Expression of PTEN and survivin in cervical cancer: promising biological markers for early diagnosis and prognostic evaluation

D. LU*, J. QIAN*, X. YIN*, Qin XIAO†, C. WANG† and Y. ZENG‡
*Department of Obstetrics and Gynecology, Yangzhou University Medical College, Yangzhou; †Department of Pathology, Affiliated Hospital of Yangzhou University, Yangzhou; and ‡Biomedical Engineering Center, Beijing University of Technology, Beijing, P. R. China

Accepted: 24 September 2012

ABSTRACT

This study aims to evaluate the expression of the anti-oncogene phosphatase and tensin (PTEN) homologue and survivin, a protein encoded by the anti-apoptotic gene baculoviral inhibitor of apoptosis repeat containing 5 (*BIRC5*), in the progression of cervical neoplasia and to elucidate the relationship between PTEN and survivin expression based on clinicopathological features in squamous cell carcinoma of the uterine cervix. A total of 20 patients with cervical ectropion and squamous metaplasia, 30 with cervical intraepithelial neoplasia, and 49 with cervical squamous cell carcinoma were enrolled in the study. Immunohistochemical staining was performed to detect PTEN and survivin expression in each group. Normal cervical epithelium from 10 people served as the control. Results showed that PTEN expression progressively decreased with the continuum from normal epithelium to squamous cell carcinoma ($P<0.05$), whereas survivin expression progressively increased ($P<0.05$). Furthermore, positive PTEN immunostaining was associated with clinical stage and tumour size ($P<0.05$). The level of PTEN expression in the metastatic pelvic lymph node group was significantly lower compared with the non-metastatic pelvic lymph node group ($P<0.01$). Positive PTEN immunostaining was not associated with age or degree of differentiation ($P>0.05$). Positive survivin immunostaining was associated with clinical stage and tumour size ($P<0.05$). Survivin-positive expression in the metastatic pelvic lymph node group was significantly higher compared with the non-metastatic pelvic lymph node group ($P<0.01$). No obvious relationship was found between survivin expression and patient age ($P>0.05$). PTEN expression negatively correlated with survivin expression in cervical intraepithelial neoplasia and cervical squamous cell carcinoma ($P<0.01$). PTEN and survivin expression correlated with incidence and progression of uterine cervical cancer. Positive expression levels of PTEN and survivin provide potential evaluation indices for early diagnosis and prognosis of uterine cervical cancer, and these biomarkers are also potentially promising therapeutic targets.

KEY WORDS: Biomarkers.
Carcinoma, squamous cell.
Cervical intraepithelial neoplasia.
PTEN protein, human.
Survivin protein, human.

Introduction

Uterine cervical cancer develops from precancerous lesions such as cervical intraepithelial neoplasia (CIN) and cervical glandular intraepithelial neoplasia (CGIN) to early invasive carcinoma to infiltrating carcinoma. The incidence and progression of cervical cancer involves the activation of multiple oncogenes and deactivation of inhibiting genes. The phosphatase and tensin (PTEN) homologue deleted on chromosome 10 was the first anti-oncogene discovered to exhibit double specific phosphatase activities; deactivation and mutation of this gene in tumours and cells has been reported to cause abnormal expression of corresponding proteins.[1] Survivin, encoded by a recently discovered oncogene, inhibits cell apoptosis, promotes cell proliferation, and participates in angiopoiesis, an important molecular event during tumourigenesis.[2]

In this study, PTEN and survivin expression in cervix tissues is detected in order to explore the underlying mechanism of PTEN and survivin in cervical cancer progression. Furthermore, it investigates a new method for early diagnosis and prognosis of cervical cancer and explores potential gene therapy biological targets for cervical cancer.

Materials and methods

A total of 49 specimens of carcinoma were obtained from patients with histologically confirmed squamous carcinoma of the cervix (age range: 33–76 years, median age: 45.3 years) in the Department of Gynecology and Obstetrics, Affiliated Hospital of Yangzhou University, between January 2010 and August 2011. In accordance with the clinical staging standards of the International Federation of Gynecology and Obstetrics, 20 cases were reported as stages I and II, while 29 were reported as stages III and IV. Six cases were well-differentiated, 29 moderately differentiated, and 14 poorly differentiated.

