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IL-29 Promotes CXCL10 Production in Human Ovarian Cancer Cells

You Li, Wanyong Qiu and Min He*

Department of Gynaecology and Obstetrics, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China

***Corresponding author:**

Min He,
Department of Gynaecology and Obstetrics, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China

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1. Abstract

1.1. Background

Ovarian cancer is one of the most common malignant tumors in the female reproductive system. CXC-motif chemokine ligand 10 (CXCL10) increases tumor infiltrating lymphocytes (TIL) infiltration and is closely related to ovarian cancer prognosis. Interleukin-29 (IL-29) is a recently discovered cytokine in the type III interferon family. Extensive progress has been made in studying IL-29 in the immune system, including inducing CXCL10 secretion in some epithelial cells. However, the interaction between IL-29 and CXCL10 in ovarian cancer is unclear.

1.2. Material and Methods

We used qPCR and Western blotting to measure IL-29 receptor expression. We measured CXCL10 production in recombinant human IL-29-stimulated OVCAR3 cells in the presence or absence of tumor necrosis factor-α (TNF-α, 20 ng/ml) by qPCR and ELISA. We investigated signal transduction involved in IL-29-induced CXCL10 production by using inhibitors and Western blotting in OVCAR3 cells.

1.3. Results

OVCAR3 cells expressed mRNA and protein for the IL-29 receptors interferon lambda receptor 1 (IFNLR1) and IL-10 receptor β (IL10Rβ). IL-29 induced CXCL10 production in OVCAR3 cells, and IL-29 (100ng/ml) significantly increased CXCL10 mRNA and protein production in synchronously stimulated with TNF-α. We found that the signal transducer and activator of transcription 1 (STAT1), P38 mitogen-activated protein kinase (MAPK), and Akt pathways are involved in IL-29-induced CXCL10 production, and the phosphorylation of these pathways is increased in IL-29- and TNF-α-stimulated OVCAR3 cells.

1.4. Conclusion

IL-29 induced CXCL10 production in ovarian cancer cells and significantly promoted CXCL10 production in TNF-α-stimulated OVCAR3 cells through the STAT1, P38 MAPK, and Akt pathways.

2. Introduction

Ovarian cancer is one of the most common malignant tumors of the female reproductive system, ranking third in incidence and second in mortality among malignant gynecological tumors [1]. High-grade serous ovarian cancer (HGSC) is the most prevalent type of ovarian cancer. In addition, due to hidden onset and a lack of screening and effective early diagnosis methods, approximately 70% of patients with ovarian cancer are already in an advanced stage when they start to seek treatment and are characterized by poor prognosis, metastasis and resistance to chemotherapy [2]. Therefore, ovarian cancer treatment is a worldwide problem. With the development of tumor immunology theory and technology, immunotherapy has begun to be used in ovarian cancer. However, the infiltration of immune cells into the tumor is difficult because ovarian cancer is solid and the tumor immune microenvironment (TME) is often immunosuppressive. Cytokines and chemokines are crucial parts of the TME. Among them, CXC-motif chemokine ligand 10 (CXCL10) is a potent chemoattractant of activated T cells, natural killer (NK) cells, and eosinophils through its receptor C-X-C motif chemokine receptor 3 (CXCR3) [3], and plays a role in tumor infiltrating lymphocyte (TIL) recruitment to the TME in multiple cancers, including ovarian, breast and colorectal cancer [4-7]. CXCL10 can be produced by both cancer cells and immune cells in the TME [8]. In addition to recruiting

TILs, CXCL10 is also an angiostatic chemokine that prevents the formation of blood vessels during tumor growth [9]. The most common type of ovarian cancer, HGSC, can be divided into four molecular subtypes: mesenchymal, immunoreactive, differentiated, and proliferative [10,11]. Among these four subtypes of HGSC, the immunoreactive subtype is characterized by increased expression of CXCL10 and CXCL11 and increased infiltration of TILs [12-14]. The immunoreactive subtype also shows the longest overall survival [15]. In conclusion, CXCL10 is closely related to the prognosis of ovarian cancer, and factors affecting the secretion of CXCL10 may play crucial roles in the prognosis of ovarian cancer [16]. Interleukin-29 (IL-29) is a new member of the Type III interferon (IFN) family, which was recently discovered in 2003 and is mainly produced by maturing dendritic cells and macrophages [17]. IL-29, IL-28A and IL-28B are Type III IFNs. Recently, of the role of IL-29 in immunological processes and the pathogenesis of various cancers have been extensively studied [18,19]. IL-29 primarily targets epithelial cells, and high IL-29 levels are therefore present during infections of the gastrointestinal tract, respiratory tract, and mucosal regions [20-24]. Studies have shown that IL-29 can promote the secretion of CXCL10 by periodontal tissue cells, thus participating in the occurrence of periodontal diseases [25]. However, the target cell population of IL-29 is more limited than that of type I IFNs, and so it is not clear whether IL-29 can target ovarian cancer cells. A large number of studies have demonstrated that tumor necrosis factor-α (TNF-α) and IFN-γ can promote the secretion of CXCL10 in cancer cells [26-28], but whether IL-29 can promote CXCL10 secretion in cancer cells such as ovarian cancer has not been reported.The purpose of this study was to investigate the effect of IL-29 on CXCL10 production in ovarian cancer cells. In addition, we investigated the signal transduction pathways involved in the production of CXCL10 in IL-29-stimulated ovarian cancer cells.

