Original Article
Potential clinical value of interleukin-31 and interleukin-33 with their receptors expression as diagnostic and predictive factors in endometrial cancer: a case-control study

Xi Zeng[1,2], Jing Li[3], Le-Ni Kang[2,4], Ming-Rong Xi[1,2], Guang-Dong Liao[1,2]

[1]Department of Gynecology and Obstetrics, The West China Second University Hospital, Sichuan University, Chengdu 610041, China; [2]Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, Chengdu 610041, China; [3]Department of Environmental and Occupational Health, West China School of Public Health, Sichuan University, Chengdu, Sichuan, China; [4]National Office for Maternal and Child Health Surveillance of China, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China

Abstract: *Aims*: To evaluate the potential role of interleukin-31 and interleukin-33 in diagnosis and prognosis from endometrial cancer. *Methods:* Tissue samples and clinical data were collected from 260 patients with endometrial cancer and 150 control patients with benign uterine diseases. Immunohistochemistry and ELISA testing quantified the expressions of interleukin-31 and interleukin-33 and their receptors. After surgery, all patients were followed up for an average of 56.3 months. Surgical effects were evaluated based on the patients' symptoms and signs. A two-sided *P* value <0.05 was considered significant. *Results:* IL-31, IL-33 and their receptors were significantly accumulated with the progression of endometrial cancer, in comparison to the controls. Moreover, the expressions were correlated with clinical characteristics, including tumor stage, differentiation, and associated with patients' disease-free survival. *Conclusions:* Limited data was available between the expressions of IL-31 and IL-33 and the receptors in patients with endometrial cancer. Our study findings suggested that the expressions of IL-31 and IL-33 might become possible biomarkers for the diagnosis and prediction in endometrial cancer.

Keywords: IL-31/IL-31R, IL-33/ST2, endometrial cancer, biomarkers

Introduction

Endometrial cancer, the tumor originating in the endometrium, is the most common gynecological cancer in developed countries, and its prevalence is increasing [1]. More than 61 880 new cases have been diagnosed, which led to 12 160 estimated deaths in America in 2018 [2]. Early detection and treatment are critical to improve the prognosis of patients with endometrial cancer. Abnormal uterine bleeding is the most frequent symptom of endometrial cancer. Only about 15% of endometrial cancer occurs in women without vaginal bleeding. Many other disorders can also lead to the same symptom [3-5]. Serum levels of some tumor markers elevated in only 20% to 30% of patients [3, 6]. Some factors perhaps could be used as new markers.

Cytokines are important immuno-regulatory proteins that mediate the interaction between immune and non-immune cells, including interleukins, tumor necrosis factor, colony-stimulating factor, chemotactic cytokines, and transforming growth factors, playing a key role in controlling innate and adaptive immune responses [7, 8].

Interleukins are produced by a variety of cells and can be used in a variety of cells. Since scientists originally thought that those cytokines were secreted by white blood cells and had physiological functions that regulated white blood cells, named Interleukin [9]. More follow-up studies reported that interleukins can also affect the proliferation, maturation, adhesion, and metastasis of many types of cells, including tumor cells [9, 10].

Interleukin 31 (IL-31) is a novel cytokine originally isolated from activated human T cell and mouse testis, Dillon et al. (2004). The mature IL-31 molecule is a 141-amino acid helix containing 4 alpha helices and belonging the Interleukin-6 (IL-6) family, which is mainly secreted by CD4+ T cells, mast cells, and mononuclear/giant cells, such as phagocytes and dendritic cells (DCs) [11]. The functional receptor of IL-31R exists in three forms (GLMR, GPL, and I31RA) and is widely distributed in activated monocytes, macrophages, myeloid progenitor cells, epidermal keratinocytes, eosinophils, and epithelium cells and lymphocytes [12-14]. Previously studies have shown that IL-31R can transmit a variety of signals in different tissues, and induce a variety of biological behaviors, including promoting vascular endothelial growth factor overexpression and promoting T cell proliferation, regulating hematopoiesis, regulating cell migration and proliferation, and inflammatory factors and immune responses [15].

Interleukin-33 (IL-33) is a cytokine discovered by Schmitz et al. in 2005 and belongs to the IL-1 family. A mature IL-33 molecule consists of 270 amino acid residues and is mainly secreted by type 2 T cells, natural killer cells, giant cells, etc., and can be continuously expressed in endothelial cells and epithelial cells [14, 16, 17]. The receptor ST2 of IL-33 is a member of the IL-1 receptor family. Its gene is called T1, DER-4 or Fit-1, locating on chromosome-2 in humans and encoded by the IL1RL1 gene [16].

