC patients and inversely correlated with circMTO1.

**Conclusions:** Serum circMTO1 is upregulated in HR-HPV–positive CC patients and has a diagnostic value for HR-HPV infection in CC.

**Key Words:** cervical cancer, high-risk human papillomavirus, serum, circMTO1, miR-199a, receiver operating characteristic, person analysis, logistic regression

(J Low Genit Tract Dis 2022;26: 212–218)

**C**ervical cancer (CC) ranks the fourth most frequent cancer and is a major cause of cancer-related mortality in women, with approximately 604,000 women diagnosed and 342,000 people dying worldwide in 2020.¹ Human papillomavirus (HPV) infection is a dominant cause of CC.² Most cases of CC develop from HPV acquisition, HPV maintenance, evolution to cervical precancer, and advancement of invasive cancer.³ Based on the oncogenic ability, HPV can be divided into low- and high-risk HPV (HR-HPV) types, among which HR-HPV 16/18 contribute to 70% of infections in invasive CC and are the most frequent carcinogenic HPV types.⁴,⁵ Most HPV infections are ephemeral, for the immunocompetent individual's immune system presumably eliminates the infection.⁶ However, the impaired immune property reduces the capability to clear HPV infection, and the persistent HR-HPV infection in the cervix increases the peril of developing CC.⁷,⁸ Over the past decades, the morbidity and mortality of invasive CC have lowered in numerous countries, primarily because of the screening program implementation in high-resource countries.⁹ The HPV test is an efficient screening strategy to prevent CC.¹⁰ Nonetheless, some CC patients have false-negative HR-HPV test results, indicating the limitation of HPV screening.¹¹,¹² Hence, finding novel and valid biomarkers of HR-HPV infection is of great magnitude for the initial diagnosis and treatment of CC.

Circular RNA (circRNA) is a particular type of covalently closed circular structure noncoding RNA, mainly existing in cytoplasm and exosome, which is generated from pre-miRNA by backsplice, including 2 prominent means of formation: intron cyclization and backsplice, which is generated from pre-miRNA by backsplice, including 2 prominent means of formation: intron cyclization and backsplice.¹³,¹⁴ Circular RNAs are imperative in epigenetically regulating transcriptional and posttranscriptional gene expression and possess tissue specificity, high stability, and conservation.¹⁵,¹⁶ Intriguingly, circRNAs can serve as oncogenic genes or tumor suppressors to modulate cancer cell chemosensitivity, metastases, differentiation, and proliferation.¹⁷ Diverse circRNAs are aberrantly expressed in CC and promise to be new markers for diagnosis and prediction of tumor episode and development.¹⁸ Circular RNA–mitochondrial tRNA translation optimization 1 (circMTO1) plays an oncogenic function in CC cell (CCC) lines and is documented to be upregulated in the tissues of CC patients.¹⁹ However, circMTO1 expression in the serum of CC patients, its clinical diagnostic value, and its correlation with HR-HPV infection in CC have rarely been reported.

Because of the absence of free ends easily digested by exonuclease, circRNAs are more steady than linear transcripts, which are considered a trait that can augment their potency as microRNA (miRNA) sponges.²⁰ MicroRNAs extensively mediate diverse biological processes, such as cell proliferation, differentiation, apoptosis, and tumorigenesis.²¹ Differentially expressed miRNAs can lead to cancer onset and progression by functioning as oncogenes or tumor suppressors.²² miR-199a is downregulated in HPV-positive CC tissues, and HDAC6 facilitates HPV-positive CC development by elevating Wnt5a and impeding miR-199a transcription.²³ Therefore, we speculated that circMTO1 may play a regulatory action in HR-HPV–positive CC by sponging miR-199a. In the present study, we determined circMTO1 expression in CC and its correlation with HR-HPV infection, expecting to exert valuable reference for early diagnosis and treatment.

