ISSN: 0067-2904

Evaluation of the level of some Interleukins in serum of Iraqi patients with Endometrial Carcinoma

Hajer S. Hameed*[1], Jabbar H. Yenzeel[1], Majeed A. Sabbah[2]
[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[2]Forensic DNA for research and training center

Received: 19/8/2022 Accepted: 25/10/2022 Published: 30/9/2023

Abstract

Endometrial Cancer (EC) is one of the most common malignancy of the female reproductive system. With an increasing incidence, it is important to improve the new prognosis ways for its pre-diagnosis that must be early, accurate and effective. This study aimed to search for biological (like some new interleukins) which could help in early diagnosis of EC before the hysterectomy. Currently not enough research is being done exploiting linking between the interleukins submitted in this study and EC. Epically IL-36 and IL-38, which have been recently described and are still under study in the world. This study is the first of its kind in Iraq. Fifty-five patients with EC (mainly in their first or second stage, due to early diagnosis and who newly have the symptoms and pain as a result of cancer) and 57 healthy controls (with ages up to 45) were involved in this study. To measure the concentration of the following interleukins: IL-27, 31, 33, 35, 36 and 38 by ELISA, blood samples were collected from women via vein puncture. The results of this study showed a highly significant ($P \leq 0.01$) increase in Interleukin 27 (IL-27), Interleukin 31 (IL-31) and Interleukin 33 (IL-33) among all studied interleukins levels in EC patient as compared with healthy controls. Interleukin 35 (IL-35), Interleukin 36 (IL-36) and Interleukin 38 (IL-38) also showed highly significant ($P \leq 0.01$) increase in EC patients as compared with healthy women. The significant increase ($P \leq 0.01$) of these interleukins can be used to help in the early diagnosis and treatment of EC, without need to hysterectomy or histological diagnosis.

KEY WORDS: Endometrial cancer, IL-27, IL-31, IL-33, IL-35, IL-36, IL-38.

تقييم مستوى بعض الانترلوكينات في مصل المرضى العراقيين المصابين بسرطان الرحم

هاجر صباح حميد[1]*, جبار حميد ينزيل[1], مجيد رشيد سباح[2]
[1]قسم علوم الحياة ، كلية العلوم ، جامعة بغداد، بغداد، العراق
[2]مركزالدنا العدلي للبحث والتدريب، جامعة النهرين، بغداد، العراق

الخلاصه:

مرض سرطان بطانة الرحم هو مرض خبيث شائع في الجهاز التناسلي الانثوي, وتشير الاحصائيات الى تزايده بين النساء، هناك حاجة ملحة لتحديد علامات بيولوجية (بعض الانترلوكينات حديثة الوصف عالميا) إضافية للتنبؤ بأمكانية حدوث مرض سرطان بطانة الرحم او التشخيص المبكر له قبل عملية الاستئصال الكلي للرحم. في الوقت الحالي ، لا توجد أبحاث كافية تم إجراؤها لاستغلال الربط بين الإنترلوكينات المقدمة في هذه

*Email: hajar.sabah91@gmail.com

الدراسة مع مرض سرطان بطانة الرحم. خاصة IL−36 و IL−38 ، والتي تم وصفها مؤخرًا ولا تزال قيد الدراسة، وتعد هذه الدراسة الاولى من نوعها في العراق. شارك في هذه الدراسة سبع وخمسون مريضة يعانون من مرض سرطان بطانة الرحم و خمس وخمسون من النساء الأصحاء (مجموعة السيطرة) (معدل اعمارهم اكبر من 45 عامًا) . تم جمع عينات الدم من النساء عن طريق الوريد لتقييم الحركيات الخلوية التالية: انترلوكين IL−27) (27، انترلوكين 31 (IL31) ، انترلوكين 33 (IL−33) ، انترلوكين 35 (IL−35) ، انترلوكين 36 (IL−36) وانترلوكين 38(IL38) في مرضى سرطان بطانه الرحم. اظهرت نتائج الدراسة حدوث زيادة معنوية عالية ($P\leq0.01$) في مستويات انترلوكين 27 و انترلوكين 31 وانترلوكين 33 لدى مريضات سرطان بطانة الرحم عند مقارنتها مع مجموعة السيطرة. كما اظهرت النتائج حدوث زيادة معنوية عالية ($P\leq0.01$) في تراكيز انترلوكين 35، انترلوكين 36 و انترلوكين 38 في مجموعة مريضات سرطان بطانة الرحم عند مقارنتها مع مجموعة السيطرة ، يمكن انيساهم تحري هذه الانترلوكينات في التشخيص المبكر لسرطان بطانة الرحم.

