Int J Clin Exp Pathol 2016;9(5):5466-5472
www.ijcep.com /ISSN:1936-2625/IJCEP0021188

Original Article

Prognostic value of interleukin-33 in cervical cancer patients who underwent radical hysterectomy

Zhiqing Wu, Huiling Zhang

Department of Gynecology, Longhua New District Central Hospital of Shenzhen, Shenzhen, China

Received December 4, 2015; Accepted February 15, 2016; Epub May 1, 2016; Published May 15, 2016

Abstract: Aim: Interleukin-33 (IL-33) is an interleukin-1-like cytokine which is involved in the immune response. The aim of this study was to investigate the expression of IL-33 in cervical cancer and explore the prognostic value of IL-33 in cervical cancer patients. Methods: The expression of IL-33 in human cervical cancer tissues was detected using quantitative reverse transcription-polymerase chain reaction and immunohistochemical analysis. The relationships of IL-33 expression with the clinical characteristics and survival of patients were analyzed. Results: The expression of IL-33 mRNA was significantly higher in cervical specimens compared with paired adjacent noncancerous tissues. IL-33 protein expression was significantly related to FIGO stage (P=0.003) and lymph node metastasis (P=0.044). Kaplan-Meier and multivariate analyses suggested that high IL-33 expression was significantly associated with unfavorable overall (P=0.035) and recurrent-free survival (P=0.011) of cervical cancer patients. Multivariate survival analysis indicated that serum IL-33 was an independent prognostic factor for cervical cancer patients. Conclusion: These results suggest that IL-33 expression is correlated with tumor development and progression of cervical cancer and may serve as an indicator of poor prognosis in patients with cervical cancer.

Keywords: IL-33, cervical cancer, prognosis

Introduction

Cervical cancer is a major cause of morbidity and mortality in women, with an age standardized mortality rate of 10/10,000 in developing countries, which is more than three times higher than in developed countries [1]. Worldwide, there are estimated 529,000 new cases and 275,000 deaths reported worldwide annually [2]. Early cervical cancer is often asymptomatic, and many patients with cervical cancer are diagnosed at an advanced stage, when patients have little prospect of effective and curative treatment [3]. The mechanisms that drive tumorigenesis, tumor invasion, metastasis and drug resistance are critical survival-influencing factors in cervical cancer, are still complex and currently, poorly understood. Therefore, identification of new biomarkers involved in the carcinogenesis and development of cervical cancer may improve our understanding of the diagnosis and treatment of cervical cancer.

Interleukin-33 (IL-33), a member of the IL-1 family of cytokine, was first discovered in nuclei of endothelial cells of the high endothelial venules of lymph nodes [4]. IL-33 contains a histone-binding domain and an IL1-like cytokine domain, and was constitutively expressed in endothelial and epithelial cells of mucosa members, keratinocytes and fibroblasts [5]. It exerts biological function by binding to a full-length membrane molecule ST2 on the surface of T-helper 2 (Th2) cells. Depending on the cellular and cytokine context, IL-33 participates in host defense and immune diseases with dual, pro-inflammatory, or protective roles [6]. In addition, IL-33 has been shown to induce immune response, and acts as a “danger signal” in necrotic cells [7]. More recently, it has been reported that disruption of ST2 signaling may enhance the anti-tumor immune response, suggesting IL-33 impedes anti-tumor immunity [8]. IL-33 has also been shown to be related to cancer development. Yamada et al. demonstrated that IL-33 administration results in the increased expression of IL-6 and the development of cholangiocarcinoma in mouse model, highlighting the role of inflammatory cytokines in the

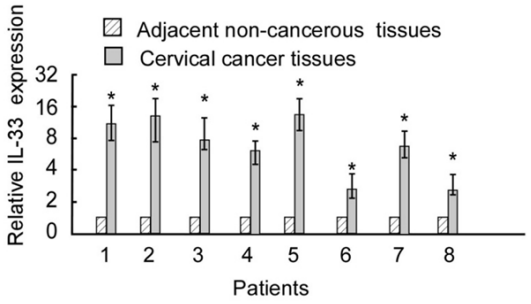

Figure 1. Increased IL-33 expression in cervical cancer specimens. Real-time PCR analysis of IL-33 expression level in cervical cancer tissues compared with paired non-cancerous tissues. asterisks, $P<0.05$.

