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Original Article

Evaluating the expression of MACC1 and c-myc in cervical cancer and their correlation

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Abstract: Objective: The association of metastasis-associated in colon cancer (MACC1) and c-myc with the clinicopathological features of cervical cancer patients is unknown. This study aimed to investigate the expression of MACC1 and c-myc in cervical cancer and the correlation of these proteins with clinicopathological features. Method: In total, 503 cervical specimens from patients undergoing gynecological surgery and out-patient biopsies were collected, including 260 cervical cancer tissue specimens (cervical cancer group), 137 out-patient biopsy cervical intraepithelial neoplasia (CIN) II-III tissue specimens (CIN II-III group), and 106 normal cervical tissue specimens. RT-PCR was used to examine the expression of MACC1 and c-myc in the tissues of the three groups, and the association between the expressions and each clinicopathological feature was analyzed. Results: MACC1 and c-myc mRNA expression in cervical cancer tissues was related to the degree of tumor pathological differentiation, pelvic lymph node metastasis, and invasion depth, and the difference was statistically significant ($P < 0.01$). The expression of both MACC1 and c-myc mRNA in tissues of the cervical cancer group was higher than that in the CIN II-III and normal control groups ($P < 0.01$), and the pairwise comparisons were statistically significant ($P < 0.01$). The expression of MACC1 and c-myc in cervical cancer tissues was positively related ($r = 0.537$, $P < 0.05$). Conclusions: Both MACC1 and c-myc were highly expressed in cervical cancer tissues. MACC1 and c-myc may facilitate the occurrence of cervical cancer.

Keywords: Cervical cancer, MACC1, c-myc, pathological feature, lymphatic metastasis

Introduction

Cervical cancer is a serious disease threatening women's health globally and ranks third in morbidity among cancers affecting women. Most patients with intraepithelial neoplasia are aged between 30 and 35 years whereas those with invasive cervical carcinoma are aged between 45 and 55 years. In recent years, cervical cancer has shown a youth-oriented tendency. About 528,000 new cases of cervical cancer occur each year worldwide, and about 266,000 patients die of cervical cancer annually [1, 2]. Lifestyle and habits change with the development of the society, thus, resulting in increasing human papillomavirus (HPV) infection in females. Genital HPV infection has become a common sexually transmitted disease (STD) in females, and most cervical cancer cases are caused due to high-risk HPV infections [3].

The formation and development of cervical cancer is a highly complex process, and the ideal biological indices predicting the occurrence, development, and prognosis of cervical cancer have not been established yet. Relevant reports show that multiple oncogenes participate in the pathological and physical development of cervical cancer [1, 4]. MACC1 is a new gene reported in human colon cancer tissues; it is located on human chromosome 7 and plays an important role in the occurrence and development of colorectal cancer, with abnormally high expression in multiple tumor tissues [5, 6]. The c-myc gene is both a translocated gene and a regulated gene that participates in the occurrence and development of multiple tumors. The c-Myc protein is closely related to human malignancies and possesses the ability to accelerate apoptosis and induce accelerated cell proliferation [7].

Table 1. Primer sequences used for MACC1, c-myc, and β-actin genes

Gene	Upstream primer sequence	Downstream primer sequence
MACC1	5'-TTCTTTTATTCCTCCTA-3'	5'-ACTCTATCATTCT-3'
c-myc	5'-CCAACAGGAGCTATGACCTC-3'	5'-CTCGGTCACCATCTCCAGCT-3'
β-actin	5'-ATCATGTTTGAGACCTTCAACA-3'	5'-CATCTCTTGCTCGAAGTCCA-3'

Presently, the expression of MACC1 and c-myc in cervical cancer and their association with the clinicopathological features of patients is not known, and examining the expression of both genes simultaneously for monitoring cervical cancer is also not reported. The association of MACC1 and c-myc expression with the occurrence and development of cervical cancer is investigated in this study by examining the expression of MACC1 and c-myc in cervical cancer tissues, thus, providing a reference for the clinical diagnosis and therapy of cervical cancer.