3. Materials and Methods

3.1. Cell Culture and Treatment

Human ovarian cell lines (A2780, ES-2, OVCAR3, and SK-OV-3) were purchased from the National Infrastructure of Cell Line Resource (Beijing, China). The IOSE-80 cell line was kindly donated by Dr. Liangdan Tang (First Affiliated Hospital of Chongqing Medical University, Chongqing, China). All cells were maintained in RPMI-1640 medium (BI, Bet, Israel) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT, USA) at 37 °C with 5% CO2.To measure the induction of CXCL10 expression, ovarian cell lines were incubated with recombinant human IL-29 (Sino Biological, Beijing, China), TNF-α (Peprotech, London, UK) or both. After 6 h of culture, the cells were harvested, and mRNA expression was analyzed by quantitative real-time polymerase chain reaction (qPCR). After 24 h of culture, the cells were harvested, and protein expression was analyzed by Western blotting, and the supernatants were collected and analyzed by enzyme-linked immunosorbent assay (ELISA).

3.2. Real-time Quantitative PCR(qPCR)

Total RNA was extracted from ovarian cells and reverse transcribed into cDNA using a reverse transcription system (Takara, Shiga, Japan). qPCR was performed to measure gene expression at the mRNA level. qPCR was carried out on a CFX Maestro Real-time PCR System (Bio-Rad, USA) using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) according to the manufacturer's instructions. All samples were normalized to GAPDH. The primers used for qPCR are listed in Table 1, and they were all designed by the Prime-Blast tool in NCBI. qPCR analysis of target molecules was performed as described previously(Schmittgen and Livak,2008).

Tabel 1: Primer sequences.

Gene ID	Protein alias	Forward primers(5'-3' sequence)	Reverse primers(5'-3' sequence)
IFNLR1	IL28RA	AGAATGTGACGCTGCTCTCC	TAGGTCACATCCTGGGGGTT
IL-10Rβ	IL-10RB	ACAACCCATGACGAAACGGT	GGGGAGAAGGCGTACTTTGT
CXCL10	CXCL10	AGCAAGGAAAGGTCTAAAAGATCTCC	GGCTTGACATATACTCCATGTAGGG

3.2. Enzyme-linked Immunosorbent Assay(ELISA)

After OVCAR3 and SK-OV-3 cells were stimulated with recombinant human IL-29 (1, 5, 10, 20, 50 or 100 ng/ml; Peprotech, London, UK) with or without TNF-α (10 ng/ml: Peprotech, London, UK) for 24 h, the supernatants were collected, and the concentrations of CXCL10 were quantitated by ELISA kits (Boster, Wuhan, China) in triplicate. All assays were performed according to the manufacturer's instructions, and the CXCL10 level was determined using the standard curve prepared for each assay.

3.3. Western Blot Analysis

The cell lysates of each sample were separated by SDS-PAGE and transferred onto a 0.2 μm PVDF membrane. The membrane was probed with primary antibodies overnight and secondary antibodies conjugated with HRP at room temperature for 1 h and then developed using an enhanced chemiluminescence system (Beyotime Biotechnology, Shanghai, China) to visualize the bands according to the manufacturer's recommended protocol. The primary antibodies included an anti-phospho-signal transducer and activator of transcription 1 (STAT1) rabbit monoclonal antibody (Cell Signaling Technology, catalog number 9167, 1:2000), anti-phospho-p38 mitogen-activated protein kinase (p38 MAPK) rabbit monoclonal antibody (Cell Signaling Technology, catalog number 8632, 1:2000), anti-phospho-AKT rabbit monoclonal antibody (Cell Signaling Technology, catalog number 4060, 1:2000), anti-STAT1 rabbit monoclonal antibody (Cell Signaling Technology, catalog number 9172, 1:2000), anti-p38 MAPK rabbit monoclonal antibody (Cell Signaling Technology, catalog number 9212, 1:2000), anti-AKT rabbit monoclonal antibody (Cell Signaling Technology, catalog number 9272, 1:2000), and anti-GAPDH mouse monoclonal antibody (Cell Signaling Technology, catalog number 97166, 1:2000). The secondary antibodies included a goat anti-rabbit IgG antibody and a goat anti-mouse IgG antibody (catalog numbers 7076 and 7074, Cell Signaling Technology, MA, USA, 1:5000).

3.4. Signal Transduction Analysis

To identify the signal transduction pathway involved in target gene expression, the following reagents (Selleckchem, Huston, USA) were used: the STAT3 inhibitor WP1066 (5 μM), the STAT1 inhibitor fludarabine (50 μM), the Akt inhibitor MK-2066 (5 μM), the p38 MAPK inhibitor SB202190 (10 μM), the extracellular signal-regulated kinase (ERK) inhibitor PD98059 (10 μM), the C-Jun N-terminal kinase (JNK) inhibitor SP600125 (10 μM) and the NF-κB inhibitor BAY11-7082 (5 μM). After 1 h of pretreatment with these inhibitors, OVCAR3 cells were cultured with IL-29 (100 ng/ml) for 24 h. Subsequently, the supernatants were collected and analyzed by ELISA. The control was treated with the drug solvent (DMSO) in the absence of IL-29.

To investigate the signaling pathways activated by IL-29 (100 ng/ml) or IL-29 plus TNF-α (20 ng/ml), OVCAR3 cells were cultured with these cytokines for 0, 15, 30, and 60 min. Then, the cells were harvested and subjected to Western blot analysis.