1. Introduction

Cancers start when the body's cells start to grow increasingly out of control. Some cancers are detected at late stage because of the rapid progression of metastasis that led to weakness in diagnosis at early stage [1]. Endometrial cancer after menopause is most common occurrence. Agents like hormones in sex-related cancers are important, e.g., estrogen hormone in EC [2]. EC is the 6th most common cancer in women all over the world. Its mortality is associated directly with the absence of prognostic factors observing or predicting the tumor recurrence. In UK, EC is the 4th commonest cancer in women. And 380,000 new EC cases were reported in 2018 in the USA [3].

EC is a cancer that starts from the endometrium. It is a result of the abnormality endometrial cells growth in which the abnormal endometrial cells can spread and invade other body parts [2]. Because of the vaginal bleeding not being associated with menstrual period, it is often the first sign in most EC cases. Around 10–15% EC in patient women is often detected at an early stage because it frequently has vaginal bleeding in abnormal time [4]. There are also other symptoms that include pain during sexual intercourse, pain with urination, pelvic pain or the uterus may also fill with pus [3]. If EC is discovered early before metastasis, surgically removing the uterus often cures EC. Through histological examinations, the degree of tumor malignancy is determined from the composite histological picture which determines the treatment plan for each patient consequently [5].

Interleukin-12 (IL-12) and Interlukin-6 (IL-6) cytokines family has recently accumulated known members each with various cancers and tumorigenesis specific immune functions related to the interaction between cytokines that lead into developing malignant tumor to enhance immune suppression [6]. Cancer and immune cells within the tumor produce irregular cytokines to the microenvironment (TME). These cytokines can be used as tools for diagnosing many types of cancer [7]. Cytokines are master regulators of inflammatory cells function. Inflammatory cells by cytokines can dampen, recruit or activate immune responses. In addition to these functions, cytokines also have a regulatory function of proliferation and differentiation of the cells [6].

Interleukin 27 (IL-27) is a member of IL-12 cytokines family. Activated antigen-presenting cells (APCs) produce IL- 27. It is also produced by monocytes, dendritic cells and macrophages following stimulation by immune stimuli or microbial products [8]. Both anti-tumor and pro-tumor functions have been recently attributed to IL-27 [6].

IL-31 is a member of the IL-6 cytokines family, mainly secreted by activated CD4+ T helper (Th) cells [4]. A significant upgrade of interlukin-31 can appear in gene expression and protein of epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) [6]. It is believed that IL-31 plays a role in chronic inflammatory diseases like atopic dermatitis (eczema), cancer, asthma, lupus erythematous, chronic urticarial, Bullous pemphigoid, etc. [9]. Interukin-33 is a resent cytokines member in family of IL-1; signal passing through suppression of tumorigenicity 2 (ST2) receptor [10]. Researches that investigate the elevation of IL-33 in serum in various tissues such as spinal cord, stomach, lung, skin and also in cells like endomaterial cells, epithelial cells lining bronchus and smooth muscle cells; serum are still ongoing [11].

Recently, IL-35 has been discovered as anti-inflammatory cytokine from family of IL-12. As an item of IL-12 family, wide range of regulatory lymphocytes produce IL-35 to play a role in immune suppression [12]. Although, regulatory T cells (Treg cells) are mostly cells expressed of IL-35, but recent evidence has unearthed that interlukin-35 has much larger distribution in the tissues [13]. Various studies indicate the presence of IL-35 in trophoblasts of placental, acute myeloid leukemia cells, cells of lung cancer, Hodgkin lymphoma cells, cancer of esophagus, hepatocellular carcinoma, colorectal and cervical cancers [14]. Other studies pointed that IL-35 plays major role in TME. The presence of Tregs in TME is considered as one of the IL-35 production mechanisms [13].

IL-36 is cytokines that is homologus to IL-1. IL-36 expression has now been assessed in multiple types of cancers such as: renal cell carcinoma, lung, colorectal, and ovarian cancer which give IL-36 great advances in cancer immunotherapy [15]. The functional effects of interlukin-36 is in condition of tumorigenesis. Some *vitro* studies have been performed to examine IL-36 role in processes of tumorigenic. Scientists have shown that IL-36 suppresses the proliferation of tumor, invasion and migration in epithelial ovarian cancer in cell lines culture [16].

A novel cytokine of IL-1 family is IL-38. Its expression was reported in skin, salivary glands, placenta, thymus, fetal liver, spleen and other lymph nodes [17]. Although IL-38 role in carcinogenesis or cancer growth is unclear, it is however thought that IL-38 may have an effect on TME or on the immunity of the person because of its function as a negative regulator that is linked to participation in human inflammation and autoimmunity and antagonists of cells receptors [18].

