CHANGES OF MIR-144-3P AND MIR-337-3P IN SERUM OF CERVICAL CARCINOMA PATIENTS AND ANALYSIS OF DIAGNOSIS AND PROGNOSIS

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ABSTRACT

Objective: *To analyze the expression changes of miR-144-3p and miR-337-3p in serum of cervical carcinoma (CC) patients and their diagnosis and prognosis.*

Methods: *Altogether 72 cases of CC patients and 63 cases of health people were selected as the research participants,with CC patients as the research group(RG) and health people as the control group(CG). miR-144-3p and miR-337-3p in serum of the CG and the RG was detected, the diagnostic value of miR-144-3p and miR-337-3p to CC was analyzed, the expression changes of miR-144-3p and miR-337-3p before and after treatment in the RG were observed, and the relationship of the expressions of miR-144-3p and miR-337-3p with the clinicopathological characteristics of CC was analyzed respectively. Human cervical carcinoma cell C33A and normal cervical epithelial cell H8 were purchased for biological behavior analysis, and the influence of miR-144-3p and miR-337-3p on the prognosis of CC patients after treatment was observed.*

Results: *The expression of miR-144-3p and miR-337-3p in the serum of patients in the RG was lower than that in the CG (P<0.05). miR-144-3p and miR-337-3p had good diagnostic value for CC. The expression levels of miR-144-3p and miR-337-3p in the RG increased evidently after treatment (P<0.05). miR-144-3p and miR-337-3p were closely related to cell differentiation, clinical stage and lymph node metastasis of CC patients (P<0.05). The cell proliferation and invasion ability of over-expression of miR-144-3p and miR-337-3p were lower than those of the other two groups, while the apoptosis rate was higher than that of the other two groups (P<0.05). The prognosis of high miR-144-3p group was better than that of low miR-144-3p group (P=0.020), and the prognosis of high miR-337-3p group was better than that of low miR-337-3p group (P=0.041).*

Conclusion: *miR-144-3p and miR-337-3p are closely related to cervical carcinoma and may be the key to the diagnosis and treatment of cervical carcinoma in the future.*

Keywords: *Cervical carcinoma, miR-144-3p, miR-337-3p.*

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Introduction

Cervical carcinoma (CC) is a common malignant neoplasm in gynecology[(1)]. The patients mainly distributed in the middle-aged and the elderly, but the incidence is showing a younger trend[(2)]. According to survey results by Huh W K[(2)] et al., there were more than 1.2 million new CC patients worldwide in 2014[(3)]. Relevant reports show that the global incidence of CC has reached 6-29%[(4)], which seriously threatens women health. At present, most of the treatment methods commonly applied in clinical use are surgery or combined with radiotherapy and chemotherapy, which have good treatment effect for early patients, while the middle and late stage patients are poor[(5)]. Now, the pathogenesis of CC has not been explored[(6)]. Therefore, exploring the pathogenesis and prevent and treat CC is a research hotspot that has never been stopped clinically. According to research, early marriage, early

pregnancy, prolificacy, sexual disorder and bacteria are correlated with CC[7]. However,many scholars start to explore the gene level.

microRNAs (miRNAs) is a non-coding small RNA found in recent years[8], with a length of only about 22 nucleotides, which generally mediates post-transcriptional adjustment of genes and participates in the adjustment of various physiological processes of the body[9]. At present, it has been confirmed that miRNA has a correlation with the progression of various diseases[10]. Other related studies have also found miRNA in blood circulation[11]. Among them, miR-144-3phas a correlation with the progression of CC[12]. miR-337-3p acted in physiological and pathological procedures, and its expression level is abnormally expressed in various neoplasms[13]. However, we have not fully understood the application of miR-144-3p and miR-337-3p in CC. Thus, we tested the changes of miR-144-3p and miR-337-3p in CC and the diagnosis and prognosis, providing new opinions for treatment of CC.

Data and methods

Patient data

Altogether 72 CC patients(research group,RG) and 63 health people(control group, CG) from June 2016 to June 2017 were obtained. This experiment was conducted with approval from the Ethics Committee, and written consent was obtained from each patient.

Inclusion and exclusion criteria

Inclusion criteria:

• Patients were diagnosed as CC;

• The diagnosis conformed to AJCC TNM staging standard[13];

• Patients had complete clinical data; patients have signed informed consent forms.