At present, the research of IL-31/IL-31R and IL-33/ST2 in endometrial cancer is quite limited; the roles have not been fully understood. Based on our literature review, this is the first article concentrating on the expression and correlation of IL-31/IL-31R and IL-33/ST2 in patients with endometrial cancer.

Materials and methods

Patients and controls

Approval from the ethics committee of West China Second University Hospital, Sichuan University, China was obtained at the beginning of the project. Two hundred and sixty patients with endometrial cancer were recruited on admission to the hospital from January 2014 to January 2015. These patients were pathological diagnosed by curettage of uterus, and confirmed by two pathologists before surgery. Post-surgery, tumors were staged according to the 2009 criteria for adenocarcinoma of the endometrium, as defined by the International Federation of Gynecology and Obstetrics. The patients did not receive prior chemotherapy or radiation. Another 150 age-matched females, who underwent hysterectomy at the hospital due to benign uterine diseases, and without history of cancers, were recruited as healthy controls.

Fresh tissue collection

With obtaining written informed consent from all patients, in compliance with the Declaration of Helsinki, a fresh sample of 1 cm × 1 cm × 1 cm was collected during the operation, within 10 min after uterus excision, and was stored at -80°C until testing.

Sectioning of tissues

Endometrium tissues were collected during surgery, and sectioned at 4 um. Clinical and histological data were collected.

ELISA

The ELISA kits (Raybio Systems) specific for human IL-31 and human IL-33 were used to detect IL-31 and IL-33 levels of fresh tissue, respectively. The sensitivity limits of the ELISA kits were separate from 4.92 pg/mL to 1200 pg/mL (IL-31) and from 2.05 pg/mL to 500 pg/mL (IL-33). The intra-assay and inter-assay reproducibility of the ELISA kits were 9%, 11% (IL-31) and 8%, and 11% (IL-33).

Immunohistochemistry

As part of routine clinical practice, three samples of primary tissue per patient were randomly selected and assayed for IL-31R and IL-33R by immunohistochemistry using rabbit anti-human (Abcam, USA). Staining pattern, intensity, and distribution were assessed by light microscopy. The receptors were considered present if distinct brownish nuclear staining was observed. Stained mononuclear cells are reported as percentage of all immune cells in the field.

The immunohistochemical staining results included the Intensity Score (IS) and the Proportion Score (PS). The IS standard: negative

Table 1. Clinical characteristics of patients and controls

Characteristics	Patients, n (%)	Controls, n (%)	*P* value
Sample size	260	150	
Mean age ± SD (range), years	59.3±10.25 (30-78)	57.22±8.89 (29-76)	0.875
BMI mean ± SD, kg/m^2	21.33±2.06	21.72±2.21	0.901
Family history of cancer			
Yes	82 (31.54%)	48 (%)	0.923
No	178 (68.46%)	102 (%)	
Menopausal status			
Premenopausal	143 (55%)	83 (55.33%)	0.948
Postmenopausal	117 (45%)	67 (44.67%)	
History of pregnancy			
Yes	218 (83.85%)	127 (84.67%)	0.827
No	42 (16.15%)	23 (15.33%)	
Uterine bleeding			
Yes	206 (79.23%)		
No	54 (20.77%)		
FIGO stage			
I	188 (72.31%)	0	
II	41 (15.77%)	0	
III	26 (10%)	0	
IV	5 (1.92%)	0	
Differentiation Stage			
G1	112 (43.08%)	0	
G2	94 (36.15%)	0	
G3	54 (20.77%)	0	
Histology			
Adenocarcinoma	260 (100%)	0	
Non-adenocarcinoma	0 (0%)	0	

(uncolored) 0 points, weak positive (light yellow) 1 point, medium positive (yellow) 2 points, and strong positive (brown) 3 points. The PS standard: 0 point of, 1 point of 1% proportion, 2 points of (1%, 10%) proportion, 3 points of (10%, 33%) proportion, 4 points of (34%, 66%) proportion, and 5 points of (67%, 100%) proportion. The final score of each slice is the sum of the IS and PS. The theoretical minimum score is 0, and maximum score is 8 [18].

