European Review for Medical and Pharmacological Sciences 2012; 16: 2044-2050

Altered expression of survivin, Fas and FasL contributed to cervical cancer development and metastasis

S.-F. WU, J.-W. ZHANG, W.-Y. QIAN, Y.-B. YANG, Y. LIU, Y. DONG, Z.-B. ZHANG, Y.-P. ZHU, Y.-J. FENG

Department of Obstetrics and Gynecology, Shanghai First People's Hospital, Shanghai Jiaotong University, Shanghai, China

Abstract. – OBJECTIVE: To evaluate the expression of apoptosis-related genes and their correlation with prognosis in cervical cancer patients.

MATERIALS AND METHODS: The expressions of Survivin, Fas and FasL in tissues of cervical cancer, cervical intraepithelial neoplasia (CIN), chronic cervicitis and normal cervix wer detected by immunohistochemical staining, and the relationship between the expression of Survivin, Fas and FasL and clinical pathologic characteristics of cervical cancer was correlation analysis.

RESULTS: The positive expression rates of Survivin and FasL in cervical cancer tissues were significantly higher than those in tissues of normal cervix, chronic cervicitis and CIN ($p < 0.05$), but lower positive expression rate of Fas was observed in cervical cancer tissues when compared with that in normal cervix, chronic cervicitis and CIN tissues ($p < 0.05$). The expression of Survivin was significantly correlated with clinical staging and lymph node metastases of cervical cancer ($p < 0.05$). The expression of FasL was correlated with lymph node metastases, clinical staging and pathological grading of cervical cancer ($p < 0.05$). The expression of Survivin was negatively correlated with that of Fas ($r = –0.517$, $p < 0.01$), but positively correlated with that of FasL ($r = 0.381$, $p < 0.01$) in tissues of cervical cancer.

CONCLUSIONS: The up-regulated expression of Survivin and FasL and down-regulated expression of Fas may be involved in the carcinogenesis and development of cervical cancer. The expression of FasL may be one of the prediction indexes for disease progression and prognosis in cervical cancer.

Key Words:
Survivin, Fas, FasL, Apoptosis, Cervical cancer.

Introduction

Apoptosis, or programmed cellular death, results from the activation of elements belonging to a family of 14 cysteine proteases called caspases, enzymes that cleave cellular proteins, including other caspases, at aspartic acid residues[1]. Apoptosis plays a key role not only in normal physiological regulation but also in the process of malignant pathological changes. It is co-regulated by the suppressor genes and the activation genes[2,3]. One class of molecules that block apoptosis by direct binding to caspases is the inhibitor of apoptosis proteins[4]. Targeting the apoptotic pathways for cancer treatment is supported by several findings emphasizing the role of aberrant apoptosis in tumorigenesis and also resistance to anticancer treatment. Evasion of apoptosis is critical for tumor growth and a hallmark of cancer cells[5]. The dysregulation of apoptosis, including the overexpression of anti-apoptotic Bcell lymphoma-2 (Bcl-2) homologues[6], the diminished expression of apoptotic protease activating factor 1[7], and the overexpression of Survivin[8] have all been reported to contribute to drug resistance.

Survivin is a bifunctional protein that acts as a suppressor of apoptosis and plays a central role in cell division. The protein is strongly expressed in the most common human neoplasms, has prognostic relevance for some of them and appears to be involved in tumor cell resistance to anticancer agents and ionizing radiation[3]. The human Survivin gene spans 14.7 kb on the telomeric position of chromosome 17 and is transcribed from a TATA-less, GC-rich promoter[9] to generate the wild-type transcript and four different splice variant mRNAs[10]. Several preclinical studies have demonstrated that down-regulation of Survivin expression or function, accomplished by means of various strategies, reduces tumor growth potential, increases the apoptotic rate and sensitize tumor cells to chemotherapeutic drugs and radiation in different human tumor models[11]. Moreover, the first Survivin inhibitors recently enter clinical trials. Recent studies suggest a possible role for Survivin in regulating the function of normal adult cells. FasL is a member of the tumor necrosis factor (TNF) superfamily of cy-