**METHODS**

**Ethics Statement**

This study was ratified by the ethics committee of our hospital. Patients or their families had voluntarily signed the informed consent form.
Study Subjects

In total, 125 CC patients (aged 26–57 years, averaged 38.04 ± 6.606 years) who were admitted to our hospital from February 2018 to February 2021 were selected as the CC group. Inclusion criteria included patients: (1) conforming to the International Federation of Gynecology and Obstetrics (FIGO) staging criteria and pathologically diagnosed with CC; (2) with no radiotherapy, chemotherapy, or biological immunotherapy before operation; and (3) cooperating with this study and with complete medical records.

Exclusion criteria were as follows: (1) non-HPV–related cancers that occur in the lower genital tract such as melanoma that are not HPV related; (2) with radiotherapy, chemotherapy, or biological immunotherapy before operation; and (3) with acute and chronic infectious diseases, hematological diseases, autoimmune diseases, and other malignant tumors.

The clinical stages of 125 CC patients included stage I (50 cases), stage IIa (41 cases), and stage IIb (34 cases). The pathological types consisted of squamous cell carcinoma (101 cases) and adenocarcinoma (24 cases). Furthermore, the CC patients were assigned into 24 cases with lymph node metastasis and 101 cases without lymph node metastasis; or 86 cases with invasion depth greater than 1/2 muscle layer; or 47 cases with negative HR-HPV infection and 78 cases with positive HR-HPV. Another 76 healthy women who took physical examination during the same period were enrolled as the normal group. There was no significant difference in age between the 2 groups (p > .05), so the 2 groups were comparable.

High-Risk HPV Assay

High-risk HPV infection was detected based on the manufacturer’s protocol of the primary Roche cobas HPV real-time PCR assay (Cobas 4800; Roche, Basel, Switzerland). This assay can differentiate following 14 HR-HPV subtypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. High-risk HPV will be judged as positive as long as any subtype of HPV is identified as positive by the machine.

Reverse Transcription–Quantitative Polymerase Chain Reaction

Fasting venous blood was collected from all participants at enrollment. The serum was separated within 2 hours, then transferred to a dedicated cryopreservation tube, and stored in a −80°C freezer for subsequent experiments. Total RNA was extracted from the serum using the TRIzol reagent (Invitrogen, Carlsbad, CA) and then reversely transcribed into the first-strand cDNA of RNA using the RT kit (Invitrogen). Reverse transcription–quantitative polymerase chain reaction was performed using the SYBR Green PCR kit (TaKaRa). Reverse transcription–quantitative polymerase chain reaction was performed using the SYBR Green PCR kit (TaKaRa, Tokyo, Japan). β-Actin and U6 were internal controls. The relative expression was computed using the 2−ΔΔCT method based on the expression of β-actin or U6. Primer sequences are presented in Table 1.

**Table 1.** Primer Sequences

| Gene       | Forward 5'-3' | Reverse 5'-3' |
|------------|---------------|---------------|
| circMTO1   | 5'-GCCTGAAACACACTGGGAAAAT-3' | 5'-CACAGATGCAGAGACAACACAGG-3' |
| miR-199a   | 5'-ACACTCCACGCTGGGCCACGAGTCAGAAT-3' | 5'-TGGTTCGTTGGAGGTGCTG-3' |
| β-actin    | 5'-TGGCATCCAGAAACTACCT-3' | 5'-TCCTCTTCGTACCTGTAGCG-3' |
| U6         | 5'-ATTGGAACACGATACAGAGAAGATT-3' | 5'-GGAACGCTTCAGATTTG-3' |

Cell Culture

Human CCC C-33A were provided by Procell (Wuhan, Hubei, China) and cultured in Dulbecco's modified Eagle medium/ nutrient mixture F-12 supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA), 100 U/mL of penicillin (Thermo Fisher Scientific), and 100 μg/mL of streptomycin (GE Healthcare Life Sciences, Marlborough, MA) in a humidified atmosphere containing 5% CO2 at 37°C until passages 2–3. The medium was replaced every 2 days and CCCs were passaged weekly, with well-grown CCCs taken for subsequent experiments.