This study is the first of its kind to describe the relationship between the levels of the above-mentioned interleukins and their association with endometrial cancer in Iraq. There are no adequate studies worldwide describing the relationship between IL-36 and IL-38 levels with endometrial cancer as it has been recently discovered and is still under study. The purpose of this study was to search about the ability to use some interleukins as a biological markers which could help in early EC diagnosis before the hysterectomy.

1. Materials and Methods

Subjects: Fifty-five women with EC with range of age between 45- 65 years and 57 blood samples from healthy women of the same age were selected. Samples were taken from participant women from Baghdad hospital of Medical City and Al-Yarmouk hospital during the period from October 2020 to December 2021. Written informal consent was obtained from all participating women and the study was approved by ethical committees: Ref.: CSEC/0122/0061, January 20, 2022, of Department of Biology, College of Science, Baghdad

University. Blood was collected to measure 6 interleukins (IL27, IL31, IL33, IL35, IL 36 and IL38) concentrations in serum of EC and healthy women by using ELISA technique.

Collection of Blood Samples and Procedures: Blood samples were collected from women by puncture of vein using disposable syringes. In gel tube, 5 ml blood was dispensed and then was left to clot for about 40 min at room temperature. Serum was separated by centrifuging the blood samples for 15 min at 3000 rpm. Serum was then stored at -20ºC in Eppendorf tubes until used for immunological assay by ELISA.

Statistical Analysis: Statistical Analysis System- SAS (2012) program was used to measure the effects of different factors in study parameters between EC patients and controls. T-test was used for significant comparison between means.

2. Results

Table 1 shows a highly significant (P≤0.01) increase in IL-27, 31 and 33 concentrations in women with EC (315.38 ±19.94 pg/ml, 298.21 ± 17.21 pg/ml and 300.26 ± 14.80 pg/ml respectively) as compared with healthy controls (26.01 ± 4.56 pg/ml, 26.42 ± 3.68 pg/ml and 28.44 ± 4.95 pg/ml respectively). Also in Table 1, the results show a significantly high (P≤0.01) increase in IL- 35, 36 and 38 concentrations in women with EC (1046.53 ± 53.52 pg/ml, 309.98 ± 21.31 pg/ml and 37.79 ± 1.77 pg/ml respectively) as compared with healthy controls (102.48 ± 16.29 pg/ml, 24.53 ± 4.06 pg/ml and 11.62 ± 5.66 pg/ml respectively).

Table 1: Interleukins concentration in Endometrial cancer patients and control

Group	Mean ± SE					
	IL-27 (pg/ml)	IL-31 (pg/ml)	IL-33 (pg/ml)	IL-35 (pg/ml)	IL-36 (pg/ml)	IL-38 (pg/ml)
Patients	315.38 ±19.94	298.21 ±17.21	300.26 ±14.80	1046.53 ±53.52	309.98 ±21.31	37.79 ±1.77
Control	26.01 ±4.56	26.42 ±3.68	28.44 ±4.95	102.48 ±16.29	24.53 ±4.06	11.62 ±5.66
T-test	39.25 **	33.75 **	30.06 **	107.63 **	41.59 **	12.14 **
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
** (P≤0.01).						

Discussion

The results show increased concentrations of some interleukins which may be due to inflammatory and immune response against malignancy cells. IL-27 increase in EC in the current study agree with the results of another study [19]. The results also agree with Chang *et al*. [20] who reported a positive correlation between differentiated stages of EC and IL-27 concentration. Much research has found that the progression of endometriosis cancer is promoted by inducing the differentiation of IL-10 and Th17 cells. All these activities are due to IL-27 secreted by macrophages and endometrial stromal cells [21]. IL-27 can use a multitude of approaches such as: antitumor immune responses activation and direct inhibition of proliferation of tumor cell, survival, angiogenesis and metastatic properties [22]. There is a linkage between IL-27 and its receptor and the activation of signaling pathways including p38 MAPK and JAK-STAT pathways [8]. Two kinds of responses involve different types of cells, pro-inflammatory and anti-inflammatory. These cells include macrophages, dendritic cells, T and B cells, and other inflammatory cells [21]. Surface expression of major histocompatibility complex I (MHCI) and major histocompatibility complex II (MHCII) in monocytes also can be enhanced by IL-27 which is co-stimulatory of CD80 and CD86, activation of adhesion molecule CD54 and activity T cells to promote the pro-inflammatory functions [7]. It is related to the

inducement of tumor specific Th1 and cytotoxic T lymphocyte (CTL) responses, as well as, to the direct inhibitory impacts on proliferation, invasion, and angiogenic potential of tumor cells [23]. IL-27 may also have an indirect antitumor role, with its direct inhibitory activity on tumor cells by targeting the TME [6].

