Relationship Between the Expression of MAP4K4 and HIF2α in Peripheral Blood of Patients with Locally Advanced Cervical Cancer and Over-Activation of Autophagy and Sensitivity to Platinum-Type Neoadjuvant Chemotherapy

Cuili N[1], Juan L[1], Dongfang L[1], Xiaolin C[1], Yanmin M[1], Chen X[1] and Ye Z[2]*

[1]Department of Gynecology, The Third Hospital of Xingtai, China

[2]Department of Gynecology, The Second Affiliated Hospital of Xingtai Medical College, China

Abstract

Objective: To investigate Mitogen Activated Protein Kinase 4 (MAP4K4) and Hypoxia-inducible in peripheral blood of patients with locally advanced cervical cancer the relationship between the expression of factors 2α, HIF-2α and autophagy overactivation and sensitivity to platinum-type neoadjuvant chemotherapy.

Methods: 230 patients with locally advanced cervical cancer admitted to our hospital from August 2017 to September 2020 were prospectively selected as research objects. The expression of MAP4K4, HIF2α and autophagy related genes (LC-3 II, LC-3 I and Beclin-1) in peripheral blood before and after chemotherapy were detected by qRT-PCR. Pearson test was used for correlation analysis. The independent risk factors of chemotherapy sensitivity was analyzed by logistics regression model.

Results: The expression of MAP4K4 and HIF2α in peripheral blood of patients with locally advanced cervical cancer was observed among subgroups of age, tumor size and histological type (P>0.05), but was observed among subgroups of differentiation, FIGO stage and lymph node metastasis (P<0.05). Before chemotherapy, MAP4K4 mRNA and HIF2α mRNA in peripheral blood of the two groups were compared (P>0.05). After chemotherapy, MAP4K4 mRNA and HIF2α mRNA in both groups were significantly decreased (P<0.05), and MAP4K4 mRNA and HIF2α mRNA in the effective group were significantly lower than those in the ineffective group (P<0.05). Before chemotherapy, LC-3 II, LC-3 I and Beclin-1 were compared between the two groups (P>0.05). After chemotherapy, LC-3 II, LC-3 I and Beclin-1 in both groups were increased, and LC-3 II, LC-3 I and Beclin-1 in ineffective group were significantly higher than those in effective group (P<0.05). MAP4K4 and HIF2α were significantly positively correlated with LC-3 II, LC-3 I and Beclin-1 (P<0.05). Differentiation degree, FIGO stage, lymph node metastasis, LC-3 II, LC-3 I, Beclin-1, MAP4K4 and HIF2α were independent risk factors for chemotherapy sensitivity in patients with locally advanced cervical cancer (P<0.05).

Conclusion: The up-regulated expression of MAP4K4 and HIF2α in patients with locally advanced cervical cancer is closely related to over-activation of autophagy during chemotherapy and sensitivity to platinum neoadjuvant chemotherapy.

Keywords: Locally Advanced Cervical Cancer; MAP4K4; HIF2α; Hyperactivation of Autophagy; Sensitivity to Platinum Neoadjuvant Chemotherapy

Introduction

Cervical cancer is a major public health problem worldwide and is a common malignant tumor of the female reproductive system [1]. Epidemiological studies [2] show that the incidence of cervical cancer is second only to breast cancer, and in recent years, the incidence group tends to develop at a younger age. Surgery is the main method for the treatment of early cervical cancer, which can effectively eradicate the disease and improve the prognosis of patients. Patients with locally advanced cervical cancer are mostly treated with concurrent chemoradiotherapy to delay the