Dual-Luciferase Assay

The binding sites of miR-199a and circMTO1 were determined through the online prediction software Starbase (http://starbase.sysu.edu.cn/). According to the prediction, the mutant (MUT) sequences and wide-type (WT) sequences of the binding sites of miR-199a and circMTO1 were, respectively, designed and cloned into the luciferase vector pGL3 (Promega, Madison, WI) to construct circMTO1-WT and circMTO1-MUT luciferase plasmids. The constructed plasmids were transfected into CCCs with miR-199a mimic or mimic negative control. After 48 hours of transfection, cells were rinsed with PBS and then lysed. Thereafter, the fluorescence activity of cells was detected using a luciferase kit (Yuanpinghao Biotechnology, Beijing, China).

Statistical Analysis

Data analysis and mapping were processed by applying SPSS 21.0 (IBM Corp, Armonk, NY) and GraphPad Prism 8 (GraphPad Software, Inc, San Diego, CA) statistical software. Enumeration data were exhibited as case numbers and percentages. Measurement data were presented as mean ± SD. Fisher test was used for comparisons of enumeration data between 2 groups and χ2 test was used for multiple group comparisons. The t test was performed for comparisons of measurement data between 2 groups. Receiver operating characteristic (ROC) curve was implemented to assess the diagnostic value of circMTO1 for CC patients and HR-HPV–positive CC patients. Binary logistic regression equation was used to evaluate the influencing factors of HR-HPV–positive CC patients, with Enter method for screening independent variables. P value was obtained through a 2-sided test, and a p value less than .05 indicated statistical significance.

RESULTS

CircMTO1 Was Upregulated in the Serum of CC Patients and Had High Diagnostic Efficacy

A total of 125 CC patients and 76 healthy controls were included in this study. We detected circMTO1 expression in the serum using RT-qPCR, which unveiled an increased circMTO1 expression in HR-HPV–positive CC patients. Binary logistic regression equation was used to evaluate the influencing factors of HR-HPV–positive CC patients, with Enter method for screening independent variables. P value was obtained through a 2-sided test, and a p value less than .05 indicated statistical significance.
circMTO1 expression to distinguish CC patients from normal healthy people \( (p < .0001; \text{see Figure 1B}) \). The area under the curve was 0.9340 and the cutoff value was 1.485 (92.8% sensitivity and 86.84% specificity). The previously mentioned results demonstrated that a serum circMTO1 level greater than 1.485 could assist CC diagnosis.

Correlation Between Serum circMTO1 and Clinicopathological Features of CC Patients

According to the median of serum circMTO1 level, CC patients were assigned to circMTO1 high expression group and circMTO1 low expression group to further observe and compare the association between circMTO1 levels and clinicopathological characteristics of CC patients. The results revealed that circMTO1 expression was prominently correlated with clinical stage, tumor differentiation degree, lymph node metastasis, and invasion depth \( (p < .05) \), but not with age, tumor size, and pathological type \( (p > .05; \text{see Table 2}) \).

High CircMOR1 Expression Assisted in Differentiating HR-HPV Infection in CC

To explore the correlation between serum circMTO1 expression and HR-HPV infection, we divided CC patients into HR-HPV–negative group \( (n = 47) \) and HR-HPV–positive group \( (n = 78) \) based