3.5. Statistical Analysis

The data are presented as the means ± standard deviation (SD). Statistical analyses were performed with GraphPad Prism software (version 8.4.0). Student’s t-test or one-way ANOVA was used to analyze the differences between groups, and p-values < 0.05 were considered significant.

4. Results

4.1. IL-29 Receptor Expression in Ovarian Cells

According to a previous report, IFN lambda receptor 1 (IFNLR1) and IL-10 receptor β (IL-10Rβ) are essential for the recognition of IL-29 by cells [29-31]. Therefore, we examined the mRNA expression levels of IL-10Rβ and IL-28Rα in the normal ovarian cell line IOSE-80 and different ovarian cancer cell lines, such as A2780, ES-2, OVCAR3, and SK-OV-3. As shown in Figure 1A, the mRNA expression of these two receptors in OVCAR3 and SK-OV-3 cells was higher than that in the other cells. Figure 1B shows the expression of IFNLR1 and IL-10Rβ in OVCAR3 and SK-OV-3 cells, as determined by Western blotting.B The extracts of unstimulated SK-OV-3 and OVCAR3 cells were subjected to SDS-PAGE. The expression of IFNLR1, IL-10Rβ, and GAPDH was determined by Western blot analysis using the corresponding antibodies. IFNLR1, IL-10Rβ and GAPDH are shown on the same gels because their molecular weights differ greatly.

4.2. Effect of IL-29 on CXCL10 in the Ovarian Cancer Cell Lines SK-OV-3 and OVCAR3

The chemokine CXCL10 has been reported to be a key factor in evaluating the effectiveness of immunotherapy in ovarian cancer. Some ovarian cancer patients show high expression of the chemokine CXCL10, while some patients show low expression. In our study, SK-OV-3 and OVCAR3 cells were cultured individually for 24 h, and the supernatant was collected for ELISA analysis. No CXCL10 secretion was detected, as shown in Fig. 2A. When TNF-α (20 ng/ml) was used to stimulate SK-OV-3 and OVCAR3 cells for 24 h, the levels of CXCL10 in the supernatants of SK-OV-3 and OVCAR3 cells increased significantly, as determined by ELISA. SK-OV-3 and OVCAR3 cells did not secrete CXCL10 in the absence of stimulating factors, but these cells increased CXCL10 secretion under certain conditions. Therefore, we analyzed the conditions affecting the production of CXCL10 to explore the key factors in the tumor microenvironment that are conducive to immunotherapy.We examined whether IL-29 could increase the secretion of CXCL10 in the ovarian cancer cell lines SK-OV-3 and OVCAR3. As shown in Figure 2B and 2C, with increasing IL-29 concentrations, CXCL10 secretion by SK-OV-3 and OVCAR3 cells also showed a gradual increasing trend at the mRNA or the protein level. When IL-29 (100 ng/ml) was used to stimulate SK-OV-3 and OVCAR3 cells for 24 h, the levels of CXCL10 in the supernatants of SK-OV-3 and OVCAR3 cells increased significantly compared with the effects of other concentrations (539.14±26.92, $P < 0.001$; 632.73±42.38, $P < 0.001$).