Statistical analysis

Data were analyzed in SPSS version 22.0 (IBM Company, USA) and are reported as mean ± standard deviation (range). Categorical variables were compared between patients and controls by Chi-Squared test. After testing for normality of the raw data, the correlation between clinical factors and levels of IL-31 and IL-33 with the receptors were analyzed by independent-samples t-test. The correlation between the tumor markers and the interleukin levels was analyzed with Student's t-test. A two-sided *p* value <0.05 was considered significant. Figures were generated in GraphPad Prism 5 (GraphPad Software Inc., USA).

Results

The comparable clinical characteristics of patients and controls were listed in **Table 1**.

IL-31 and IL-33 expressions in endometrial tissues

The tissue levels of IL-31 and IL-33 in tumor patients were more elevated than the counterparts in controls, *P*<0.001 (**Table 2**).

Furthermore, the expressions of IL-31 and IL-33 in endometrial adenocarcinoma tumor tissues increased with the degree of differentiations,

Table 2. The tissue expressions of IL-31 and IL-33 in patients and controls (pg/ml)

Interleukins	Patients	Controls	P Value
IL-31	175.80±39.03	57.93±25.08	<0.001
IL-33	101.31±51.14	44.74±24.41	<0.001

Note: Data are expressed as mean ± standard deviation (range).

Table 3. The expressions of IL-31 and IL-33 in patients with differentiations (pg/ml)

Interleukins	G1	G2	G3	P Value
IL-31	88.16±36.31	155.68±46.51	221.83±59.06	<0.001
IL-33	65.71±26.05	110.55±39.25	160.17±41.32	<0.001

Note: Data are expressed as mean ± standard deviation (range).

and the P values were both <0.001, which was statistically significant (**Table 3**).

The expressions of IL-31R in tissues

By immunohistochemistry, IL-31R protein was the least expressed in normal endometrial cells, and the highest expression was found in G3 endometrial adenocarcinoma cells. The average scores of the groups were as follows: control group: 2.1 points, tumor group: 4.9 points.

In the control group, the staining score <2.1 was divided into IL-31R weak expression group with 82 cases. The staining score ≥2.1 was divided into IL-31R strong expression group with 68 cases. The difference in staining scores between the four groups was statistically significant (P=0.021*).

In the tumor group, the staining score <6.1 was further divided into IL-31R weak expression group with 123 cases. The staining score ≥6.1 was divided into IL-31R strong expression group with 137 cases. We further conducted an intra-group stratification analysis of the disease group specimens: 3.4 points in the G1 group, 5.6 points in the G2 group, and 7.8 points in the G3 group (**Figure 1**).

Combined with the case data of the study subjects, we found that in the patients' group, the intensity of IL-31R staining expression was related to the presence of diabetes (P=0.032), clinical stage (P=0.004) and differentiation level of the patients (P=0.011). While, in the control group, the intensity of IL-31R staining expression was not significantly associated with clinical characteristics including meno-

pause, obesity, diabetes, or hypertension status (**Table 4**).

The correlations between IL-31 and IL-31R

We further verified the distribution of IL-31 and IL-31R staining results in the control, G1, G2, and G3 groups, confirming that all data were in a normal distribution. Pearson correlation analysis was performed on the results of IL-31 and IL-31R staining in each group. The results showed that there was a positive correlation between IL-31 and IL-31R staining in each group (**Figure 2**).

The relationship between the expression of IL-31R and the prognosis of patients

In the IL-31R strong expression group, 11 patients lost follow-up, the average follow-up time of the rest was 54.04 months, and 19 patients suffered recurrence, with the earliest recurrence time of 7 months and 55 months of the latest. The median recurrence time was 28.95 months after surgery.

In the IL-31R weak expression group there were 14 patients lost follow-up and another 14 patients had recurrence. Among the rest of the patients, the average follow-up time was 57.1 months, the earliest recurrence time was 14 months, and the latest recurrence time was 52 months. The median recurrence time was 31.79 months.

After analyzing the free survival (FS) of 235 patients, the difference of FS between IL-31R weak and strong expression group was statistically significant (**Figure 3**).

The expressions of ST2 in tissues

By immunohistochemistry, ST2 protein was the lowest expressed in normal endometrial cells, and the highest expression was found in G3 endometrial adenocarcinoma cells. The average scores of the groups were as follows: control group: 2.7 points, tumor group: 5.2 points, the difference was statistically significant.

