Expression of survivin, caspase-3 and p53 in cervical cancer assessed by tissue microarray: Correlation with clinicopathology and prognosis

H. Lu[1], M. Gan[2], G. Zhang[2], T. Zhou[2], M. Yan[3], S. Wang[1]

[1]Department of Pathology, Taizhou Central Hospital of Taizhou Enze Medical Center
[2]Department of Pathology, [3]Department of Obstetrics and Gynecology, Taizhou Hospital of Taizhou Enze Medical Center Zhejiang (China)

Summary

Objective: The aim of this study was to determine the role of survivin, caspase-3 and p53 expression in cervical cancer, and their correlation with clinicopathological features and prognosis. *Methods:* Two hundred and twenty-eight cases of cervical disease were analyzed retrospectively between February 2003 and May 2007 at Taizhou Hospital of Taizhou Enze Medical Center. The expressions of survivin, caspase-3 and p53 were detected by immunohistochemistry (EnVision), assessed by tissue microarray. The correlation of the three genes and clinicopathological factors as well as prognosis were statistically analyzed. *Results:* The results showed that the positive expression rate of survivin, caspase-3 and p53 in cervical cancer was significantly higher than in the CIN group and cervicitis group ($p < 0.05$). The expression of survivin was related with clinical staging, stromal involvement and lymph node metastasis ($p < 0.05$). The positive ratio of caspase-3 was significantly different from histological grading ($p < 0.05$). The positive expression of p53 was correlated with histological type and grading ($p < 0.05$). The expression of survivin in cervical cancer was negatively associated with that of caspase-3 ($p < 0.01$). The positive expression of survivin in the survival group and non-survival group was significantly statistically difference ($p < 0.01$). There was a significant difference between survivin expression and survival duration by the log-rank method. whereas no association with survival was seen for caspase-3 and p53 positivity. *Conclusion:* Survivin, caspase-3 and p53 may play an important role in the occurrence and development of cervix carcinoma. It has been suggested that the high expression of survivin or p53 and low expression of caspase-3 are closely correlated with cervical cancer. They could be used as markers for malignant degree and invasiveness of cervix cancer. Survivin can also be used in the estimation of prognosis and survival time of cervix carcinoma.

Key words: Survivin; Caspase-3; p53; Cervical cancer; Survival analysis.

Introduction

Cervical cancer is the second most common cancer among women worldwide. More than 500,000 cases of cervical cancer occur every year, and 75-80% of the cases are in developing countries.

Apoptosis, often referred to as programmed cell death, is a physiologic process for the elimination of specific types of cells, occurring extensively in embryonic development, metamorphosis, and differentiation. Abnormal regulation of apoptosis is likely to contribute to the pathogenesis of autoimmune diseases and malignant tumors [1]. The acquired ability to resist apoptotic stimuli is shared by many types of malignant diseases, and genetic alteration in the components of apoptotic pathways is a pivotal mechanism in the development of cancer [2]. With regard to cervical cancer, only a few reports concerning survivin and caspase-3 expression have been published. The overexpression of survivin can inhibit apoptosis, resulting in abnormal cell proliferation and transduction towards to malignancy [3]. Although Yamada *et al*. [4]. and Mahotka *et al*. [5]. succeeded in quantifying gene expression levels of surviving splice variants using glioblastoma and renal cell carcinoma specimens, the expression pattern of these variants in various human cancers has not been extensively studied. Caspase-3 could lead to antiapoptotic phenotype. Most factors trigger apoptosis via a signal way mediated by caspase-3 [6]. p53 protein, encoding on chromosome 17p13, has a central role in regulation of the cell cycle and apoptosis. Mutation of the p53 gene has been quoted as one of the most common markers in human cancers. Multiple trials have investigated the role of the p53 gene in the carcinogenesis of cervical cancer [7, 8]. In this study, the expression and distribution of survivin, caspase-3 and p53 was detected in 107 cases of cervical cancer with an immunohistochemical EnVision method to study the relationship between these markers and development of cervical cancer.