progression of the disease, but the recurrence rate of locally advanced cervical cancer patients after concurrent chemoradiotherapy is high, resulting in the improvement of the prognosis of locally advanced cervical cancer patients is still not as expected [3]. Recently, platinum-based neoadjuvant chemotherapy can effectively reduce tumor volume, thus providing surgical opportunities for locally advanced cervical cancer and improving prognosis [4]. Studies have confirmed [5] that patients with locally advanced cervical cancer differ in their sensitivity to chemotherapy due to inter-individual heterogeneity. A number of recent *in vitro* studies have confirmed that the overactivation of autophagy is an important reason affecting the sensitivity of cervical cancer cells to chemotherapy [6]. Mitogen Activated Protein Kinase Kinase-4 (MAP4K4) is an important factor involved in regulating the proliferation and invasion of cervical cancer cells. Recent *in vitro* studies have found that the up-regulated expression of MAP4K4 can activate autophagy and thus affect the chemotherapy sensitivity of cervical cancer patients [7]. Hypoxia is a common feature of solid tumors, and Hypoxia-Inducible Factors (HIF) are important factors involved in regulating the adaptation of solid tumors to hypoxia. Among them, HIF2α has been confirmed to be upregulated in cervical cancer patients, and is associated with resistance to platinum chemotherapy drugs regulated by autophagy [8]. However, at this stage, studies on MAP4K4 and HIF2α still remain at the cellular level *in vitro*, and there is still a lack of high level of clinical cohort evidence, especially neoadjuvant chemotherapy for locally advanced cervical cancer has not been reported. Based on this background, this study aims to study the expression of MAP4K4 and HIF2α in patients with locally advanced cervical cancer, and clarify the influence of the expression changes of MAP4K4 and HIF2α on autophagy activation and chemotherapy sensitivity, so as to provide new ideas for the subsequent early evaluation of chemotherapy efficacy for locally advanced cervical cancer.

Data and Methods

General Information: 230 patients with locally advanced cervical cancer admitted to our hospital from August 2017 to September 2020 were prospectively selected as the study objects.

Inclusion criteria: 1. Did not receive any antitumor therapy before admission; 2. Age 40 to 60 years old; 3. After enrolment, he received 3 cycles of neoadjuvant chemotherapy in our hospital; 4. Pathological diagnosis of cervical cancer; 5. Patients voluntarily sign informed consent; 6. The stage of the International Union of Obstetrics and Gynecology is IB2-IIA2 [9]; the pathological type was confirmed as squamous cell carcinoma and adenocarcinoma.

Exclusion criteria: 1. Recurrent or metastatic cervical cancer; 2. Cognitive dysfunction, difficult to cooperate with the completion of the test; 3. combined with malignant tumors in other parts of the body; 4. Patients with immunodeficiency or immune-related diseases; 5. had received neoadjuvant chemoradiotherapy before entering the group; 6. combined with blood system diseases; this study was approved by the Ethics Committee of our hospital. The mean age of 161 patients in the effective group was (51.85 ± 6.54) years, the tumor size was <5 cm in 77 cases, ≥ 5 cm in 84 cases, lymph node metastasis was 106 cases, no 55 cases, histological type was 95 cases of adenocarcinoma, 66 cases of squamous cell carcinoma, clinical stage was 90 cases of b2, 71 cases of a1~ a2, differentiation degree: There were 65 cases with low differentiation and 96 cases with medium/high scores. The mean age of 69 patients in the invalid group was (52.95 ± 6.78) years, the tumor size was <5 cm in 33 cases, ≥ 5 cm in 36

cases, lymph node metastasis was 106 cases, no 55 cases, histological type was 40 cases of adenocarcinoma, 29 cases of squamous cell carcinoma, clinical stage was 39 cases of b2, 30 cases of II A1-II A2, differentiation degree: There were 28 cases with low differentiation and 41 cases with medium/high scores.

Methods

Collection of patients' baseline data: After enrollment, the responsible nurses of the department collected the baseline information of all patients, including age and basic medical history, etc., and conducted routine enrollment education for patients. After pathological examination, the relevant information of the patient's disease was retained, including tumor size, lymph node metastasis, clinical stage, pathological type, and degree of tissue differentiation.

Neoadjuvant chemotherapy regimen: All patients were treated with carboplatin combined with paclitaxel neoadjuvant chemotherapy before operation. The specific chemotherapy regimen was as follows: d1, 175 mg/m² paclitaxel, d2, carboplatin: area under the curve 4~5, intravenous infusion, repeated every 3 weeks, and all patients were treated for 3 cycles.

Peripheral blood sample collection: 5 ml of peripheral venous blood was collected in the morning of the next day after admission (fasting 8 h) and in the morning after 3 cycles of chemotherapy (fasting 8 h). After peripheral blood samples were obtained, they were centrifuged 1000 r/min for 15 min (with a centrifugation radius of 10 cm), the upper serum was left to separate, and then centrifuged again at 1200 r/min for 5 min (with a centrifugation radius of 10 cm). After centrifugation, 1.5 mL of serum was absorbed and stored in an enzyme-free centrifuge tube and placed in a refrigerator at -80°C for testing.