\[ \begin{array}{c|c|c|c|c|c}
\text{Clinical pathological features} & n & \text{Low expression (n = 63)} & \text{High expression (n = 62)} & p \\
\hline
\text{Age, y} & & & & \\
<35 & 62 & 32 & 30 & \\
\geq35 & 63 & 31 & 32 & \\
\text{Tumor size, cm} & & & & \\
\leq4 & 65 & 31 & 34 & \\
>4 & 60 & 32 & 28 & \\
\text{Clinical stage} & & & & <.0001 \\
\text{Stage I} & 50 & 35 & 15 & \\
\text{Stage IIa} & 41 & 21 & 20 & \\
\text{Stage IIb} & 34 & 7 & 27 & \\
\text{Differentiation degree} & & & .0028 \\
\text{High} & 36 & 26 & 10 & \\
\text{Moderate/low} & 89 & 37 & 52 & \\
\text{Lymph node metastasis} & & & .0002 \\
\text{Yes} & 24 & 4 & 20 & \\
\text{No} & 101 & 59 & 42 & \\
\text{Pathological type} & & & & \\
\text{Squamous cell carcinoma} & 101 & 50 & 51 & \\
\text{Adenocarcinoma} & 24 & 13 & 11 & \\
\text{Invasion depth} & & & <.0001 \\
\leq1/2 muscle layer & 86 & 57 & 29 & \\
>1/2 muscle layer & 39 & 6 & 33 & \\
\end{array} \]
on the HPV assay. The RT-qPCR results showed that serum circMOR1 was higher in HR-HPV–positive CC patients than in HR-HPV–negative CC patients ($p < .001$; see Figure 2A). The ROC curve was further plotted to distinguish HR-HPV–positive CC patients from HR-HPV–negative CC patients based on the circMTO1 expression ($p < .0001$; see Figure 2B), which illustrated that the area under the curve was 0.8511 and the cutoff value was 2.480 (56.41% sensitivity and 100% specificity). Altogether, a serum circMTO1 level greater than 2.480 possessed certain auxiliary diagnostic values for HR-HPV–positive CC.

**CircMTO1 Was Independently Associated With HR-HPV Infection in CC**

We compared and analyzed the incidence of HR-HPV infection in the circMTO1 high/low expression groups. The results unraveled that the incidence of positive HR-HPV was remarkably elevated in the circMTO1 high expression group (52 cases, 82.54%) relative to the circMTO1 low expression group ($p < .01$; see Table 3), indicating that circMTO1-upregulated CC patients had a higher incidence of HR-HPV infection.

To further investigate whether circMTO1 was independently correlated with positive HR-HPV, we first analyzed the relevance between clinical indexes of CC patients and HR-HPV infection using univariate logistic regression analysis. Based on the results, clinical stage, tumor differentiation degree, lymph node metastasis, invasion depth, and circMTO1 (all $p < .1$) were included as independent variables in multivariate logistic regression analysis, which revealed that serum circMTO1 was independently associated with HR-HPV infection in CC patients after adjusting clinical stage, tumor differentiation degree, and invasion depth ($p = .019$, OR = 2.587, 95% CI = 1.168–5.733; see Table 4). Overall, CC patients with elevated circMTO1 expression had a higher risk of HR-HPV infection than CC patients with low circMTO1 expression.

**Serum mir-199a Was Downregulated and Inversely Correlated With CircMTO1 in HR-HPV–Positive CC Patients**

Circular RNAs can exert regulatory roles via the ceRNA mechanism. To preliminarily investigate the possible regulatory mechanism of circMTO1 in HR-HPV–positive CC, the binding sites between miR-199a and circMTO1 were predicted through the Starbase Web site ($p < .001$; see Figure 3A), and the binding relation was validated using dual-luciferase assay ($p < .001$; see Figure 3B). Furthermore, RT-qPCR was implemented to detect miR-199a expression in the serum of HR-HPV–negative/positive CC patients to explore the relationship between miR-199a and HR-HPV infection, which delineated a markedly decreased miR-199a in HR-HPV–positive CC patients compared with HR-HPV–negative ones ($p < .001$; see Figure 3C). Subsequently, a prominent negative relationship between circMTO1 and miR-199a was revealed by Pearson correlation ($p < .001$; see Figure 3D). In a word, serum miR-199a was weakly expressed and negatively linked with circMTO1 in HR-HPV–positive CC patients, and circMTO1 had the potential to function as a ceRNA of miR-199a in regulating HR-HPV–positive CC.