Materials and Methods

Materials

Two hundred and twenty-eight cases and specimens of cervical diseases were obtained from Taizhou Hospital of Taizhou Enze Medical Center from February 2003 to May 2007. There were 35 females with cervicitis, 86 females with CIN (including 28 cases of CIN I, 26 cases of CIN II and 32 cases of CIN III) IV and 107 females with cervical cancer (including 81 cases of squamous cancer and 26 cases of adenocarcinoma). Of the

Revised manuscript accepted for publication March 1, 2010

107 cervical cancer cases, the mean age was 43.5 years old (27-71 years old). All patients with cancer were operated before and were subjected to chemotherapy or radiotherapy. According to the pathological differentiation of squamous cancer, the cervical cancer was graded as: high differentiation (22 cases), middle differentiation (43 cases), and low differentiation (16 cases). For clinical pathological stages (FIGO, 2000), there were 37 cases of Stage I, 44 cases of Stage II, and 26 cases of Stage III or IV. With stromal involvement, 28 cases had infiltration no more than superficial myometrium and 79 cases had infiltration more than deep myometrium. Twenty-six cases had lymph node metastasis and 81 cases had no metastasis. Follow-up was carried out through telephone calls or letters. Survival time was defined as the time from diagnosis to death or to the final examination. Postoperative follow-up lasted over three years: 72 cases were followed-up more than one year and 44 cases more than three years, including 28 cases of survival and 16 cases of death due to tumor recurrence and/or metastasis.

Reagents and methods

The specimens were obtained from surgical resection. All tissues were formalin-fixed and paraffin-embedded. The cervical disease tissue microarrays containing 228 case specimens were used to determine survival and caspase-3 and p53 expression by immunohistochemistry (EnVision). Survivin rabbit-anti-human monoclonal antibody, caspase-3 mouse-anti-human monoclonal antibody and p53 mouse-anti-human monoclonal antibody were purchased from Gene Company Ltd. The EnVision detection kit was bought from Beijing Zhongshan Biological Reagent Company Ltd.. The working concentration of the primary antibody was 1: 50, and that of the remaining two antibodies was 1: 100. EnVision staining was performed. PBS was substituted for the primary antibody as a negative control, and the known positive slips served as positive controls.

Judgment of the results

Positive staining of survivin and caspase-3 expression was mainly located in the cytoplasm, while p53 was in the nucleus with brown-yellow granules. In each section, 5 high-power visual fields were randomly selected and observed. Two hundred cells in each visual field were counted. The staining was judged according to the percentage of the positive cells; no or positive cells < 5%, negative; positive cells 5%-20%, weakly positive; positive cells 20%-50%, moderately positive; positive cells > 50%, strongly positive.

Statistical Analysis

The SPSS 10.0 statistical software package was used to analyze the data. The chi-square test was performed to investigate the correlation between clinical variables and immunostaining. Kaplan-Meier's method was used to illustrate the survival curve and the log-rank method test was used to test the difference of survival rate; $p < 0.05$ was considered statistically significant.

Results

Table 1 shows the expression of survivin, caspase-3 and p53 in different cervical tissue samples.

The results showed a significant correlation between the expressions of survivin and clinical staging, stromal involvement and lymph node metastasis ($p < 0.05$). However, there was no significant difference in survivin expression between different histological grading or types. The positive rate of caspase-3 was significantly different from histological grading ($p < 0.05$) and the positive expression of p53 was correlated with histological type and grading ($p < 0.05$). The expression of both caspase-3 and p53 was not related to clinical staging, stroma involvement and lymph node metastasis (Table 2, Figures 1-6).

Table 1. — *Expression of survivin, caspase-3, p53 in different cervical samples [n(%)].*

Pathological type	n	Survivin		Caspase-3		p53	
Cervicitis	35	0	0	32	91.4%	1	2.8%
CIN	86	42	35.7%	48	55.8%	35	28.6%
Squamous cell carcinoma	81	72	88.9%*	29	35.8%*	68	83.9%*

Compared with cervicitis CIN, *$p < 0.05$.