Exclusion criteria:

• Patients had multiple neoplasms; patients have received neoplasm-related treatment within half a year;

• Patients had other congenital diseases, autoimmune defects, hepar and renal insufficiency, low treatment compliance for mental disorders,and drug allergy;

• Patients died during treatment;

• Transferred patients.

Inclusion criteria of the CG:

• The health people in our hospital were included;

• Patients had no major medical history before;

• All the results of the physical check-ups were normal;

• Patients agreed to participate in this investigation.

Cell data

Both human cervical carcinoma cell C33A (BNCC341097) and H8 (BNCC340657) were obtained from Bena Culture. DMEM medium including 10% FBS and 1% penicillin/streptomycin mixture was applied at 37°C with 5%CO_2.

When the fusion degree grows to 80%, the cells were digested with trypsin for about 1min, the medium was replaced with 1: 3, and the cells were sub-cultivated once every 2 days.

Cell transfection

miR-144-3p-mimics, miR-144-3p-inhibition, miR-NC,miR-337-3p-mimics, miR-337-3p-inhibition, and miR-NC were transfected into C33A using Lipofectamine 2000 reagent.

Detection method

PCR detection

Total RNA was extracted using EasyPure miRNA Kit, and detected for purity, concentration and integrity by UV spectrophotometer and agarose gel electrophoresis. Reverse transcription of RNA into cDNA was performed by 2X TS miRNA Reaction Mix in TransScript Green miRNA Two-Step qRT-PCR SuperMix kit, and the specific operation steps were performed. Then PCR amplification was performed, with the system: 1μL of cDNA, 0.4μL of upstream and downstream primers, 10μL of 2×TransTaq® Tip Green qPCR SuperMix, 0.4μL of Passive Reference Dye (50X),20μLddH2O. PCR reaction conditions: 94°C for 30s, 94°C for 5s, 60°C for 15s, and 60°C for 10s, with a total of 40 cycles. Each sample was provided with 3 repeated wells, and the experiment was performed 3 times. This data were analyzed using $2^{-\triangle\triangle qct}$.

CCK-8 detection

Cells were obtained 24h after transfection(with 4*106 cells), cultivated on 96-well plates, and then cultivated for 0h, 24 h, 48h, 72h. A 10μL of CCK solution (Beyotime Biotechnology, Shanghai, China, C0037) and 90μL of basal medium (DMEM) were put into each well, cultivated at 37°C for 2h, and then OD value at 450nm was tested by an enzyme reader.

	F (5'-3')	R (5'-3')
miR-144-3p	ACACTCCAGCTGGGTA-CAGTATAGATGAT-GTACT	CTCAACTGGTGTCGT-GGA
GAPDH	GTCTC-CTCTGACTTCAA-CAGCG	AC-CACCCTGTTGCTGTAGCCAA
miR-337-3p	CGCTTCAGCTCCTATAT-GA	CGCTTCACTAACGGCTGGGG
U6	CTCGCTTCGGCAGCA CA	AACGCTTCACGAATTTGC GT

Table 1: Primer sequence.

CCK-8 detection

Cells were obtained 24h after transfection(with $4*10^6$ cells), cultivated on 96-well plates, and then cultivated for 0h, 24 h, 48h, 72h. A 10μL of CCK solution (Beyotime Biotechnology, Shanghai, China, C0037) and 90μL of basal medium (DMEM) were put into each well, cultivated at 37°C for 2h, and then OD value at 450nm was tested by an enzyme reader.

Cell invasion

The cells were obtained and cell suspension was prepared. The cell density was adjusted to 4×10^4 cells and suspended in serum-free medium including 1μg/ ml mitomycin C to inhibit cell proliferation.

The cells were cultivated into transwell upper chamber and 10% bovine fetal serum was added into lower chamber.

After culturing at 37°C for 24h, the substrate and cells in the upper chamber that did not go through the membrane were removed by washing with PBS for 3 times, immobilized with paraformaldehyde for 10 min, washed with double distilled water 3 times, dyed through 0.5% crystal violet, and the invasion was tested with a microscope.

