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Original Article

Survivin, MMP-2, and MMP-9 expression in different types of cervical lesions and correlation analysis

Jin Yu[1,2*], Qingsheng Xie[1*], Hui Zhou[1], Yongpai Peng[1], Huaiwu Lu[1], Tingting Yao[1], Zhongqiu Lin[1,2]

[1]Department of Gynecological Oncology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China; [2]Key Laboratory of Malignant Tumor Gene Regulation and Target, Therapy of Guangdong Higher Education Institutes, Sun Yat-sen University, Guangzhou, China. [*]Equal contributors.

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Abstract: To analyze Survivin, MMP-2, and MMP-9 expression in different types of cervical lesions and discuss their correlation and clinical significance. Patients with chronic cervicitis, cervical intraepithelial neoplasia, or cervical cancer in our hospital were collected. ELISA was applied to detect the level of Survivin, MMP-2 and MMP-9 in serum. Immunohistochemistry was used to measure Survivin, MMP-2 and MMP-9 expression in tissue. Their correlation was analyzed. Survivin, MMP-2, and MMP-9 were overexpressed in chronic cervicitis, cervical intraepithelial neoplasia, and cervical cancer ($P < 0.05$). Their levels were related to pathological grade, clinical stage, differentiation, vascular invasion, and lymph node metastasis ($P < 0.05$), but not age, gross type, and histological type ($P > 0.05$). Their levels were elevated significantly following increased pathologic classification, clinical upstage, lower differentiation degree, vascular invasion and lymph node metastasis emergence. Survivin, MMP-2, and MMP-9 showed escalating trend in the serum and cervical tissue of chronic cervicitis, cervical intraepithelial neoplasia, and cervical cancer patients. They were highly expressed in cervical cancer patients' serum and tissue. Their levels were correlated with pathological grade, clinical stage, differentiation, vascular invasion, and lymph node metastasis, and showed obvious elevation.

Keywords: Cervical cancer, Survivin, MMP-2, MMP-9

Introduction

Cervical cancer is a common malignant tumor in female. There were 500,000 new cases around the world every year, and developing countries accounted for 80%. Its mortality rate is high with 233,000 patients died every year. In China, new cases accounted for about 28. 8% of the total amount, which are about 140,000 cases every year [1]. Recent survey showed that cervical cancer presented young trend. Because of its inconspicuous symptoms in early stage and low attention, cervical cancer is often misdiagnosed and missed diagnosed seriously affects the prognosis. In the process of cervical cancer development, there is a type of lesion called time precancerous lesions characterized as long interval and reversible. That is from benign lesions becoming cervical intraepithelial neoplasia (CIN) after a series of pathological development. Cancer development has multiple stages and steps. Oncogenes and tumor suppressor genes work together to regulate cell apoptosis and participate in neovascularization [2]. Survivin is an important member of apoptosis inhibiting protein family that is associated with tumor occurrence and development. It can effectively inhibit apoptosis and promote cell proliferation and differentiation [3]. Matrix metalloproteinases (MMPs) are the main enzymes in the human body that involve in extracellular matrix degradation. MMP-2 affects tumor invasion and metastasis through degrading type IV and V collagen [4]. As the largest molecule in the MMPs, MMP-9 participates in the basement membrane destruction, leading to metastasis and neovascularization, which affect tumor cell invasion [5]. In this study, we collected chronic cervicitis, cervical intraepithelial neoplasia, and cervical cancer patients in our hospital, and detected Survivin, MMP-2, and MMP-9 level in serum and tissue through enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry to analyze

Table 1. ELISA detection of Survivin, MMP-2, and MMP-9 content in serum

Group	Cases	Survivin (ng/ml)	MMP-2 (ng/ml)	MMP-9 (ng/ml)
Cervical cancer	60	1.16 ± 0.04*,#	1.47 ± 0.07*,#	1.58 ± 0.04*,#
CIN	50	0.09 ± 0.03#	0.08 ± 0.01#	0.07 ± 0.02#
Chronic cervicitis	30	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.02

*P < 0.05, compared with CIN; #P < 0.05, compared with chronic cervicitis.