In the control group, the staining score <2.7 was divided into ST2 weak expression group, a
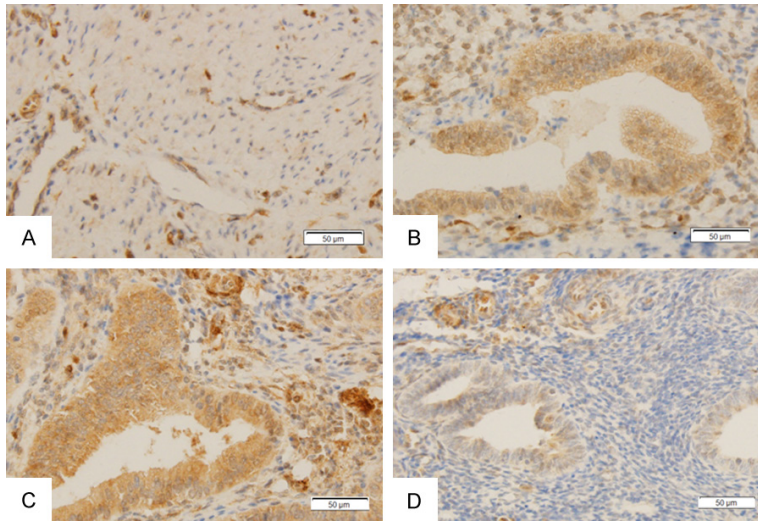
Figure 1. Immunohistochemical staining of IL-31R in cancer and normal tissues. A. Well-differentiated endometrial cancer. B. Moderately differentiated endometrial cancer. C. Poorly differentiated endometrial cancer. D. Normal endometrial tissue. According to the staining criteria, the average scores of the groups were as follows: 3.4 points in the high-differentiation group, 5.6 points in the moderately differentiated group, 7.8 points in the poorly differentiated group, and 2.1 points in the normal group. The difference was statistically significant (P=0.021*).

Table 4. Relationship between IL-31R expression and clinical features in patient group

Character	Number (n=260)	IL-31R Expression		P Value
		Weak (n=123)	Strong (n=137)	
Menopausal Status				
Premenopausal	117	52	65	0.403
Postmenopausal	143	71	72	
BMI				
<28	156	64	92	0.013
≥28	104	59	45	
Diabetes				
Yes	51	31	20	0.032
No	209	92	117	
Hypertension				
Yes	77	39	38	0.484
No	183	84	99	
FIGO stage				
I	188	76	112	0.004
II	41	27	14	
III	26	16	10	
IV	5	4	1	
Differentiation				
G1	112	41	71	0.011
G2	94	52	42	
G3	54	30	24	

total of 67 cases; the staining score ≥2.7 was divided into ST2 strong expression group with 83 cases.

In the tumor group, staining score <5.2 was divided into ST2 weak expression group, a total of 108 cases, the staining score ≥5.2 was divided into ST2 strong expression group with 152 cases. We further conducted an intra-group stratification analysis of disease group specimens: G1 group: 3.3 points, G2 group 4.9 points, and G3 group 6.5 points (**Figure 4**).

Combined with the clinical data, we found that the intensity of ST2 staining expression was related to the presence of obesity (P=0.012), diabetes (P=0.013) tumor stage (P< 0.001) and differentiation levels (P=0.017), and the difference was statistically significant (**Table 5**).

In the control group, combining with the case data of the study subjects, the intensity of ST2 staining expression was not significantly associated with whether the patient had menopause, obesity, presence of diabetes, or hypertension.

The correlations between IL-33 and ST2

We further verified the distribution of IL-33 and ST2 staining results in each group, and there was a positive correlation the staining in each group (**Figure 5**).

The relationship between the expression of ST2 and the prognosis of patients

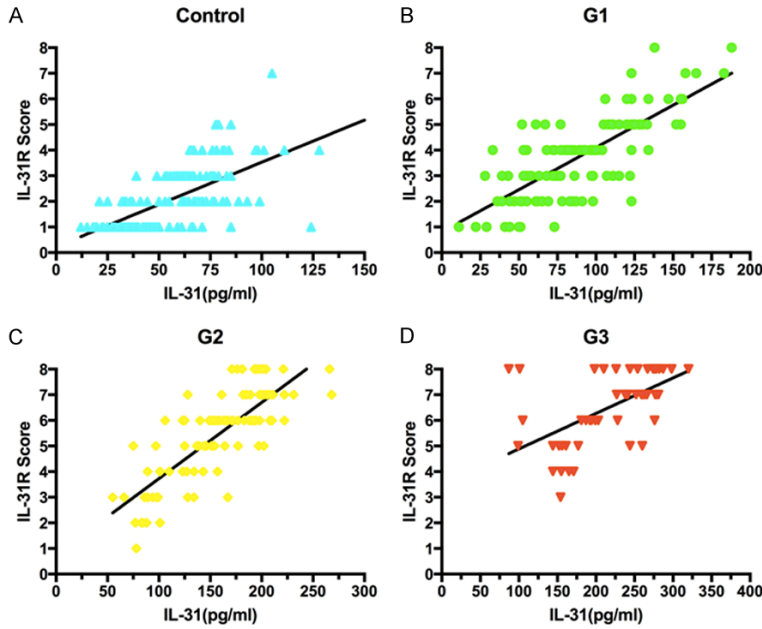Nine patients were lost follow-up in the ST2 strong expres-