Materials and methods

General information

In total, 503 cervical specimens from patients undergoing gynecological surgery admitted to Department of Obstetrics and Gynecology, The People's Hospital of Mianyang City, as well as out-patient biopsies were collected, including 260 cervical cancer tissue specimens (cervical cancer group), 137 out-patient biopsies of cervical intraepithelial neoplasia (CIN) II-III tissue specimens (CIN II-III group), and 106 normal cervical tissue specimens from hospitalized patients undergoing hysteromyomectomy (normal control group). The patients were 24-68 years old with an average age of 42.36 ± 7.26 years. According to the WHO female genital organ cervical cancer TNM 2014 and FIGO classification standard [8], the patients in the cervical cancer group were classified as follows: on the basis of (i) tissue typing: 63 cases of adenocarcinoma and 197 cases of squamous carcinoma; (ii) clinical stages: 118 cases in phase I and 142 cases in phase II; (iii) degree of pathological differentiation: 112 cases of high differentiation, 83 cases of intermediate differentiation, and 65 cases of low differentiation; 81 cases of lymphatic metastasis and 179 cases of no lymphatic metastasis; and 236 cases of positive high-risk HPV and 24 cases of negative high-risk HPV infection. The inclusion criteria were as follows: The patients did not receive any treatment prior to extracting cervical tissue specimens; all tissue slices were confirmed and diagnosed by the chief physician in the pathology department; and the clinical data of the patients were complete. All cervical tissues after extraction were stored at -80°C. The study was approved by the Ethics Committee of our hospital. All the subjects included in this study provided signed informed consent.

Main instruments and reagents

The Agilent Mx3000P/3005P real-time fluorescence quantitative PCR system was purchased from Beijing Keyu Xingye Science and Technology Development Co., Ltd. GelDoc-It-TS3 automatic gel-imaging system was purchased from Shanghai Yuansheng Instrument and Equipment Co., Ltd. Total RNA extraction kit (Trizol method) was purchased from Applied Biosystems, USA. M-MLV reverse transcription kit was purchased from Promega (Beijing) Biotechnology Co., Ltd. MACC1 and c-myc PCR kits were purchased from Biomiga Company. Primers for the real-time fluorescence quantitative PCR for MACC1, c-myc, and the β-actin internal reference gene were synthesized by Dalian Kakara Company. The primer sequences are listed in **Table 1**.

Test method

From cervical tissue specimens stored at -80°C, 100 mg tissues were sliced, placed in liquid nitrogen, and completely grinded. The prepared tissues were mixed with Trizol reagent, placed at room temperature, and allowed to stand for 30 min to ensure complete lysis. Total RNA was extracted in strict accordance with the manufacturer's instructions, and the concentration and purity of extracted RNA was determined by ultraviolet spectrophotometry and protein electrophoresis. The extracted RNA was reverse transcribed, according to the manufacturer's instructions. The extracted cDNA samples were stored at -20°C. The PCR samples were prepared, according to the manufacturer's instructions in a 12.62-μL volume made up to 20 μL with DEPC-treated water. The PCR

Table 2. Relationship between relative expression of MACC1 and c-myc mRNA in cervical cancer tissues and clinicopathological parameters ($\bar{x}\pm s$)

