European Review for Medical and Pharmacological Sciences 2017; 21: 2596-2603

Expression levels of PTEN, HIF-1α, and VEGF as prognostic factors in ovarian cancer

W. SHEN[1], H.-L. LI[1], L. LIU[2], J.-X. CHENG[1]

[1]Department of Obstetrics and Gynecology, the Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei, China
[2]Tumor Institute, the Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei, China

Abstract. – OBJECTIVE: Epithelial ovarian cancer is associated with high mortality, mostly because of delayed diagnosis, necessitating the search for novel diagnostic and prognostic markers. Here we evaluated the association between expression levels of phosphatase and tensin homolog (PTEN), hypoxia-inducible factor (HIF)-1α, and vascular endothelial growth factor (VEGF), and ovarian cancer.

PATIENTS AND METHODS: Expressions of these proteins were assessed by immunohistochemistry in 21 specimens of normal ovary tissues and 76 specimens of ovarian cancer tissues. Associations with pathological characteristics and prognosis were determined using chi-square test, Kaplan-Meier method, log-rank test, and Cox regression model.

RESULTS: Expression of PTEN in ovarian cancer tissue was negatively associated with clinical stage and differentiation degree. A reverse trend was observed in association between expression of HIF-1α and VEGF, and the clinical stage of the disease. PTEN expression negatively correlated with HIF-1α and VEGF expression levels, whereas both latter positively correlated with each other. The overall survival of patients with positive PTEN expression was significantly longer than that of those with negative expression; the opposite trend was observed with HIF-1α and VEGF. The differentiation degree and expressions of HIF-1α and PTEN were dependent predictors, whereas VEGF expression, clinical stage and lymph node metastasization were independent prognostic factors in these patients.

CONCLUSIONS: PTEN, HIF-lα, and VEGF were found to be prognostic markers in ovarian cancer, with VEGF also being as an independent prognostic factor. Combined detection of their expression levels may be useful for determination of the degree of malignancy, metastasis, and prognosis of ovarian cancer.

Key Words:
Ovarian cancer, PTEN, HIF-1α, VEGF, Prognosis.

Introduction

Epithelial ovarian cancer is a common gynaecological malignancy. Its incidence rate is the third highest among other gynaecological malignancies, following cervical cancer and endometrial cancer. Furthermore, ovarian cancer is associated with the highest mortality, mostly because clinical symptoms are not manifested clearly during the early stages of cancer. When manifested, the cancer is usually spread into the abdominal cavity and may even cause pleural effusions. Because of delayed diagnosis, the 5-year survival rate of these patients is very low[1].

At present, ovarian cancer markers, such as CA125 and CEA, are used for cancer screening[2]. Their diagnostic value is not sufficient, though, and more precise markers are needed to diagnose ovarian cancer, and determine its metastasization or recurrence.

Cancers develop because of inactivation of tumor suppressor genes, activation of proto-oncogenes, or because of modulation of apoptosis-related genes. These processes lead to an imbalance between cell proliferation, differentiation, and apoptosis[3]. Some of these genes hold potential as future markers for ovarian cancer.

Located on the chromosome 10, the phosphatase and tensin homolog (PTEN) is the first identified tumor suppressor gene with phosphorylase activity that is closely related to tumor development. PTEN expression is absent or reduced in many cancers or precancerous lesions[4].

The hypoxia-inducible factor (HIF)-1α is the transcription factor activated by hypoxic stress. This transcription factor regulates a panel of downstream genes, including the vascular endothelial growth factor (VEGF). HIF-1α has been documented as a contributor to the cancer deve-

Corresponding Author: Shen Wei, MD; e-mail: shenwei1980112@163.com

lopment and occurrence[5]. The aforementioned downstream target gene VEGF is a mitogen for vascular endothelial cells. Cancer cells are known to secrete high levels of VEGF, thereby promoting tumor angiogenesis and production of tumor stroma[6].

These three factors have been associated by previous studies. Thus it was found that, in gastric cancer, PTEN negatively regulates HIF-lα through the PI3K/AKT/FARP signalling pathway, leading to decreased expression of VEGF[7]. There are no similar findings about ovarian cancer. It is possible that PTEN, HIF-lα, or VEGF can be useful as markers of ovarian cancer.

The aim of this study was to evaluate a potential association between PTEN, HIF-1α, and VEGF, and clinicopathological characteristics and prognosis of patients with ovarian cancer.