Figure 2. Correlation analysis of IL-31 and IL-31R expressions in controls and patients with different differentiation. According to the Shapiro-Wilk test, each group of data is in normal distribution. A. Control group, $R^2=0.47$, $P<0.01$, 95% CI: (0.27, 0.59). B. G1 group, $R^2=0.57$, $P<0.01$, 95% CI: (0.28, 0.48). C. G2 group, $R^2=0.64$, $P<0.01$, 95% CI: (0.75, 0.89). D. G3 group, $R^2=0.3441$, $P<0.01$, 95% CI: (0.38, 0.74).
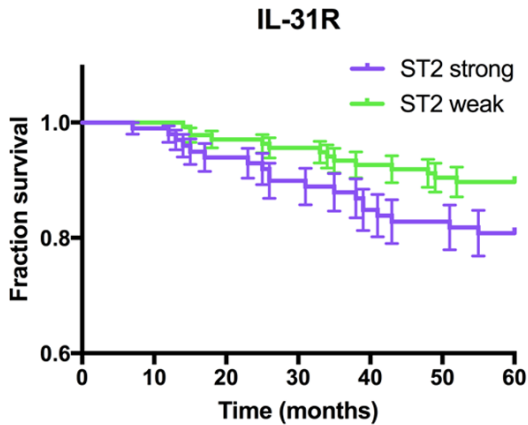

Figure 3. Relationship between IL-31 expression and prognosis in endometrial adenocarcinoma. The difference was statistically significant ($P=0.034*$).

sion group, the average follow-up time of the rest of the 99 patients was 54.3 months, and 18 patients had recurrence. The earliest recurrence time was 7 months and the latest recurrence time was 51 months after operation with the median recurrence time of 28.7 months.

In the ST2 weak expression group, 15 patients were lost, the average follow-up time of the rest

of the 136 patients was 56.9 months, and 15 patients suffered recurrence. The earliest recurrence time was 14 months and the latest time was 55 months. The median recurrence time was 31.9 months postoperative.

After analyzing the free survival (FS) of 235 patients, the difference of FS between ST2 weak and strong expression group was statistically significant (**Figure 6**).

Discussion

Previous literature reports that the occurrence and development of multiple solid tumors are closely related to IL-31/IL-31R and IL-33/ST2 pathways. Low concentration of IL-31/IL-31R can significantly inhibit the proliferation of intestinal epithelial cells. If the cell concentration is increased, IL-31/IL-31R will lose its inhibitory activity and even promote cell proliferation and cell migration. IL-31/IL-31R is highly expressed in patients with follicular lymphoma. Its expression level is closely related to the tumor progression and prognosis [16]. Further studies also claimed IL-31/IL-31R can activate AKT pathway, a classical cellular pathway associated with deep cell proliferation, suggesting that IL-31/IL-31R may have an activity that regulates cell proliferation [14-16].