**DISCUSSION**

Cervical cancer occupies approximately 5% of global cancer, contributing to high morbidity and death rates. The persistence of HR-HPV infection remains the primary root of CC and precursor lesion. Plentiful circular RNAs are reported to engage in CC pathogenesis and as potential biomarkers. This study analyzed the correlation between circMTO1 and HR-HPV–positive CC. Aberrant expressions of numerous circular RNAs are observed in tumors, and this dysregulation is implicated in the tumorigenesis and metastasis of various cancer. As gene regulators, circular RNAs...
perform crucial impacts in cancer evolution and are considered prospective markers in diagnosing and treating cancer. Hence, we measured the circMTO1 expression and observed an elevated serum circMTO1 level in CC patients. In addition, through the ROC curve, we found that a serum circMTO1 level greater than 1.485 (92.8% sensitivity and 86.84% specificity) could assist the diagnosis of CC. The tumor-promoting effect of circMTO1 is also documented in oral squamous cell carcinoma. The circMTO1 knockdown represses chemoresistance, migration, and invasion of CCCs. Our study initially analyzed the diagnostic value of circMTO1 for CC.

The FIGO stage can provide risk stratification for operable CC patients and predict survival time. Lymph node metastasis remains an important contributor to the poor survival in CC patients. Subsequently, we divided CC patients into circMTO1 high/low expression cases to further assess the relevance of circMTO1 and clinicopathological indicators, which unraveled the correlation of circMTO1 with clinical stage, tumor differentiation grade, lymph node metastasis, and invasion depth. The association between circMTO1 and lymph node metastasis is also presented in bladder cancer and colorectal cancer. CircMTO1 acts as an oncogene in CC and circMTO1-upregulated CC patients have short overall survival relative to the circMTO1-downregulated patients. To sum up, our findings could exert certain significance for distinguishing CC individuals based on serum circMTO1 expression.

Interestingly, a similar highly expressed ciRS-7 is interrelated to large tumor size, metastatic lymph node, deep invasion, advanced FIGO stage, and HPV infection in CC patients. Because of the primary pathogenic effect of oncogenic HR-HPV infection in CC, we further explored the correlation between circMTO1 and HR-HPV infection. Based on our results, circMTO1 was much higher in HR-HPV–positive CC patients and its serum level greater than 2.480 (56.41% sensitivity and 100% specificity) exerted a diagnostic value for HR-HPV–positive CC. Endogenous circRNAs are reported to be highly expressed in HPV-positive tumors. In HPV-related malignancies, circRNAs are documented to participate in tumorigenesis, cancer evolution, and drug resistance, among which some are regarded as effective diagnostic

## Table 4. CircMTO1 Was Independently Associated With HR-HPV Infection in CC

|                      | Univariate logistic regression analysis |                      | Multivariate logistic regression analysis |
|----------------------|----------------------------------------|----------------------|------------------------------------------|
|                      | p      | OR 95% CI        | p      | OR 95% CI        |
| Age, y               | .744   | 1.009 0.955–1.067| —      | —                |
| Tumor size, cm       | .345   | 1.421 0.685–2.946| —      | —                |
| Clinical stage       | .029   |                   | .09    |                   |
| Clinical stage (1)   | .319   | 1.538 0.659–3.589| .211   | 1.835 0.708–4.751|
| Clinical stage (2)   | .008   | 3.846 1.424–10.389| .034   | 3.557 1.102–11.486|
| Differentiation degree | .003 | 3.392 1.517–7.586| .004   | 3.846 1.548–9.556|
| Lymph node metastasis | .025  | 3.707 1.181–11.633| .677   | 1.338 0.341–5.253|
| Pathological type    | .162   | 0.488 0.178–1.334| —      | —                |
| Invasion depth       | .003   | 3.975 1.582–9.986| .049   | 2.821 1.003–7.933|
| CircMTO1             | .001   | 3.187 1.578–6.437| .019   | 2.587 1.168–5.733|

**TABLE 4.** CircMTO1 Was Independently Associated With HR-HPV Infection in CC
and prognostic targets.\textsuperscript{42} Furthermore, we found that circMTO1- upregulated CC individuals had a higher occurrence of HR-HPV infection and circMTO1 was independently related to HR-HPV infection in CC, pointing to the strong relevance of circMTO1 and HR-HPV infection.