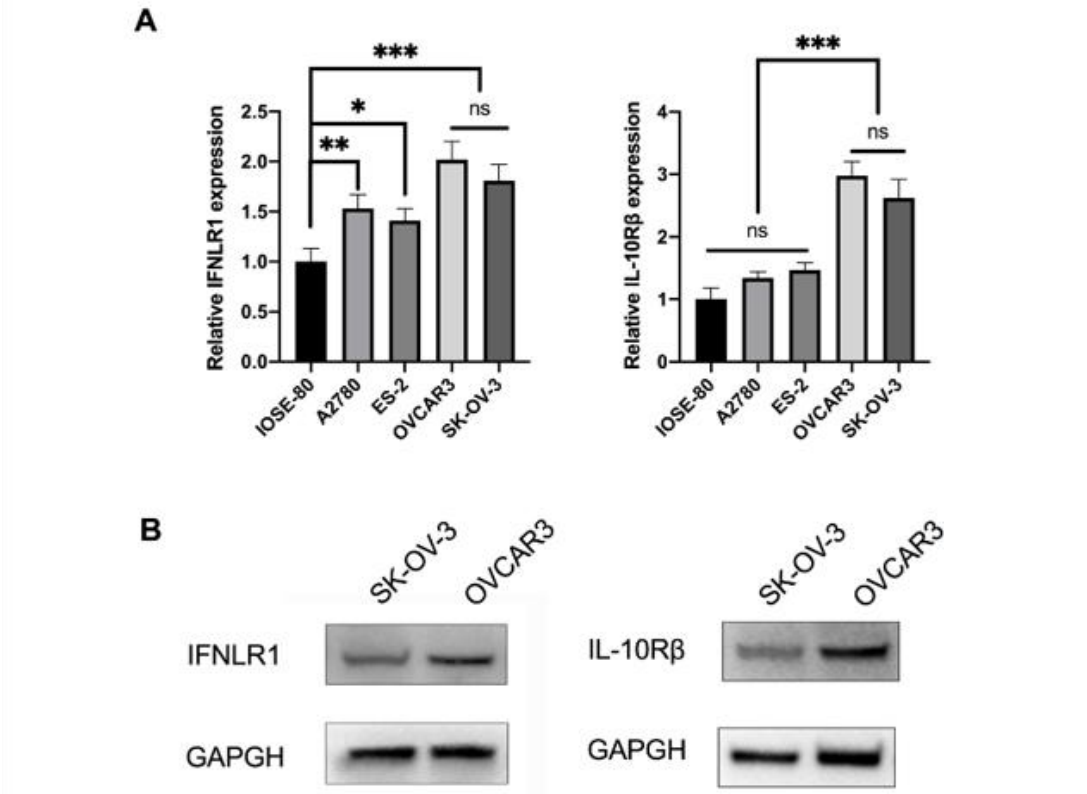

Figure 1: Interferon lambda receptor 1 (IFNLR1) and IL-10 receptor β (IL-10Rβ) expression in SK-OV-3 and OVCAR3 cells. **A** IFNLR1 and IL-10Rβ expression in normal ovarian cells (IOSE-80) and different ovarian cancer cell lines (A2780, ES-2, OVCAR3, SK-OV-3) was measured by qPCR. The results are from a representative experiment that was performed in triplicate, and the data are shown as the means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, NS, not significant. ***P < 0.001, IFNLR1 and IL-10Rβ expression in OVCAR3 and SK-OV-3 cells was significantly different from that in other cells.

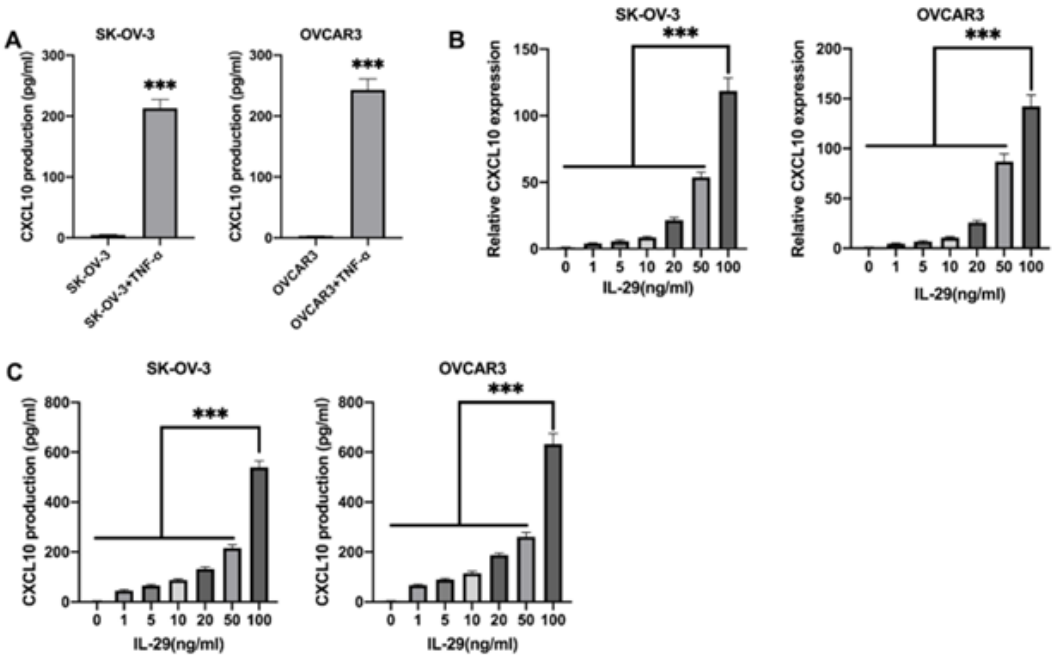

Figure 2: CXCL10 production in OVCAR3 cells stimulated with TNF-α or IL-29. **A** SK-OV-3 and OVCAR3 cells were stimulated with TNF-α (20 ng/ml). Their supernatants were collected after 24 h. The concentrations of CXCL10 in the supernatants were measured by ELISA. **B, C** SK-OV-3 and OVCAR3 cells were stimulated with different concentrations of IL-29 (0, 1, 5, 10, 20, 50, and 100 ng/ml). **B** CXCL10 gene expression in SK-OV-3 and OVCAR3 cells after 6 h measured by qPCR. **C** The concentrations of CXCL10 in the supernatants after SK-OV-3 and OVCAR3 cells were stimulated with different concentrations of IL-29 (0, 1, 5, 10, 20, 50, 100 ng/ml) for 24 h were measured by ELISA. The results are shown as the mean and SD of a representative experiment performed in triplicate. ***P < 0.001, After stimulation with IL-29 (100 ng/ml), the production of CXCL10 in SK-OV-3 and OVCAR3 cells increased significantly compared with the effects of the other concentrations.

4.3. IL-29 Regulates TNF-α-Induced CXCL10 Production in Ovarian Cancer Cells

TNF-α is known to induce CXCL10 production in epithelial cells, including ovarian cancer cells. Therefore, we hypothesized that IL-29 may regulate TNF-α-induced CXCL10 production in ovarian cancer cells. As shown in Figure 1 and Figure 2, the protein expression of the IL-29 receptor was higher in OVCAR3 cells than in SK-OV-3 cells, and OVCAR3 cells secreted more CXCL10 in response to IL-29 or TNF-α stimulation. Therefore, we chose OVCAR3 cells for subsequent validation. Figure 3A shows that IL-29 (100 ng/ml) and TNF-α (20 ng/ml) cooperatively enhanced relative CXCL10 gene expression in OVCAR3 cells ($P< 0.001$). Figure 3B shows that TNF-α (20 ng/ml) induced CXCL10 production (217.37 ± 18.32 pg/ml) and that IL-29 (100 ng/ml) induced CXCL10 production (632.13 ± 42.68 pg/ml). IL-29 (100 ng/ml) significantly enhanced CXCL10 production in TNF-α (20 ng/ml)-stimulated OVCAR3 cells (1596.2 ± 92.1 pg/ml, $P< 0.001$).