oncogenesis of cholangiocarcinoma [9]. Fang el al. proposed that induced IL-33 expression enhances the tumorigenic activity and microglia/macrophage infiltration of glioma cells [10]. Liu et al. found that expression of IL-33 was higher than in normal breast tissues, and was also associated with more lymph nodes involvement of breast cancer, suggesting that IL-33 may play an important role in the progress and metastasis of breast cancer [11]. However, the role of IL-33 in patients with cervical cancers is not elucidated.

To confirm the expression pattern of IL-33 in cervical cancer tissues and evaluate its associations with tumor progression and patients' prognosis, immunohistochemistry was performed using 102 formalin-fixed and paraffin-embedded cancerous tissues from patients with cervical cancer. Then, the associations between IL-33 expression, clinicopathological characteristics, and prognosis of cervical cancer patients were statistically evaluated. The results indicate that IL-33 has a key role in tumor progression and that IL-33 expression is associated with the clinical prognosis in patients with cervical cancer.

Materials and methods

Patients and sample collection

A total of 102 primary cervical cancer cases were histopathologically and clinically diagnosed in the Longhua New District Central Hospital of Shenzhen between 2000 and 2007. All the patients received radical hysterectomy, while none of the patients had been treated with any tumor-specific therapy before surgery. The follow-up period ranged from 2 to 60 months after surgical resection (average: 43.1 months; median: 51.4 months). Patients who died of other causes were excluded from the analysis. Tissue samples from non-cancerous oral lesions were also collected during the study period and served as the normal controls. Prior patient's written informed consent was obtained for use of the tissue samples for research purposes. This study was approved by the institutional ethics committee of the Longhua New District Central Hospital of Shenzhen.

Real-time reverse transcription (RT)-PCR

Total RNA from 8 pairs of frozen cervical cancer and paired adjacent non-cancerous cervical tissues were extracted using Trizol reagent (Invitrogen). Reverse transcription reactions were performed according to the manufacturer's protocol. Quantitative PCR was carried out with SYBR Premix Ex Taq (TaKaRa) in the ABI-7300 Real-Time PCR System (Applied Biosystems) for 40 cycles under the following conditions: 95°C 10 s, then 60°C 10 s, and 72°C 20 s. The results were normalized to those of the GAPDH control. The sequences for sense and antisense primers are as follows: IL-33, forward, 5'-AATCAGGTGACGGTGTTG-3', reverse, 5'-ACACTCCAGGATCAGTCTTG-3'; GAPDH, forward, 5'-GAAGGTGAAGGTCGGAGTC-3', reverse, 5'-GAGATGGTGATGGGATTTC-3'.

Immunohistochemistry

Immunohistochemical analysis was performed to study IL-33 expression in 102 paraffin-embedded tissues which had been processed into 5-mm serial sections. Briefly, the sections were dewaxed in xylene and rehydrated in grade alcohol, and then they were boiled in 10 mmol/L of citrate buffer (pH 6.0) for antigen retrieval. After inhibition of endogenous peroxidase activities by 3% hydrogen peroxide in methanol, slides were treated with 1% bovine serum albumin to block non-specific binding. Next, the slides were incubated with a rabbit monoclonal anti-IL-33 antibody (1:150; Abcam). After washing, the tissue sections were then incubated with the biotinylated secondary antibody followed by further incubation with streptavidin-horseradish peroxidase complex. Negative control slides were prepared by omitting the primary antibody under the same experi-