Accumulating reports unveil that circRNAs exert biological functions by functioning as miRNA sponges.\textsuperscript{43}-\textsuperscript{46} For instance, circMTO1 can trigger gallbladder cancer progression by sponging miR-219a-5p.\textsuperscript{32} CircMTO1 sponges miR-6893 to facilitate CC development and chemoresistance.\textsuperscript{33} Consequently, we verified this regulatory regime. The binding sites of circMTO1 and miR-199a were predicted and ascertained. Moreover, miR-199a-3p was sponged by circMTO1.\textsuperscript{19,47} Consistently, miR-199a, as a tumor suppressor, is weakly expressed in CC, which suppresses CCC and HR-HPV infection in CC, pointing to the strong relevance of circMTO1.\textsuperscript{19} The above observations are consistent with the idea that miR-199a-3p is sequestered by circMTO1.\textsuperscript{19,47} Collectively, circMTO1 may regulate HR-HPV–positive CC by sponging miR-199a.

As a species-specific DNA virus, HPV possesses high tissue and host specificity, causing various lesions from mucosal hyperplasia progressing to malignant tumors. After the body is infected with HPV, the immune system will be altered and produce numerous inflammatory cytokines, resulting in inflammatory responses and tissue damages.\textsuperscript{51} Most individuals have CC due to long-term HR-HPV infection, so it is pivotal to find new biomarkers associated with HR-HPV infection for the early diagnosis and treatment of CC. Circular RNAs have a closed-loop structure with high stability and can perform regulatory properties through the ceRNA mechanism. The previous studies mostly detected circRNA levels in tissues from CC patients, rarely in the serum. Determining the serum expression of circRNAs in CC patients assists in accurately assessing the disease and adopting reasonable therapeutic measures. This study innovatively elicited the close relevance of circMTO1 with CC onset and HR-HPV infection and verified its diagnostic value, and identified the shunt function of circMTO1 in HR-HPV–positive patients when HPV detection is used as a primary screening, which poses imperative reference significance to cancer therapeutics.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49.
2. Marima R, Hull R, Lolas G, et al. The catastrophic HPV/HIV dual viral oncogenicity in concert with dysregulated alternative splicing in cervical cancer. Int J Mol Sci 2021;22.
3. Castle PE, Einstein MH, Sahasrabuddhe VV. Cervical cancer prevention and control in women living with human immunodeficiency virus. CA Cancer J Clin 2021;71:505–26.
4. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev 2003;16:1–17.
5. de Sanjose S, Diaz M, Castellsague X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007;7:453–9.
6. Alhamlan FS, AlFageeh MB, Al Moushait MA, et al. Human papillomavirus–associated cancers. Adv Exp Med Biol 2021;1313:1–14.
7. Denisow SA, Rositch AF, Firnhaber C, et al. Incidence and progression of cervical lesions in women with HIV: a systematic global review. Int J STD AIDS 2014;25:163–77.
8. Padilla-Mendoza JR, Gomez-Lopez LA, Lopez-Casamichana M, et al. Human papillomavirus coinfection in the cervical intraepithelial lesions and cervical cancer of Mexican patients. Biomed Res Int 2020;2020:454220.
9. Vaccarella S, Lortet-Tieulent J, Plummer M, et al. Worldwide trends in cervical cancer incidence: impact of screening against changes in disease risk factors. Eur J Cancer 2013;49:2262–73.
10. Cho HW, Shin SR, Lee JK, et al. Accuracy of human papillomavirus tests on self-collected urine versus clinician-collected samples for the detection of cervical precancer: a systematic review and meta-analysis. J Gynecol Oncol 2022;33:e4.
11. Jiang W, Marshall Austin R, Li L, et al. Extended human papillomavirus genotype distribution and cervical cytology results in a large cohort of Chinese women with invasive cervical cancers and high-grade squamous intraepithelial lesions. Am J Clin Pathol 2018;150:43–50.
12. Zhao C, Li Z, Nayar R, et al. Prior high-risk human papillomavirus testing and Papancicolou test results of 70 invasive cervical carcinomas diagnosed in 2012: results of a retrospective multicenter study. Arch Pathol Lab Med 2015;139:184–8.
13. Belousova EA, Filipenko ML, Kushlinskii NE. Circular RNA: new regulatory molecules. Bull Exp Biol Med 2018;164:803–15.
14. Jiang L, Wang X, Zhan X, et al. Advance in circular RNA modulation effects of heart failure. Gene 2020;763S:100036.
15. Chen LL, Yang L. Regulation of circRNA biogenesis. RNA Biol 2015;12:381–8.
16. Zeng X, Yuan X, Cai Q, et al. Circular RNA as an epigenetic regulator in chronic liver diseases. Cell 2021;10.
17. Zhang X, Zhong B, Zhang W, et al. Circular RNA circMTO1 inhibits proliferation of glioblastoma cells via miR-92/WWOX signaling pathway. Med Sci Monit 2019;25:6454–61.
18. Wang H, Wei M, Kang Y, et al. Circular RNA circ_PVT1 induces epithelial-mesenchymal transition to promote metastasis of cervical cancer. Aging (Albany NY) 2020;12:20139–51.
19. Ghafouri-Fard S, Khoshbakht T, Bahramian A, et al. CircMTO1: a circular RNA with roles in the carcinogenesis. Biomed Pharmacother 2020;121:112025.
20. Thomson DW, Dinger ME. Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet 2016;17:272–83.
21. Adil MS, Khulood D, Somanath PR. Targeting Akt-associated microRNAs as monotherapy or adjuvant therapy in cancer. Nucleic Acids Res 2019;47:7753–66.
22. Shao Y, Zhu F, Zhu S, et al. HDAC6 suppresses microRNA-199a transcription and augments HPV-positive cervical cancer progression through Wnt5a upregulation. Int J Biochem Cell Biol 2021;136:106000.
23. Liu X, Tong Y, Xia D, et al. Circular RNAs in prostate cancer: biogenesis, biological functions, and clinical significance. Mol Ther Nucleic Acids 2021;26:1130–47.
24. Saliciccioli I, Zhou CD, Okonji EC, et al. European trends in cervical cancer mortality in relation to national screening programs, 1985–2014. Cancer Epidemiol 2021;74:102002.
26. Zhang Z, Zhang J, Xia N, et al. Expanded strain coverage for a highly successful public health tool: prophylactic 9-valent human papillomavirus vaccine. *Hum Vaccin Immunother* 2017;13:2880–91.