Category	*n*	MACC1 mRNA	*F/t value*	*P* value	c-myc mRNA	*F/t* value	*P* value
Age			1.453	0.147		0.829	0.407
≤45	70	18.653±13.624			7.362±4.163		
> 45	190	16.654±8.021			6.939±3.443		
Menopause			1.425	0.155		1.204	0.229
Yes	76	13.294±6.208			8.216±3.298		
No	184	12.134±5.871			7.563±4.224		
Tissue typing			1.006	0.315		1.145	0.253
Adenocarcinoma	63	13.834±8.627			9.163±5.428		
Squamous carcinoma	197	15.209±9.683			8.305±5.097		
Clinical stages			1.533	0.126		0.654	0.513
Phase I	118	13.297±8.134			7.674±4.079		
Phase II	142	14.983±9.371			8.011±4.175		
Degrees of differentiation			12.630	< 0.001		11.020	< 0.01
High differentiation	112	11.263±7.539			7.476±5.364		
Intermediate differentiation	83	14.046±8.336			9.357±4.987		
Low differentiation	65	17.537±8.476			11.687±7.229		
Lymphatic metastasis			8.307	< 0.001		6.714	< 0.001
Yes	81	20.167±9.241			13.249±7.509		
None	179	12.078±6.186			8.267±4.378		
HPV results			0.948	0.343		0.755	0.450
Positive	236	13.249±9.076			8.216±4.672		
Negative	24	11.421±8.162			7.453±5.137		
Tumor size			1.277	0.202		0.679	0.497
≤3 cm	136	14.089±9.149			6.267±3.715		
> 3 cm	124	12.753±7.552			5.943±3.970		
Vessel invasion			1.214	0.225		1.350	0.178
Yes	22	10.754±5.076			11.373±4.578		
None	238	12.517±6.628			9.697±5.652		
Depth of invasion			3.891	< 0.001		2.638	0.008
≤1/2 muscular layer	143	13.337±7.284			7.383±3.984		
> 1/2 muscular layer	117	16.466±5.253			9.358±7.796		

conditions were as follows: Initial denaturation at 94°C for 10 min followed by 40 cycles of 94°C for 45 s, 60°C for 45 s and 72°C for 45 s. The experiment was repeated three times and the mean results were considered. The results were analyzed using the $2^{-\Delta\Delta CT}$ method.

Statistical analyses

SPSS19.0 (SPSS China) was used for statistical analysis. All measurement data are presented as mean ± standard deviation ($\bar{x}\pm s$). ANOVA was used for comparison among multiple groups and t-test was used for two groups' comparison. Pearson correlation analysis was performed to determine the correlation between MACC1 and c-myc expression in cervical cancer tissues. $P < 0.05$ was considered as statistically significant.

Results

Association between MACC1 and c-myc mRNA in cervical cancer tissues and clinicopathological features

MACC1 and c-myc mRNA expression in cervical cancer tissues was related to the degree of tumor pathological differentiation, pelvic lymph node metastasis, and invasion depth, with a statistically significant difference ($P < 0.01$). The expression was unrelated to age, meno-

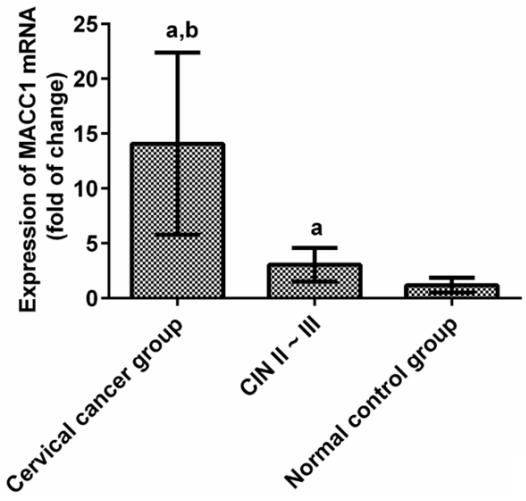

Figure 1. Comparison of MACC1 mRNA expression results in tissues of the cervical cancer group, CIN II-III group, and the normal control group. Note: [a]*P* < 0.01 compared with the normal control group; [b]*P*<0.01 compared with CIN II-III.