IL-33 and ST2 were not detected in normal tissues, but high expression in malignant tissues, considering that IL-33 may promote metastasis, invasion and spread of ovarian cancer [19]. Jovanovic found that exogenous IL-33 can promote malignant mammary tumor growth and induce tumor lung and liver metastasis [20, 21]. Similar situations were also found in epithelial squamous cell carcinoma and breast cancer. IL-33 plays an important role in tumorigenesis and progression through its receptor ST2 [22-24]. In gastric cancer, the level of IL-33 expression is closely related to the clinical features such as depth of invasion, tumor stage, and distant metastasis [25, 26]. In lung cancer,
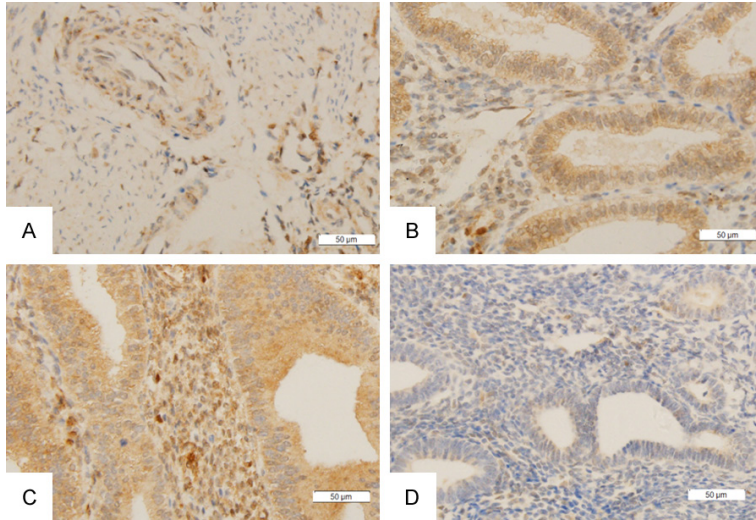
Figure 4. The expression of ST2 in cancer and normal tissues. A. Highly differentiated endometrial cancer. B. Moderately differentiated endometrial cancer. C. Poorly differentiated endometrial cancer. D. Normal endometrial tissue. According to the staining criteria, the average scores of the groups were as follows: 3.3 in the highly differentiated group, 4.9 in the moderately differentiated group, 6.5 in the poorly differentiated group, and 2.1 in the normal group (P=0.009*).

Table 5. Relationship between ST2 expression and clinical features in patient group

Character	Number (n=260)	ST2 Expression		P Value
		Weak (n=108)	Strong (n=152)	
Menopausal Status				
Premenopausal	117	53	64	0.266
Postmenopausal	143	55	88	
BMI				
<28	156	55	101	0.012
≥28	104	53	51	
Diabetes				
Yes	51	29	22	0.013
No	209	79	130	
Hypertension				
Yes	77	36	41	0.268
No	183	72	111	
FIGO stage				
I	188	81	107	<0.001
II	41	19	22	
III	26	16	10	
IV	5	3	2	
Differentiation				
G1	112	47	65	0.017
G2	94	47	47	
G3	54	14	40	

with the disease progresses, the normal vascular endothelium gradually decreases and IL-33 increases [27, 28]. In colorectal cancer, IL-33 can promote the growth and metastasis of colorectal cancer, and play an important role in the recruitment of CD4+ T cells and tumor immune escape [28]. In ovarian cancer, IL-33 has similar effects [19, 29, 30]. In mouse models of breast cancer, the expression level of IL-33 in primary and metastatic gradually increased with time. After artificial knockout of the gene of interleukin receptor, the model mouse primary solid tumor incidence slowed down significantly, delaying the growth of the tumor. The lung and liver metastasis were also significantly inhibited [21]. Thus, activated IL-31R/ST2 may trigger rapid tumor progression by inhibiting the immune function of T lymphocytes and NK cells [31].

Through our research, we found that the expression of IL-31 and its receptors IL-31R, IL-33 and its receptor ST2 were higher in the tumor patients than in the normal group. At the same time, the higher tumor differentiation level, the later the stage, the higher the expression levels of IL-31, IL-33 and their receptors.