27. Bonin-Jacob CM, Almeida-Lugo LZ, Puga MAM, et al. IL-6 and IL-10 in the serum and exfoliated cervical cells of patients infected with high-risk human papillomavirus. *PLoS One* 2011;6:e2048639.

28. Lee HJ, Kim MJ, Kim YS, et al. UHRF1 silences gelsolin to inhibit cell death in early stage cervical cancer. *Biochem Biophys Res Commun* 2020;526:1061–8.

29. Cai H, Zhang P, Xu M, et al. Circular RNA hsa_circ_0000263 participates in cervical cancer development by regulating target gene of miR-150-5p. *J Cell Physiol* 2019;234:11391–400.

30. Huang Y, Zhu Q. Mechanisms regulating abnormal circular RNA biogenesis in cancer. *Cancers (Basel)* 2017;13:4185.

31. Wang M, Gu B, Yao G, et al. Circular RNA expression profiles and the pro-tumorigenic function of CircRNA_10156 in hepatitis B virus-related liver cancer. *Int J Med Sci* 2019;17:1351–65.

32. Zou C, Li X, Lv X, et al. Circular RNA mitochondrial translation optimization 1 homologue (CircMTO1) induced by zinc finger protein 460 (ZNF460) promotes oral squamous cell carcinoma progression through the microRNA miR-320α/alpha thalassemia/mental retardation, X-linked (ATRX) axis. *Bioengineered* 2021;12:9585–97.

33. Chen X, Ai G, Zhou J, et al. CircMTO1 promotes tumorigenesis and chemoresistance of cervical cancer via regulating miR-6893. *Biomed Pharmacother* 2019;117:109064.