Flow cytometry

Cells were treated with 0.25% trypsin, rinsed with PBS twice, put with 100μL binding buffer, made into suspension with $1*10^6$ cells/mL, added with AnnexinV-FITC and PI, cultivated at indoor temperature for 5 min, and tested with FC500MCL.

Outcome measures

miR-144-3p and miR-337-3p in the CG and the RG, the diagnostic value of the two to CC, the changes of the two before and after treatment in the RG, the relationship of the two with the clinicopathological characteristics of CC, and the role of the two on the prognosis of CC patients after treatment were analyzed.

Statistical method

SPSS24.0 was applied for analysis, and all figures were visualized by Graphpad8. Counting data were represented by (%) and compared by chi-square test. Measurement data were presented as (mean± SD) and compared using the t test.

The diagnostic value was determined using ROC. The survival was tested by the Kaplan-Meier and compared using the Log-rank test. $P<0.050$ was statistically significant.

Result

miR-144-3p and miR-337-3p in serum of the CG and the RG

Both serum miR-144-3p and miR-337-3p in the RG were evidently lower than those in the CG (both $P<0.05$). See Figure 1.

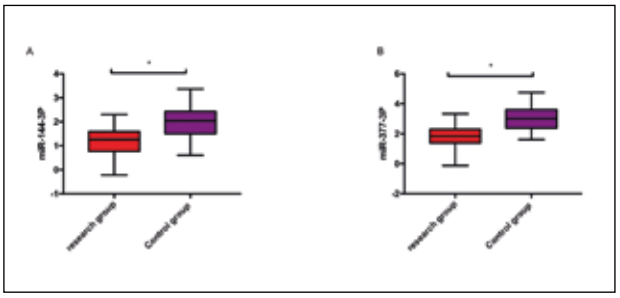

Figure 1: Expression of miR-144-3p and miR-337-3p in serum of the CG and the RG.
A) Expression of miR-144-3p in serum of the CG and the RG. B) Expression of miR-337-3p in serum of the CG and the RG.

Diagnostic value of miR-144-3p and miR-337-3p for CC

When miR-144-3p>1.83, the diagnostic sensitivity and specificity for predicting CC occurrence were 63.49% and 90.28%, respectively. When miR-337-3p>2.565, the diagnostic sensitivity and specificity for predicting CC occurrence were 68.25% and 90.28%, respectively. See Figure 2 and Table 2.

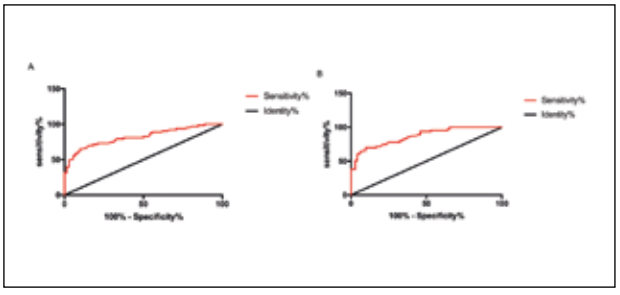

Figure 2: Diagnostic value of miR-144-3p and miR-337-3p to CC.
A) ROC curve of diagnostic value of miR-144-3p for CC. B) ROC curve of diagnostic value of miR-337-3p to CC.

	miR-144-3p	miR-337-3p
AUC	0.820	0.866
Std.Error	0.037	0.030
95%CI	0.747~0.893	0.807~0.925
cut-off	>1.83	>2.565
Sensitivity (%)	63.49	68.25
Specificity (%)	90.28	90.28
Youden index (%)	53.77	58.53
P	<0.001	<0.001

Table 2: Diagnostic effects of miR-144-3p and miR-337-3p on CC.

Changes in miR-144-3p and miR-337-3p before and after treatment in the RG

miR-144-3p and miR-337-3p in the RG increased evidently after treatment (P<0.05). See Figure 3.