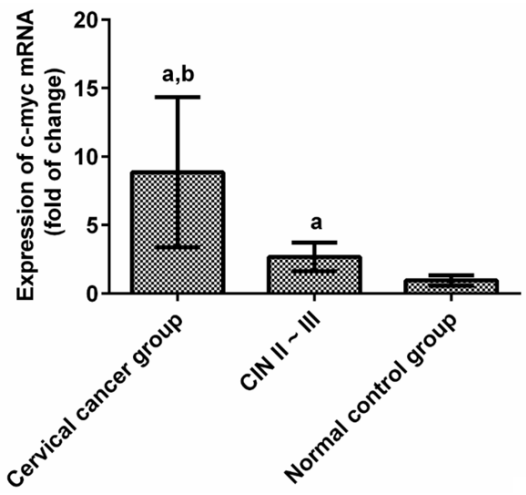

Figure 2. Comparison of c-myc mRNA expression results in tissues of the cervical cancer group, CIN II-III group, and the normal control group. Note: [a]*P* < 0.01 compared with the normal control group; [b]*P* < 0.01 compared with CIN II-III.

pause, tumor tissue typing, clinical stages, high-risk HPV infection test results, tumor size, and vessel invasion ($P > 0.05$). The details are summarized in **Table 2**.

Expression of MACC1 mRNA in tissues of the cervical cancer group, CIN II-III group, and the normal control group

The expression levels of MACC1 mRNA in tissues of the cervical cancer group, CIN II-III group, and the normal control group were 14.079 ± 8.297, 3.046 ± 1.531, and 1.186 ± 0.671, respectively. Compared with those in the normal control group, the expression levels of MACC1 mRNA in tissues of the cervical cancer group and CIN II-III group were significantly higher ($P < 0.01$); compared with that in the CIN II-III group, the expression level of MACC1 mRNA in tissues of the cervical cancer group was significantly higher ($P < 0.01$). The trend of MACC1 mRNA expression among the three groups was as follows: cervical cancer group > CIN II-III group > normal control group, and the pairwise comparisons among the three groups were statistically significant ($P < 0.01$, **Figure 1**).

Expression of c-myc mRNA in tissues of the cervical cancer group, CIN II-III group, and the normal control group

The expression levels of c-myc mRNA in tissues of the cervical cancer group, CIN II-III group, and the normal control group were 8.857 ± 5.479, 2.673 ± 1.047, and 0.951 ± 0.376, respectively. Compared with those in the normal control group, the expression levels of c-myc mRNA in tissues of the cervical cancer group and CIN II-III group were significantly higher ($P < 0.01$); compared with that in CIN II-III group, the expression level of c-myc mRNA in tissues of the cervical cancer group was significantly higher ($P < 0.01$). The trend of c-myc mRNA expression among the three groups was as follows: cervical cancer group > CIN II-III group > normal control group, and the pairwise comparisons among the three groups were statistically significant ($P < 0.01$, **Figure 2**).

Correlation between MACC1 mRNA and c-myc mRNA expression in cervical cancer tissues

The expression of MACC1 and c-myc in cervical cancer tissue was positively related ($r = 0.306$, $P < 0.05$), as indicated by Pearson linear regression analysis. See **Table 3** and **Figure 3**.

Discussion

Globally, cervical cancer is the most common malignancy among women's cancers, besides breast cancer and colorectal cancer. Cervical cancer is a unique female malignancy with an identified pathogen that is closely related to high-risk HPV infection [9]. HPV infection plays an important role in the occurrence and devel-

Table 3. Correlation of expression quantity between MACC1 and c-myc mRNA in cervical cancer tissues

Gene	Relative expression quantity	*R* value	*P* value
MACC1	14.079±8.297	0.306	$P < 0.01$
c-myc	8.857±5.479		

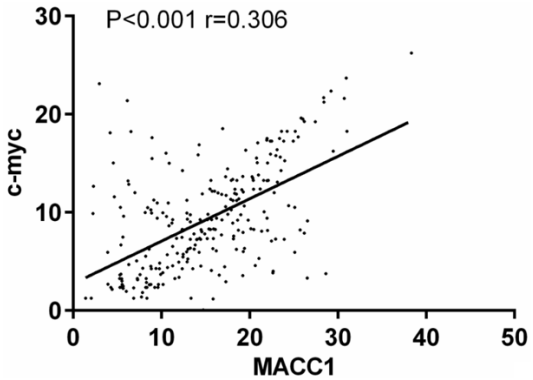

Figure 3. Logistics regression analysis of the expression levels of MACC1 and c-myc in cervical cancer tissue.

