HAYDARPAŞA NUMUNE MEDICAL JOURNAL

DOI: 10.14744/hnhj.2024.93206
Haydarpasa Numune Med J 2024;64(3):416–426

ORIGINAL ARTICLE

Disruption of The CXC Chemokine Network in Endometrioid Endometrial Adenocarcinoma: Novel Therapeutic Targets and Prognostic Markers

Hatice Güngör[1], Roberto Dina[2]

[1]Department of Internal Medicine, Medical Oncology, University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital, Istanbul, Türkiye
[2]Department of Metabolism, Digestion and Reproduction, Hammersmith Campus, DuCane Road, London, United Kingdom

Abstract

Introduction: Endometrioid endometrial adenocarcinoma is one of the most common gynecological cancers in women, with an increasing incidence in recent years. CXC chemokines and their receptors, which play a critical role in regulating immune and angiogenic processes in the tumor microenvironment, are thought to have significant functions in endometrial carcinogenesis.

Methods: In this study, we comprehensively examined the expression profiles of five CXC chemokines (CXCL9, CXCL10, CXCL11, CXCL4, and CXCL4L1) and three CXCR3 receptor variants (CXCR3A, CXCR3B, and CXCR3-alt) in 50 normal endometrium and 50 endometrioid endometrial adenocarcinoma samples using quantitative real-time PCR and immunohistochemical analyses.

Results: Quantitative real-time PCR analyses revealed significant upregulation of CXCR3A, CXCR3-alt, CXCL9, and CXCL11 in endometrioid endometrial adenocarcinoma, whereas CXCR3B, CXCL10, CXCL4, and CXCL4L1 were downregulated. These findings indicate that the pro-proliferative CXCR3A/CXCR3-alt axis becomes hyperactive in tumor tissue, while the angiostatic CXCR3B axis is suppressed. Furthermore, we observed distinct chemokine correlation patterns in normal endometrium and tumor tissue, suggesting a reorganization of the chemokine network during disease progression. Immunohistochemical analyses confirmed the tissue-level localization and cell-specific expression of these molecules. When integrated with clinical data, certain chemokine expression patterns showed significant correlations with tumor stage and grade. Notably, high CXCR3A and low CXCR3B expression were associated with a more aggressive tumor phenotype and poor prognosis.

Discussion and Conclusion: This study provides a comprehensive view of the disruption of the CXC chemokine network in endometrioid endometrial adenocarcinoma. Our findings demonstrate that these molecules not only play a role in the pathogenesis of the disease but also have potential as prognostic markers and therapeutic targets. Targeting CXCR3 receptor variants and their ligands may lead to the development of novel strategies for endometrial cancer treatment in the future. Further studies should focus on translating these findings into clinical applications and evaluating the therapeutic efficacy of CXC chemokine modulation.

Keywords: Angiogenesis; Cancer progression; CXC chemokines; CXCR3 receptor variants; Endometrioid endometrial adenocarcinoma; Immunoangiostasis; Tumor microenvironment.

Correspondence: Hatice Güngör, M.D. Department of Internal Medicine, Medical Oncology, University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital, Istanbul, Türkiye
Phone: +90 553 429 92 44 **E-mail:** hhgungor@gmail.com
Submitted Date: 14.07.2024 **Revised Date:** 07.08.2024 **Accepted Date:** 23.08.2024
Haydarpaşa Numune Medical Journal

Endometrial cancer is one of the most common gynecological malignancies in women worldwide, with an increasing incidence[1,2]. Particularly in developed countries, a significant increase in endometrial cancer cases is observed in conjunction with the prevalence of obesity and lifestyle changes[3,4]. Endometrioid endometrial adenocarcinoma is the most common histological subtype of endometrial cancer, accounting for approximately 80% of cases[5,6]. This type is generally estrogen-dependent and has a better prognosis[7].

Despite advancements in early diagnosis and treatment methods, the prognosis for advanced-stage and recurrent cases remains poor[7,8]. While traditional treatment approaches such as surgery, radiotherapy, and chemotherapy are effective for many patients, new treatment strategies are needed for resistant and metastatic cases[5,6]. Therefore, understanding the molecular mechanisms of endometrial carcinogenesis and identifying new therapeutic targets is of great importance[9].