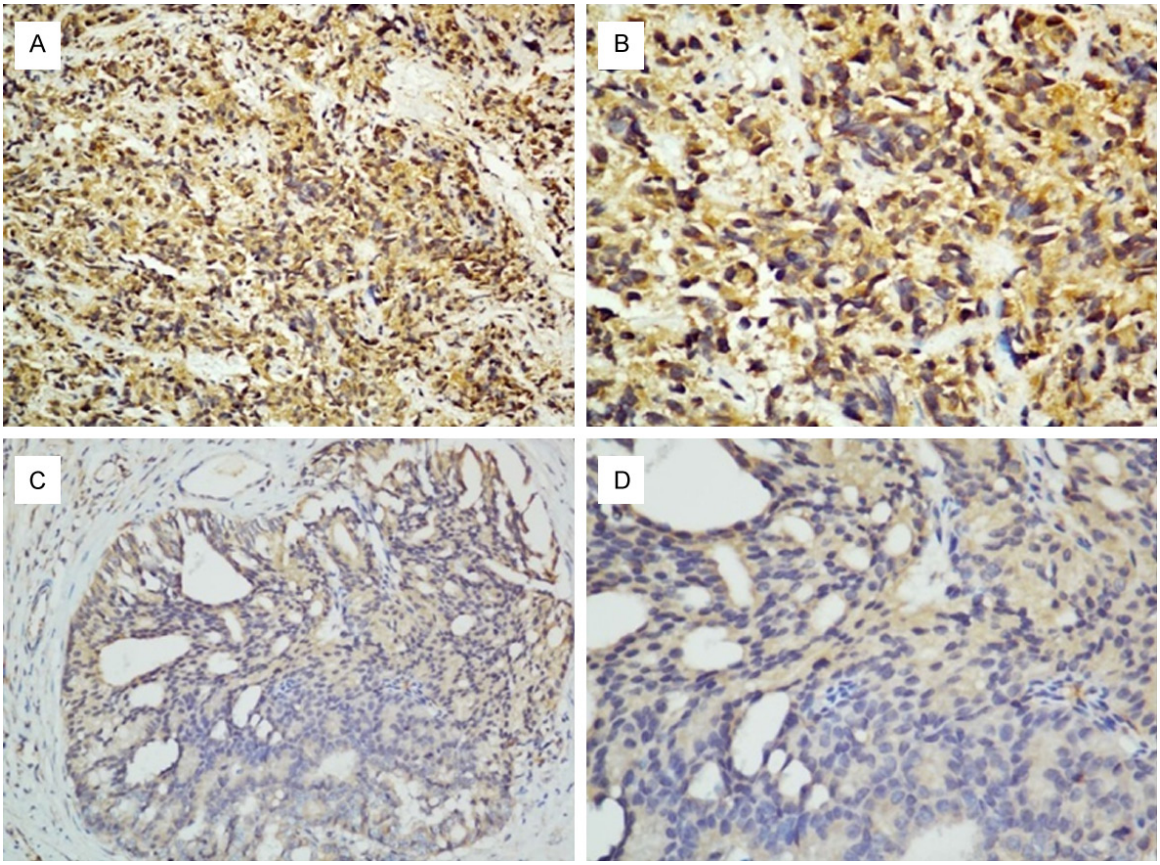

Figure 2. Representative immunostaining of IL-33 in cervical cancer tissues. High (A. 200×; B. 400× magnification) and low (C. 200×; D. 400× magnification) expression of IL-33 in cervical cancer tissues.

mental conditions, and the absence of non-specific immunoreactive staining was confirmed.

Immunoreactivity was semi-quantitatively evaluated on the basis of staining intensity and distribution scores. The intensity score was defined as 0, negative; 1, weak; 2, moderate; or 3, strong, and the proportion score was defined as 0, negative; 1, <10%; 2, 11-50%; 3, 51-80%; or 4, >80% positive cells. The immunoreactivity score, which defined as the product of the proportion score multiplied by the intensity score, ranged from 0 to 12. The sections were divided into the following three groups based on the immunoreactivity score: negative immunoreactivity was defined as low expression was defined as a total score of <4 and high expression was defined as a total score >4.