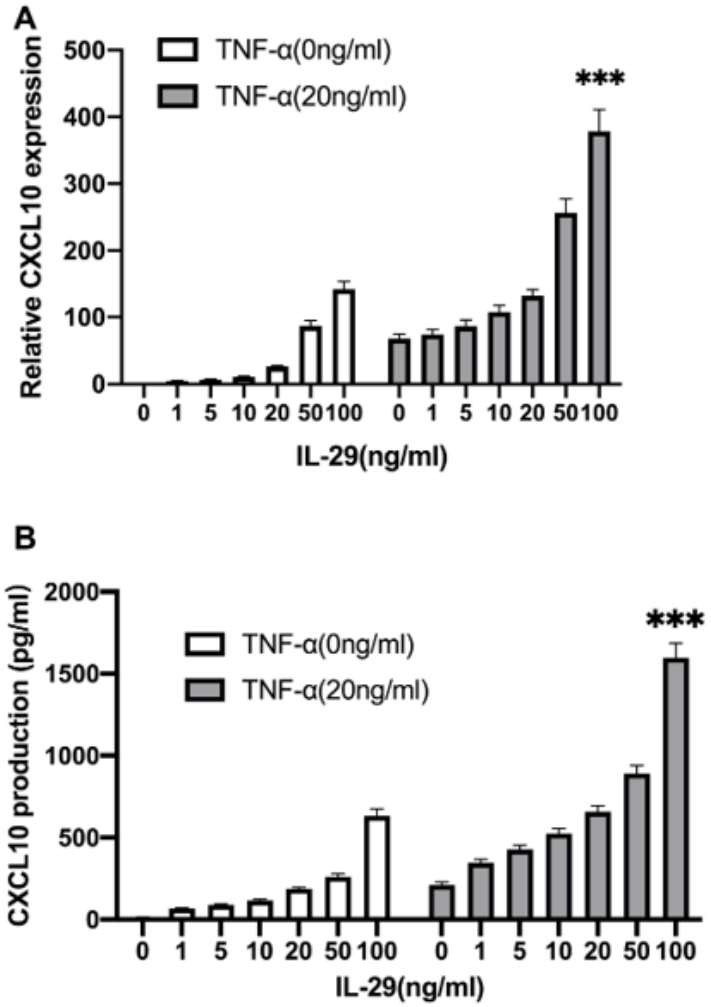

Figure 3: CXCL10 production in IL-29-induced OVCAR3 cells. OVCAR3 cells were stimulated with IL-29 (0, 1, 5, 10, 20, 50, 100 ng/ml) with or without TNF-α (20 ng/ml). **A** CXCL10 gene expression in OVCAR3 cells treated with different concentrations of IL-29 with or without TNF-α for 6 h was measured by qPCR. ***$P < 0.001$ compared with all other groups. **B** OVCAR3 cells were stimulated with different concentrations of IL-29 with or without TNF-α. The supernatants were collected after 24 h. The concentrations of CXCL10 in the supernatants were measured by ELISA.

4.4. Effect of IL-29 on CXCL10 Secretion in OVCAR3 Cells Treated with Signal Transduction Inhibitors

Next, we used inhibitors to study the signal transduction mechanism of IL-29-induced CXCL10 secretion in OVCAR3 cells. We focused on the MAPK, STAT, Akt, and NF-κB pathways, which are known to be associated with CXCL10 secretion. In the absence of inhibitors, IL-29 (100 ng/ml) induced OVCAR3 cells to secrete CXCL10 (632.4± 43.1 pg/ml). In this study, fludarabine (STAT1 inhibitor), MK-2206 (Akt inhibitor), and SB203580 (P38 MAPK inhibitor) inhibited CXCL10 production (66.23 ± 5.89 pg/ml). $P < 0.001$; 107.47 ± 13.87 pg/ml, $P < 0.001$; 115.16 ± 8.72 pg/ml, $P < 0.001$). Conversely, PD98059, an ERK inhibitor, and SP600125, a JNK inhibitor, increased CXCL10 production (824.14 ± 55.92 pg/ml, $P < 0.001$; 731.18 ±62.16 pg/ml, $P = 0.007$). WP1066 (STAT3 inhibitor) and BAY 11-7082 (NF-κB inhibitor) did not regulate CXCL10 production (578.23 ±37.16 pg/ml, $p = 0.221$; 568.25±64.92 pg/ml, $P = 0.186$). These findings suggest that the STAT1, AKT, and P38 MAPK pathways positively regulate CXCL10 secretion in OVCAR3 cells stimulated by IL-29, while the ERK and JNK pathways negatively regulate CXCL10 in IL-29-stimulated OVCAR3 cells. Figure 4 A CXCL10 production was examined in the presence of signal transduction inhibitors in IL-29-stimulated OVCAR3 cells after 24 h. These effects were measured in the presence or absence of the following signaling inhibitors: the STAT3 inhibitor WP1066 (5 μM), the STAT1 inhibitor fludarabine (50 μM), the Akt inhibitor MK-2066 (5 μM), the p38 MAPK inhibitor SB202190 (10 μM), the extracellular signal-regulated kinase (ERK) inhibitor PD98059 (10 μM), the JNK inhibitor SP600125 (10 μM) and the NF-κB inhibitor BAY11-7082 (5 μM). Fludarabine, MK-2206, and SB203580 all inhibited CXCL10 production ($P < 0.001$). PD98059 and SP600125 increased CXCL10 production ($P < 0.001$; $P < 0.05$). WP1066 and BAY 11-7082 did not regulate CXCL10 production ($P > 0.05$). B CXCL10 gene expression was quantified by qPCR in OVCAR3 cells treated with IL-29 for 6 h in the presence or absence of signaling inhibitors. Ctrol, Control. ***$P < 0.001$ comparing IL-29 +fludarabine vs. IL-29, IL-29 +MK-2066, IL-29 +SB202190.