Tissue sections from 30 cases of CIN exhibiting representative pathological changes were selected from the

Correspondence to: D Lu or Y. Zeng
Email: ludan1968@yahoo.com.cn
yjzeng@bipu.edu.cn

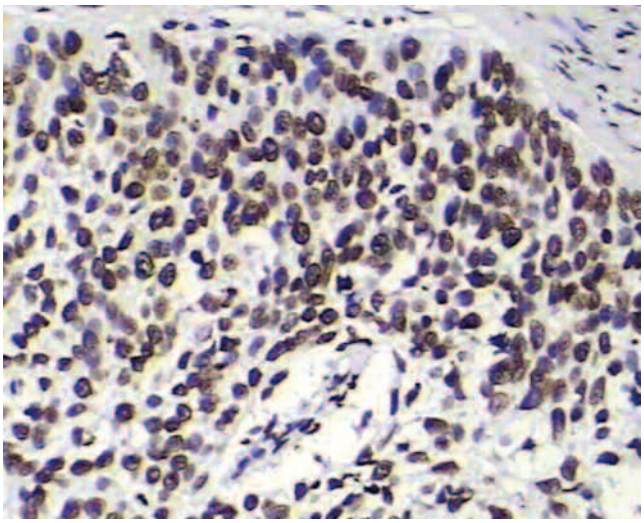

Fig. 1. Immunohistochemical staining of PTEN in the nuclei of malignant cells (original magnification x200).

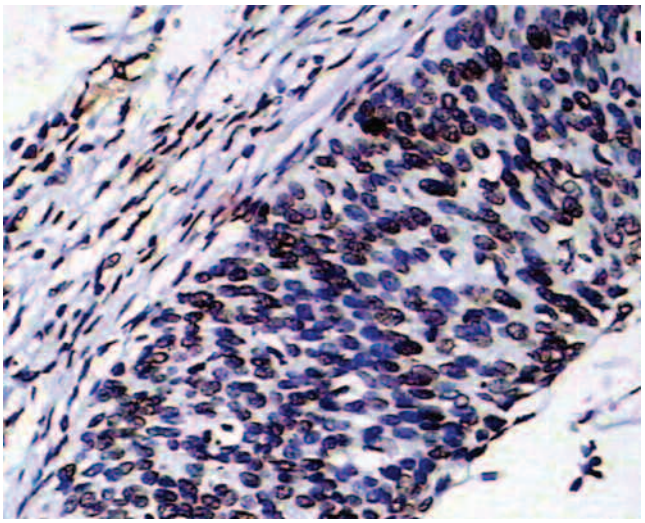

Fig. 2. Immunohistochemical staining of PTEN in the nuclei of CIN3 cells (original magnification x200).

Department of Gynecology and Obstetrics, Clinical College of Yangzhou University, including seven CIN1, 10 CIN2 and 13 CIN3. Sections from 20 cases of cervical ectropion with squamous metaplasia were also selected, and normal cervical tissues from 10 people were used as controls.

All patients had not previously received radiotherapy, chemotherapy or hormone therapy. The diagnosis in all cases was confirmed histologically.

Immunohistochemical staining and identification

All tissue specimens were fixed in 10% formalin and processed to paraffin wax. Four serial sections (4 μm) were prepared. One was stained with haematoxylin and eosin (H&E), two were used for immunohistochemistry (IHC) and the last was a negative control. A streptavidin-peroxidase kit method was used for IHC (Zhongshan Goldenbridge Biotechnology, Beijing, China). The IHC reaction was visualised using diaminobenzidine (DAB). The negative control was treated with PBS instead of the primary antibody. Sections containing cervical cancer deposits were used as positive controls. The specimens were evaluated blind and independently by two pathologists.

In each section, 10 high-power fields were examined at random, and the immunoreactivity of 100 tumour cells was assessed in each field. The PTEN-positive particles appeared dark brown and were localised to the nuclei. Survivin particles were brown/yellow and were primarily expressed in the cytoplasm. Positive expression was stratified into four categories as follows: (–) marker-positive cancer cells <10%; (+) moderate number (10–25%) of marker-positive cancer cells; (++) marker-positive cancer cells in the range 25–75%; and (+++) marker-positive cancer cells >75%.

Statistical analysis

Data management was performed using SPSS 11.5 software. Clinical and pathological data were correlated with biomarker (PTEN and survivin) expression and cervical cancer stage using the χ^2 test and Fisher's exact probability method. Correlation between PTEN and survivin in CIN and cervical cancer was analysed using binomial distribution statistical analysis and Spearman correlation analysis. Statistical significance was regarded as $P<0.05$.

Table 1. Correlation between PTEN and survivin expression and clinicopathological features of cervical cancer.