Corresponding Author: Wu Su-fang, MD; e-mail: sfang_wu@hotmail.com

tokines. Similar to other members of the TNF superfamily, FasL is synthesized as a type 2 transmembrane protein and acts in a juxtacrine manner. As with TNF-α, FasL can be cleaved by several matrix metalloproteinases, giving rise to a soluble form of FasL[12]. Fas is a signaling molecule which facilitates apoptosis. It is a type I transmembrane protein and a member of the TNF receptor (TNFR) superfamily. The crosslinking and signal transduction of FasL and Fas play important roles in inducing apoptosis, maintaining body's stability and keeping the environment in balance[13]. Once Fas combines with FasL, the apoptosis signal would be started, and then Fas positive cell apoptosis with few hours[2,3,14-16]. Survivin and Fas act together on Caspase 3 and Caspase 7 which play key roles in apoptosis.

We investigated the expression of Survivin and Fas/FasL system in tissues of normal cervix, chronic cervicitis, cervical intraepithelial neoplasia (CIN) and cervical cancer, and the clinical significance in cervical cancer occurrence and development process.

Materials and Methods

Case and Sample

The cervical tissues were obtained from the Obstetrics and Gynecology Department of The First People's Hospital of Shanghai Jiaotong University. The tissue samples were obtained from uterine surgery or cervical biopsy between Jan. 2001 and Dec. 2008, and were tested and confirmed by hematoxylin-eosin (HE) staining. There were 47 cases of cervical cancer, 25 cases of CIN, 20 cases of chronic cervicitis, and 20 cases of normal cervix. The patients of cervical cancer were 25-52 years old, median age was 38 years old, 42 cases were squamous carcinoma and the other 5 cases were adenocarcinoma. Divided by the differentiation degree, there were 15 cases of well differentiated, 29 cases of moderately differentiated, and 3 cases of poorly differentiated. According to clinical stages (Federation Internationale de Gynecologie et d'Obstetrique: FIGO, 2000), there were 5 cases of 1a stage, 39 cases of 1b-IIa stage, and 3 cases of IIb-III stage. Thirty cases of them suffered from lymph node metastases, and the other 17 cases didn't (The operated samples were tested by pathological diagnosis, and non-operated samples were based on magnetic resonance imaging (MRI) diagnosis). All patients had no history of radiotherapy or chemotherapy. The patients of CIN were 25-49 years old, median age was 37 years old. There were 9 cases of CINI, 11 cases of CINII, and 5 cases of CINIII. The patients of chronic cervicitis were 30-55 years old, median age was 42 years old. The patients of normal cervix were 25-51 years old, median age was 38 years old. No significant differences of age distribution were found between groups ($p > 0.05$).

Reagent and Immunohistochemistry Test

Cervical samples were cut into 4 μm thick section, and stained with immunohistochemistry self-potential method (SP method). The primary antibodies of rabbit anti human polyclonal antibody to Survivin, Fas and FasL were from Santa Cruz Biotechnology, Santa Cruz, CA, USA. The immunohistochemistry reagent was purchased from Beijing Zhongshan Biotechnology Co., Ltd. The concentration of primary antibody was 1:100. The experimental procedures were performed strictly according to the kit's instructions: dewaxed the paraffin section, repaired the antigen with citrate, color developed with diaminobenzidine (DAB), re-stained with hematoxylin, dehydration, transparence, mount sections, then observed the sections with optical microscope. The breast cancer section was used as positive control, and phosphate buffered saline (PBS) as negative control.