opment of cervical cancer. At present, an HPV vaccine has been popularized and used to reduce the occurrence of cervical cancer to some extent. However, with the changing social lifestyle, the rate of HPV infection has remained high, and the prevalence of cervical cancer also tends to be greater in the younger population [10]. The main clinical therapy for cervical cancer is surgery assisted by chemotherapy or radiotherapy. Although the mortality rate of patients has been reduced greatly, metastasis, invasion, and local recurrence of cervical cancer are still the major factors resulting in patient death [11]. Therefore, to investigate the occurrence and development of cervical cancer, latent genes that inhibit tumor growth and reduce tumor metastasis and invasion are of great importance clinically.

In this study, the expression levels of MACC1 and c-myc mRNA in normal cervical tissues, CIN II-III, and cervical cancer tissues were found to be gradually elevated and were related to the degree of tumor pathological differentiation, pelvic lymph node metastasis, and invasion depth, indicating that MACC1 and c-myc may participate in the occurrence and development of cervical cancer and play an important role in its invasion and metastasis. MACC1 is a recently identified gene that is closely related to colon cancer metastasis and can regulate tumor growth and metastasis with a key role in the process of cell proliferation and differentiation [12]. MACC1 can be expressed independently of c-Met, and is a regulator of c-Met. High c-Met expression can induce autocrine expression of HGF, resulting in invasion and malignant transformation of cells. Increased expression of MACC1 can induce c-Met expression and HGF overexpression, which can cause c-Met activation in the cell nucleus by MACC1, thus, forming a feedback pathway involving the three proteins and facilitating invasion, metastasis, and malignant transformation of tumor cells [13, 14]. MACC1 may also be involved in the occurrence and development of cervical cancer through this feedback pathway. These observations are similar to the conclusions of the study conducted by Chandrasinghe et al. [15], which were that high expression of MACC1 in colon cancer can promote the invasion and metastasis of tumor cells; MACC1 can also be used as an independent test index of prognosis and life expectancy in patients with colon cancer.

c-myc is a common cancer-promoting gene involved in cell proliferation, differentiation, apoptosis, and metastasis, and many other biological functions are mediated by the transcription factor encoded by this gene [16]. High expression of c-myc can induce the evolution of cells from normal cells to malignant tumor cells. Studies indicate that when body cells are stimulated, the stability of c-myc is altered, which results in c-myc activation, leading to over-proliferation of cells that are, thus, immortalized and form tumor cells [17]. c-myc is activated in the evolution process in the normal cervix uteri and participates in the development of cervical cancer [18]. This observation is similar to that reported by Cui et al. [19]. In this study, MACC1 and c-myc expression in cervical cancer tissues was found to be positively related, indicating that the joint detection of both has better clinical predictive value for the occurrence and development of cervical cancer.

This study includes a large number of specimens, which can suitably indicate the expression of MACC1 and c-myc in different cervical tissues and ensure the reliability of the study.

The limitations of this study are that patients in phase III-IV were not included in this study, the analyses for prognosis and life expectancy were not performed. Tumor occurrence, development, invasion, and metastasis constitute a complex process regulated by multiple factors. The proliferation and apoptosis of tumor cells can be promoted by cytokines secreted from tumor cells [20]. MACC1 and c-myc are also highly expressed in other tumors. The mechanism of action of both in female malignancies is not yet known. We, therefore, hope that the prognosis and survival of patients with cervical cancer can be analyzed in the next study and that the mechanism of action of the proteins encoded by both genes in cervical cancer can be elucidated.

In conclusion, both MACC1 and c-myc are highly expressed in cervical cancer tissues and may facilitate its occurrence, development, invasion, and metastasis; thus, MAAC1 and c-myc expression may have an important predictive value clinically.

Disclosure of conflict of interest

None.

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