Table 2. Immunohistochemistry detection of Survivin expression in cervical tissue

Groups	Cases	Expression intensity			Positive rate (%)
		-	+-++	+++	
Cervical cancer	60	17	33	10	71.7*,#
CIN	50	32	12	6	36#
Chronic cervicitis	30	27	3	0	10

*P < 0.05, compared with CIN; #P < 0.05, compared with chronic cervicitis.

their correlation with different types of cervical lesions.

Materials and methods

General information

60 patients with early cervical invasive cancer between in January 2014 and January 2015 in Sun Yat-sen Memorial Hospital were enrolled. The mean age of patients was 52.5 ± 4.8 (30-70) years old. There were 11 cases in stage Ia, 17 cases in stage Ib, Including 11 cases in stage I, 18 cases in stage IIa, and 14 cases in stage IIb. 10 patients were in G1, 12 patients in G2, and 38 patients in G3. 43 cases were squamous cell carcinoma and 19 cases were adenocarcinoma, with 16 cases of vascular invasion and 10 cases of pelvic lymph node metastasis. 50 CIN patients received surgical treatment in Sun Yat-sen Memorial Hospital were selected with mean age at 51.8 ± 4.0 (35-65) years old. 30 patients with chronic cervicitis were chose as control with average age at 52.6 ± 4.2 (35-70) years old. The enrolled patients showed no significant difference in gender, age, and weight among different groups (P > 0.05). All of the patients had signed the informed consent, and the study was approved by Sun Yat-sen Memorial Hospital medical ethics committee.

Inclusion criteria: All patients were diagnosed by pathology without combining with connective tissue diseases or immune system disease. No patients received preoperative radiotherapy, chemotherapy, immune therapy, freezing or laser treatment.

Reagents and instruments

Na_2EDTA anticoagulation, Survivin ELISA kit, MMP-2 ELISA kit, MMP-9 ELISA kit, RPMI1640 medium, PBS, Survivin blocking buffer + primary antibody, MMP-2 blocking buffer + primary antibody, MMP-9 blocking buffer + primary antibody, rabbit anti-rat immunohistochemical secondary antibody kit, DAB kit, hydrogen peroxide, polylysine, 0.01% sodium citrate buffer, xylene, anhydrous ethanol, paraffin wax, hematoxylin, neutral balsam (Changzhou Mountain Chemical co., LTD, China).

Clean bench (Formal, USA), centrifuge (Pigeon, Shanghai), inverted microscope (Olympus), microplate reader (TECNA, UK), tissue embedder (SAKURA, Japan); dehydrator (TIYODA, Japan), ultramicrotome (Leica, Germany), temperature vibrator (Jinghong, Shanghai), image analysis system (HP, USA).

ELISA

Fasting venous blood was extracted from patients and centrifuged to collect supernatant and store in refrigerator. ELISA was applied to test Survivin, MMP-2, and MMP-9 content in blood: the kit was put in room temperature for 30 min. standard substance was diluted and added to five repeat holes at each concentration. After sample adding, dosing fluid, washing, developing, and termination, the plate was read absorbance on microplate reader at 450 nm. Linear regression equation was drawn to calculate the sample concentration.

Immunohistochemistry

Formalin fixation; conventional dehydration, hyalinization; paraffin, embedding; slicing, patch, baking; dewaxing, dehydration; high pressure hot repair; hydrogen peroxide blocking; goat serum blocking; 50 μl primary antibody incubation at room temperature for 1 h; 50 μl horse radish peroxidase labeled second-