Pearson correlation analysis further showed that there was a positive correlation between IL-31/IL-31R and IL-33/ST2 expression: the expression level of interleukin was increased, and the receptor level was also increased accordingly. Inter-group analysis
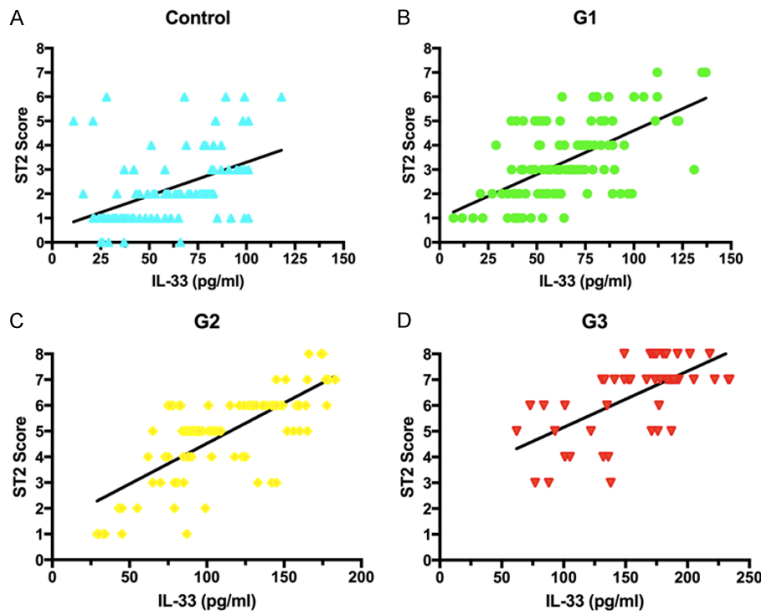
Figure 5. Correlation analysis of IL-33 and ST2 expressions in controls and patients with different differentiations. According to the Shapiro-Wilk test, each group of data is in normal distribution. A. Control group, R^2=0.51, P<0.01, 95% CI: (0.27, 0.59). B. G1 group, R^2=0.36, P<0.01, 95% CI: (0.27, 0.45). C. G2 group, R^2=0.53, P<0.01, 95% CI: (0.25, 0.37). D. G3 group, R^2=0.4, P<0.01, 95% CI: (0.14, 0.29).
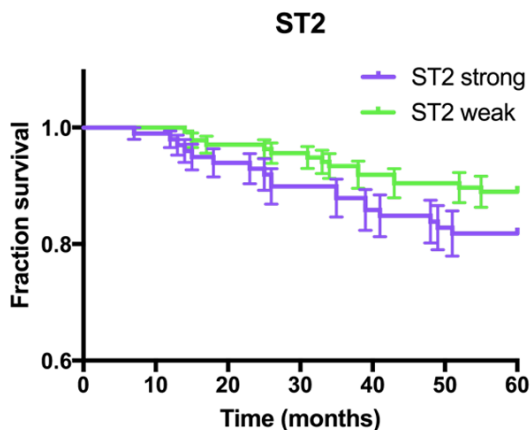

Figure 6. Relationship between ST2 expression and prognosis in endometrial adenocarcinoma. The difference was statistically significant (P=0.041*).

also showed that the expression of IL-31/IL-31R and IL-33/ST2 increased with the increase of cell atypical and tumor differentiation level. The difference was statistically significant, indicating that it was related to the differentiation level.

Survival analysis showed that patients with strong IL-31/IL-31R and IL-33/ST2 expressions had a slightly worse prognosis than those with high expression, which is consistent with the situation reported in the previous literature.

Conclusion

Combining the above experimental results and a systematic review of previous literature, we believe that IL-31/IL-31R and IL-33/ST2 may be involved in the progression of endometrial adenocarcinoma in some way, and with the prognosis of patients. Thus, the potential regulation mechanism of IL-31/IL-31R and IL-33/ST2 in endometrial adenocarcinoma is worthy of further investigation.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Guang-Dong Liao, Department of Gynecology and Obstetrics, The West China Second University Hospital, Sichuan University, Chengdu 610041, China. E-mail: liaoguangdong2018@126.com

References

[1] Morice P, Leary A, Creutzberg C, Abu-Rustum N and Darai E. Endometrial cancer. Lancet 2016; 387: 1094-108.

[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. CA-Cancer J Clin 2019; 69: 7-34.

[3] Zeng X, Zhang Z, Gao QQ, Wang YY, Yu XZ, Zhou B and Xi MR. Clinical significance of serum interleukin-31 and interleukin-33 levels in patients of endometrial cancer: a case control study. Dis Markers 2016; 2016: 9262919.

[4] Gredmark T, Kvint S, Havel G and Mattsson LÅ. Histopathological findings in women with postmenopausal bleeding. BJOG-Int J Obstet Gy 1995; 102: 133-6.

[5] Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E and Vergote I. Endometrial cancer. Lancet 2005; 366: 491-505.

[6] Angioli R, Plotti F, Capriglione S, Montera R, Damiani P, Ricciardi R, Aloisi A, Luvero D, Cafà

EV, Dugo N, Angelucci M and Benedetti-Panici P. The role of novel biomarker HE4 in endometrial cancer: a case control prospective study. Tumour Biol 2013; 34: 571-6.

[7] Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L and Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004; 10: 942-9.