Table 2. — *Relationship between the expression of survivin, caspase-3, p53 and pathological features of cervical cancer (positive %).*

Pathological factors		n	Survivin		Caspase-3		p53	
			+	%	+	%	+	%
Age	≤ 35	27	23	85.2	11	40.7	20	74.1
	> 35	80	67	83.8	33	41.3	64	80.0
Pathological types	Squamous carcinoma	81	72	88.9	29	35.8	68	83.9
	Adenocarcinoma	26	18	69.2	15	57.7	16	61.5
Pathological grades (SC)	I	22	20	90.9	13	59.1	15	68.2
	II	43	39	90.7	14	32.6	38	88.4
	III	16	13	81.3	2	12.5	15	93.8
Clinical stages	I	37	26	70.3	19	51.4	27	73.0
	II	44	39	88.6	16	36.4	37	84.1
	III-IV	26	25	96.2	9	34.6	20	76.9
Stromal involvement	≤ superficial myometrium	28	19	67.9	15	53.6	19	67.9
	≥ deep myometrium	79	71	89.9	29	36.7	65	82.2
Lymphatic metastasis	no	81	64	79.0	35	43.2	64	79.0
	yes	26	26	100.0	9	34.6	20	76.9

Fig. 1

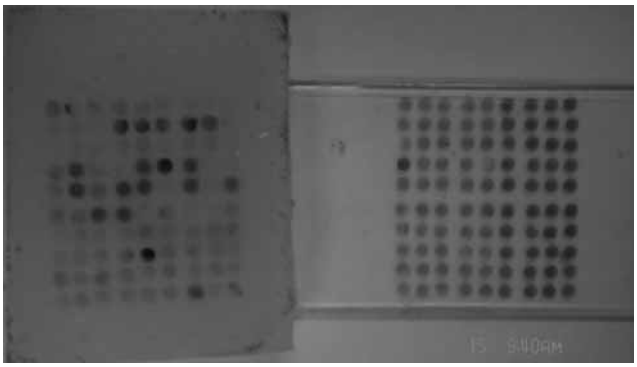

Fig. 2