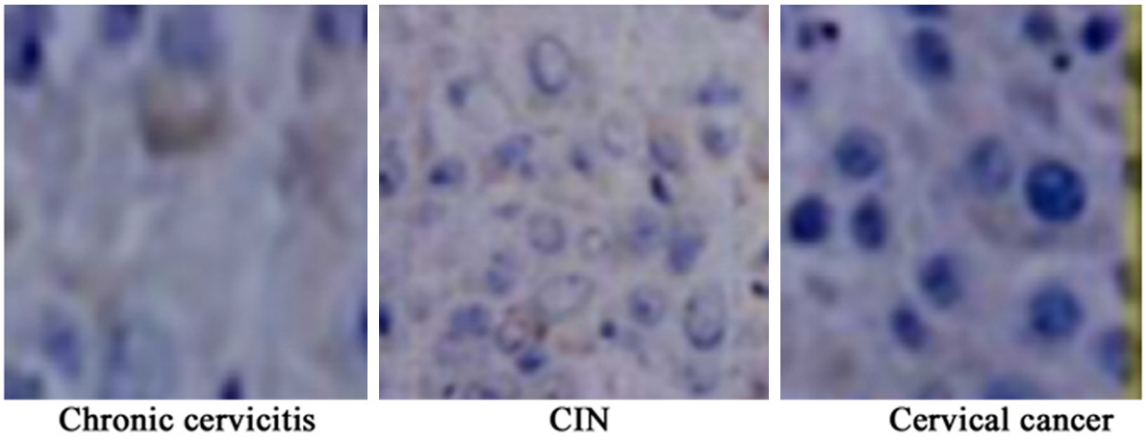

Figure 1. Immunohistochemical detection of Survivin expression in cervical tissue (×400).

ary antibody incubation for 10 min; 50 μl streptavidin-peroxidase incubation for 10 min; DAB development; termination; hematoxylin restain, hydrochloric acid and alcohol differentiation; conventional dehydration, hyalinization; sealing; image system collected 5 random fields from each section and recorded.

Criteria

The known immunohistochemical staining slice was treated as positive control and PBS buffer instead of primary antibody was used as negative control to observe HE staining slice.

MMP-2 and MMP-9 positive results were judged negative (-) as no staining in nucleus, while brown or tan particles in cell membrane or cytoplasm. Weak positive (+), staining cells less than 10%; positive (++), staining cells were 26%-50%; strong positive (+++), staining cells were > 50%.

Survivin positive results were judged negative (-) as no staining in nucleus, while brown or tan particles in cell membrane or cytoplasm. Weak positive (+), staining cells were ≤ 10%; positive (++), staining cells were 26%-50%; strong positive (+++), staining cells were > 50%.

(-) was deemed as negative, while (+)-(+++) were positive. Image system collected 5 random fields from each section and recorded.

Statistical analysis

All statistical analysis was performed on SPSS19.0 software. Data were expressed as Mean ± SD. Enumerate data was tested by chi-square test, whereas measurement data was presented as mean ± standard deviation and tested by ANOVA. Logistic regression model was applied for multivariate analysis. $P < 0.05$ was considered as statistically significant.

Results

ELISA detection of Survivin, MMP-2, and MMP-9 content in serum

Peripheral venous blood was extracted from the patients to detect serum level of Survivin, MMP-2 and MMP-9 using ELISA. The results showed that the level of Survivin was 1.16 ± 0.04 ng/ml, MMP-2 was 1.47 ± 0.07 ng/ml, and MMP-9 was 1.58 ± 0.04 ng/ml in cervical cancer patients, which was obviously higher than that in CIN group and chronic cervicitis group, with statistically significant difference ($P < 0.05$). Serum Survivin, MMP-2 and MMP-9 levels in CIN group were significantly higher than that in chronic cervicitis group ($P < 0.05$) (**Table 1**).

Immunohistochemistry detection of Survivin expression in cervical tissue

Survivin expression in cervical tissue was detected using immunohistochemistry. The results showed that expression of Survivin was strongly positive in 10 cases and positive expressed in 33 cases of cervical cancer. The positive rate reached 71.7%, which was significantly higher compared with CIN and chronic cervicitis ($P < 0.05$). Furthermore, its expression in CIN was obviously higher than in chronic cervicitis ($P < 0.05$) (**Table 2**; **Figure 1**).

Table 3. Immunohistochemistry detection of MMP-2 and MMP-9 expression in cervical tissue

Groups	Cases	MMP-2 expression intensity				MMP-9 expression intensity			
		-	+-++	+++	Positive rate (%)	-	+-++	+++	Positive rate (%)
Cervical cancer	60	12	38	10	80*,#	13	36	11	78.3*,#
CIN	50	35	14	1	30#	40	10	0	20#
Chronic cervicitis	30	27	3	0	10	27	3	0	10

*P < 0.05, compared with CIN; #P < 0.05, compared with chronic cervicitis.