Increased concentration of IL-31 in the current study agrees with other studies[4], [11]. IL-31 and many other interleukins participate in EC cell growth and metastasis in epithelial mesenchymal transition (EMT) or is connected with cancer diseases development [3]. Moreover, some studies have noted that IL-31 may play an important role in diseases related with cancer, including human malignant lymphomas of T-cell lineage, lung cancer, human follicular lymphoma and EC [24]. Low concentration of IL-31/IL-31R can significantly inhibit the proliferation of intestinal epithelial cells [25]. If the cell concentration is increased, IL-31/IL-31R will lose its inhibitory activity and even promote cell proliferation and cell migration [24]. Its expression concentration is related closely to the tumor prognosis, progression and development [4]. Further studies have also claimed that IL-31/IL-31R can activate pathway of AKT that leads to suggest that IL-31/IL-31R may have an activity of regulating cell proliferation [26]. IL-31 concentration increase has been detected in tissues or serum in patients with several kinds of cancers and/or many chronic diseases [11]. Recently, a lot of research suggest that there may be a close relationship between IL-31 and pathogenesis of tumors [23].

Increasing the concentration of IL-33 were significantly accumulated with the progression of EC which agrees with other studies [4], [27]. Some interleukins like IL-33 help in EC cells growth and metastasis or EMT in the advanced cancer [3]. TME played an important role in invasion and spread of cancer and is constituted of cancer-associated fibroblasts (CAFs) and other of stromal cells diseases groups, including endothelial and inflammatory cells [10]. Biological processes tumor regulated by CAFs contribute to progression of cancer by different ways, such as remodeling affectivity of ECM [27]. Exosomes secreted by CAFs can also transport mediators that are involved in cell-cell communication in a paracrine style, including non-coding RNA molecules or some inflammatory mediators like TNF-α, IL-1β and IL-33. High concentration of IL-33 IL-1β and IL-18, in contrast, contributed to mediation of anti-tumorigenic effects by the activation responses of immunity against tumors [28]. Interleukin-33 is a cytokine with a dual function that also acts as a nuclear factor; typically, IL-33 is expressed in the cell's nucleus and resides there, there were correlation between clinical characteristics and these expressions, including occurrence, differentiation, development, stage of multiple solid tumors and patients' survival [10]. However, upon tissue necrosis, damage or injury; IL-33 is released rapidly into extracellular space to bind to its cognate suppression receptor of tumorigenicity 2 (ST2 or also named as IL-1RL1), which is found on the target cells membrane and potently activate Th2 immune response [29]. In normal tissues, IL-33 and ST2 were not detected, while in malignant tissues, a high expression IL-33 and ST2 was found; which reveals that IL-33 may promote invasion, metastasis and spread of ovarian cancer [27]. Researchers have found that exogenous IL-33 can promote malignant mammary tumor growth of EC and metastasis and plays an important role in CD4+ T cells recruitment and escape of tumor immunity [30]. In EC, IL-33 has effects; thus, activated IL-31R/ST2 may trigger rapid tumor progression by inhibiting the immune role of T lymphocytes and NK cells [4]. IL-33 acts as a cytokine extracellularly and as a nuclear factor intracellularly [26]. Ali *et al*. [33] suggested that nuclear NF-κB is locked by nuclear IL-33, the gene expression is triggered by reduction of the transcription factor NF-κB. All these events lead to dampen proinflammatory signaling. Multiple aspects are regulated by NF-κB on adaptive and innate immune functions and serves as inflammatory responses of pivotal mediators [31]. Furthermore, NF-κB takes on a critical role in regulating the activation, survival and differentiation of inflammatory T cells and cells

of innate immunity [32]. Expression of a large number of genes is regulated by NF-κB, which carry important functions in apoptosis, necrosis, inflammation, proliferation, and angiogenesis [33].