	Group	Number of cases	PTEN-positive expression (*n*)	*P*	Survivin-positive expression (*n*)	*P*
Age (years)	50	34	13	>0.05	26	>0.05
	>50	15	6		9	
Differentiation	Well	6	2	>0.05	2	<0.05
	Moderate	29	12		20	
	Poor	14	5		13	
Clinical stage	I, II	20	12	<0.05	11	<0.05
	III, IV	29	7		24	
Tumour size (cm)	<4	30	15	<0.05	18	<0.05
	≥4	19	4		17	
Pelvic lymph node metastasis	Yes	21	3	<0.01	20	<0.01
	No	28	16		15	

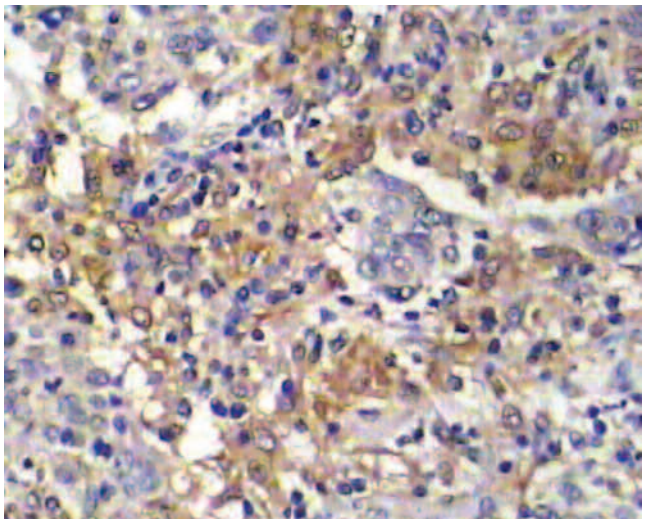

Fig. 3. Immunohistochemical staining of survivin in the cytoplasm of malignant cells (original magnification x200).

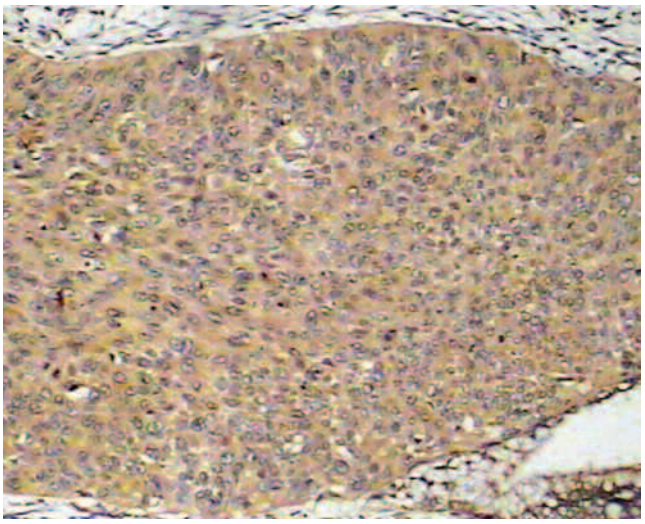

Fig. 4. Immunohistochemical staining of survivin in the cytoplasm of CIN3 cells (original magnification x200).

Results

PTEN and survivin expression

The PTEN-positive expression rate was 100% (10/10), 75% (15/20), 53.3% (16/30) and 38.8% (19/49) in samples of normal cervix, cervical ectropion with squamous metaplasia, CIN and cervical squamous cell carcinoma, respectively ($P<0.05$). The survivin-positive expression rate was 10% (1/10), 30% (6/20), 53.3% (16/30) and 71.4% (35/49) in samples of normal cervix, cervical ectropion with squamous metaplasia, CIN and cervical squamous cell carcinoma, respectively ($P<0.05$). PTEN-positive particles appeared dark brown and were localised primarily in the cell nuclei (Figs. 1 and 2). Survivin-positive particles were distributed primarily in the cytoplasm (Figs 3 and 4).

Correlation between biomarker expression and clinicopathological features

PTEN-positive expression in cervical cancer was associated with clinical stage and tumour size ($P<0.05$). The level of PTEN-positive expression in the metastatic pelvic lymph node group was significantly lower compared with the non-metastatic pelvic lymph node group ($P<0.01$). Positive PTEN immunostaining did not correlate with age or degree of tumour differentiation ($P>0.05$). Positive survivin immunostaining was associated with clinical stage, tumour size and degree of differentiation ($P<0.05$). Survivin-positive expression in the metastatic pelvic lymph node group was significantly higher compared with the non-metastatic pelvic lymph node group ($P<0.01$). No obvious relationship was found between survivin expression and patient age ($P>0.05$; Table 1).

Table 2. Correlation between PTEN and survivin expression in cervical intraepithelial neoplasia.

PTEN	Number of case	Survivin Positive	Survivin Negative	*P*	r_1
Positive	9	1	8	0.000	–0.488
Negative	21	20	1		

Table 3. Correlation between PTEN and survivin expression in cervical cancer.

PTEN	Number of case	Survivin Positive	Survivin Negative	*P*	r_2
Positive	19	7	12	0.000	–0.526
Negative	30	28	2		

Correlation between biomarker expression in CIN and cervical cancer

PTEN expression negatively correlated with survivin expression in CIN ($r_1= –0.488$, $P<0.01$; Table 2).