Statistical analysis

All the experiments were repeated at least 3 times, and data were analyzed for statistical significance by using the SPSS software (SPSS 16.0). Result of quantitative data was expressed as the mean ± SD and evaluated using the Student's t-test. The survival index was obtained using the Kaplan-Meier method and compared using the log-rank test. In all comparisons, differences are considered statistically significant at $P<0.05$.

Results

IL-33 is overexpressed in human cervical cancer tissues

To examine the expression pattern of IL-33 in cervical cancer, we used real-time RT-PCR analysis to assess the difference mRNA levels of IL-33 in cervical cancer tissues and paired adjacent non-cancerous tissues. As anticipated, IL-33 expression was significantly upregulated in cervical cancer tissues compared with that of adjacent non-cancerous tissues (**Figure 1**). The representative immunostaining of IL-33

Table 1. Association of cervical cancer clinicopathological parameters and IL-33 expression

Clinicopathologic Characteristic	Category	No.	IL-33 expression		*P value*
			High	Low	
Age (y)	≤50	63	41	22	0.167
	>50	39	20	19	
FIGO stage	Ib1	67	33	34	0.003
	>Ib1	35	28	7	
Differentiation	1/2	44	24	20	0.345
	3	58	37	21	
Tumor size	≤4 cm	65	39	26	0.167
	>4 cm	37	22	25	
LN Metastasis	No	81	44	37	0.044
	Yes	21	17	4	

protein in cervical cancer tissues was shown in **Figure 2**.

Relationship between IL-33 expression and clinical features

Table 1 shows the relationship between IL-33 expression and the clinical features. IL-33 expression did not vary significantly with gender, age or tumor differentiation.

However, we found a significant correlation between IL-33 expression and FIGO stage (P=0.003) and lymph nodes metastasis (P= 0.044).

Relationship between IL-33 expression and prognosis

For the survival analysis, the median duration of follow-up after surgery was 43.1 months (range 2-60 months). The patients' survivals of all 102 cases were significantly influenced by FIGO stage, tumor size, lymph node metastasis as well as IL-33 expression. As shown in **Figure 3**, patients with higher IL-33 expression had worse overall survival (P=0.035) and recurrent-free survival (P=0.011) than those with lower IL-33 expression. Multivariate analysis indicated that IL-33 expression was an independent prognostic factor of patient's overall survival (P=0.042) and recurrent-free survival (P=0.014), as shown in **Table 2**.

Discussion

Although several molecular factors and histological features have been reported to be associated with the progression and prognosis of cervical cancer, more effective biomarkers are necessary for the early-stage diagnosis and the accurate prediction of the clinical outcome of cervical cancer patients. In this study, we investigated the expression pattern and the potential prognostic role of IL-33 in cervical cancer. Our data showed that the expression of IL-33 was upregulated in human cervical cancer tissues compared with that in paired adjacent non-cancerous tissues. In addition, the relationship between clinicopathological features and IL-33 expression showed that IL-33 expression was positively correlated with FIGO stage and lymph nodes metastasis. To evaluate the prognostic value of IL-33 expression in cervical cancer patients, we divided them into two subgroups (high IL-33 expression and low IL-33 expression) and compared outcome between the two groups. The Kaplan-Meier survival analysis revealed that patients with high IL-33 expression had shorter overall and recurrent-free survival than those with low IL-33 expression. To our knowledge, this is the first study to demonstrate IL-33 as a candidate prognostic biomarker for cervical cancer.