The results of IL-35 increase agree with those by Mirlekar and Pylayeva-Gupta [7]. Also, Xue *et al*. [14] who highlighted function of cytokines like IL-27, 30, and 35 in the TME by examining their indirect activity during regulatory mechanics of immune cells that behave as either inhibit or instigate progression of tumor, as well their direct effects on cells of cancer itself. First evidence of involvement of IL-35 in the pathogenesis of endometriosis was provided by study of Zhang *et al*. [34] through suppressing immunoreaction and promoting proliferation of Endometrial Stromal Cells (ESCs); thus, IL-35 may serve as a potential biomarker for endometriosis. Similar to the expression of IL-27, IL-35 can immediately impact the survival of cancer cells via affecting tumor cells and due to its non-covalent heterodimeric nature, it is important to consider how IL-35 is delivered in the model system [12]. Nicholl *et al*. [35] spotted that IL-35 recombination enhancement of survival, proliferation and spread of the pancreas adenocarcinoma cell line in a dose-dependent manner. Also, IL-35 identical to IL-27 in the TME influenced of immune responses [12]. In both immune deficient and competent mice, tumor cells expression of IL-35 engineered to exhibit increased accumulation of myeloid cell in the TME, which resulting an increased angiogenesis of tumor cells [6]. This indicates the involvement of tumor-produced of IL-35 is going to promote survival of tumor [14]. As well, immune competent mice missed unprompted responses of CTL to tumors via promoting favoring tumor conditions [13]. Although IL-27 and IL-35 share subunits, these cytokines have indirect and direct roles on the tumor resulting in either tumor elimination or progression [12]. IL-35 has been embroiled in promoting advancement of tumor by increasing cancer cell proliferation, angiogenesis, metastasis and T cell exhaustion, immune suppression; in constant, IL-35 may have anti-tumor effects attributed to its potential impacts in decreasing migration and invasion cancer cells [6].

IL-36 increased concentration in this study agrees with Chelvanambi *et al*. who remarked that the interleukin-36 families of pro-inflammatory cytokines play essential roles in multiple diseases, attempting with instigator psoriasis of autoimmune diseases to an initiator of therapeutic immune responses against tumor cells [36]. So that, inflammation is now recognized as a signature of cancer, it is not unexpected that IL-36 is presently being researched in more depth in its implication in many kinds of cancer [15]. In the context of tumorigenesis, the functional effect of IL-36 in *vitro* studies was carried out to examine the job of IL-36 in tumorigenic processes. IL-36 has shown to suppress proliferation and migration process of tumor in epithelial ovarian cancer cell lines [37]. Also, it seems that the pro-inflammatory implications of IL-36 and related cytokines may be essential for anti-tumor immunity response [15]. Interestingly, there has been observed positively correlation between Interlukin-36α expression and overall survival for patients, tumor size, differentiation degree and growth of tumor [38]. Thus, IL-36α could have tumor-suppressive effects [15]. IL-36γ may also have anti-tumor activity which is similar to IL-36α effects [16]. The role of Interlukin-36γ in cancer research began in breast cancer and melanoma where the expression of IL-36γ was inversely correlated with the disease progression [15]. IL-36γ was found within the TME in various types of cells such as: macrophages, monocytes, tumor cells and vasculature cells including smooth muscle cells and high endothelial venules, which are connected to upgrade the structures of tertiary lymph nodes [37]. In particular, expression of interlukin-36γ by macrophages is associated with inflammation signs, such as: fibrosis, T cell infiltration and increased density of B cells in tertiary lymphoid structures; also, IL-36 might change in the TME and promote the differentiation of type 1 effector lymphocytes to a crucial aspect of the reaction of anti-tumor immune [38]. Expression of IL-36γ is found to correlate with the number of lymphocytes

infiltrating in tumor such as CD8+ and NK cells; the adaptive tumor antigen-specific CD8+ T cell immune response is enhanced by IL-36γ; which further corroborates its activity as anti-tumor cytokines [15].

IL-38 increases in EC patients. It is possible that apoptotic cells is releasing of IL-38. In this case, IL-38 will have an antagonistic activity on induction of inflammatory cytokine [40]. IL-38 is considered to maintain the homeostasis of the microenvironment in these tissues by suppressing inflammatory responses [41]. The anti-inflammatory effect of IL-38 includes the release of IL-38 from apoptotic cells to limit inflammatory responses of macrophage [39]. Still only a small number of studies has looked into the function of IL-38 in cancer. IL-38 might seriously impact the cancer cells themselves and directly affect their growth [41]. As an example, homeostatic of IL-38 expression might be assisting in controlling the expansion of epithelial cells of colon, as recommended by in *vitro* anti-proliferative and pro-apoptotic roles of IL-38 in human colorectal cancer cells [17]. However, IL-38 may act in the TME to modulate the statement of inflammation in the tumor and the anti-tumoral immune response; appears to be detrimental roles for the patients with cancer, due to its roles as immunosuppressive [39]. Limited research suggested that direct inhibitory activity of IL-38 on proliferation of tumor cell might assist in its anti-tumoral potential in several kinds of malignant tumor, while modulation of immune responses in the TME might confer pro-tumoral properties to this cytokine [18]. Further studies may help to explain a pattern linking anti- or pro-tumoral functions of expression IL-38 in producing cell types, organs or tissues in inflammatory background and/or the tumors immunogenicity [17].