Chemokines are small chemotactic cytokines that play critical roles in various biological processes such as immune cell migration, angiogenesis, and tumor progression[8,9]. These molecules have important functions in normal physiological processes as well as in cancer development and progression[10,11]. CXC chemokines are an important subgroup of the chemokine family and exhibit angiogenic or angiostatic properties depending on the presence or absence of the ELR motif[10,11].

Non-ELR CXC chemokines (CXCL9, CXCL10, CXCL11, CXCL4, and CXCL4L1) exert their effects through the CXCR3 receptor and play important roles in regulating the immune response and inhibiting angiogenesis[12,13]. These chemokines have a critical role in the formation of anti-tumor immune responses by regulating the infiltration and activation of immune cells in the tumor microenvironment[9,14].

Three different splice variants of the CXCR3 receptor have been identified: CXCR3A, CXCR3B, and CXCR3-alt[14,15]. These variants lead to different cellular responses; CXCR3A and CXCR3-alt generally promote cell proliferation and migration, while CXCR3B has angiostatic and apoptotic effects[13,15]. In the context of cancer, disruption of the expression balance of these receptor variants has been associated with tumor growth and metastasis[16,17].

Recent studies have shown that CXC chemokines and their receptors play important roles in various types of cancer[18,19]. For example, increased expression of CXCR3A and CXCR3-alt and decreased expression of CXCR3B have been reported in ovarian cancer[20]. Similarly, CXC chemokines have been shown to be associated with tumor progression and metastasis in breast cancer and melanoma[19,21].

The role of the CXC chemokine network in endometrial cancer has not yet been fully elucidated. However, some studies have shown that the CXCL12/CXCR4 axis is important in the proliferation and metastasis of endometrial cancer cells[22]. Additionally, CXCL14 has been reported to promote invasion of endometrial cancer cells and angiogenesis[23].

In this study, we aimed to comprehensively examine the expression profiles of CXC chemokines (CXCL9, CXCL10, CXCL11, CXCL4, and CXCL4L1) and CXCR3 receptor variants (CXCR3A, CXCR3B, and CXCR3-alt) in normal endometrium and endometrioid adenocarcinoma tissues. Understanding the role of changes in expression patterns of these molecules in endometrial carcinogenesis is critical for identifying potential prognostic markers and developing new therapeutic targets.

Materials and Methods

This study was conducted in accordance with the ethical standards of the Helsinki Declaration.

Tissue Samples and Patient Data

This study utilized fifty frozen normal endometrium samples, fifty endometrioid adenocarcinoma samples, and one thymus surgical tissue sample obtained from the Imperial College NHS Trust Tissue Bank following approval of the written application in accordance with project proposal submissions to NRES. All samples were procured with Imperial College ethical committee approval (R11043) and patient's consents. Tissue samples were immediately frozen in liquid nitrogen after surgical resection and stored at -80°C. Comprehensive clinical data for endometrioid endometrial adenocarcinoma tissue samples were obtained from the Imperial College NHS Database. This data included diagnosis date, age at diagnosis, prognosis, stage, grade, diagnosis, treatments administered (surgery, chemotherapy, and radiotherapy), current status, platelet counts, and histological data. Histological details of diseases affecting normal endometrium patients were also collected.

Primer Design

Coding sequences for CXCL9 (NM_002416.1), CXCL10 (NM_001565.3), CXCL11 (NM_005409.4), CXCL4 (NM_002619.2), CXCL4L1 (NM_002620.2), CXCR3A

(NM_001504.1), and CXCR3B (NM_001142797.1) were obtained from the PubMed Nucleotide database. Primer pairs for each chemokine ligand and receptor variant were created using Primer3 Output (http://frodo.wi.mit.edu/primer3/). Primers were carefully selected based on criteria such as the presence of exon-exon junctions, 21-25 nucleotide length, 60°C melting temperature, 40% G-C content, and absence of poly-tails or hairpin loops. Additionally, chemokine and receptor variant sequences were carefully compared, and primer pairs covering non-overlapping, specific regions were selected.