IL-33 is a member of the interleukin family of cytokines that regulates a wide variety of cellular functions. Its receptor is ST2, an IL-1 receptor family member that also acts as a negative regulator of TLR-IL-1R signaling and the IL-1R accessory protein [12]. By binding to the ST2 receptor, IL-33 can activates NF-κB and MAP kinases, which in turn stimulate the downstream expression of TH2-associated cytokines such as IL-4, IL-5 and IL-6 [13]. More recently, IL-33 has been shown to participate in many diseases with dual proinflammatory or protective roles depending on the cellular and cytokine context. Carlock et al. revealed that high level expression of IL-33 during ovulation and fluctuated IL-33 expression during estrous cycle are associated with ovarian tissue homeostasis [14]. Byrne et al. found that IL-33 is significantly upregulated in inflamed skin samples of atopic dermatitis patients, and hypothesized that IL-33 acts as a novel "alarmin" that is released in the full-length active form after tissue damage. In this way, IL-33 can mediate the recruitment of innate immune cells to sites of infection or cellular damage [15]. In support of this hypothesis, Lee et al. observed a positive correlation of IL-33 expression level in pleural and the existence of tuberculous pleurisy, fur-

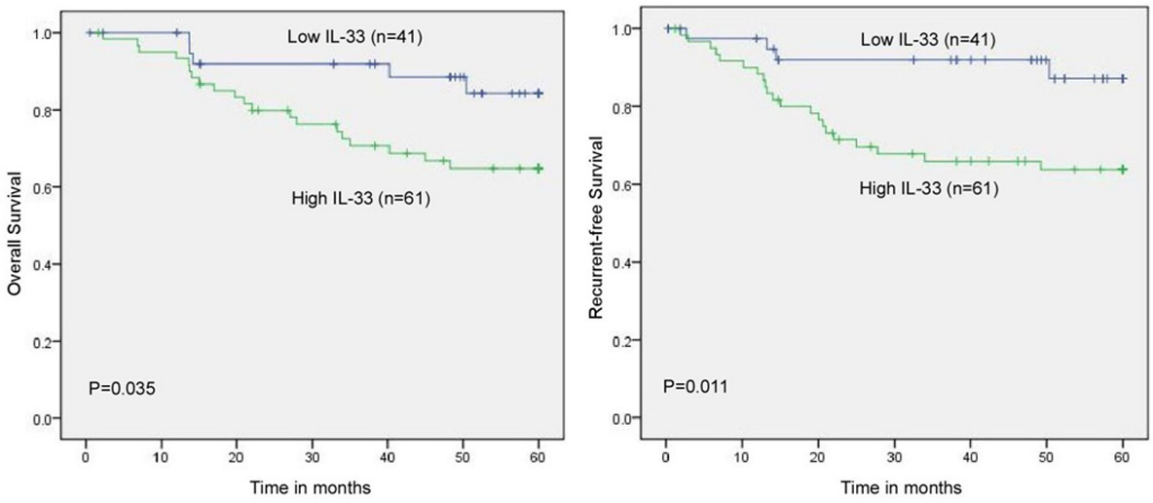

Figure 3. Survival analysis of IL-33. Kaplan-Meier curves for overall and recurrent-free survival of IL-33 low vs high expression in patients with cervical cancer.

Table 2. Multivariate Analyses of Parameters Associated with OS and RFS in cervical cancer patients

Parameter	Overall survival		Recurrent-free survival	
	HR (95% CI)	*P value*	HR (95% CI)	*P value*
Age (>50 vs ≤50)	1.084 (0.211-3.654)	0.128	1.424 (0.146-5.102)	0.115
Tumor Stage (>Ib1 vs Ib1)	2.495 (0.138-7.376)	0.036	1.258 (0.059-6.138)	0.274
Differentiation (Grade 3 vs 1/2)	1.282 (0.575-4.852)	0.740	1.247 (0.063-4.746)	0.869
Tumor size (>4 cm vs ≤4 cm)	1.426 (0.274-5.246)	0.092	1.307 (0.155-6.614)	0.075
LN Metastasis (+ vs -)	2.446 (0.100-4.512)	0.029	2.153 (0.527-6.726)	0.064
IL-33 expression (High vs Low)	3.126 (0.754-5.164)	0.042	3.752 (1.175-7.615)	0.014

ther indicating the roles of IL-33 in inflammatory responses and pathogenesis [16].