4.5. Effects of IL-29 on STAT1, Akt and P38 MAPK Pathway Phosphorylation in TNF-α-Stimulated OVCAR3 Cells

Figure 4 shows that the STAT1, AKT and P38 MAPK pathways positively regulated CXCL10 secretion in IL-29-stimulated OVCAR3 cells. To further verify this finding, we examined the phosphorylation of STAT1, AKT, and P38 MAPK in OVCAR3 cells stimulated with IL-29 with or without TNF-α to explore the signaling pathways involved in CXCL10 secretion. Figure 5 shows that the phosphorylation levels of STAT1, AKT and P38 MAPK in OVCAR3 cells were increased after IL-29 stimulation ($P < 0.001$), and the phosphorylation levels of STAT1, AKT and P38 MAPK were significantly increased by IL-29 plus TNF-α ($P < 0.001$) compared with IL-29 alone.

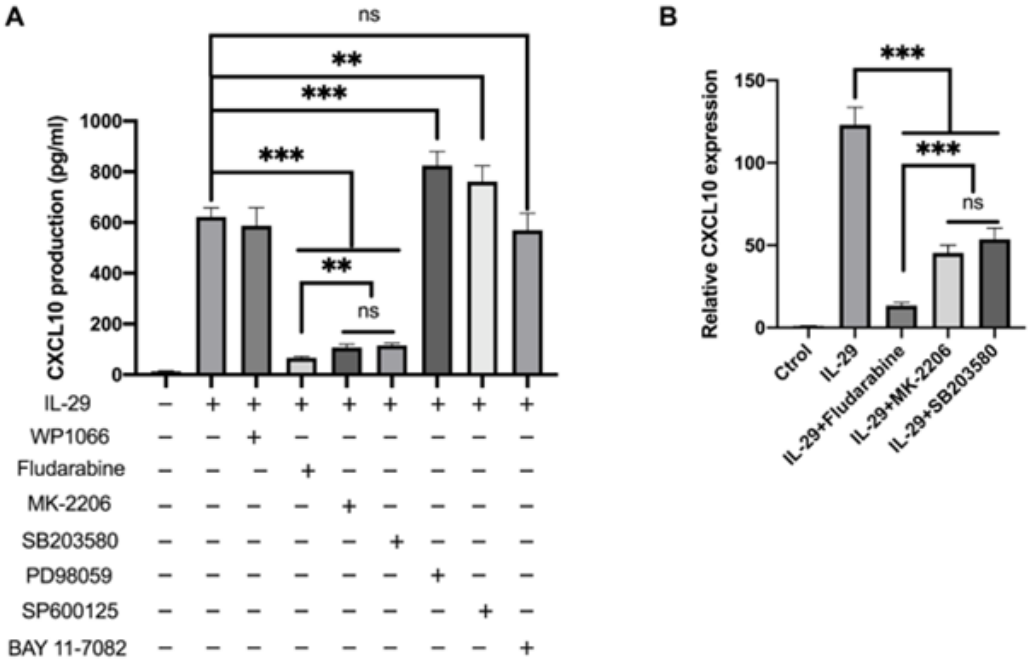

Figure 4 A:CXCL10 production was examined in the presence of signal transduction inhibitors in IL-29-stimulated OVCAR3 cells after 24 h. These effects were measured in the presence or absence of the following signaling inhibitors: the STAT3 inhibitor WP1066 (5 μM), the STAT1 inhibitor fludarabine (50 μM), the Akt inhibitor MK-2066 (5 μM), the p38 MAPK inhibitor SB202190 (10 μM), the extracellular signal-regulated kinase (ERK) inhibitor PD98059 (10 μM), the JNK inhibitor SP600125 (10 μM) and the NF-κB inhibitor BAY11-7082 (5 μM). Fludarabine,MK-2206, and SB203580 all inhibited CXCL10 production ($P < 0.001$). PD98059 and SP600125 increased CXCL10 production ($P < 0.001$; $P < 0.05$). WP1066 and BAY 11-7082 did not regulate CXCL10 production ($P > 0.05$). **B**CXCL10 gene expression was quantified by qPCR in OVCAR3 cells treated with IL-29 for 6 h in the presence or absence of signaling inhibitors. Ctrol, Control. ***$P < 0.001$ comparing IL-29 +fludarabine vs. IL-29, IL-29 +MK-2066, IL-29 +SB202190.