Criterion

The sections were confirmed by pathologist that they had antigen expression and localization. The positive cells of Survivin, Fas, and FasL were identified by claybank particle. Semi-quantitative analysis was taken to analyze overall intensity of cell staining under high power microscope (×400). The staining intensity was 0 (negative), 1 (yellow), 2 (claybank) or 3 (tawny). Each section was observed 5 fields randomly, calculated the percentage of positive cells in 500 cells and scored. The percentage of positive cells was scored as follows: 0, < 10% labeled tumor cells; 1, 10%-40% labeled tumor cells; 2, 10%-70% labeled tumor cells; and 3, > 70% labeled tumor cells. A composite score was obtained by multiplying the grade by the intensity, that is, – (0-1); + (2); ++ (3-4); +++ (5-6). (–) represented negative, (+) represented positive.

Statistical Analysis

All statistical procedures were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL,

USA). Enumeration data were performed using χ^2 test and Fisher exact test as appropriate. Correlation analysis was performed using Sperrman rank correlation analysis. $p < 0.05$ was considered to be statistically significant.

Results

The Expression of Survivin, Fas, and FasL in Tissues of Different Cervical Lesions

The positive cell of Survivin was located in the cytoplasm (Figure 1), while Fas and FasL were located in cytoplasm and cytomembrane (Figures 2, 3). The expressions of Survivin, Fas, and FasL in tissues of different cervical lesions are shown in Table I. The positive expression rates of Survivin and FasL in tissues of cervical cancer were significantly higher than those in tissues of normal cervix, chronic cervicitis and CIN ($p < 0.05$), and they were not expressed in normal tissues. The positive expression rate of Survivin in tissues of CIN had no significant difference compared to chronic cervicitis ($p > 0.05$), and had significant differences compared to normal cervix ($p < 0.05$). However, the positive expression rate of Survivin had no significant difference between chronic cervicitis and normal cervix ($p > 0.05$). The positive expression rate of FasL had no significant difference between CIN and chronic cervicitis ($p > 0.05$), and both of them were higher than those in tissues of normal cervix ($p < 0.05$). The positive expression rate of Fas in tissues of cervical cancer was significantly lower than that in tissues of normal cervix, chronic cervicitis and CIN ($p < 0.05$); however, there were no differences among normal cervix, chronic cervicitis and CIN ($p > 0.05$).

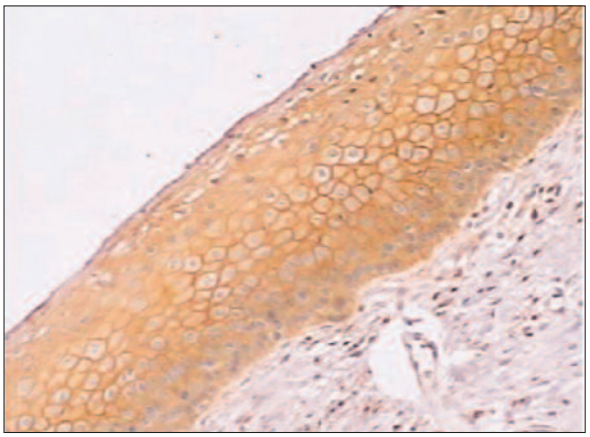

Figure 2. Expression of Fas in tissues of normal cervix (SP method, × 100).

The Relationship Between the Expression of Survivin, Fas and FasL and Clinical Pathologic characteristics of Cervical Cancer

The expressions of Survivin, Fas, and FasL cervical cancer were analyzed using Fisher exact test (Table II). The positive expression rate had no significant difference between patients of < 35 years old aged ≥ 35 years old ($p > 0.05$). The expression of Survivin was correlated with clinical staging and lymph node metastases of cervical cancer ($p = 0.043$, $p = 0.008$), and had no significant relationship with pathological grading, tissue type and tumor growth type of cervical cancer ($p > 0.05$). The expression of Fas had no rela-