The expression of MAP4K4, HIF2α and autophagy related factors: LC-3 II, LC-3 I and Beclin-1 in peripheral blood was detected by qRT-PCR Trizol method (Shanghai Shangbao Biotechnology Co., LTD.) was used to extract total RNA, and SYBRR Green fluorescent dye kit (Shanghai Win-win Biotechnology Co., LTD.) and ABI StepOne PCR instrument (Shanghai Liuli Intelligent Technology Co., LTD.) were used for reverse transcription operation. The reverse transcription conditions were as follows: Reverse transcription was performed at 98°C, 50°C and 37°C, and RT-PCR amplification was performed after reverse transcription into cDNA, and GAPDH was used as the internal reference. The primer sequence was: HIF2α: Forward 5'-GAACATGG CCCCCGATGAAT-3'; reverse-5'-CCCCTGAGCTCCTGGTAGAT-3'. MAP4K4-Forward-5'-CGGAA TTCATGGCGAACGACTCCCCTGCAAAAAGTCTG-3'; Reverse-5'-CCGCTCGAGCCAGCTCAGAAGAGAAGTCCTGCC-3'. The reaction conditions were as follows: 65°C for 1min, 95°C for 15 sec, 95°C for 10 min. Finally, the relative mRNA expression levels of MAP4K4, HIF2α, LC-3 II, LC-3 I and Beclin-1 were calculated by reference to $2^{-\Delta\Delta CT}$.

Evaluation criteria of curative effect

With reference to the evaluation criteria for solid tumors [10], all patients included in this study were evaluated with the assistance of B-ultrasound or MRI, and were divided into: Complete Response (CR): All target lesions disappeared; Partial Response (PR): Reduction of the total length and diameter of the target lesion by 50% or more; Stable Disease (SD): Target lesion enlargement of less than 25% or reduction of less than 50%; Progressive Disease (PD): The target lesion increases by more than 25% or a new lesion appears. CR and PR were

included in the effective group, and PD and SD were included in the ineffective group. There were 161 CR+PR cases in this study. There were 69 cases with PD+SD.

Statistical methods

SPSS20.0 software was used to analyze the data, and GraphPad Prism 8 was used to draw graphs to represent the measurement data. Independent sample t test was used for comparison between two groups, and paired sample t test was used for comparison between the same group. The statistical data were expressed as percentages and χ^2 test was used to compare the two groups. P<0.05 was considered to be statistically significant.

Results

Relationship between the expression of MAP4K4 and HIF2α in peripheral blood and pathological characteristics of cervical cancer patients before chemotherapy

There were no significant differences in the expression of MAP4K4 and HIF2α in peripheral blood of patients with locally advanced cervical cancer among subgroups in age, tumor size and histological type (P>0.05), but there were significant differences in differentiation degree, FIGO stage and lymph node metastasis among subgroups (P<0.05). See Table 1 for details.

Expression of MAP4K4 and HIF2α in peripheral blood of patients before and after chemotherapy in two groups

Before chemotherapy, there were no significant differences in MAP4K4 mRNA and HIF2α mRNA in peripheral blood between the two groups (P>0.05). After chemotherapy, MAP4K4 mRNA and HIF2α mRNA in both groups were significantly decreased (P<0.05), and MAP4K4 mRNA and HIF2α mRNA in the effective group were significantly lower than those in the ineffective group (P<0.05). See Table 2 for details.

Expression of autophagy related factors LC-3 II, LC-3 I and Beclin-1 in two groups

Before chemotherapy, there were no significant differences in LC-3 II, LC-3 I and Beclin-1 between the two groups (P>0.05). After chemotherapy, LC-3 II, LC-3 I and Beclin-1 in both groups were increased, and LC-3 II, LC-3 I and Beclin-1 in the ineffective group were significantly higher than those in the effective group (P<0.05). See Table 3 for details.

Relationship between the expression of MAP4K4 and HIF2α in peripheral blood and autophagy related factors LC-3 II, LC-3 I and Beclin-1 after chemotherapy

MAP4K4 and HIF2α were significantly positively correlated with LC-3 II, LC-3 I and Beclin-1 (P<0.05), as shown in Table 4 for details.