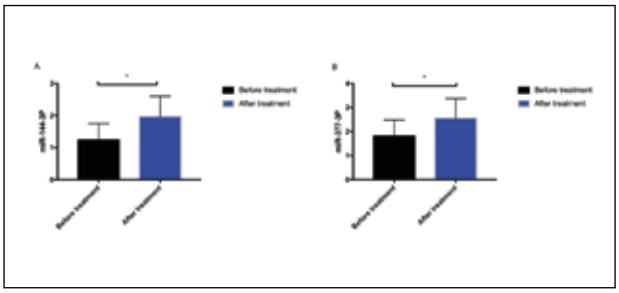

Figure 3: Expression changes of miR-144-3p and miR-337-3p before and after treatment in RG.
A) The expression changes of miR-144-3p in the RG before and after treatment. B) The expression changes of miR-337-3p in the RG before and after treatment.

Correlation of miR-144-3p with CC clinicopathological characteristics

miR-144-3p in CC patients was not related to age, pregnancy history and family history (p>0.05), but had a correlation with the differentiation, clinical stage, as well as lymph node metastasis (P<0.05). See Table 3.

Correlation ofmiR-337-3pwith CC clinicopathological characteristics

miR-337-3p was not related to age, pregnancy history and family history (p>0.05), but had a correlation with cell differentiation, clinical stage, as well as lymph node metastasis (P<0.05). See Table 4.

Effect of miR-144-3p on CC cells

After transfecting miR-144-3p into C33A, the proliferation and invasion of miR-144-3p-mimics group were lower than those of the other two groups, while the apoptosis rate was higher than that of the other two groups (P<0.05). The proliferation and invasion of miR-144-3p-inhibition group were higher than those of miR-NC group, and the apoptosis was lower than that of miR-NC group (P<0.05). See Figure 4.

Clinical features	miR-144-3p	F/t	P
Age		0.190	0.850
<60 (31)	1.23±0.46		
≥60 (41)	1.21±0.43		
Pregnant history		0.073	0.942
Yes (57)	1.22±0.48		
None (15)	1.23±0.45		
Family history		0.071	0.943
Yes (13)	1.20±0.44		
No (59)	1.21±0.46		
Cell differentiation		3.631	0.032*
Highly differentiated (33)	1.57±0.50		
Moderately differentiated (26)	1.34±0.47		
Poorly differentiated (13)	1.18±0.43		
Clinical stage		2.015	0.048*
I~II (55)	1.47±0.48		
III~IV (17)	1.21±0.41		
Lymph node metastasis		2.187	0.032*
Yes (39)	1.20±0.44		
No (33)	1.43±0.45		

Table 3: Correlation of miR-144-3p with CC clinicopathological features.
*Note: *indicates P<0.05.*

Clinical features	miR-337-3p	F/t	P
Age		0.066	0.948
<60 (31)	1.82±0.63		
≥60 (41)	1.81±0.64		
Pregnant history		0.054	0.958
Yes (57)	1.78±0.65		
None (15)	1.79±0.62		
Family history		0.078	0.923
Yes (13)	1.77±0.66		
No (59)	1.79±0.67		
Cell differentiation		3.655	0.031*
Highly differentiated (33)	2.16±0.62		
Moderately differentiated (26)	1.82±0.61		
Poorly differentiated (13)	1.68±0.66		
Clinical stage		2.715	0.008*
I~II (55)	2.13±0.63		
III~IV (17)	1.67±0.54		
Lymph node metastasis		2.165	0.034*
Yes (39)	1.68±0.61		
No (33)	1.99±0.60		

Table 4: Correlation of miR-337-3pwith CC clinicopathological features.
*Note: *indicates P<0.05.*

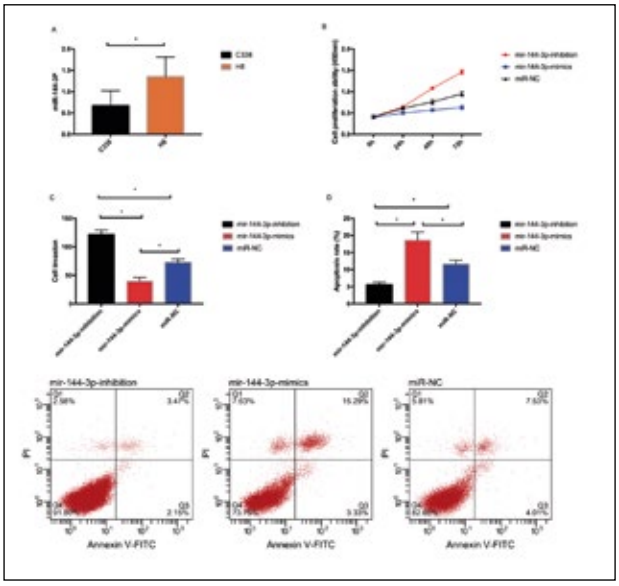

Figure 4: Effect of miR-144-3p on CC cells.
A) miR-144-3p expression level in C33A and H8 cells. B) Proliferation of C33A cell. C) Invasion of C33A cells. D) Apoptosis rate of C33A cells.