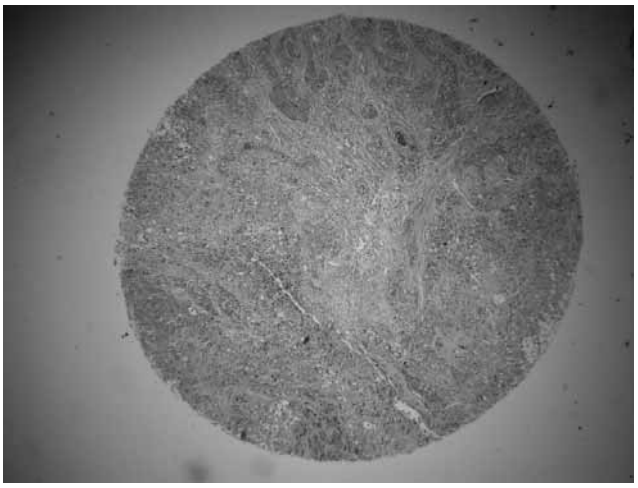

Fig. 3

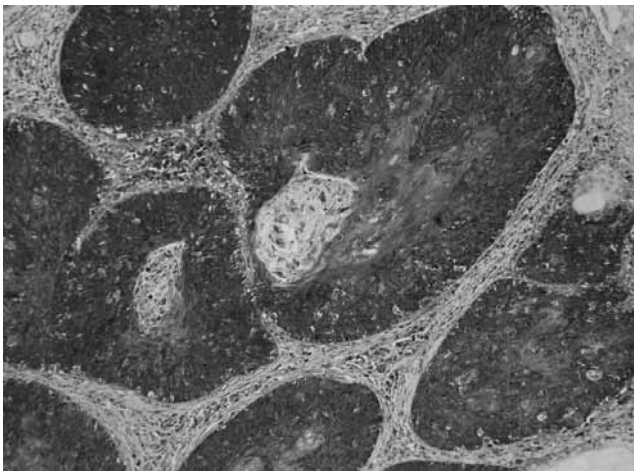

Fig. 4

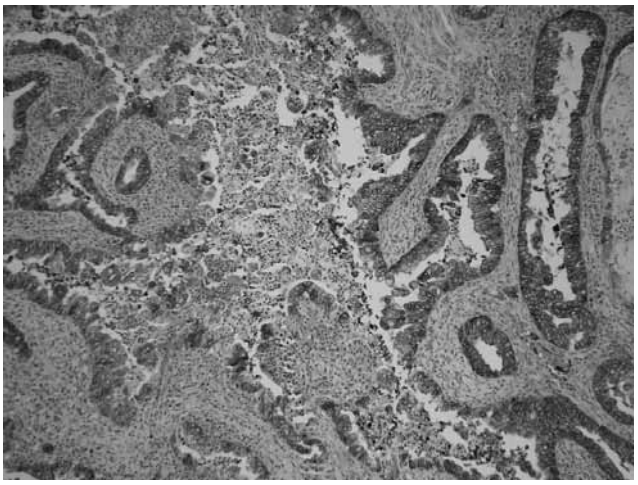

Fig. 5

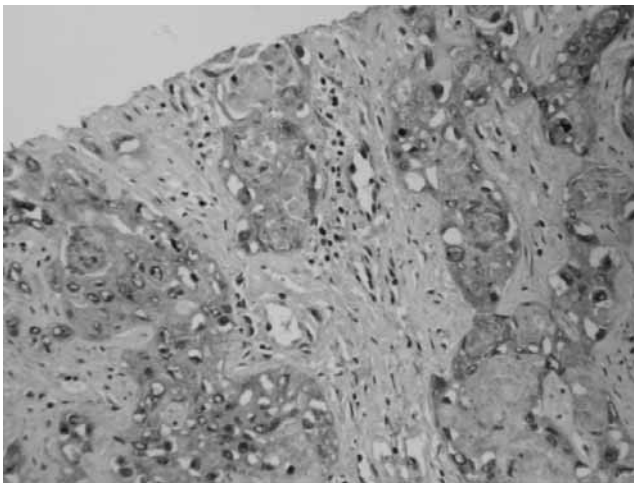

Fig. 6

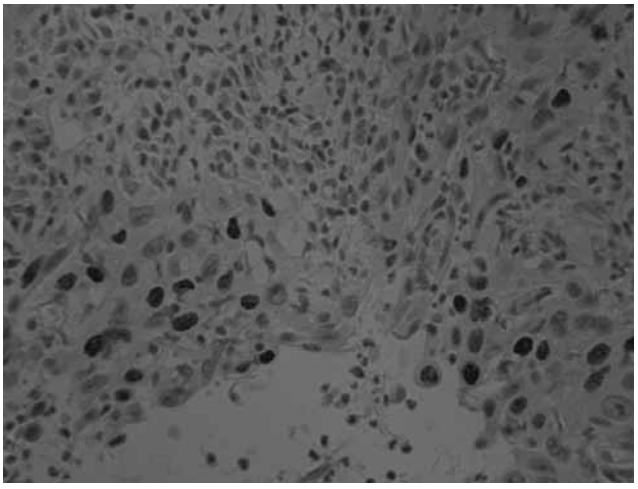

Figure 1. — Tissue microarray paraffin block and section of cervical cancer.
Figure 2. — H&E section of cervical squamous cell cancer by tissue microarray.
Figure 3. — Invasive squamous cell carcinoma grade 2 strongly positive for survivin in the nucleus and cytoplasm (survivin immunostaining).
Figure 4. — Cervical adenocarcinoma positive for survivin in the cytoplasm (survivin immunostaining).
Figure 5. — Squamous cell carcinoma grade 1 weakly positive for caspase-3 in the cytoplasm (caspase-3 immunostaining).
Figure 6. — Squamous cell carcinoma grade 2 interspersed positive for p53 in the nucleus (p53 immunostaining).