Patients and Methods

Patients' Specimens

Seventy-six tissue specimens were obtained from patients with primary ovarian cancer who had been admitted from October 2007 to September 2010 to our hospital. The study was approved by the Ethical Committee of the Fourth Hospital of Hebei Medical University. In addition, we procured specimens from 21 patients with uterine fibroids; these specimens were used as normal ovarian tissue controls. Control patients had undergone hysterosalpingo-oophorectomy in the same time period as patients with ovarian cancer.

Patients with ovarian cancer patients had a median age of 52.9 years (range 32 to 72 years; 34 patients ≤ 50 years old and 42 patients >50 years old). None of these patients had received radiotherapy or chemotherapy before the operation, and their clinical and pathological files were complete. All patients were followed up until September 2015. Among them, there were 34 cases of serous cystadenocarcinoma, 24 cases of mucinous cystadenocarcinoma, and 18 cases of endometrial carcinoma. Furthermore, 26 specimens represented well differentiated cancer, whereas 50 specimens were from moderately or poorly differentiated ovarian cancer.

Patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO) 2000 staging system. The histopathological grade of the cancer was determined by the World Health Organization (WHO) classification standard. Thirty-eight cases were in stages I and II, and the remaining 38 cases in stages III and IV of the disease. There were 40 cases presenting with lymph node metastasis, and 36 cases with no lymph node metastasis.

Immunohistochemistry

Rabbit anti-human monoclonal VEGF and HIF-1α antibodies, rabbit anti-human PTEN polyclonal antibody, PV-6001 detection reagents, and DAB kit were purchased from Beijing Zhongshan Jinqiao Reagent Co., Ltd (Beijing, China).

The specimens were fixed in 10% formalin, embedded in 5 μm sections of paraffin, processed by conventional dewaxing to water, and further soaked for 5 min in a 3% hydrogen peroxide solution at room temperature to eliminate endogenous peroxidase activity. Anti-PTEN, HIF-1α, and VEGF antibodies were added, and slides were incubated overnight at 4°C. This was followed by three washes with phosphate-buffered saline, each wash going for 2 min. Then, the PV-6001 reagent was added, and slides were incubated at 37°C for 15-20 min, with three more washes afterwards. The slides were finally stained with DAB for approximately 5 min to achieve the desired staining intensity, which was verified under a microscope. The stain was removed with distilled water, and the slides were counterstained with hematoxylin, dehydrated, cleared in xylene, sealed with neutral gum, and observed under a light microscope.

As per literature recommendations[8], five fields were randomly selected from each section at high magnification. A semi-quantitative analysis was carried out to rank the slides based on staining intensity and number of positive cells. The staining intensity was ranked as cells with no color (0 points), cells stained pale yellow (1 point), cells stained yellow-brown (2 points), or cells stained brown (3 points). The number of positive cells was ranked as 0 points (positive cells constituting <10% of total cells), 1 point (10-49% positive cells), 2 points (50-74% positive cells), and 3 points (≥75% positive cells). These two scores were combined. The threshold was 2 points (*i.e.* scores 0-2 were considered negative, and >2 were considered positive).

Statistical Analysis

All statistical analyses were performed using the SPSS13.0 (SPSS Inc., Chicago, USA) statistical software. Qualitative data were analyzed using the chi-square test. The Kaplan-Meier method was used to explore the correlations among expressions of PTEN, HIF-1α, and VEGF. The difference in survival curves was evaluated by a log-rank test. The Cox regression model was