Univariate analysis of neoadjuvant chemotherapy sensitivity in patients with locally advanced cervical cancer

Chemotherapy results of the two groups of patients were incorporated into the logistics regression model as dependent variables (effective =1, ineffective =0). The results showed that differentiation degree, FIGO stage, lymph node metastasis, LC-3 II, LC-3 I, Beclin-1, MAP4K4 and HIF2α were risk factors (P<0.05). See Table 5 for details.

Discussion

Neoadjuvant chemotherapy sensitivity is an important factor affecting the further improvement of prognosis in patients with locally advanced cervical cancer. The results of this study showed that 30% (69/230) patients had poor sensitivity to neoadjuvant chemotherapy, which was similar to the results of Yang Yongbo *et al.* [11]. Therefore, it is of great significance to further search for markers of sensitivity to neoadjuvant chemotherapy in the early stage

Table 1: The relationship between the expression of MAP4K4 and HIF2α in peripheral blood and the pathological characteristics of cervical cancer patients before chemotherapy.

Pathological feature	MAP4K4 mRNA	t	P VALUE	HIF2α mRNA	t	P Value
Age (years)						
<50 (n=125)	3.84 ± 0.31	1.26	0.21	3.87 ± 0.35	1.76	0.07
≥ 50 (n=105)	3.89 ± 0.29			3.80 ± 0.25		
Tumor size						
<5 cm (n=110)	3.87 ± 0.21	1.61	0.10	3.85 ± 0.26	1.86	0.06
≥ 5 cm (n=120)	3.82 ± 0.26			3.78 ± 0.31		
Histological type						
adenocarcinoma (n=135)	3.85 ± 0.21	1.59	0.11	3.81 ± 0.28	0.50	0.61
Squamous carcinoma (n=95)	3.80 ± 0.25			3.79 ± 0.31		
Degree of differentiation		26.5	0.00		31.85	0.00
Poorly differentiated (n=93)	4.72 ± 0.35	1		4.67 ± 0.29		
Medium/high differentiation (n=137)	2.98 ± 0.64			2.87 ± 0.56		
FIGO staging		41	0.00		28.01	0.00
b2 (n=129)	3.01 ± 0.31	2		3.11 ± 0.29		
a1~ a2 (n=101)	4.61 ± 0.28			4.53 ± 0.44		
Lymph node metastasis		17.8	0.00		24.04	0.00
NO (n=152)	3.41 ± 0.38	6		3.35 ± 0.28		
Yes (n=78)	4.26 ± 0.32			4.31 ± 0.29		

Table 2: Expression of MAP4K4 and HIF2α in peripheral blood of patients before and after chemotherapy in two groups.

Group	MAP4K4 mRNA		HIF2α mRNA	
	Post-chemotherapy	Pre-chemotherapy	Pre-chemotherapy	Post-chemotherapy
Active group (n=161)	3.85 ± 0.31	1.75 ± 0.21	3.72 ± 0.22	1.63 ± 0.15
Invalid (n=69)	3.79 ± 0.35	2.98 ± 0.15	3.69 ± 0.18	2.78 ± 0.16
T value	1.23	50.21	1.08	50.88
P value	0.22	0.00	0.28	0.00

Note: a $P<0.05$ was compared with that before chemotherapy

Table 3: Expression of autophagy related factors LC-3II, LC-3 I and Beclin-1 in the two groups.

Group (n)	LC-3 II mRNA		LC-3 I mRNA		Beclin-1 mRNA	
	Pre-chemotherapy	post-chemotherapy	Pre-chemotherapy	post-chemotherapy	Pre-chemotherapy	post-chemotherapy
Active group (n=161)	1.39 ± 0.21	2.02 ± 0.12a	1.12 ± 0.31	1.87 ± 0.18a	1.23 ± 0.17	1.91 ± 0.23[a]
Invalid group (n=69)	1.43 ± 0.15	2.98 ± 0.12a	1.20 ± 0.30	2.38 ± 0.15a	1.21 ± 0.21	2.68 ± 0.22[a]
T value	1.63	52.49	1.83	22.21	0.70	23.99
P value	0.10	0.00	0.07	0.00	0.48	0.00