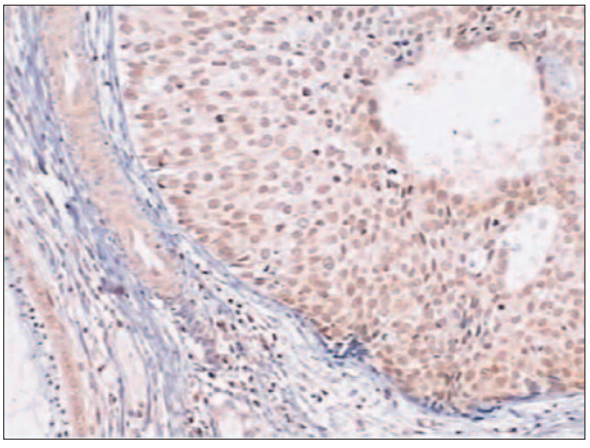

Figure 1. Expression of Survivin in tissues of cervical cancer (SP method, × 100).

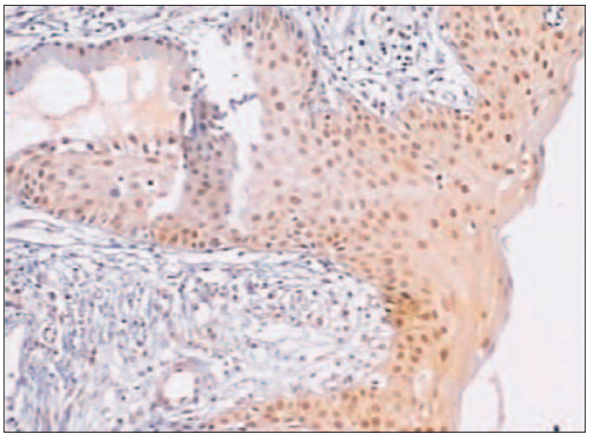

Figure 3. Expression of FasL in tissues of CIN (SP method, × 100).

Table I. Expression of survivin, Fas and FasL in different cervical tissues (n, %)

Groups	Survivin	Fas	FasL
Normal cervix (N = 20)	0 (0)[b]	18 (90.0)[b]	0 (0)[b]
Chronic cervicitis (N = 20)	3 (15.0)[b]	17 (85.0)[b]	10 (50.0)[a,b]
CIN (N = 25)	9 (36.0)[a,b]	20 (80.0)[b]	17 (68.0)[a,b]
Cervical cancer (N = 47)	32 (68.1)	23 (48.9)	44 (93.6)

[a]Compared to normal cervix, $p < 0.05$; [b]Compared to cervical cancer, $p < 0.05$.

tionship with clinical staging, pathological grading, tissue type, tumor growth type, and lymph node metastases of cervical cancer ($p < 0.05$). The expression of FasL was correlated with clinical staging, pathological grading, and lymph node metastases of cervical cancer ($p = 0.034$, $p = 0.049$, $p = 0.042$), but had no significant relationship with tissue type and tumor growth type ($p > 0.05$).

The Relationship Between the Expression of Survivin, Fas, and FasL in Tissues of Cervical Cancer

Among the 32 cases of cervical cancer that Survivin expressed positively, there were 10 cases that Fas expressed positively, and 32 cases that FasL expressed positively. Among the 15 cases of cervical cancer that Survivin expressed negatively, there were 13 cases that Fas expressed positively, and 12 cases that FasL expressed positively. The expression of Survivin was negatively correlated with that of Fas in tissues of cervical cancer ($r = –0.517$, $p < 0.01$), and was positively correlated with that of FasL ($r = 0.381$, $p < 0.01$). Among the 44 cases of cervical cancer that FasL expressed positively, there were 21 cases that Fas expressed positively. Among the 3 cases of cervical cancer that FasL expressed negatively, there were 2 cases that Fas expressed positively. The expression of Fas was not significantly correlated with that of FasL ($r = –0.093$, $p > 0.05$).