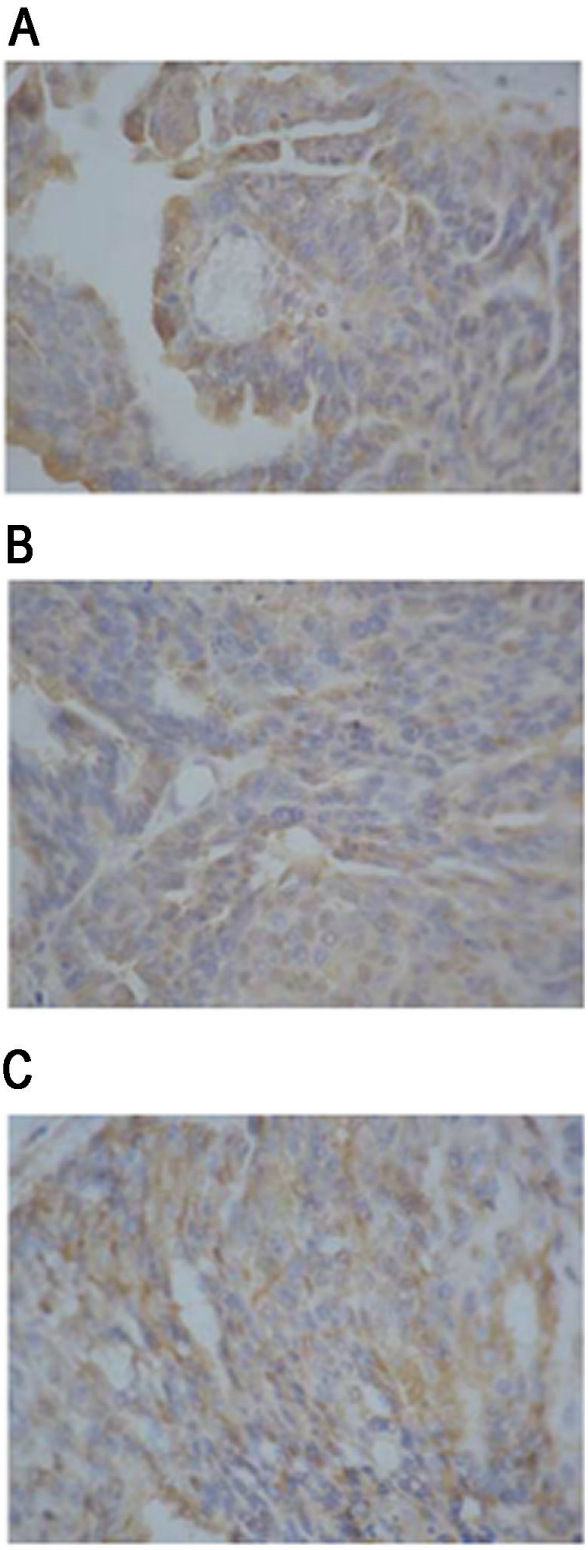

Figure 1. Positive expression of PTEN, HIF-1α, and VEGF in ovarian cancer tissue. A. PTEN; B. HIF-1α; C. VEGF. Magnification ×400.

used for multivariate analysis. The $p < 0.05$ was considered statistically significant.

Results

Expressions of PTEN, HIF-1α, and VEGF in Ovarian Cancer and Normal Ovarian Tissue

PTEN was primarily expressed in the cytoplasm, HIF-1α in the cytoplasm or nucleus, and VEGF in the cytoplasm or cytomembrane (Figure 1). The percentages of specimens positive for PTEN, HIF-1α, and VEGF were, respectively, 100, 0, and 14.29% in normal ovarian tissue specimens, and 36.84, 60.53, and 68.42% in specimens of ovarian cancer tissue ($p < 0.01$, ovarian tissue *vs.* normal tissue).

Relationships Between PTEN, HIF-1α, and VEGF Expressions, and Clinicopathological Parameters in Ovarian Cancer Tissue

Expression of PTEN in ovarian cancer tissue was associated with clinical stage or differentiation degree, but not with histological type, age, or lymph node metastasization (Table I). Furthermore, PTEN expression levels were higher in stages I/II than in stages III/IV, as well as in G1 stage *vs.* G2/G3 stage (Table I). These differences were statistically significant (Table I).

About HIF-1α and VEGF, their expressions were associated with clinical stage, differentiation degree, and lymph node metastasization, but not with histological type or age (Table I). In contrast to PTEN, HIF-1α and VEGF expression levels were higher in stages III/IV *vs.* stages I/II (Table I), and in G2/G3 stage *vs.* G1 stage (Table I). Also, expressions of both these proteins were higher in specimens with lymph node metastases, compared with specimens without lymph node metastasization (Table I). As with PTEN, these differences reached statistical significance (Table I).

Correlations Among PTEN, HIF-1α and VEGF Expressions in Ovarian Cancer Tissue

The occurrence of ovarian cancer is a result of a concerted effect of multiple genes[9]. Therefore, we assumed that expressions of the three genes of interest might be related to each other. To test for this association, a Spearman rank correlation analysis was performed (Table II). We observed that PTEN expression was negatively correlated to HIF-1α or VEGF expressions (Table II), while HIF-1α expression was positively correlated with VEGF expression (Table II).