RNA Isolation

Total RNA was isolated from 50 normal endometrium and 50 endometrioid endometrial adenocarcinoma tissue samples using the trizol (phenol-chloroform) extraction protocol. This protocol had been previously optimized by the Gungor laboratory. Tissue samples were cut into small pieces on a sterile plastic plate using a disposable scalpel and transferred to an Eppendorf tube containing glass beads. Samples were homogenized using a Micro Dismembrator U (Sartorius) at 2000 rpm for 5 minutes. The isolation process was performed using trizol reagent (Invitrogen) and chloroform. The RNA pellet was washed with 75% ethanol and dissolved in DEPC-treated water. The quality and quantity of the obtained mRNA were measured using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific).

Reverse Transcription and Quantitative Real-Time PCR

RNA isolation was performed using the trizol (phenol-chloroform) extraction protocol. Tissue samples were homogenized using a Micro Dismembrator U, and RNA was isolated using trizol reagent and chloroform. The quality and quantity of the isolated RNA were measured using a NanoDrop 1000 Spectrophotometer. cDNA synthesis was performed using M-MLV reverse transcriptase enzyme. The isolated mRNA was converted to cDNA using M-MLV reverse transcriptase enzyme (Promega). Expression levels of CXC chemokines and CXCR3 receptor variants were measured using SYBR Green PCR Master Mix (Applied Biosystems) on a 7900 HT Fast Real-Time PCR System (Applied Biosystems). Optimized primer concentrations were used for each gene. Results were normalized using the HPRT reference gene and analyzed using the ΔCt method.

DNA Isolation and Mutation Analysis

Genomic DNA was isolated from both normal endometrium and endometrioid adenocarcinoma samples using the TRI Reagent protocol. Mutation analysis was planned using Sequenom for a panel of 150 genes but could not be performed due to time constraints.

Statistical Analysis

Data were analyzed using two-tailed unpaired t-tests, Pearson correlation coefficient (r), and one-way ANOVA. A p-value<0.05 was considered statistically significant. Additionally, fold changes in chemokine ligand or receptor expression were determined by calculating 2-ΔΔCt values and analyzed using SPSS software.

This comprehensive methodology allowed us to examine and compare the expression profiles of CXC chemokines and CXCR3 receptor variants in normal endometrium and endometrioid adenocarcinoma in detail.

Results and Discussion

Expression of CXC Chemokines and CXCR3 Receptor Variants

In the first stage of our study, we examined the expression profiles of CXC chemokines and CXCR3 receptor variants in different cell types. This analysis was important for understanding which cell types express these molecules under normal physiological conditions.

In the study, different cell types were used as positive controls: unstimulated and stimulated T cells (with CD3/CD28 and ionomycin/PMA), IFN-γ/TNF-α stimulated HUVECs, PBMCs, and platelets. These cell types are important for understanding the physiological expression patterns of CXC chemokines and CXCR3 receptor variants.

Expression of CXCR3 Receptor Variants

We examined the expression of three different variants of the CXCR3 receptor—CXCR3A, CXCR3B, and CXCR3-alt—in various cell types. These cell types included unstimulated T cells, CD3/CD28 stimulated T cells, ionomycin/PMA stimulated T cells, thymus tissue, IFN-γ/TNF-α stimulated HUVECs (human umbilical vein endothelial cells), and PBMCs (peripheral blood mononuclear cells). Expression levels were measured by qRT-PCR and normalized using the HPRT reference gene.

Our results demonstrated that CXCR3A is highly expressed particularly in T cells (Fig. 1a). CXCR3B expression was more prominent in ionomycin/PMA stimulated T cells and PBMCs (Fig. 1b). The CXCR3-alt variant showed higher expression in unstimulated T cells and ionomycin/PMA stimulated T cells (Fig. 1c).