of locally advanced cervical cancer and guide the optimization of early treatment. MAP4K4, located in 2q11.2 of human chromosome, contains 1,200 amino acids and has a molecular weight of 140 KDa. Initially, MAP4K4 was confirmed to be up-regulated in brain tissue and involved in the occurrence and development of atherosclerosis and cerebrovascular diseases [12]. Recently, MAP4K4 has been found to play a particularly important role in the development of cancer and is expected to be an important potential therapeutic target [13]. MAP4K4 can inhibit the proliferation of tumor cells and block the progression of the disease by affecting the G0/G1 cell phase of tumor cells, which is an important idea for clinical intervention in the progressive development of breast cancer. Targeted regulation of MAP4K4 signaling pathway can effectively inhibit the proliferation of breast cancer cells and promote apoptosis [14]. The change of immune microenvironment is an important pathological basis leading to the progression of malignant tumors, and the change of MAP4K4 expression can effectively regulate the immune microenvironment and thus affect the development of breast cancer [15]. The important role of MAP4K4 in the progression of malignant tumors has also attracted the attention of cervical cancer researchers. By constructing an *in vitro* model of cervical cancer, Mei *et al.* [16] found that the upregulation of MAP4K4 could effectively promote the proliferation and migration of cervical cancer cells, suggesting that the upregulation of MAP4K4 might be related to the malignant development of cervical cancer. As an important member of the HIF family, HIF2α is an important factor involved in regulating the adaptation of tumor cells and tissues to hypoxic environment. The expression of HIF2α in cervical cancer tissues is significantly higher than that in adjacent tissues, and the high expression of HIF2α is closely related to lymph node metastasis and FIGO staging [17]. Studies have confirmed [18] that inhibiting the expression of HIF2α can effectively inhibit the G1 phase stagnation of cervical cancer cells and promote cell apoptosis. The results of this study showed that the expression of MAP4K4 and HIF2α was closely related to the differentiation degree, FIGO stage and lymph node metastasis of patients with locally advanced cervical cancer, suggesting that the upregulation of MAP4K4 and HIF2α was closely related to the disease deterioration of patients with locally advanced cervical cancer.

The results of this study showed that after chemotherapy, the expression levels of MAP4K4 mRNA and HIF2α mRNA in the

Table 4: Relationship between the expression of MAP4K4 and HIF2α in peripheral blood and autophagy related factors LC-3II, LC-3I and Beclin-1 after chemotherapy.

Group	MAP4K4 mRNA		HIF2α mRNA	
	r	p	r	p
LC-3 II	0.52	<0.01	0.45	<0.01
LC-3 I	0.49	<0.01	0.51	<0.01
Beclin-1	0.51	<0.01	0.49	<0.01

effective group were significantly lower than those in the ineffective group, suggesting that the up-regulated expression of MAP4K4 and HIF2α in patients with locally advanced cervical cancer was not only involved in the progressive development of the disease, but also related to the sensitivity of neoadjuvant chemotherapy during peri-chemotherapy. Autophagy is a kind of non-apoptotic cell death, which is a highly conserved process in eukaryotic cells and has been proved to be a "double-edged sword" that plays a protective or harmful role in tumorigenesis [19]. Studies have found [20] that chemotherapy can activate autophagy of tumor cells, and moderate activation of autophagy can effectively promote the death of cancer cells and thus improve the effect of chemotherapy. However, there is evidence that autophagy is over-activated in some patients after chemotherapy, resulting in tumor cells becoming resistant to chemotherapy and thus affecting the efficacy of neoadjuvant chemotherapy. The results of this study showed that the expressions of autophagy related genes LC-3 II, LC-3 I and Beclin-1 in the effective group were significantly lower than those in the ineffective group, suggesting that the over-activation of autophagy in patients with locally advanced cervical cancer is closely related to chemotherapy sensitivity. *In vitro* studies by Jiang *et al.* [21] showed that upregulation of HIF2α enhanced autophagy of cervical cancer cells under hypoxia conditions. In addition, the latest *in vitro* study by Jiang *et al.* [8] confirmed that the up-regulated expression of HIF2α can participate in the regulation of the sensitivity of cervical cancer cells to cisplatin. Huang *et al.* [22] showed *in vitro* studies that up-regulated expression of MAP4K4 could enhance autophagy of cervical cancer cells and reduce their sensitivity to platinum-based chemotherapy drugs. The Pearson test results showed that the expression of MAP4K4 and HIF2α was positively correlated with the levels of LC-3 II, LC-3 I and Beclin-1, confirming that the overexpression of MAP4K4 and HIF2α was related to chemotherapy

Table 5: Univariate analysis of neoadjuvant chemotherapy sensitivity in patients with locally advanced cervical cancer.