Table I. Expressions of PTEN, HIF-1α, and VEGF, and clinicopathological parameters in 76 specimens of ovarian cancer.

Parameters	No.	PTEN Positive (%)	PTEN p	HIF-1α Positive (%)	HIF-1α p	VEGF Positive (%)	VEGF p
Histological type							
Serous	34	14 (41.18)	0.633	18 (52.94)	0.389	21 (61.76)	0.367
Mucinous	24	9 (37.50)		17 (70.83)		19 (79.17)	
Endometrial	18	5 (27.78)		11 (61.11)		12 (66.67)	
Age							
≤50	34	13 (38.24)	0.821	22 (64.71)	0.502	25 (73.53)	0.389
>50	42	15 (35.71)		24 (57.14)		27 (64.29)	
Clinical stage							
I/II	38	19 (50.00)	0.017	18 (47.37)	0.019	21 (55.26)	0.014
III/IV	38	9 (23.68)		28 (73.68)		31 (81.58)	
Differentiation degree							
G1	26	15 (57.69)	0.007	10 (38.46)	0.005	13 (50.00)	0.013
G2/G3	50	13 (26.00)		36 (72.00)		39 (78.00)	
Lymph node metastases							
Negative	36	16 (44.44)	0.192	17 (47.22)	0.024	20 (55.56)	0.022
Positive	40	12 (30.00)		29 (72.50)		32 (80.00)	

Table II. Relationship among expression levels of PTEN, HIF-1α, and VEGF.

		PTEN	HIF-1α	VEGF
PTEN	Correlation coefficient	1.000	-0.276	-0.244
	p (2-tailed)		0.016	0.034
	N	76	76	76
HIF-1α	Correlation coefficient	-0.276	1.000	0.320
	p (2-tailed)	0.016		0.005
	N	76	76	76
VEGF	Correlation coefficient	-0.244	0.320	1.000
	p (2-tailed)	0.034	0.005	
	N	76	76	76

Association Between PTEN, HIF-1α and VEGF Expressions, and Clinical Prognosis in Patients with Ovarian Cancer

Here we analyzed the association between PTEN, HIF-1α, and VEGF expression levels, and clinical prognosis in patients with ovarian cancer. This association was probed by the Kaplan-Meier method and log-rank test. Our analyses indicate that the overall survival of patients with positive PTEN expression was significantly longer than that of patients with negative PTEN expression (Table III; Figure 2A). In contrast, positive expression levels of HIF-1α or VEGF were associated with markedly shorter overall patient survival (Table III; Figure 2B and 2C). The aforementioned associations were statistically significant.

In addition, the survival benefit was observed in patients with lower clinical stage ($p = 0.001$), better differentiation degree ($p = 0.022$), and without lymph node metastasization ($p = 0.009$).

Multivariate Analysis of Prognostic Factors

To further explore the prognostic factors in ovarian cancer, the factors with statistical significance in the Kaplan-Meier survival analysis were subjected to a multivariate Cox survival analysis. The latter analysis revealed that differentiation degree and expression levels of HIF-1α and PTEN were dependent predictors of prognosis of ovarian cancer, whereas VEGF expression, clinical stage, and lymph node metastasization were independent prognostic factors (Table IV).

Discussion

Cancer is a polygenic disease, whose occurrence and development involve multiple pathways and numerous genes[10]. Combined detection of several markers of ovarian cancer can provide more

Table III. Analysis of prognostic value of proteins of interest in ovarian cancer patients.

Factors	Cases	Survival	Survival rate[1] (%)	Median overall survival (months)	p[2]
PTEN					
Negative	48	9	18.75	26.33	0.008
Positive	28	12	42.86	43.27	
HIF-1α					
Negative	30	13	43.33	48.13	0.003
Positive	46	8	17.39	25.90	
VEGF					
Negative	24	10	41.67	51.80	0.005
Positive	52	11	21.15	24.30	

Footnote: [1]5-year survival rate; [2]log-rank test.

information about underlying mechanisms of cancer proliferation, invasion, and metastasization[11].