Conclusion

It can be concluded that the increase in the level of some interleukins like IL-27, 31, 33, 35, 36 and 38 could be used in the early diagnosis for EC and in accurate prognostic diagnosis.

Acknowledgements: This study was supported by the Department of Biology, College of Sciences, University of Baghdad. Many thanks to all participant patients and control women. A great thanks to Baghdad hospital of Medical City and Al-Yarmouk hospital including doctors and nurses who were supportive in accomplishing this study.
Conflict of interest: The author declares no conflicts of interest.

References

[1] B.J. Mohamad, F. A. Zghair, and Z.T. Fadhil, "Clinical and Histopathological Features of Ovarian Cancer in Iraq, Baghdad Between 2014-2020", *eijs*, vol. 63, no. 6, pp. 2354–2361, Jun. 2022.

[2] E. Coll-de la Rubia, E. Martinez-Garcia, G.Dittmar, A. Gil-Moreno, S. Cabrera, and E. Colas, "Prognostic Biomarkers in Endometrial Cancer: A Systematic Review and Meta-Analysis" , *J. Clin. Med*, vol. 9, no. 6, pp. 1900, Jun. 2020.

[3] I. Ray, L.B. Meira, A. Michael, and P. E. Ellis, "Adipocytokines and disease progression in endometrial cancer: a systematic review" *Cancer Metastasis Rev*, vol. 41, no.1, pp. 211-242, Mar. 2022.

[4] X. Zeng, J. Li, L.N. Kang, M.R. Xi, and G.D. Liao, "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" *Int J Clin Exp Pathol.* Vol. 13, no. 6, pp. 1324–1332. Jun. 2020.

[5] J. Tarannum, P. Manaswini, C. Deekshitha, B.P. Reddy, and A.S. Sunder, "Elucidative Histopathological Study in Female Cancer Patients: Histopathology in Female cancers", *eijs*, vol. 61, no. 4, pp. 720–726, Apr. 2020.

[6] O. Kourko, K. Seaver, N. Odoardi, S. Basta, and K. Gee, "IL-27, IL-30, and IL-35: A Cytokine Triumvirate in Cancer" *Front Oncol*. vol. 9, no. 969. Oct. 2019.

[7] B. Mirlekar and Y. Pylayeva-Gupta, "IL-12 Family Cytokines in Cancer and Immunotherapy" *Cancers.* vol. 13, no. 2, pp. 167. Jan. 2021.

[8] R.R. Meka, S. H. Venkatesha, S. Dudics, B. Acharya, and K. D. Moudgil, "IL-27-induced modulation of autoimmunity and its therapeutic potential" *Autoimmun Rev*. vol. 14, no. 12, pp. 1131-1141. Dec. 2015.

[9] J. M. Nemmer, M. Kuchner, A. Datsi, P. Oláh, V. Julia, U. Raap, and B. Homey, "Interleukin-31 Signaling Bridges the Gap Between Immune Cells, the Nervous System and Epithelial Tissues". *Front. Med.* vol. 8, 639097. Feb. 2021.

[10] K.M. Larsen, M.K. Minaya, V. Vaish, and M. Peña, "The Role of IL-33/ST2 Pathway in Tumorigenesis" *Int J Mol Sci*. vol. 19, no. 9, pp. 2676. Sep. 2018.

[11] X. Zeng, Z. Zhang, Q.Q. Gao, Y.Y. Wang, X. Z. Yu, B. Zhou, and M. R. Xi, "Clinical Significance of Serum Interleukin-31 and Interleukin-33 Levels in Patients of Endometrial Cancer: A Case Control Study" *Dis Markers*. vol. 2016. .9262919. May. 2016.

[12] K. Liu, A. Huang, J. Nie, J. Tan, S. Xing, Y. Qu, and K. Jiang, "IL-35 Regulates the Function of Immune Cells in Tumor Microenvironment". *Front. Immunol*. vol. 12, 683332. May. 2021.

[13] C. Ye, H. Yano, C. J. Workman, and D. Vignali, "Interleukin-35: Structure, Function and Its Impact on Immune-Related Diseases" *J Interferon Cytokine Res.* vol. 41, no. 11, pp. 391-406. Nov. 2021.

[14] W. Xue, D. Yan, and Q. Kan, "Interleukin-35 as an Emerging Player in Tumor Microenvironment" *J Cancer*. vol. 10, no.9, pp. 204-2082. May. 2019.