PTEN expression negatively correlated with survivin expression in cervical cancer tissues ($r_2=–0.526$, $P<0.01$; Table 3).

Discussion

Uterine cervical cancer is a malignant tumour of the female reproductive tract. The pathogenesis potentially involves proto-oncogene activation, anti-oncogene deactivation, and abnormality of the apoptosis-related gene and anti-metastasis gene. Elucidation of the molecular pathology of cervical cancer would help to clarify the mechanisms underlying the incidence and progression of this disease, and facilitate early diagnosis and intervention.

The *PTEN* gene-encoded protein exhibits double phosphoesterase activity and dephosphorylates phospholipids and lipoprotein lipases. The PTEN protein plays a key role in the regulation of the phosphatidylinositol-3-kinase (PI3K/AKT) signal conduction pathway, and it inhibits cell cycle progression, arrests cell growth, and mediates apoptosis by decreasing phosphatidylinositol (3,4,5)-triphosphate (PIP3)-induced phosphokinase B (PKB) activation.[3] In addition, PTEN inhibits cell attachment and migration through

dephosphorylation of focal adhesion kinases, thus inhibiting tumour diffusion.

Mayo *et al.* reported that the *PTEN* gene inhibits tumour cell growth by suppressing mitogen-activated protein (MAP) kinase phosphorylation.[4] In the present study, PTEN content in normal tissue, in mild CIN, in late-stage CIN and tumour tissues was assessed, and the results indicated that PTEN was positively expressed in normal tissues and in mild CIN, but was decreased in late-stage CIN and in tumour tissue.

Immunohistochemistry was used to determine PTEN expression level in order to investigate the correlation between PTEN expression and the incidence, progression and clinical pathological features of cervical cancer. The results indicated a gradual decrease in PTEN expression among normal tissues, cervical ectropion with squamous metaplasia, CIN and cervical squamous cell carcinoma, indicating that normal PTEN expression regulates cervical epithelial growth and differentiation. The decrease in PTEN expression during carcinomatous changes decreases regulation of the PIP3-PKB signal conduction pathway, resulting in over-proliferation of cervical epithelial cells. In the present study, PTEN-positive expression was associated with tumour size, clinical stage and metastatic potential, demonstrating that PTEN plays an important role in regulating cell growth as well as tumour infiltration and metastasis, and could serve as a biological marker to evaluate cervical cancer progression and prognosis.

As one of the inhibitors of apoptosis, surviving-positive expression exhibits tissue selectivity and inhibits apoptosis by suppressing caspase 3 and caspase 7 activity.[6] In addition, survivin interacts with various blood vessel regulatory factors, such as angiogenin-1, vascular endothelial growth factor and basic fibroblast growth factor to inhibit vascular endothelial cell apoptosis and stabilise blood vessel structures. Kim *et al*.[7] examined 41 samples of CIN and squamous cell carcinoma using a reverse transcription polymerase chain reaction (RT-PCR) method and concluded that the rate of survivin-positive expression gradually increased with disease progression. In the present study, a gradual increase in survivin expression was observed in normal tissues, cervical ectropion with squamous metaplasia, CIN, and cervical squamous cell carcinoma, indicating that survivin expression in cervical tissue lesions progresses with disease transformation from precancerous change to cancer. Survivin expression occurs as an early event in cervical cancer and could serve as a biomarker to predict progression of pathological change prior to cervical cancer. Results from the present study demonstrated that positive survivin expression correlated positively with clinical stage, tumour size and lymph node metastasis, indicating the important role of survivin in the invasiveness of tumours.

In the present study, PTEN and survivin were shown to be involved in cell cycle regulation and apoptosis, although with opposing biological effects. PTEN-positive expression decreased with disease progression and clinical stage, whereas survivin expression increased. PTEN expression negatively correlated with survivin expression in CIN and squamous cell carcinoma. PTEN deactivation potentially upregulates survivin expression through the PI3K/AKT pathway and inhibits tumour and vascular endothelial cell apoptosis, thus promoting tumour cell proliferation and invasion.[8]

In conclusion, PTEN and survivin have been used as targets for the prevention and treatment of tumours,[9–11] but play different roles in cervical cancer. PTEN deactivation and survivin over-expression are both important for progression and metastasis of cervical squamous cell carcinoma; however, further understanding of the interactive regulation of PTEN and survivin, and the further elucidation of the genes involved, may provide new tumour markers for early prevention, diagnosis and prognosis of cervical cancer. PTEN and survivin may also represent new approaches for gene therapy targets as well as the development of antitumour therapies. □

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