Discussion

Survivin, the smallest member of the inhibitors of apoptosis proteins (IAP) family, is a

Table II. Relationship between clinical pathologic characteristics and expression of Survivin, Fas and FasL in 47 patients with cervical cancer (n, %).

Index	Survivin	Fas	FasL
Age			
< 35 (n=20)	14 (70.0)	12 (60.0)	19 (95.0)
≥ 35 (n=27)	18 (66.7)	11 (40.7)	25 (92.6)
Tumor growth type			
Exogenous type (n=25)	17 (68.0)	11 (44.0)	24 (96.0)
Endogenous type (n=14)	10 (71.4)	8 (57.1)	12 (85.7)
Ulcerative type (n=8)	5 (62.5)	4 (50.0)	8 (100.0)
Clinical staging			
Ia (n=5)	1 (20.0)	3 (60.0)	3 (60.0)
Ib-IIa (n=39)	28 (71.8)	19 (48.7)	38 (93.7)
IIb-III (n=3)	3 (100.0)	1 (33.3)	3 (100.0)
Tissue type			
Squamous carcinoma (n=40)	28 (70.0)	21 (52.5)	38 (95.0)
Adenocarcinoma (n=7)	4 (57.1)	2 (28.6)	6 (85.7)
Pathological grading			
Well differentiated (n=15)	10 (66.7)	10 (66.7)	12 (80.0)
Moderately differentiated (n=29)	20 (69.0)	12 (41.4)	29 (100.0)
Poorly differentiated (n=3)	2 (66.7)	1 (33.3)	3 (100.0)
Lymph node metastases			
Yes (n=30)	25 (83.3)	12 (36.7)	30 (100.0)
No (n=17)	7 (41.2)	11 (70.6)	14 (82.4)

142-amino acid, 16.5-kDa protein coded by a single-copy gene located on the human 17q25 chromosome, and it acts as a suppressor of apoptosis and plays a central role in cell division[2].

The protein of Survivin is strongly expressed in the most common human solid tumor and hematologic malignancy, while expressed at low level in most of undifferentiated tissues, and it has closely related with tumor development, infiltration and prognosis[3,14]. Our study showed that there was relevance between Survivin expression and cervical clinical staging and lymph node metastases, which is consistent with the above discussion. This suggests that Survivin, as a specific factor of cervical cancercan be used to predict disease risk.

There are two major pathways for Survivin to inhibit apoptosis: (1) Survivin regulating apoptosis by cyclin-dependent kinase (CDK). It promotes CDK4 to release p21, and inhibits apoptosis. (2) Survivin binds to active Caspase3 and Caspase7, preventing accumulation of Asp-Glu-Val-Asp (DEVD)-cleaving suicide enzyme which induces by Caspase activator or apoptosis inducer, and Caspase3 is an important pathway mediated by Fas. The p21 is triggered to release when Survivin/CDK4 complex is formed, and the p21 interacts with Caspase3 in mitochondria and inhibits apoptosis mediated by Fas[2,3,14,15]. However, report[2] suggests that Survivin inhibits Caspase9 but doesn't inhibit Caspase3 and Caspase7, and this may explain why the results of Survivin and Fas were not consistent in our experiment.

Fas is a 45-kDa, type I transmembrane glycoprotein, which is encoded by Fas gene, located on the human 10q23 chromosome, and plays a regulatory role in apoptosis. FasL is a member of TNF family. It is a 40-kDa, type II transmembrane protein, which is located on the human 1q23 chromosome, and mediates apoptosis via binding with its receptor. The Fas/FasL interaction plays an important role in immune system adjustment and tumor progression[16-18]. Recent researches[17,19] suggest that many tumor cells not only can effectively escape from the body's lymphocyte attacks through Fas and FasL expression on the surface of them, but also actively kill the immunocompetent cells that come into contact with them, which ultimately leads to failure of immunotherapy. Our study found that the expression of FasL increased gradually in the tissues of normal cervix, chronic cervicitis, CIN and cervical cancer, and the difference was statistically significant. Especially the expression of FasL in chronic cervicitis cells increased compared to normal cells suggesting that FasL can promote cell to escape the attack from lymphocytes effectively. This is enough to explain the clinical phenomenon that patients of chronic cervicitis are susceptible to CIN and cervical cancer, and verify the role of chronic inflammation in the occurrence and development of cervical cancer.