[15] M. Yang, E. Giehl, C. Feng, M. Feist, H. Chen, E. Dai, Z. Liu, C. Ma, R. Ravindranathan, D. L. Bartlett, B. Lu, and Z.S. Guo, "IL-36γ-armed oncolytic virus exerts superior efficacy through induction of potent adaptive antitumor immunity" *Cancer Immunol Immunother*. vol. 70, no. 9, pp. 2467-2481. Sep. 2021.

[16] L. Chang, R. Guo, and Z. Yuan, "IL-36α suppresses proliferation of ovarian cancer cells" *Tumour Biol.* vol. 39, no. 6, 1010428317706918. Jun. 2017.

[17] A. Esmaeilzadeh, N. Bahmaie, E. Nouri, M. J. Hajkazemi, and M. Zareh Rafie, "Immunobiological Properties and Clinical Applications of Interleukin-38 for Immune- Mediated Disorders: A Systematic Review Study" *Int J Mol Sci.* vol. 22, no. 22, pp. 12552. Nov. 2021.

[18] K. Takada, T. Okamoto, M. Tominaga, K. Teraishi, T. Akamine, S. Takamori, M. Katsura, G. Toyokawa, F. Shoji, M. Okamoto, Y. Oda, T. Hoshino, and Y. Maehara, "Clinical implications of the novel cytokine IL-38 expressed in lung adenocarcinoma: Possible association with PD-L1 expression" *PloS one.* vol. 12, no. 7, e0181598. Jul 2017.

[19] G. Carbotti, G. Barisione, I. Airoldi, D. Mezzanzanica, M. Bagnoli, S. Ferrero, A. Petretto, M. Fabbi, and S. Ferrini, "IL-27 induces the expression of IDO and PD-L1 in human cancer cells" *Oncotarget.* vol. 6, no. 41, pp. 43267–43280. Dec. 2015.

[20] K. K. Chang, L. B. Liu, L. P. Jin, B. Zhang, J. Mei, H. Li, C. Y. Wei, W. J. Zhou, X. Y. Zhu, J. Shao, D. J. Li, and M.Q. Li, "IL-27 triggers IL-10 production in Th17 cells via a c-Maf/RORγt/Blimp-1 signal to promote the progression of endometriosis" *Cell Death Dis.*, vol. 8, no. 3, e2666. Mar. 2017.

[21] M. S. Li, Z. Liu, J. Q. Liu, X. Zhu, Z. Liu, and X.F. Bai, "The Yin and Yang aspects of IL-27 in induction of cancer-specific T-cell responses and immunotherapy" *Immunotherapy*., vol. 7, no. 2, pp. 191–200. 2015.

[22] S. Dhaiban, Al-Ani, M.; N.M. Elemam, M.H. Al-Aawad, Z. Al-Rawi, and A.A. Maghazachi, "Role of Peripheral Immune Cells in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis". *Sci*, vol. 3, no. 12. 2021.

[23] M. Fabbi, G. Carbotti, and S. Ferrini, "Dual Roles of IL-27 in Cancer Biology and Immunotherapy" *Mediators Inflamm*., vol. 2017, 3958069. 2017.

[24] E. Ferretti, A. Corcione, and V. Pistoia, "The IL-31/IL-31 receptor axis: general features and role in tumor microenvironment" *J Leukoc Biol*., vol. 102, no. 3, pp. 711-717. Sep. 2017.

[25] Z. Lan, Y. Wang, X. Yu, H. Song, Q. Li, D. You, M. Yuan, X. Zeng, B. Zhou, Y. Song, M. Su, Y. Zhang, L. Zhang, and M. Xi, "Interleukin-31 single nucleotide polymorphisms are significantly associated with endometrial cancer in Chinese Han women" *Int J Clin Exp Pathol. vol.*, 11, no. 2, pp. 894-903. Feb. 2018.

[26] S. Koontongkaew, "The tumor microenvironment contribution to development, growth, invasion and metastasis of head and neck squamous cell carcinomas" *J Cancer.*, vol .4, no., pp.66-83. Jan. 2013.

[27] P. Santulli, M. Even, S. Chouzenoux, A. E. Millischer, B. Borghese, D. de Ziegler, F. Batteux, and C. Chapron, "Profibrotic interleukin-33 is correlated with uterine leiomyoma tumour burden" *Hum Reprod.*, vol. 28. no. 8, pp. 2126–2133. Aug. 2013.