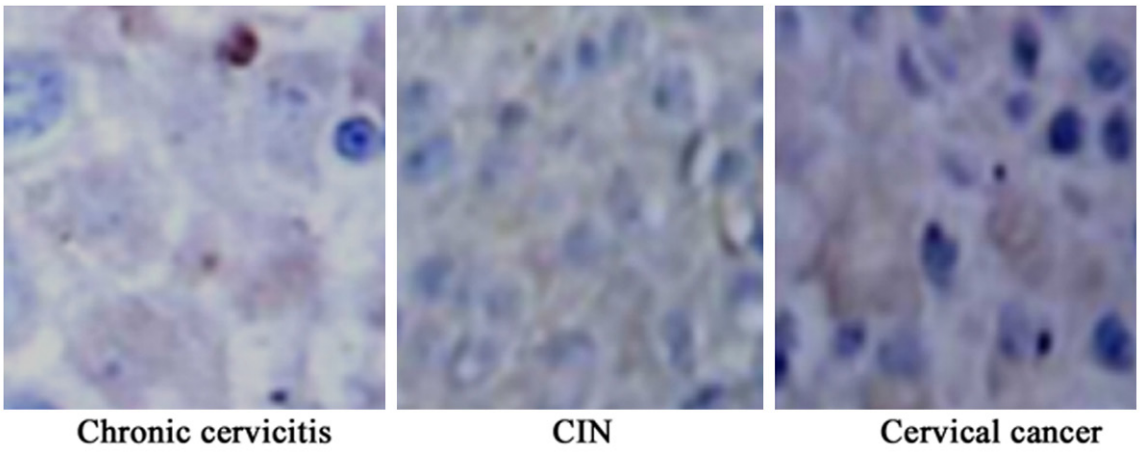

Figure 2. Immunohistochemical detection of MMP-2 expression in cervical tissue (×400).

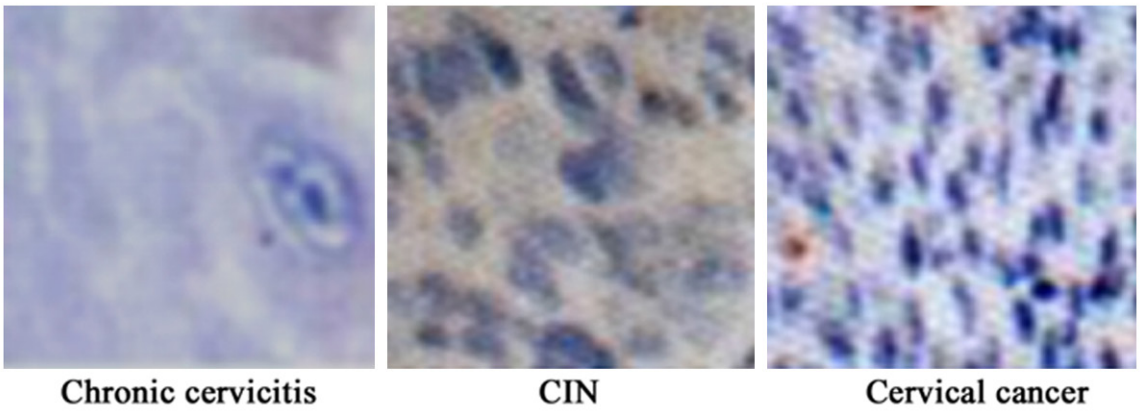

Figure 3. Immunohistochemical detection of MMP-9 expression in cervical tissue (×400).

Immunohistochemistry detection of MMP-2 and MMP-9 expression in cervical tissue

MMP-2 and MMP-9 expression in cervical tissue were tested using immunohistochemistry. The results showed that MMP-2 expression was strongly positive in 10 cases and positive expressed in 38 cases of cervical cancer, while MMP-9 expression was strongly positive in 11 cases and positive in 36 cases of cervical cancer. Their positive rate achieved 80% and 78.3%, which were obviously higher compared with CIN and chronic cervicitis ($P < 0.05$). Furthermore, their expressions in CIN were markedly higher than that in chronic cervicitis ($P < 0.05$) (**Table 3**; **Figures 2** and **3**).