On the other hand, mounting evidence has suggested the role of IL-33 beyond immune responses. Namely, it may serve as a potential prognostic biomarker and a valuable therapeutic target for a variety of human cancers. Hu et al. demonstrated that circulating levels of IL-33 were elevated in patients with non-small-cell lung cancer (NSCLC), and that IL-33 could be used as a promising potential diagnostic and prognostic marker in NSCLC [17]. Santulli et al. unveiled that serum IL-33 is elevated in women with uterine leiomyoma and correlated with features of uterine leiomyoma tumor burden [18]. Tong et al. reported that interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways [19]. In the present study, our data show that high expression of IL-33 was significantly correlated to lymph node metastasis and poor prognosis in cervical cancer patients. These finding are partially consistent with those of previous reports, and together indicate the utility of IL-33 as a prognostic marker in human cancers.

Previous study has shown that IL-33 protein and mRNA levels in cervical tissues were significantly lower in severe CIN patients than that of mild CIN or no CIN patients. However, cervical cancer tissues did not show reduced IL-33 expression compared with severe CIN tissues [20]. The data was not in agreement with our findings, where the expression of IL-33 was upregulated in cervical cancer tissues compared with that in paired adjacent non-cancerous tissues, and IL-33 expression level was higher in advanced stage cervical cancer tissues compared with that in early stage diseases. The difference may be due to the different roles of IL-33 in proliferative and cancerous diseases.

In conclusion, this is the first study to show a significant correlation of the expression level of IL-33 and clinical survival in cervical cancer patients. In view of these data, analysis of IL-33 expression in surgical tissue samples from cervical cancer patients may provide additional information to the decision-making process regarding appropriate treatment strategies in cervical cancer. Further studies assessing the correlation of IL-33 expression with prognosis and metastasis in a larger cohort of patients are necessary to confirm the utility of IL-33 as a viable prognostic marker and target of cervical cancer therapies.

Disclosure of conflict of interest

None.

Address correspondence to: Zhiqing Wu, Department of Gynecology, Longhua New District Central Hospital of Shenzhen, Shenzhen 518110, China. Tel: +86 137-1381-0682; Fax: +86 755-2801-5466; E-mail: zhiqing1984@yeah.net

References

[1] Colombo N, Carinelli S, Colombo A, Marini C, Rollo D, Sessa C; ESMO Guidelines Working Group. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23 Suppl 7: vii27-32.

[2] Arbyn M, Castellsague X, de Sanjose S, Bruni L, Saraiya M, Bray F and Ferlay J. Worldwide burden of cervical cancer in 2008. Ann Oncol 2011; 22: 2675-2686.

[3] Resbeut M, Fondrinier E, Fervers B, Haie-Meder C, Bataillard A, Lhommé C, Asselain B, Basuyau JP, Brémond A, Castaigne D, Dubois JB, Houvenaeghel G, Lartigau E, Leblanc E, Sastre-Garau X, Sarradet A, Guastalla JP, Chauvergne J; Fédération Nationale des Centres de Lutte contre le Cancer; Standards, Options and Recommandations; Lutte contre le Cancer Ligue 101 Comité; Société Française d'Oncologie Gynécologique; Fédération Hospitalère de France; Fédération de Cancérologie des CHR & U. [Standards, Options and Recommendations for the management of invasive cervical cancer patients (non metastastic)]. Bull Cancer 2003; 90: 333-346.

[4] Baekkevold ES, Roussigne M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, Brandtzaeg P, Erard M, Haraldsen G and Girard JP. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. Am J Pathol 2003; 163: 69-79.

[5] Lingel A, Weiss TM, Niebuhr M, Pan B, Appleton BA, Wiesmann C, Bazan JF and Fairbrother WJ. Structure of IL-33 and its interaction with the ST2 and IL-1RAcP receptors--insight into heterotrimeric IL-1 signaling complexes. Structure 2009; 17: 1398-1410.