Currently, the occurrence and development of cervical cancer is considered as a continuous process: cervical dysplasia (light → medium → heavy) → carcinoma *in situ* → early invasive carcinoma → invasive carcinoma. Consequently, we compared the expression difference of Survivin, Fas, and FasL in the tissues of normal cervix, chronic cervicitis, CIN and cervical cancer, and the expression difference of the three indicators according to the occurrence and development process of cervical cancer, which were different from other researches. We found that the positive expression rate of Fas in tissues of cervical cancer was significantly lower than that in tissues of normal cervix. This result suggests that there may be antigenic loss of Fas in the occurrence of cervical cancer, which may be one of the pathogenesis mechanisms of cervical cancer. The expression of FasL was correlated with lymph node metastases, clinical staging and pathological grading of cervical cancer. This result suggests that the positive expression rate of FasL in tissues of cervical cancer can reflect the malignant degree of pathological changes and disease progression. Another result of our study was that the positive expression rate of FasL in the tissues of cervical cancer and CIN was significantly higher than that in tissues of normal cervix. This reveals that the occurrence of cervical cancer is related to the up-regulated expression of FasL. It is suggested that tumor cells expresses FasL, and evades the immune surveillance by promoting apoptosis of Fas-positive lymphocytes[17,19]. The positive expression rate of FasL increased gradually in the tissues of chronic cervicitis, CIN and cervical cancer, and the high expression in the tissues of CIN, suggesting that the influence of FasL appeared in the early period of disease process.

When the FasL expression can't be down-regulated, CIN will develop into invasive carcinoma by inhibiting the immune system of host. This is consistent with and closely related to the currently accepted process of cervical cancer. Our study also found that the expression of FasL was correlated with clinical staging and pathological grad-

ing of cervical cancer, the positive expression rate of the tissues with lymph node metastases was significant higher than the tissues without lymph node metastases, suggesting that the prognosis was poor in the tissues with high expression of FasL. More importantly, FasL is closely related to the disease process, however, the expression of Survivin was only related to clinical staging and lymph node metastases, this suggesting that the expression of FasL may be one of the prediction indexes for disease progression and prognosis in cervical cancer.

The physiological function of Fas/FasL system is to limit excessive expansion or contraction of certain cell populations, involve normal cell proliferation, differentiation and apoptosis to maintain homeostasis, and it plays an important role in the clinical course of many diseases. Fas, as a receptor molecule that expresses prevalently, can occur on the surface of many kinds of cells, however, FasL is only expressed in the activated T cells and natural killer (NK) cells, so the activated NK cells tend to be the most effective way to kill the target cells by apoptosis[2,3,17]. Studies find that the expressions of Fas of most benign tumors are similar to the normal tissue where it is derived from. But the expressions of Fas of malignant tumor are significantly down-regulated or loss expressed to escape from the elimination of Fas/FasL system[15,19]. We investigated the relevance of the three indicators above and found that Survivin was correlated to the expression of Fas/FasL system, and this is consistent with the mechanism above that Survivin can resist against apoptosis. Although Fas and FasL is a pair of indicator, the relevance between them isn't significant, and this may be because of the involvement of other mechanisms. Besides, the positive expression rate of FasL increased gradually in the tissues of chronic cervicitis, CIN and cervical cancer, and the high expression in the tissues of CIN, suggesting that the influence of FasL appeared in the early period of disease progression, and this provides a better prediction value of prognosis. Especially in the tissues of chronic cervicitis and CIN, different from Survivin and Fas, the positive expression rate of FasL was significant different from the normal cervix. This suggests that the down-regulation of Fas antigen in tissues of cervix is only one of the factors resulting in tumor occurrence and development, while the up-regulation of FasL and the immune factors involved may be the more important factors.