34. Matsuoka K, Machida H, Mandelbaum RS, et al. Validation of the 2018 FIGO cervical cancer staging system. *Gynecol Oncol* 2019;152:87–93.

35. Zheng RR, Huang XX, Jin C, et al. Preoperative platelet count improves the preoperative platelet count improves the serum and exfoliated cervical cells of patients infected with high-risk human papillomavirus. *PLoS One* 2011;6:e2048639.

36. Wu H, Song S, Yan A, et al. Circular RNA hsa_circ_0000263 participates in cervical cancer development by regulating target gene of miR-150-5p. *J Cell Physiol* 2019;234:11391–400.

37. Huang Y, Zhu Q. Mechanisms regulating abnormal circular RNA biogenesis in cancer. *Cancers (Basel)* 2017;13:4185.

38. Ge Z, Li JF, Wang CY, et al. CircMTO1 inhibits cell proliferation and invasion by regulating Wnt/b-catenin signaling pathway in colorectal cancer. *Eur Rev Med Pharmacol Sci* 2018;22:8203–9.

39. Zhou Y, Shen L, Wang YZ, et al. The potential of cirR-7 for predicting onset and prognosis of cervical cancer. *Neoplasma* 2020;67:312–22.

40. Fan Q, Huang T, Sun X, et al. HPV-16/18 E6-induced APOBEC3B expression associates with proliferation of cervical cancer cells and hypomethylation of Cyclin D1. *Mol Carcinog* 2021;60:313–30.

41. Kolitz E, Lucas E, Hosler GA, et al. Human papillomavirus-positive and –negative vulvar squamous cell carcinoma are biologically but not clinically distinct. *J Invest Dermatol* 2021;S0022-202X:2381–2.

42. Bonelli P, Borrelli A, Tuccillo FM, et al. The role of circRNAs in human papillomavirus (HPV)-associated cancers. *Cancers (Basel)* 2021;13.

43. Liu W, Ma W, Yuan Y, et al. Circular RNA hsa_circRNA_103809 promotes lung cancer progression via facilitatig ZNF121-dependent MYC expression by sequestering miR-4302. *Biochem Biophys Res Commun* 2018;500:846–51.

44. Lv W, Liu S, Zhang Q, et al. Circular RNA CircCOL5A1 sponges the MiR-7-5p/Epica1 axis to promote the progression of keoids through regulating PI3K/Akt signaling pathway. *Front Cell Dev Biol* 2021;9:626027.

45. Qu S, Zhong Y, Shang R, et al. The emerging landscape of circular RNA in life processes. *RNA Biol* 2017;14:992–9.

46. Zhu X, Han J, Lan H, et al. A novel circular RNA hsa_circRNA_103809/miR-377-3p/GOT1 pathway regulates cisplatin-resistance in non-small cell lung cancer (NSCLC). *BMC Cancer* 2020;20:1190.

47. Song R, Li Y, Hao W, et al. Circular RNA MTO1 inhibits gastric cancer progression by elevating PAWR via sponging miR-199a-3p. *Cell Cycle* 2020;19:3127–39.

48. Wang X, Lin Y, Liu J. Long non-coding RNA DLX6-AS1 promotes proliferation by acting as a ceRNA targeting miR-199a in cervical cancer. *Mol Med Rep* 2019;19:1248–55.

49. Yang X, Feng KX, Li H, et al. MicroRNA-199a inhibits cell proliferation, migration, and invasion and activates AKT/mTOR signaling pathway by targeting B7-H3 in cervical cancer. *Technol Cancer Res Treat* 2020;19:153303820942245.

50. Pulati N, Zhang Z, Gulimilamu A, et al. HPV16+ -miRNAs in cervical cancer and the anti-tumor role played by miR-5701. *BMC Cancer* 2020;20:1190.

51. Dang YP, Yuan XY, Tian R, et al. Curcumin improves the paclitaxel-induced apoptosis of HPV-positive human cervical cancer cells via the NF-κB-p53-caspase-3 pathway. *Exp Ther Med* 2015;9:1470–6.