[28] E. Sahai, I. Astsaturov, E. Cukierman, D. G. DeNardo, M. Egeblad, R.M. Evans, D. Fearon, F.R. Greten, S.R. Hingorani, T. Hunter, R. O. Hynes, R. K. Jain, T. Janowitz, C. Jorgensen, A.C. Kimmelman, M.G. Kolonin, R.G. Maki, R.S. Powers, D.C. Puré, E., Ramirez, R. Scherz-Shouval, M.H. Sherman, S. Stewart, T.D. Tlsty, D.A. Tuveson, F.M. Watt, V. Weaver, A.T. Weeraratna, and Z. Werb, "A framework for advancing our understanding of cancer-associated fibroblasts" *Nat Rev Cancer.*, vol.20, no.3, pp. 174-186. Mar. 2020.

[29] C. Feng, L. Kou, P. Yin, and Y. Jing, "Excessive activation of IL-33/ST2 in cancer-associated fibroblasts promotes invasion and metastasis in ovarian cancer" *Oncol Lett.*, vol. 23, no. 5, pp. 158. May. 2022

[30] X. Tong, M. Barbour, K. Hou, C. Gao, S. Cao, J. Zheng, Y. Zhao, R. Mu, and H.R. Jiang, "Interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways" *Mol Oncol.*, vol.10, no. 1, pp. 113–125. Jan. 2016.

[31] S. Ali, A. Mohs, M. Thomas, J. Klare, R. Ross, M. L. Schmitz, and M. U. Martin, "The dual function cytokine IL-33 interacts with the transcription factor NF-κB to dampen NF-κB-stimulated gene transcription" *J Immunol.*, vol. 187, no. 4, pp. 1609-16. Aug 2011

[32] T. Liu, L. Zhang, D. Joo, and S.C. Sun, "NF-κB signaling in inflammation" *Signal Transduct Target Ther.*, *vol.* 2: 17023. Jul. 2017.

[33] M.H. Park, and J.T. Hong, "Roles of NF-κB in Cancer and Inflammatory Diseases and Their Therapeutic Approaches" *Cells.*, vol. 5, no. 2, pp.15. Mar. 2016.

[34] C. Zhang, Z. Peng, D. Ban, and Y. Zhang, "Upregulation of Interleukin 35 in Patients With Endometriosis Stimulates Cell Proliferation" *Reprod Sci.*, vol. 25, no. 3, pp. 443-451. Mar. 2018.

[35] M.B. Nicholl, C.L. Ledgewood, X. Chen, Q. Bai, C. Qin, K.M. Cook, E.J. Herrick, A. Diaz-Arias, B.J. Moore, and Y. Fang, "IL-35 promotes pancreas cancer growth through enhancement of proliferation and inhibition of apoptosis: evidence for a role as an autocrine growth factor" *Cytokine.*, vol. 70, no. 2, *pp.* 126–133. Dec. 2014.

[36] M. Chelvanambi, A.M. Weinstein, and W.J. Storkus, "IL-36 Signaling in the Tumor Microenvironment" *Adv Exp Med Biol.*, vol. 1240, pp. 95–110. 2020.

[37] D. Queen, C. Ediriweera, and L. Liu, "Function and Regulation of IL-36 Signaling in Inflammatory Diseases and Cancer Development" *Front Cell Dev Biol.*, vol. 7, pp. 317. Dec. 2019.

[38] X. Wang, X. Zhao, C. Feng, A. Weinstein, R. Xia, W. Wen, Q. Lv, S. Zuo, P. Tang, X. Yang, X. Chen, H. Wang, S. Zang, L. Stollings, T. L. Denning, J. Jiang, J. Fan, G. Zhang, X. Zhang, Y. Zhu, W. Storkus, and B. Lu, "IL-36γ Transforms the Tumor Microenvironment and Promotes Type 1 Lymphocyte-Mediated Antitumor Immune Responses" *Cancer Cell.*, vol. 28, no. 3, pp. 296–306. Sep. 2015.

[39] J. Mora, A. Schlemmer, I. Wittig, F. Richter, M. Putyrski, A.C. Frank, Y. Han, M. Jung, A. Ernst, A. Weigert, and B. Brüne, "Interleukin-38 is released from apoptotic cells to limit inflammatory macrophage response" *J Mol Cell Biol.*, vol. 8, no. 5, pp. 426–438. Oct. 2016.

[40] J. Dang, Z. He, X. Cui, J. Fan, D. J. Hambly, B. D. Hambly, X. Li, and S. Bao, "The Role of IL-37 and IL-38 in Colorectal Cancer" *Front Med.*, vol. 9, 811025. Feb. 2022.

[41] A. Diaz-Barreiro, A. Huard, and G. Palmer, "Multifaceted roles of IL-38 in inflammation and cancer" *Cytokine.*, vol. 151. 155808. 2022.