Group	Assign		MMSE score		
			OR	95% CI	P value
Tumor size	<5 cm=0	≥ 5 cm=1	1.031	0.897~2.131	0.261
Histological type	Adenocarcinoma =0	Squamous carcinoma =1	1.123	0.789~1.987	0.535
Degree of differentiation	Poorly differentiated =1	Medium/high differentiation =0	2.363	1.351~4.654	<0.001
FIGO staging	b2=0	a1 ~ a2=1	3.123	1.365~5.654	<0.001
Lymph node metastasis	NO=0	YES=1	2.356	1.365~6.578	<0.001
LC-3 II	<=2.51	≥ 2.51=1	3.231	1.657~5.654	<0.001
LC-3 I	<2.43=	≥ 2.43=1	4.652	1.654~6.763	<0.001
Beclin-1	<2.39=	≥ 2.39=1	3.654	1.785~5.987	<0.001
MAP4K4	<2.60=	≥ 2.60=1	3.546	1.657~5.684	<0.001
HIF2α	<2.55=	≥ 2.55=1	3.756	1.726~6.551	<0.001

Note: LC-3 II, LC-3I, Beclin-1, MAP4K4 and HIF2α were all expressed after chemotherapy

resistance in patients with locally advanced cervical cancer. Multivariate regression analysis showed that MAP4K4 and HIF2α were independent risk factors for chemotherapy resistance in patients with locally advanced cervical cancer. These results suggest that the expression of MAP4K4 and HIF2α is up-regulated with the malignant progression of cervical cancer, and the up-regulated expression of the two can promote the progressive development of the disease by promoting the proliferation of tumor cells and inhibiting apoptosis. At the same time, the high expression of MAP4K4 and HIF2α can promote the over-activation of autophagy during chemotherapy, thus reducing the sensitivity of cervical cancer cells to chemotherapy and ultimately affecting the chemotherapy efficacy.

In summary, based on the clinical cohort-level, this study further clarified that the up-regulation of MAP4K4 and HIF2α expression is closely related to the deterioration of patients with locally advanced cervical cancer, and is closely related to the resistance to platinum neoadjuvant chemotherapy, thus providing evidence reference for the two as reliable serum markers for early evaluation of chemotherapy efficacy in patients with locally advanced cervical cancer for clinical transformation.

References

1. Hu Z, Ma D. The precision prevention and therapy of HPV-related cervical cancer: New concepts and clinical implications. Cancer Med. 2018;7(10):5217-36.

2. Shrestha AD, Neupane D, Vedsted P, Kallestrup P. Cervical cancer prevalence, incidence and mortality in low- and middle-income countries: A systematic review. Asian Pac J Cancer Prev. 2018;19(2):319-24.

3. Gadducci A, Cosio S. Neoadjuvant chemotherapy in locally advanced cervical cancer: Review of the literature and perspectives of clinical research. Anticancer Res. 2020;40(9):4819-28.

4. Gupta S, Maheshwari A, Parab P, Mahantshetty U, Hawaldar R, Chopra SS, et al. Neoadjuvant chemotherapy followed by radical surgery versus concomitant chemotherapy and radiotherapy in patients with stage IB2, IIA, or IIB squamous cervical cancer: A randomized controlled trial. J Clin Oncol. 2018;36(16):1548-55.

5. Benson R, Pathy S, Kumar L, Mathur S, Dadhwal V, Mohanti BK. Locally advanced cervical cancer - neoadjuvant chemotherapy followed by concurrent chemoradiation and targeted therapy as maintenance: A phase II study. J Cancer Res Ther. 2019;15(6):1359-64.