Effect of miR-337-3p on CC cells

miR-337-3p was lower in CC cells (P<0.05). Detection of cell biological behavior after transfection of mir-377-3p into C33A revealed that miR-337-3p-mimics group cells proliferated, with lower invasion ability than the other two groups, while the apoptosis was higher than the other two groups (P< 0.05). The proliferation and invasion of miR-337-3p-inhibition group were stronger than those of miR-NC group, and the apoptosis was weaker than that of miR-NC group (P<0.05). See Figure 5.

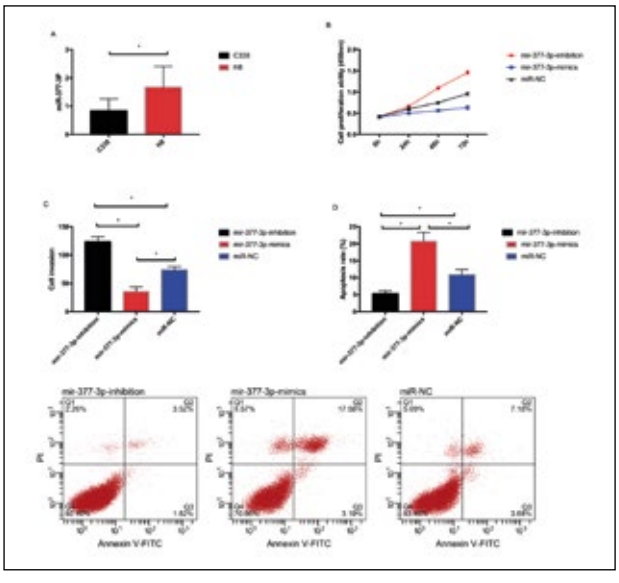

Figure 5: Effect of miR-337-3p on CC cells.
A) miR-377-3p expression level in C33A and H8 cells. B) Proliferation of C33A cell. C) Invasion of C33A cells. D) Apoptosis rate of C33A cells.

Influence of miR-144-3p and miR-337-3p on prognosis of CC patients after treatment

According to the content of miR-144-3p and miR-337-3p after treatment, the patients were grouped into high miR-144-3p (miR-144-3p>2.00, n=39), low miR-144-3p (miR-144-3p≤2.00, n=33), high miR-337-3p (miR-337-3p>2.56, n=36), and low miR-337-3p (miR-337-3p≤2.56, n=36). The prognosis of high miR-144-3p was more favorable than that of low miR-144-3p (P=0.020), and that of high miR-337-3p was more favorable than that of low miR-337-3p (P=0.041). See Figure 6.

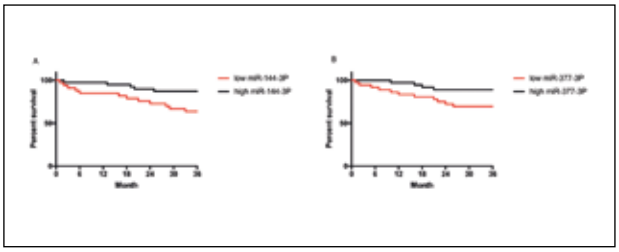

Figure 6: Effect of miR-144-3p and miR-337-3p on prognosis of CC patients after treatment.
A) Three-year survival curve of prognosis in high miR-144-3p group and low miR-144-3p group. B) Three-year survival curve of high miR-337-3p group and low miR-337-3p group.