Relationship between Survivin, MMP-2, and MMP-9 expression levels in cervical cancer group and clinicopathological feature

The relationship between Survivin, MMP-2, and MMP-9 expression levels in cervical cancer group and clinicopathological features were

Table 4. The relationship between Survivin, MMP-2, and MMP-9 expression levels in cervical cancer group and clinicopathological feature

Item	Cases	Survivin	MMP-2	MMP-9
Age				
< 45	27	21 (77.8)	23 (85.2)	24 (88.9)
≥ 45	33	22 (66.7)	22 (66.7)	23 (69.7)
P		> 0.05	> 0.05	> 0.05
Pathological grade				
G1	10	6 (60)	7 (70)	7 (70)
G2	12	11 (91.7)	10 (83.3)	9 (75)
G3	38	38 (100)	34 (89.5)	38 (100)
P		P < 0.05	P < 0.05	P < 0.05
Clinical stage				
Ia	11	4 (36.4)	4 (36.4)	4 (36.4)
Ib	17	12 (70.6)	13 (76.5)	14 (82.4)
IIa	18	16 (88.9)	17 (94.4)	16 (88.9)
IIb	14	14 (100)	14 (100)	14 (100)
P		P < 0.05	P < 0.05	P < 0.05
Gross type				
Endophytic	28	25 (89.3)	24 (85.7)	24 (85.7)
Exophytic	32	28 (87.5)	27 (84.3)	28 (87.5)
P		> 0.05	> 0.05	> 0.05
Pathological classification				
Squamous cell cancer	43	37 (86.0)	38 (88.4)	38 (88.4)
Adenocarcinoma	19	17 (89.5)	15 (78.9)	16 (84.2)
P		> 0.05	> 0.05	> 0.05
Differentiation				
Poorly	23	22 (95.7)	22 (95.7)	22 (95.7)
Middle to well	37	30 (81.1)	28 (75.7)	29 (78.4)
P		P < 0.05	P < 0.05	P < 0.05
Vascular invasion				
With	16	16 (100)	15 (93.8)	15 (93.8)
Without	44	36 (81.8)	35 (79.5)	36 (81.8)
P		P < 0.05	P < 0.05	P < 0.05
Lymph node metastasis				
With	10	10 (100)	10 (100)	10 (100)
Without	50	42 (84.0)	40 (80)	41 (82)
P		P < 0.05	P < 0.05	P < 0.05

analyzed, including age, pathologic grade, clinical stage, gross type, histological type, differentiation degree, vascular invasion, and lymph node metastasis. Their levels were found to be related to pathological grade, clinical stage, differentiation, vascular invasion, and lymph node metastasis ($P < 0.05$), but not age, gross type, and histological type ($P > 0.05$). Their levels elevated significantly following the pathologic classification increase, clinical upstage, lower differentiation degree, vascular invasion and lymph node metastasis emergence (**Table 4**).

Discussion

Chronic cervicitis is a common gynecological disease with high incidence and younger trend. It serious impacts on women's physical and mental health by causing pelvic inflammatory disease and adverse pregnancy that are high risk factors of cervical cancer [6]. CIN showed high canceration rate, containing cervical erosion, cervical polyp, and cervical adenocele. It developed to malignancy through multiple oncogenes and tumor suppressor genes, leading to neovascularization and cell apoptosis [7, 8]. Cervical cancer is the most common female malignant diseases in developing countries. In recent years, its incidence gradually increased especially in women younger than 35. It accounts for the leading type in female malignancy in our country and showed younger trend [9]. Early cervical cancer can appear invasion, metastasis, and poor prognosis without timely treatment. Finding and treating cervical cancer in time to prevent tumor invasion and metastasis can prolong the survival time [10]. This study selected chronic cervicitis, CIN, and cervical cancer patients in our hospital to detect Survivin, MMP-2, and MMP-9 in serum and tissue, and further analyzed their correlation with different types of cervical lesions.