One of candidate markers is PTEN. It is a recently discovered tumor suppressor gene that regulates cell cycle, induces apoptosis in tumor cells, and inhibits their growth, invasion, and metastasization[12]. PTEN inhibits proliferation of cancer cells by activating the FAK pathway, suppresses cancer cell growth by negatively regulating the MAPK pathway, and induces cancer cell apoptosis by suppressing the PI3K pathway[13]. Genetic abnormalities of PTEN gene (mutation, heterozygous loss, hypermethylation, microsatellite instability, transcription modification) lead to a "silencing" of gene expression[14]. Furthermore, low PTEN expression on protein level plays a role in the progression of prostate cancer, endometrial cancer, and ovarian cancer[15-17]. Loss of heterozygosity in PTEN was reported in as high as 45% of primary ovarian malignancies. Abnormal PTEN gene leads to diminished levels of this protein in the cell[18]. Our findings showed that PTEN protein expression in ovarian cancer was significantly lower than that in normal ovarian tissues, indicating that down-regulation of PTEN protein is closely associated with progression of ovarian cancer, which is consistent with previous reports[19,20]. Furthermore, we observed a negative relationship between the differentiation degree of ovarian cancer and PTEN protein expression levels, which is also consistent with previous reports[21]. Thereby, absent or low expression of PTEN may be a marker of ovarian cancer. In addition, PTEN protein expression was associated with the clinical stage, but not with lymph node metastases. It can be speculated that gene deletion may be a late event in the pathogenesis of ovarian cancer and, therefore, may occur on a specific stage of cancer.

HIF-1α plays an important role in cancer cell metabolism, angiogenesis, and metastasization; its expression is regulated by multiple factors[22]. HIF-1α is expressed in most cancers, and its expression is associated with proliferation, invasion, and metastasization. The high HIF-1α expression is most commonly associated with poor prognosis[23,24], as has been shown in colon, breast, and pancreatic cancers[25-27]. In our work, expression of HIF-1α was markedly higher in ovarian cancer than in normal ovarian tissue. Other studies demonstrated similar findings[28]. This suggests that HIF-1α could be used as an early diagnostic marker for ovarian cancer screening. HIF-1α was overexpressed at advanced clinical stages, in poorly differentiated ovarian cancer and patients with lymph node metastases. Therefore, high expression levels of HIF-1α may serve as a prognostic marker in ovarian cancer.

Table IV. Independent prognostic factors for overall survival in patients with ovarian cancer.

Determinants	Wald	p	RR	95% CI
VEGF expression	4.960	0.026	2.016	1.088-3.737
Clinical stage	10.117	0.001	2.456	1.412-4.271
Lymph node metastasis	7.696	0.006	2.180	1.257-3.780

Footnote: RR, relative risk; CI, confidence interval.