Discussion

Cervical carcinoma is a common malignant neoplasm in women gynecologic diseases[15]. As there is no obvious feature in the early stage, most patients have reached the middle and late stage after being diagnosed[16]. At present, surgery is still the most effective method for cervical carcinoma treatment. Hysterectomy or hysterectomy is generally performed according to the patient's reproductive needs and severity of the disease[17]. However, cervical carcinoma patients usually have large local lesions, parauterine infiltration, and lymph node metastasis. After surgery, the best effect cannot be achieved and the recurrence rate is extremely high[18]. Therefore, with the development of medical technology, studies have confirmed the correlation of miRNAs with neoplasm diseases, which is a major research topic[19]. This research is meaningful to treat CC by exploringmiR-144-3p and miR-337-3p.

miR-144-3p and miR-337-3p are both low expressed in CC patients, suggesting that they may be participated in the progression of CC. However, the exploration on miR-144-3p in glioma by Cheng Z x[20], and the exploration on miR-337-3p in liver carcinoma by Zuo X L[21] had the same results.

However, ROC revealed that the sensitivity and specificity of miR-144-3p for CC occurrence in detection serum were 63.49% and 90.28%, those of miR-337-3p were 68.25% and 90.28% respectively. All of them have good diagnostic efficacy, suggesting that miR-144-3p and miR-337-3p may be neoplasm markers for CC screening. However, compared with traditional neoplasm markers (CEA and CA125), miR-144-3p and miR-337-3pare better in specificity, help clinical diagnosis of CC, and can improve the prognosis. miRNAs have been found to be expressed in many species in the medical field and acted in cardiovascular, infection, autoimmune diseases and metabolic disorders[22]. However, miR-144-3p was found to act in various neoplasms[23]. For example, Li B[24] proposed that miR-144-3p could hinder the progression of gastric carcinoma through targeting PBX3 to inhibit epithelial-mesenchymal transition. Therefore, we speculated that miR-144-3p may also affect the occurrence and development of CC by regulating various cell functions. However, miR-337-3p also belongs miRs with abnormal expression in many neoplasm diseases. At present, the specific situation of cervical carcinoma has not been explored. miR-337-3p and miR-144-3p were closely related to cell differentiation, clinical staging and lymphatic metastasis of cervical carcinoma, which further revealed that miR-144-3p and miR-337-3p were connected with the development of cervical carcinoma. Moreover, the two are lower in patients with more severe clinical pathology, indicating that the lowmiR-144-3p and miR-337-3p may be closely related the severity of cervical carcinoma.

In the future, we can objectively understand the disease development of patients by observing the changes of miR-144-3p and miR-337-3p. We transfected miR-144-3p and miR-337-3p into CC cells to further explore the role of the two on CC. HighmiR-144-3p and miR-337-3p proliferated and had lower invasion ability than the other two groups, while the apoptosis was higher than the other two groups. These results suggested that miR-144-3p and miR-337-3p acted as neoplasm suppressor genes in CC, which can support our results. Finally, through follow-up investigation, we knew that the decrease of miR-144-3p and miR-337-3p also indicated the poor prognosis, which further revealed the latent clinical application value of miR-144-3p and miR-337-3p. The above also preliminarily revealed that miR-144-3p and miR-337-3p may become latent therapeutic targets for cervical carcinoma in the future, and in-depth analysis of their mechanisms of action will be of great significance for cervical carcinoma. There are still some deficiencies in this experiment. Firstly, this experiment did not collect data on benign CC patients, so it is impossible to judge the specific roles of miR-337-3p and miR-144-3p on benign CC lesions. In addition, the experimental period was short, it is difficult to evaluate the long-term prognosis of miR-337-3p and miR-144-3p on cervical carcinoma. We will conduct more in-depth research on the mechanism of the two in cervical carcinoma to obtain the best results.

To sum up, miR-337-3p and miR-144-3p are closely related to cervical carcinoma and may be helpful for the treatment of cervical carcinoma in the future.