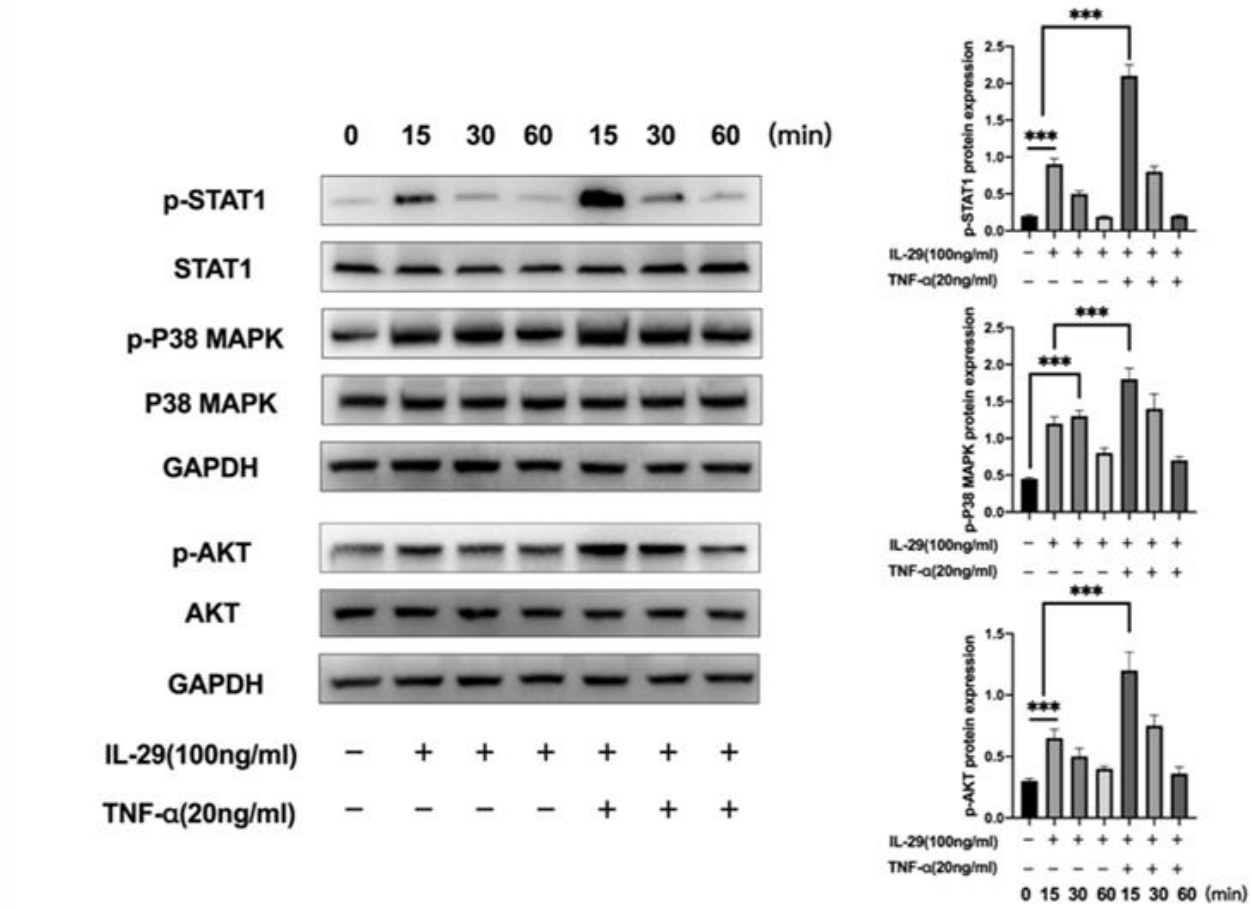

Figure 5: The STAT1, P38 MAPK and AKT signaling pathways mediated IL-29- or IL-29/TNF-α-induced CXCL10 expression in OVCAR3 cells. Activation of STAT1, AKT or P38 MAPK signaling in OVCAR3 cells treated with IL-29 (100 ng/ml) with or without TNF-α (20 ng/ml) for 0, 15, 30, or 60 min was analyzed by Western blotting. The same samples were examined on parallel gels to examine the bands of phosphorylated protein, total protein or GAPDH. p-STAT1, p-P38 MAPK and GAPDH were probed on the same gels since their molecular weights differed greatly. ***P < 0.001 comparing the phosphorylation levels of STAT1, AKT and P38 MAPK after IL-29 plus TNF-α stimulation and IL-29 stimulation alone.