Fig. 7

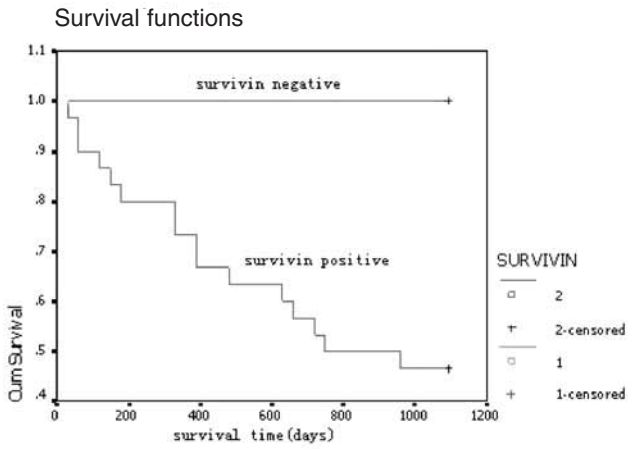

Fig. 8

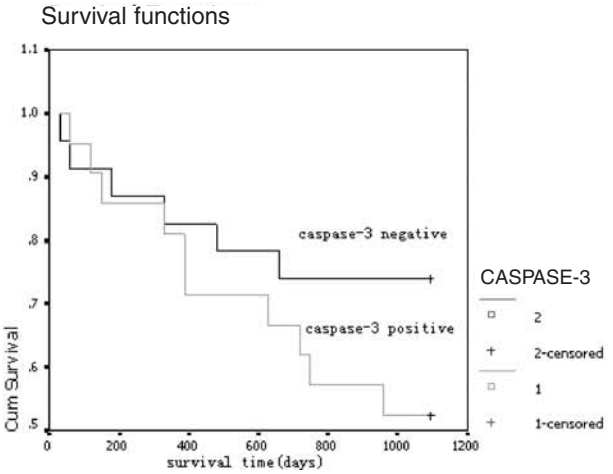

Fig. 9

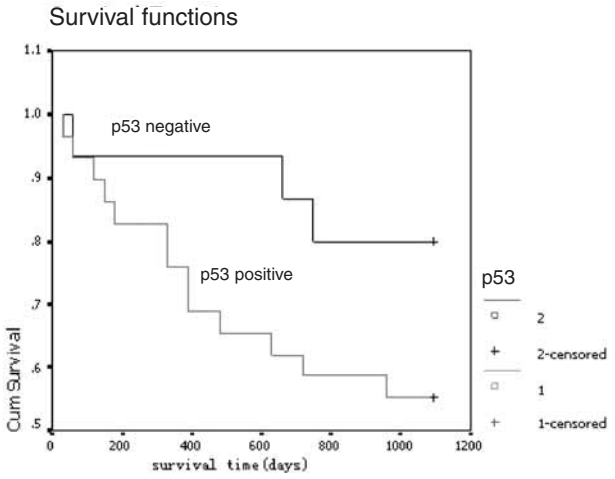

Figure 7. — Log-rank showed that from the expression of the survivin positive and negative group, there were statistically significant differences.
Figure 8. — Log-rank showed that from the expression of the caspase-3 positive and negative group, there were no statistically significant differences.
Figure 9. — Log-rank showed that from the expression of the p53 positive and negative group, there were no statistically significant differences.

In 107 cervical cancer cases 29 cases were positive for the expression of caspase-3 in 90 cases of survivin-positive expression. In 17 cases of survivin-negative expression, 15 cases were positive for the expression of caspase-3. There was a significant difference between them ($p < 0.05$). In 90 cases positive for survivin expression, 74 cases were positive for p53 expression. In 17 cases negative for survivin expression, ten cases was positive for p53 expression. There was no significant difference between them ($p < 0.05$).

The relationship between the expression of survivin, caspase-3 and p53 in cervical cancer and 3-year survival rate are listed in Table 3 and Figures 7-9. The positive expression of survivin in the survival and deceased group showed a significant statistical difference ($p < 0.01$). There was also a significant difference in survival time between survivin expression and duration of survival by the log-rank method, whereas, the positive expression of caspase-3 and p53 showed no statistical difference in the follow-up data for three years and were unrelated to survival.