References

1) Fidler M M, Gupta S, Soerjomataram I, et al. Cancer incidence and mortality among young adults aged 20–39 years worldwide in 2012: a population-based study[J]. The Lancet Oncology, 2017, 18(12): 1579-1589.
2) Zhang Y, Zheng T, Zhang W. Report of cancer incidence and mortality in China, 2012[J]. Advances in Modern Oncology Research, 2018, 4(3): 1-7.
3) Bobdey S, Sathwara J, Jain A, et al. Burden of cervical cancer and role of screening in India[J]. Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical & Paediatric Oncology, 2016, 37(4): 278.
4) Lynge E, Lönnberg S, Törnberg S. Cervical cancer incidence in elderly women-biology or screening history?[J]. European Journal of Cancer, 2017, 74: 82-88.
5) Small Jr W, Bacon M A, Bajaj A, et al. Cervical cancer: a global health crisis[J]. Cancer, 2017, 123(13): 2404-2412.
6) Lees B F, Erickson B K, Huh W K. Cervical cancer screening: evidence behind the guidelines[J]. American journal of obstetrics and gynecology, 2016, 214(4): 438-443.
7) Kessler T A. Cervical cancer: prevention and early detection[C]//Seminars in oncology nursing. WB Saunders, 2017, 33(2): 172-183.
8) Boen J R A, Gevaert A B, De Keulenaer G W, et al. The role of endothelial miRNAs in myocardial biology and disease[J]. Journal of Molecular and Cellular Cardiology, 2020, 138: 75-87.
9) Devi K P, Rajavel T, Daglia M, et al. Targeting miRNAs by polyphenols: Novel therapeutic strategy for cancer[C]//Seminars in cancer biology. Academic Press, 2017, 46: 146-157.

10) Burwinkel B, Cuk K, Zucknick M, et al. Circulating miRNAs as markers for breast cancer: U.S. Patent 10, 316, 367[P]. 2019-6-11.
11) Paul P, Chakraborty A, Sarkar D, et al. Interplay between miRNAs and human diseases[J]. Journal of cellular physiology, 2018, 233(3): 2007-2018.
12) Wu J, Zhao Y, Li F, et al. miR-144-3p: A novel tumor suppressor targeting mapk6 in cervical cancer[J]. Journal of physiology and biochemistry, 2019, 75(2): 143-152.
13) Tang D, Zhao L, Peng C, et al. LncRNA CRNDE promotes hepatocellular carcinoma progression by upregulating SIX1 through modulating miR-337-3p[J]. Journal of cellular biochemistry, 2019, 120(9): 16128-16142.
14) Durand M, Murphy S, Leidhin C N, et al. Cervical cancer staging: Pearls & pitfalls[C]. European Congress of Radiology 2017, 2017.
15) Choi S, Hsu I C J. Cervical cancer[M]//Handbook of Evidence-Based Radiation Oncology. Springer, Cham, 2018: 631-652.
16) Roh H J, Kim K B, Lee J H, et al. Early cervical cancer: predictive relevance of preoperative 3-tesla multiparametric magnetic resonance imaging[J]. International journal of surgical oncology, 2018, 2018.
17) Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis[J]. The Lancet Global Health, 2020, 8(2): e191-e203.
18) Ramirez P T, Frumovitz M, Pareja R, et al. Minimally invasive versus abdominal radical hysterectomy for cervical cancer[J]. New England Journal of Medicine, 2018, 379(20): 1895-1904.
19) Cancer Genome Atlas Research Network. Integrated genomic and molecular characterization of cervical cancer[J]. Nature, 2017, 543(7645): 378-384.
20) Cheng Z X, Song Y X, Wang Z Y, et al. miR-144-3p serves as a tumor suppressor by targeting FZD7 and predicts the prognosis of human glioblastoma[J]. Eur Rev Med Pharmacol Sci, 2017, 21(18): 4079-4086.
21) Zuo X L, Chen Z Q, Wang J F, et al. miR-337-3p suppresses the proliferation and invasion of hepatocellular carcinoma cells through targeting JAK2[J]. American journal of cancer research, 2018, 8(4): 662.
22) Mori M A, Ludwig R G, Garcia-Martin R, et al. Extracellular miRNAs: from biomarkers to mediators of physiology and disease[J]. Cell metabolism, 2019.
23) Nothnick W B, Swan K, Flyckt R, et al. Human endometriotic lesion expression of the miR-144-3p/miR-451a cluster, its correlation with markers of cell survival and origin of lesion content[J]. Scientific reports, 2019, 9(1): 1-10.
24) Li B, Zhang S, Shen H, et al. MicroRNA-144-3p suppresses gastric cancer progression by inhibiting epithelial-to-mesenchymal transition through targeting PBX3[J]. Biochemical and biophysical research communications, 2017, 484(2): 241-247.

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