5. Discussion

IL-29 is predominantly produced by maturing dendritic cells and macrophages. IL-29 has been implicated in numerous immunological responses and has shown antiviral activity similar to that of type I IFNs. However, IL-29 primarily targets epithelial cells and does not target as many cell types as type I IFNs. IL-29 levels are increased during infection in the gastrointestinal tract, respiratory tract, and mucosal regions. Multiple studies have indicated that IL-29 is involved in the pathogenesis of cancer, as well as its antitumor effects. Studies have indicated the ability of IL-29 to serve as a mediator of innate and adaptive immune responses. In previous studies, IL-29 has been shown to increase macrophage reactivity to IFN-γ, thereby promoting the immune response. Multiple studies also indicate the involvement of IL-29 in the pathogenesis of cancer, as well as its antitumor effects. Furthermore, compared with type I IFN therapies, IL-29 is likely to be used for immunotherapy with fewer side effects, such as cytokine release syndrome, because the target cells of IL-29 are more limited. Therefore, IL-29 may be used as an adjuvant for immunotherapy.CXCL10 interacts with its receptor CXCR3 through paracrine and/or autocrine signaling to mediate immune cell chemotaxis, differentiation and activation, thereby inhibiting tumorigenesis. Interestingly, CXCL10 has also been reported to promote tumor growth and metastasis in gastric cancer and colon cancer. indicated that CXCL10/CXCR3 overexpression was a biomarker of poor prognosis in patients with stage II colorectal cancer. However, in contrast, the positive prognosis of ovarian cancer is associated with high levels of TNF-α and McP-1, which are immune mediators of Th1 and Th2 immunity, respectively. McP-1 recruits monocytes to differentiate into M1-type macrophages, which may suppress tumor growth and development(Italiani and Boraschi,2014). M1-type macrophages have both direct and indirect tumor destruction effects: direct effects include the release reactive oxygen and nitrogen intermediates to exert cytotoxicity and inhibit the proliferation of tumor cells and indirect effects are mediated by the release of cytokines such as CXCL9, CXCL10, CXCL11, IL-23 and TNF-α to promote T cell- and NK cell-driven antitumor immunity(Nardin and Abastado,2008). Moreover, the immunoreactive subtype of ovarian cancer is characterized by high expression of CXCL10(,2011). CXCL10 is one of the indicators of the efficacy of immunotherapy for ovarian cancer. Previous

experiments by our group have confirmed that CXCL10 is not secreted by unstimulated ovarian cancer cell lines such as SK-OV-3 and OVCAR3. Therefore, exploring the factors that cause the increase in CXCL10 secretion in ovarian cancer is expected to play a role in immunotherapy for ovarian cancer.In recent years, researchers have conducted many studies on the biological effects of TNF-α and found that it is a major proinflammatory cytokine. TNF-α has a variety of biological activities, including promoting inflammation, apoptosis, and cell proliferation and differentiation. Previous studies have confirmed that TNF-α can increase the secretion of CXCL10 in various cancers, such as breast cancer and colon cancer. found that IL-29 increases CXCL10 in human keratinocyte cells and promotes T lymphocyte infiltration. indicated that IL-29 could enhance the secretion of CXCL10 in human oral epithelial cells and further promote CXCL10 secretion after TNF-α stimulation. Based on this finding, the present study investigated the effect of IL-29 on the production of CXCL10 in ovarian cancer, explored whether IL-29 had a synergistic stimulatory effect in the presence of TNF-α and discovered the mechanism involved.In this study, we demonstrated that IL-29 (100 ng/ml) could significantly increase the secretion of CXCL10 in OVCAR3 ovarian cancer cells in conjunction with TNF-α (20 ng/ml). This is the first paper to report that IL-29 promotes CXCL10 secretion in ovarian cancer, especially in combination with TNF-α. IL-29 has previously been reported to affect the secretion of other cytokines. IL-29 induces high levels of IL-6 and IL-8 in peripheral blood monocytes, accompanied by moderate levels of IL-10; IL-6 and IL-8 are involved in proinflammatory responses. Studies have shown that IL-29 can inhibit IL-5 and IL-13 in allergic asthma to reduce the inflammatory response in asthma. IL-29 can also inhibit a cytokine involved in asthma known as IL-19, which is thought to induce Th2 cells to secrete proinflammatory cytokines.We used pathway inhibitors and found that IL-29 activated the STAT1, P38 MAPK, Akt, ERK, and JNK pathways in ovarian cancer. Several previous studies have examined the activation of signaling pathways induced by IL-29. Several studies have demonstrated that IL-29 mainly activates the JAK/STAT signaling pathway via STAT1 and STAT2. reported that IL-29 induces the phosphorylation of P38 MAPK, ERK, JNK and Akt in human intestinal epithelial cells. demonstrated that IL-29 enhanced STAT1 phosphorylation in human melanoma cell lines. reported that IL-29 induced the phosphorylation of MAPK, ERK, JNK, STAT1 and NF-κB P65 in human synovial fibroblasts. suggested that IL-29 activated the MAPK, STAT3, Akt, ERK, JNK and NF-κB pathways. Based on previous studies and our experimental results, we believe that IL-29 can activate the P38 MAPK, ERK, JNK and STAT1 pathways. IL-29 may activate the Akt and NF-κB pathways in a cell type-dependent manner.In this study, we demonstrated that the p38 MAPK, STAT1, and Akt pathways positively regulated CXCL10 production in IL-29-stimulated ovarian cancer cells. demonstrated that the P38 MAPK, STAT3 and NF-κB pathways play critical roles in the secretion of CXCL10 by human oral epithelial cells, while the JNK and ERK pathways inhibit CXCL10 production. Zaheer et al reported that the ERK pathway inhibited the production of CXCL10 in human airway epithelial cells induced by human rhinovirus. Based on previous studies and our experimental results, we believe that the P38 MAPK and STAT1 pathways positively regulate CXCL10 production, while the JNK and ERK pathways negatively regulate CXCL10 production. IL-29 may activate the Akt and NF-κB pathways in a cell type-dependent manner to promote CXCL10 secretion.In conclusion, IL-29 enhances the production of CXCL10 in ovarian cancer cells and synergistically interacts with TNF-α to significantly increase the production of CXCL10 in ovarian cancer cells. IL-29 promotes the production of CXCL10 in ovarian cancer cells through the p38 MAPK and STAT1 pathways. As high expression of CXCL10 indicates good efficacy and prognosis of ovarian cancer immunotherapy, IL-29 may be used as a new adjuvant in immunotherapy in the future.

6. Funding

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