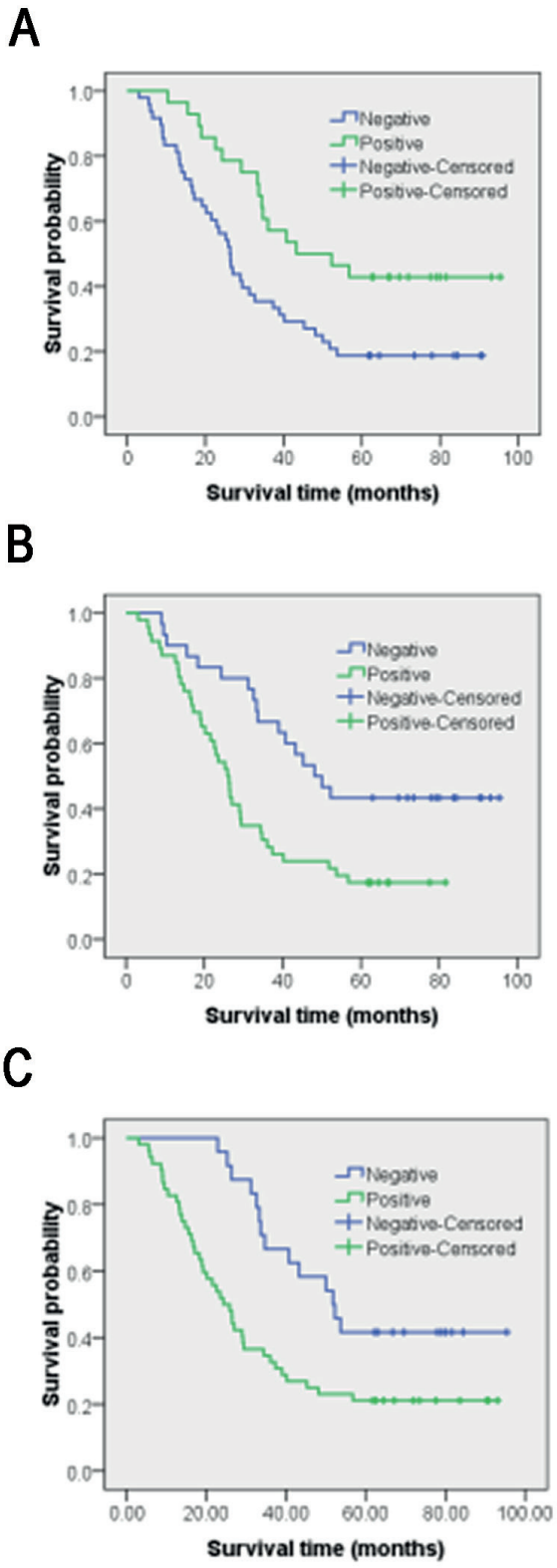

Figure 2. Association between overall survival of patients with positive expression PTEN, HIF-1α, and VEGF probed by the Kaplan-Meier method and log-rank test. A. PTEN; B. HIF-1α; C. VEGF.

VEGF is a vascular growth factor that promotes the growth of vascular endothelial cells[29]. It can also promote proliferation of vascular endothelial cells, improve vascular permeability, modulate the extracellular matrix, induce angiogenesis, and promote tumor growth and metastases. Moreover, VEGF has an impact on proliferation and apoptosis of tumor cells[30,31]. Increased VEGF expression promotes proliferation of tumor cells, and accelerates invasion and metastasis of tumor cells. Generally, VEGF is overexpressed in malignant tumors and vascular endothelial cells[32]. In our study, VEGF expression levels positively correlated with clinical stage, differentiation degree, and lymph node metastasis. These observations indicate that VEGF expression is increased in the process of tumor growth, which leads to the formation of blood vessels, the appearance of a large number of structural and functional abnormalities in tumor blood vessels, and the promotion of tumor progression and metastases. Notably, VEGF expression was also observed in normal ovarian tissues in our research, although this expression was not strongly positive. Indeed, expression levels of VEGF were significantly lower in normal ovarian tissue than in ovarian cancer specimens, which is consistent with literature reports[33]. These results suggest that VEGF plays an important role in the progression of ovarian cancer and is closely related to malignancy, invasion, and metastasis. Therefore, detection of VEGF expression could be used as a clinical indicator of the clinical stage of ovarian cancer.

Despite many reports being published on expression levels of PTEN, HIF-lα, and VEGF in various cancers, the observations have not been consistent. By inducing the expression of VEGF and glycolysis-related enzymes, HIF-1α can increase the supply of blood, oxygen, and energy to the tumor, and attenuate hypoxia[34]. In our study, HIF-lα expression was positively correlated with VEGF expression, similar to previous publications[35]. PTEN inhibits expression of HIF-lα[36]. Therefore, loss of PTEN gene upregulates HIF-1α expression and activates the P13K/Akt pathway to induce VEGF expression and tumor angiogenesis[37,38]. Not surprisingly, PTEN was negatively correlated with both HIF-lα and VEGF in our study. Other investigators reported that deletion of PTEN gene increases HIF-lα expression[37], supporting the role of PTEN as a negative regulator of carcinogenesis.

Our univariate and multivariate survival analyses demonstrated that positive expressions

of PTEN, HIF-lα, and VEGF were prognostic factors in ovarian cancer patients, with VEGF expression also being an independent prognostic factor. Low expression of PTEN and high expressions of HIF-lα and VEGF were associated with poor prognosis and significantly lower survival rate.