[6] Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, Nambu A, Abe T, Kiyonari H, Matsumoto K, Sudo K, Okumura K, Saito H and Nakae S. IL-33 is a crucial amplifier of innate rather than acquired immunity. Proc Natl Acad Sci U S A 2010; 107: 18581-18586.

[7] Enoksson M, Lyberg K, Moller-Westerberg C, Fallon PG, Nilsson G and Lunderius-Andersson C. Mast cells as sensors of cell injury through IL-33 recognition. J Immunol 2011; 186: 2523-2528.

[8] Jovanovic IP, Pejnovic NN, Radosavljevic GD, Pantic JM, Milovanovic MZ, Arsenijevic NN and Lukic ML. Interleukin-33/ST2 axis promotes breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells. Int J Cancer 2014; 134: 1669-1682.

[9] Yamada D, Rizvi S, Razumilava N, Bronk SF, Davila JI, Champion MD, Borad MJ, Bezerra JA, Chen X and Gores GJ. IL-33 facilitates oncogene-induced cholangiocarcinoma in mice by an interleukin-6-sensitive mechanism. Hepatology 2015; 61: 1627-1642.

[10] Fang KM, Yang CS, Lin TC, Chan TC and Tzeng SF. Induced interleukin-33 expression enhances the tumorigenic activity of rat glioma cells. Neuro Oncol 2014; 16: 552-566.

[11] Liu J, Shen JX, Hu JL, Huang WH and Zhang GJ. Significance of interleukin-33 and its related cytokines in patients with breast cancers. Front Immunol 2014; 5: 141.

[12] Gangemi S, Allegra A, Profita M, Saitta S, Gerace D, Bonanno A, Alonci A, Petrungaro A, Russo S and Musolino C. Decreased plasma levels of IL-33 could contribute to the altered function of Th2 lymphocytes in patients with polycythemia vera and essential thrombocythemia. Cancer Invest 2013; 31: 212-213.

[13] Ali S, Mohs A, Thomas M, Klare J, Ross R, Schmitz ML and Martin MU. The dual function cytokine IL-33 interacts with the transcription factor NF-kappaB to dampen NF-kappaB-stimulated gene transcription. J Immunol 2011; 187: 1609-1616.

[14] Carlock CI, Wu J, Zhou C, Tatum K, Adams HP, Tan F and Lou Y. Unique temporal and spatial expression patterns of IL-33 in ovaries during ovulation and estrous cycle are associated with ovarian tissue homeostasis. J Immunol 2014; 193: 161-169.

[15] Byrne SN, Beaugie C, O'Sullivan C, Leighton S and Halliday GM. The immune-modulating cy-

tokine and endogenous Alarmin interleukin-33 is upregulated in skin exposed to inflammatory UVB radiation. Am J Pathol 2011; 179: 211-222.

[16] Lee KS, Kim HR, Kwak S, Choi KH, Cho JH, Lee YJ, Lee MK, Lee JH, Park SD and Park DS. Association between elevated pleural interleukin-33 levels and tuberculous pleurisy. Ann Lab Med 2013; 33: 45-51.

[17] Hu LA, Fu Y, Zhang DN and Zhang J. Serum IL-33 as a diagnostic and prognostic marker in non- small cell lung cancer. Asian Pac J Cancer Prev 2013; 14: 2563-2566.

[18] Santulli P, Even M, Chouzenoux S, Millischer AE, Borghese B, de Ziegler D, Batteux F and Chapron C. Profibrotic interleukin-33 is correlated with uterine leiomyoma tumour burden. Hum Reprod 2013; 28: 2126-2133.

[19] Tong X, Barbour M, Hou K, Gao C, Cao S, Zheng J, Zhao Y, Mu R and Jiang HR. Interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways. Mol Oncol 2016; 10: 113-25.

[20] Wang L, Li H, Liang F, Hong Y, Jiang S and Xiao L. Examining IL-33 expression in the cervix of HPV-infected patients: a preliminary study comparing IL-33 levels in different stages of disease and analyzing its potential association with IFN-gamma. Med Oncol 2014; 31: 143.