Acknowledgements

This study was funded [in part] by [Shanghai Science and Technology Committee Foundation], grant number [09411962500]. This study was funded [in part] by [Shanghai Municipal Health Bureau Foundation], grant number [054019]. This study was funded [in part] by [Foundation from The first People's Hospital, Shanghai Jiaotong University], grant number [06B27].

References

1) Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-257.
2) Mita AC, Mita MM, Nawrocki ST, Giles FJ. Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. Clin Cancer Res 2008; 14: 5000-5005.
3) Pennati M, Folini M, Zaffaroni N. Targeting survivin in cancer therapy: fulfilled promises and open questions. Carcinogenesis 2007; 28: 1133-1139.
4) Schimmer AD. Inhibitor of apoptosis proteins: translating basic knowledge into clinical practice. Cancer Res 2004; 64: 7183-7190.
5) Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
6) Reed JC. Dysregulation of apoptosis in cancer. J Clin Oncol Rep 1999; 17: 2941-2953.
7) Soengas MS, Capodieci P, Polsky D. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. Nature 2001; 409: 207-211.
8) Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, Reed JC. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas(cd95), Bax, caspases, and antocancer drugs. Cancer Res 1998; 58: 5315-5320.
9) Monzó M, Rosell R, Felip E, Astudillo J, Sánchez JJ, Maestre J, Martín C, Font A, Barnadas A, Abad A. A novel anti-apoptosis gene: re-expression of survivin messenger rna as a prognosis marker in non-small-cell lung cancers. J Clin Oncol 1999; 17: 2100-2104.
10) Li F, Ling X. Survivin study: an update of "What is the next wave?". J Cell Physiol 2006; 208: 476-486.
11) Jiang G, Li J, Zeng Z, Xian L. Lentivirus-mediated gene therapy by suppressing survivin in BALB/c nude mice bearing oral squamous cell carcinoma. Cancer Biol Ther 2006; 5: 435-440.
12) Mitsiades N, Yu WH, Poulaki V, Tsokos M, Stamenkovic I. Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug. Cancer Res 2001; 61: 577-581.

13) Schneider P, Holler N, Bodmer JL, Hahne M, Frei K, Fontana A, Tschopp J. Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. J Exp Med 1998; 187: 1205-1213.

14) Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature 2001; 411: 342-348.

15) Choi C, Benveniste EN. Fas Ligand/Fas system in the brain: regulator of immune and apoptotic responses. Brain Res Rev 2004; 44: 65-81.

16) Espinosa M, Cantú D, Herrera N, Lopez C, De la Garza J, Maldonado V, Melendez-Zajgla J. Inhibitors of apoptosis proteins in human cervical cancer. BMC Cancer 2006; 6: 45.

17) Ibrahim R, Frederickson H, Parr A, Ward Y, Moncur J, Khleif SN. Expression of FasL in squamous cell carcinomas of the cervix and cervical intraepithelial neoplasia and its role in tumor escape mechanism. Cancer 2006; 106: 1065-1077.

18) Morino H, Ohashi K, Watanabe O, Munakata S. Expression of Fas ligand and bcl-2 in cervical carcinoma and their prognostic significance. Am J Clin Pathol 2005; 123: 879-885.

19) Hsiao HL, Wang WS, Chen PM, Su Y. Overexpression of thymosin-4 renders SW480 colon carcinoma cells more resistant to apoptosis triggered by FasL and two topoisomerase II inhibitors via downregulating Fas and upregulating Survivin expression, respectively. Carcinogenesis 2005; 27: 936-944.