6. Guo J, Chen M, Ai G, Mao W, Li H, Zhou J. Hsa_circ_0023404 enhances cervical cancer metastasis and chemoresistance through VEGFA and autophagy signaling by sponging miR-5047. Biomed Pharmacother. 2019;115:108957.

7. Mei J, Wang DH, Wang LL, Chen Q, Pan LL, Xia L. MicroRNA-200c suppressed cervical cancer cell metastasis and growth via targeting MAP4K4. Eur Rev Med Pharmacol Sci. 2018;22(3):623-31.

8. Jiang L, Shi S, Li F, Shi Q, Zhong T, Zhang H, et al. miR-519d-3p/HIF-2α axis increases the chemosensitivity of human cervical cancer cells to cisplatin via inactivation of PI3K/AKT signaling. Mol Med Rep. 2021;23(5):353.

9. Matsuo K, Machida H, Mandelbaum RS, Konishi I, Mikami M. Validation of the 2018 FIGO cervical cancer staging system. Gynecol Oncol. 2019;152(1):87-93.

10. Coleman RL, Lorusso D, Gennigens C, González-Martín A, Randall L, Cibula D, et al; innovaTV 204/GOG-3023/ENGOT-cx6 Collaborators. Efficacy and safety of tisotumab vedotin in previously treated recurrent or metastatic cervical cancer (innovaTV 204/GOG-3023/ENGOT-cx6): A multicentre, open-label, single-arm, phase 2 study. Lancet Oncol. 2021;22(5):609-19.

11. Yongbo Y. Expression of osteopontin and cyclooxygenase-2 in cervical cancer and their correlation with chemotherapy sensitivity [J]. J Bengbu Med Coll. 2021;46(4):5.

12. Chuang HC, Wang X, Tan TH. MAP4K family kinases in immunity and inflammation. Adv Immunol. 2016;129:277-314.

13. Gao X, Gao C, Liu G, Hu J. MAP4K4: An emerging therapeutic target in cancer. Cell Biosci. 2016;6:56.

14. Xie D, Li S, Wu T, Wang X, Fang L. MiR-181c suppresses triple-negative breast cancer tumorigenesis by targeting MAP4K4. Pathol Res Pract. 2022;230:153763.

15. Zhang Q, Xin H, Fen T. Function of microRNA-141 in human breast cancer through cytotoxic CD4+ T cells regulated by MAP4K4 expression. Mol Med Rep. 2018;17(6):7893-901.

16. Mei J, Wang DH, Wang LL, Chen Q, Pan LL, Xia L. MicroRNA-200c suppressed cervical cancer cell metastasis and growth *via* targeting MAP4K4. Eur Rev Med Pharmacol Sci. 2018;22(3):623-31.

17. Zhang L, Chen Q, Hu J, Chen Y, Liu C, Xu C. Expression of HIF-2α and VEGF in cervical squamous cell carcinoma and its clinical significance. Biomed Res Int. 2016;2016:5631935.

18. Kawanaka T, Kubo A, Ikushima H, Sano T, Takegawa Y, Nishitani H. Prognostic significance of HIF-2alpha expression on tumor infiltrating macrophages in patients with uterine cervical cancer undergoing radiotherapy. J Med Invest. 2008;55(1-2):78-86.

19. Yang Y, Wang Q, Song D, Zen R, Zhang L, Wang Y, et al. Lysosomal dysfunction and autophagy blockade contribute to autophagy-related

cancer suppressing peptide-induced cytotoxic death of cervical cancer cells through the AMPK/mTOR pathway. J Exp Clin Cancer Res. 2020;39(1):197.

20. Esfandyari YB, Doustvandi MA, Amini M, Baradaran B, Zaer SJ, Mozammel N, et al. MicroRNA-143 sensitizes cervical cancer cells to cisplatin: A promising anticancer combination therapy. Reprod Sci. 2021;28(7):2036-49.

21. Jiang L, Shi S, Shi Q, Zhang H, Hu R, Wang M. Similarity in the functions of HIF-1α and HIF-2α proteins in cervical cancer cells. Oncol Lett. 2017;14(5):5643-51.

22. Huang H, Han Q, Zheng H, Liu M, Shi S, Zhang T, et al. MAP4K4 mediates the SOX6-induced autophagy and reduces the chemosensitivity of cervical cancer. Cell Death Dis. 2021;13(1):13.