Conclusions

PTEN, HIF-lα and VEGF were found to be prognostic markers in ovarian cancer, with VEGF also being as an independent prognostic factor. Combined detection of expression levels of PTEN, HIF-lα, and VEGF may be useful for determination of the degree of malignancy, metastasis, and prognosis of ovarian cancer.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.
2) Maeda T, Inoue M, Koshiba S, Yabuki T, Aoki M, Nunokawa E, Seki E, Matsuda T, Motoda Y, Kobayashi A, Hiroyasu F, Shirouzu M, Terada T, Hayami N, Ishizuka Y, Shinya N, Tatsuguchi A, Yoshida M, Hirota H, Matsuo Y, Tani K, Arakawa T, Carninci P, Kawai J, Hayashizaki Y, Kigawa T, Yokoyama S. Solution structure of the SEA domain from the murine homologue of ovarian cancer antigen CA125 (MUC16). J Biol Chem 2004; 279: 13174-13182.
3) Kuo L, Thengchaisri N, Hein TW. Regulation of coronary vasomotor function by reactive oxygen species. Mol Med Ther 2012; 1: 1000101.
4) Freihoff D, Kempe A, Beste B, Wappenschmidt B, Kreyer E, Hayashi Y, Meindl A, Krebs D, Wiestler OD, von Deimling A, Schmutzler RK. Exclusion of a major role for the PTEN tumour-suppressor gene in breast carcinomas. Br J Cancer 1999; 79: 754-758.
5) Brown JM. Exploiting the hypoxic cancer cell: mechanisms and therapeutic strategies. Mol Med Today 2000; 6: 157-162.
6) Nishida N, Yano H, Komai K, Nishida T, Kamura T, Kojiro M. Vascular endothelial growth factor C and vascular endothelial growth factor receptor 2 are related closely to the prognosis of patients with ovarian carcinoma. Cancer 2004; 101: 1364-1374.
7) Park GS, Joo YE, Kim HS, Choi SK, Rew JS, Park CS, Kim SJ. Expression of PTEN and its correlation with angiogenesis in gastric carcinoma. Korean J Gastroenterol 2005; 46: 196-203.
8) Jin C, Wang A, Chen J, Liu X, Wang G. Relationship between expression and prognostic ability of PTEN, STAT3 and VEGF-C in colorectal cancer. Exp Ther Med 2012; 4: 633-639.
9) Szabova L, Yin C, Bupp S, Guerin TM, Schlomer JJ, Householder DB, Baran ML, Yi M, Song Y, Sun W, McDunn JE, Martin PL, Van Dyke T, Difilippantonio S. Perturbation of Rb, p53, and Brca1 or Brca2 cooperate in inducing metastatic serous epithelial ovarian cancer. Cancer Res 2012; 72: 4141-4153.
10) Zhang M, Zhou YF, Gong JY, Gao CB, Li SL. Expression of autophagy-related protein LC3B, p62, and cytoplasmic p53 in human retinoblastoma tissues. Eur Rev Med Pharmacol Sci 2016; 20: 3152-3160.
11) Kong WM. The regulation of gene therapy research in competent adult patients, today and tomorrow: implications of EU Directive 2001/20/EC. Med Law Rev 2004; 12: 164-180.
12) Stiles B, Gilman V, Khanzenzon N, Lesche R, Li A, Qiao R, Liu X, Wu H. Essential role of AKT-1/protein kinase B alpha in PTEN-controlled tumorigenesis. Mol Cell Biol 2002; 22: 3842-3851.
13) Gu J, Tamura M, Yamada KM. Tumor suppressor PTEN inhibits integrin- and growth factor-mediated mitogen-activated protein (MAP) kinase signaling pathways. J Cell Biol 1998; 143: 1375-1383.
14) Yang L, Kuang LG, Zheng HC, Li JY, Wu DY, Zhang SM, Xin Y. PTEN encoding product: a marker for tumorigenesis and progression of gastric carcinoma. World J Gastroenterol 2003; 9: 35-39.
15) Waite KA, Eng C. Protean PTEN: form and function. Am J Hum Genet 2002; 70: 829-844.
16) Dahia PL. PTEN, a unique tumor suppressor gene. Endocr Relat Cancer 2000; 7: 115-129.
17) Chu EC, Tarnawski AS. PTEN regulatory functions in tumor suppression and cell biology. Med Sci Monit 2004; 10: RA235-RA241.
18) Kurose K, Zhou XP, Araki T, Cannistra SA, Maher ER, Eng C. Frequent loss of PTEN expression is linked to elevated phosphorylated Akt levels, but not associated with p27 and cyclin D1 expression, in primary epithelial ovarian carcinomas. Am J Pathol 2001; 158: 2097-2106.
19) Depowski PL, Rosenthal SI, Ross JS. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. Mod Pathol 2001; 14: 672-676.
20) Rustia A, Wierzbicki V, Marrocco L, Tossini A, Zamponi C, Lista F. Is exon 5 of the PTEN/MMAC1 gene a prognostic marker in anaplastic glioma? Neurosurg Rev 2001; 24: 97-102.
21) Chen R, Agarwal K, Sheppard CJ, Chen X. Imaging using cylindrical vector beams in a high-numerical-aperture microscopy system. Opt Lett 2013; 38: 3111-3114.
22) Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003; 3: 721-732.

23) Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 2010; 29: 625-634.

24) Marín-Hernández A, Gallardo-Pérez JC, Ralph SJ, Rodríguez-Enríquez S, Moreno-Sánchez R. HIF-1alpha modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms. Mini Rev Med Chem 2009; 9: 1084-1101.

25) Mizukami Y, Li J, Zhang X, Zimmer MA, Iliopoulos O, Chung DC. Hypoxia-inducible factor-1-independent regulation of vascular endothelial growth factor by hypoxia in colon cancer. Cancer Res 2004; 64: 1765-1772.

26) Bos R, van der Groep P, Greijer AE, Shvarts A, Meijer S, Pinedo HM, Semenza GL, van Diest PJ, van der Wall E. Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer 2003; 97: 1573-1581.

27) Büchler P, Reber HA, Büchler M, Shrinkante S, Büchler MW, Friess H, Semenza GL, Hines OJ. Hypoxia-inducible factor 1 regulates vascular endothelial growth factor expression in human pancreatic cancer. Pancreas 2003; 26: 56-64.

28) Daponte A, Ioannou M, Mylonis I, Simos G, Minas M, Messinis IE, Koukoulis G. Prognostic significance of Hypoxia-Inducible Factor 1 alpha (HIF-1 alpha) expression in serous ovarian cancer: an immunohistochemical study. BMC Cancer 2008; 8: 335.

29) Möbius C, Demuth C, Aigner T, Wiedmann M, Wittekind C, Mössner J, Hauss J, Witzigmann H. Evaluation of VEGF A expression and microvascular density as prognostic factors in extrahepatic cholangiocarcinoma. Eur J Surg Oncol 2007; 33: 1025-1029.

30) Mercurio AM, Lipscomb EA, Bachelder RE. Non-angiogenic functions of VEGF in breast cancer. J Mammary Gland Biol Neoplasia 2005; 10: 283-290.

31) Yabushita H, Shimazu M, Noguchi M, Kishida T, Narumiya H, Sawaguchi K, Noguchi M. Vascular endothelial growth factor activating matrix metalloproteinase in ascitic fluid during peritoneal dissemination of ovarian cancer. Oncol Rep 2003; 10: 89-95.

32) Nishida N, Yano H, Komai K, Nishida T, Kamura T, Kojiro M. Vascular endothelial growth factor C and vascular endothelial growth factor receptor 2 are related closely to the prognosis of patients with ovarian carcinoma. Cancer 2004; 101: 1364-1374.

33) Abu-Jawdeh GM, Faix JD, Niloff J, Tognazzi K, Manseau E, Dvorak HF, Brown LF. Strong expression of vascular p ermeability factor (vascular endothelial growth factor) and its receptors in ovarian borderline and malignant neoplasms. Lab Invest 1996; 74: 1105-1115.

34) Yasuda S, Arii S, Mori A, Isobe N, Yang W, Oe H, Fujimoto A, Yonenaga Y, Sakashita H, Imamura M. Hexokinase II and VEGF expression in liver tumors: correlation with hypoxia-inducible factor 1 alpha and its significance. J Hepatol 2004; 40: 117-123.

35) Wang L, Chen ZJ, Wang QT, Cao WF, Jian Y, Wang SX, Zhang BH. Expression of hypoxia-inducible factor 1 alpha and vascular endothelial growth factor in prostate cancer and its significance. Zhonghua Nan Ke Xue 2006; 12: 57-59.

36) Mizukami Y, Li J, Zhang X, Zimmer MA, Iliopoulos O, Chung DC. Hypoxia-inducible factor-1-independent regulation of vascular endothelial growth factor by hypoxia in colon cancer. Cancer Res 2004; 64: 1765-1772.

37) Emerling BM, Weinberg F, Liu JL, Mak TW, Chandel NS. PTEN regulates p300-dependent hypoxia-inducible factor 1 transcriptional activity through Forkhead transcription factor 3a (FOXO3a). Proc Natl Acad Sci USA 2008; 105: 2622-2627.

38) Blancher C, Moore JW, Robertson N, Harris AL. Effects of ras and von Hippel-Lindau (VHL) gene mutations on hypoxia-inducible factor (HIF)-1alpha, HIF-2alpha, and vascular endothelial growth factor expression and their regulation by the phosphatidylinositol 3'-kinase/Akt signaling pathway. Cancer Res 2001; 61: 7349-7355.