[8] Walsh PT, Buckler JL, Zhang J, Gelman AE, Dalton NM, Taylor DK, Bensinger SJ, Hancock WW and Turka LA. PTEN inhibits IL-2 receptor–mediated expansion of CD4+ CD25+ Tregs. J Clin Invest 2006; 116: 2521-31.

[9] Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR and Katze MG. Into the eye of the cytokine storm. Microbil Mol Biol R 2012; 76: 16-32.

[10] Yoshimoto T, Morishima N, Okumura M, Chiba Y, Xu M and Mizuguchi J. Interleukins and cancer immunotherapy. Immunotherapy 2009; 1: 825-44.

[11] Qian Y, Corum L, Meng Q, Blenis J, Zheng JZ, Shi X, Flynn DC and Jiang BH. PI3K induced actin filament remodeling through Akt and p70S6K1: implication of essential role in cell migration. Am J Physiol Cell Physiol 2004; 286: C153-63.

[12] Wang RC, Wei Y, An Z, Zou Z, Xiao G, Bhagat G, White M, Reichelt J and Levine B. Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science 2012; 338: 956-9.

[13] Xu L and Brink M. mTOR, cardiomyocytes and inflammation in cardiac hypertrophy. Biochim Biophys Acta 2016; 1863: 1894-903.

[14] Koontongkaew S. The tumor microenvironment contribution to development, growth, invasion and metastasis of head and neck squamous cell carcinomas. J Cancer 2013; 4: 66-83.

[15] McKnight NC and Yue Z. Beclin 1, an essential component and master regulator of PI3K-III in health and disease. Curr Pathobiol Rep 2013; 1: 231-8.

[16] Allavena P, Sica A, Solinas G, Porta C and Mantovani A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol 2008; 66: 1-9.

[17] Liotta LA and Kohn EC. The microenvironment of the tumour-host interface. Nature 2001; 411: 375.

[18] Allred DC, Clark GM, Elledge R, Fuqua SA, Brown RW, Chamness GC, Osborne CK and McGuire WL. Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. J Natl Cancer Inst 1993; 85: 200-6.

[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] Jovanovic I, Radosavljevic G, Mitrovic M, Lisnic Juranic V, McKenzie AN, Arsenijevic N, Jonjic S and Lukic ML. ST2 deletion enhances innate and acquired immunity to murine mammary carcinoma. Eur J Immunol 2011; 41: 1902-12.

[21] 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: 1669-82.

[22] Shtilbans V. Role of stromal-epithelial interaction in the formation and development of cancer cells. Cancer Microenviron 2013; 6: 193-202.

[23] Gershon R and Kondo K. Infectious immunological tolerance. Immunol 1971; 21: 903.

[24] Gershon RK and Kondo K. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunol 1970; 18: 723.

[25] Sun P, Ben Q, Tu S, Dong W, Qi X and Wu Y. Serum interleukin-33 levels in patients with gastric cancer. Dig Dis Sci 2011; 56: 3596-601.

[26] Kim MS, Kim E, Heo JS, Bae DJ, Lee JU, Lee TH, Lee HJ, Chang HS, Park JS, Jang AS, Koh ES, Hwang HG, Lim G, Kim S and Park CS. Circulating IL-33 level is associated with the progression of lung cancer. Lung Cancer 2015; 90: 346-51.

[27] Zhang Y, Davis C, Shah S, Hughes D, Ryan JC, Altomare D and Peña MM. IL-33 promotes growth and liver metastasis of colorectal cancer in mice by remodeling the tumor microenvironment and inducing angiogenesis. Mol Carcinog 2017; 56: 272-87.

[28] Kolodin D, van Panhuys N, Li C, Magnuson AM, Cipolletta D, Miller CM, Wagers A, Germain RN, Benoist C and Mathis D. Antigen-and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. Cell Metab 2015; 21: 543-57.

[29] Deng K, Wang H, Shan T, Chen Y, Zhou H, Zhao Q and Xia J. Tristetraprolin inhibits gastric cancer progression through suppression of IL-33. Sci Rep 2016; 6: 24505.

[30] Yu XX, Hu Z, Shen X, Dong LY, Zhou WZ and Hu WH. IL-33 promotes gastric cancer cell invasion and migration via ST2–ERK1/2 pathway. Dig Dis Sci 2015; 60: 1265-72.

[31] Schmieder A, Multhoff G and Radons J. Interleukin-33 acts as a pro-inflammatory cytokine and modulates its receptor gene expression in highly metastatic human pancreatic carcinoma cells. Cytokine 2012; 60: 514-21.