This study extracted peripheral venous blood and tested Survivin, MMP-2, and MMP-9 expression using ELISA. It was found that levels of Survivin, MMP-2, and MMP-9 were elevated in cervical cancer compared with CIN and chronic cervicitis. They also increased in CIN compared with chronic cervicitis. We further collected cervical tissue and tested Survivin, MMP-2, and MMP-9 expression by immunohistochemistry. The results presented that Survivin, MMP-2, and MMP-9 positive rates were elevated in cervical cancer com-

pared with CIN and chronic cervicitis, while their levels increased in CIN compared to chronic cervicitis. It is suggested that Survivin, MMP-2, and MMP-9 showed a rising trend in the serum and cervical tissue of chronic cervicitis, CIN, and cervical cancer. Survivin locates in 17q25 and encodes cytoplasm protein containing 142 amino acids. It showed strong antiapoptosis ability with the molecular weight reached 16.5 kd [11]. Survivin was overexpressed in solid tumor and hematological malignancy. It was related to tumor invasion and metastasis and affected prognosis [12]. MMPs can degrade ECM, destroy basement membrane barrier, and decompose local tissue structure, leading to tumor cells invasion and metastasis gradually to the surrounding tissue. Moreover, it also can promote neovascularization to provide favorable condition for tumor cell migration through blood and lymphatic channel [13]. As the most important members of MMPs superfamily, MMP-2 mainly degrades ECM and fibronectin to destroy basement membrane integrity. It also can promote neovascularization to supply oxygen and nutrients for tumor cells. MMP-9 showed circumscribed function compared with MMP-2 as only decomposing nestin [14, 15]. Under physiological condition, MMP-9 is able to maintain balance through gene expression changes, potential enzyme activation, and MMP synthesis [16].

This study also analyzed the relationship between clinicopathological characteristics and Survivin, MMP-2, and MMP-9 expressions, including age, pathologic grade, clinical stage, gross type, histological type, differentiation degree, vascular invasion, and lymph node metastasis. Their levels were found to be related to pathological grade, clinical stage, differentiation, vascular invasion, and lymph node metastasis, but not age, gross type, and histological type. Their levels elevated significantly following the pathologic classification increase, clinical upstage, lower differentiation degree, vascular invasion and lymph node metastasis emergence. Survivin is hardly expressed in normal mature tissues, while it can be detected in embryonic period, fetal period, and tumor tissues. Survivin affects polymerization and depolymerization in microtubule through inhibiting caspase-3 and -7 to block G2/M phase and suppress apoptosis. In addition, Survivin participates in tumor angiogenesis and enhances tumor cell drug resistance [17]. Researchers discovered that Survivin overexpressed in cervical cancer with diameter > 4 cm, lymphatic metastasis, and positive SCC, and can predict prognosis [18]. MMP-2 was found to be overexpressed in cervical cancer tissues at CIN III and with infiltration and metastasis. Regulating MMP-2 expression can adjust cancer cells' invasive and metastatic abilities [19]. MMP-9 and MMP-2 were overexpressed in recurrent patients and significantly associated with lymph node invasion and metastasis [20], which was similar with our results.

To sum up, Survivin, MMP-2, and MMP-9 showed a escalating trend in the serum and cervical tissue of chronic cervicitis, CIN, and cervical cancer patients. They were highly expressed in cervical cancer patients' serum and tissue. Their levels were correlated with pathological grade, clinical stage, differentiation, vascular invasion, and lymph node metastasis, and showed obvious elevation. Survivin, MMP-2, and MMP-9 combined action promoted cervical cancer occurrence and development. Cervical lesion biopsy and Survivin, MMP-2, and MMP-9 detection have important clinical significance and may provide guidance on patients' prognosis and treatment.

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Disclosure of conflict of interest

None.

Address correspondence to: Zhongqiu Lin and Tingting Yao, Department of Gynecological Oncology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, 107 Yan Jiang Rd West, Guangzhou 510120, China. Tel: +86 13802921545; Fax: +86 20 81332853; E-mail: linzhongqiul@126.com (ZQL); Tel: +86 13265991356; Fax: +86 20 81332853; E-mail: tingtingyao@126.com (TTY)

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