Table 3. — *Relationship between the expression of survivin, caspase-3 and p53 in cervical cancer and 3-year survival rate [n(%)].*

	n	Survivin	%	*p*	Caspase-3	%	*p*	p53	%	*p*
Alive	28	14	50.0		11	39.3		16	57.1	
Dead	16	16	100.0	< 0.01	10	62.5	> 0.05	13	50.0	> 0.05

Discussion

Survivin, a novel apoptosis suppressive gene, is selectively expressed in tumors. It is closely correlated with apoptosis and cell cycle modulation. In addition, the overexpression of survivin could speed up the shift of cells from S G1 towards S, promoting cell proliferation. Survivin is highly expressed in most tumor tissues [9, 10]. Kim *et al*. and Yamamoto *et al*. found a gradually increased positive expression rate of survivin in the different tissues of invasion and metastasis of cervival cancer [11, 12]. In this study, the positive expression rate of survivin in the cervical cancer group was significantly higher than in the CIN group and cervicitis group. The significant correlations between survivin status and clinical staging, stromal involvement and lymph node metastasis were consistent with Kim and Yamamoto' findings. Indications were that the survivin gene was involved in the development of cervical cancer, and closely related to the invasion and metastasis of cancer. However, the expression of survivin was not associated with the pathological types and pathological grade.

Caspase-3 is the most important member of the caspase family. It induces apoptosis. Shin *et al*. and others suggested that the development of cervical cancer was related to decreased caspase-3 expression inducing blockage of apoptosis [6, 13]. In the present study, the positive rate of caspase-3 was not associated with clinical staging, stromal involvement or lymph node metastasis, but was associated with histological grading. The positive inactivation of caspases might result in failing or delaying apoptosis, therefore the survival of damaged cells becomes prone to further genetic damage. Apparently, the defect of caspase-expression plays an important role in the tumorigenic process. Meanwhile, our study also revealed the expression of survivin in cervical cancer was negatively associated with that of caspase-3, meaning an adverse effect on cervical cancer.

p53 protein, the tumor suppressor, contributes to the controlling of cell cycle checkpoints and apoptosis. It is frequently lost or mutated in multiple types of human cancers [14]. In our study, the positive expression of p53 was correlated with histological type and grading in different clinicopathological characteristics. It indicated that the p53 gene is related to tumor-related anaplastic extent. The expression rate in clinical stage, stromal involvement and lymphatic metastasis has continued to increase, but is not statistically significant.

The positive expression rate of survivin in the deceased patient group was statistically significantly higher than that of the surviving group ($p < 0.05$), which indicates tumors with survivin over-expression have an unfavorable prognosis. There was a significant difference in survival time between survivin expression and length of survival by the log-rank method, whereas the expression level of caspase-3 and p53 in either the survival group or positive-control group showed no significant difference in comparision with the negative-control group. This demonstrates that caspase-3 and p53 were not related to tumor prognosis. Therefore, in our opinion, there is a correlation between survivin expression and tumor prognosis or the survival duration.

In conclusion, we have documented the expression of survivin, caspase-3 and p53 in the different clinicopathological features of cervical carcinoma, and the correlation with clinicopathological features and prognosis. The positive expression rate of survivin, caspase-3 and p53 in cervical cancer was significantly higher than in the CIN group and cervicitis group. These three genes play an important role in cervical carcinogenesis and could be very useful markers in identifing degree of malignancy and invasiveness of cervix cancer. Additionally, the log-rank test revealed that the expression of survivin was an independent prognostic indicator, suggesting a new target for the diagnosis of cervical cancer. Further studies are needed to investigate the role and mechanism of action of survivin, caspase-3 and p53 in cervical cancer development.

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Address reprint requests to:
H.S. LU, M.D.
Department of Pathology
Taizhou Central
Hospital of Taizhou Enze Medical Center
999 Donghai Street
Taizhou, Zhejiang, 318000 (China)
e-mail: luhongsheng00@163.com