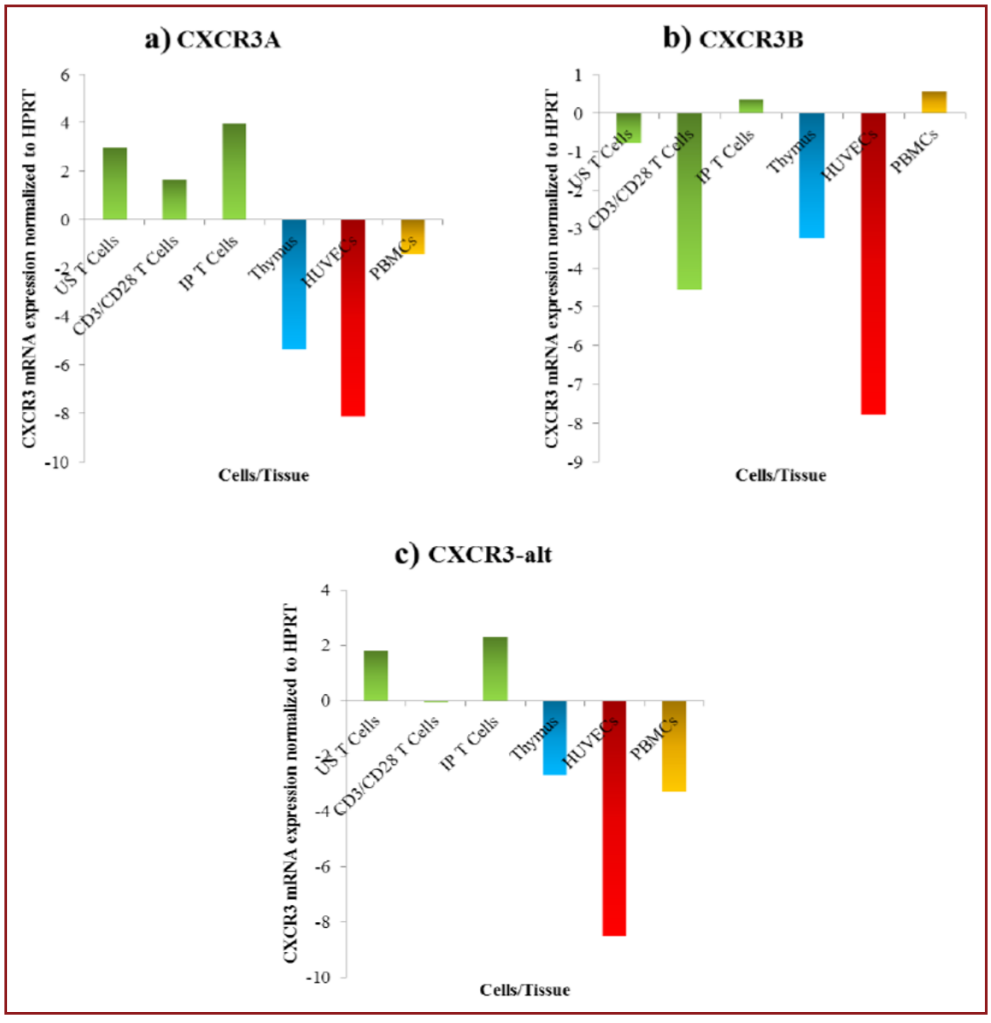

Figure 1. Expression of CXCR3 receptor variants in different cell types **(a)** CXCR3A, **(b)** CXCR3B, **(c)** CXCR3-alt expression levels. Data show ΔCt values normalized to HPRT. Bars represent mean±standard deviation (n=3).

Expression of CXC Chemokines

We examined the expression of CXCL9, CXCL10, CXCL11, CXCL4, and CXCL4L1 chemokines in the same cell types and additionally in platelets. This analysis helped us understand which chemokines are produced by which cell types.

The expression patterns of CXCL9, CXCL10, and CXCL11 showed similarity, and these chemokines were highly expressed particularly in T cells and HUVECs (Fig. 2a-c). This finding confirms that these chemokines are produced by immune cells and endothelial cells[9,14].

On the other hand, the expression of CXCL4 and CXCL4L1 was almost entirely limited to platelets (Fig. 2d-e). This result confirms that these two chemokines are platelet-derived factors and is consistent with previous findings in the literature[11,17].

CXCR3 Expression in Normal Endometrium and Endometrioid Endometrial Adenocarcinoma

In the second stage of our study, we compared the expression of CXCR3 receptor variants in normal endometrium and endometrioid endometrial adenocarcinoma tissues. This analysis helped us understand how the expression balance of CXCR3 receptor variants changes in tumor tissue.

CXCR3A, CXCR3B, and CXCR3-alt expression were measured in triplicate by qRT-PCR in 50 normal endometrium and 50 endometrioid endometrial adenocarcinoma tissue samples and analyzed using the ΔCt method.

Our results showed that CXCR3A and CXCR3-alt expression significantly increased in endometrioid endometrial adenocarcinoma compared to normal endometrium (Figs 3a and 3c, $p<0.05$). In contrast, CXCR3B expression tended to decrease in tumor tissue, but this difference was not

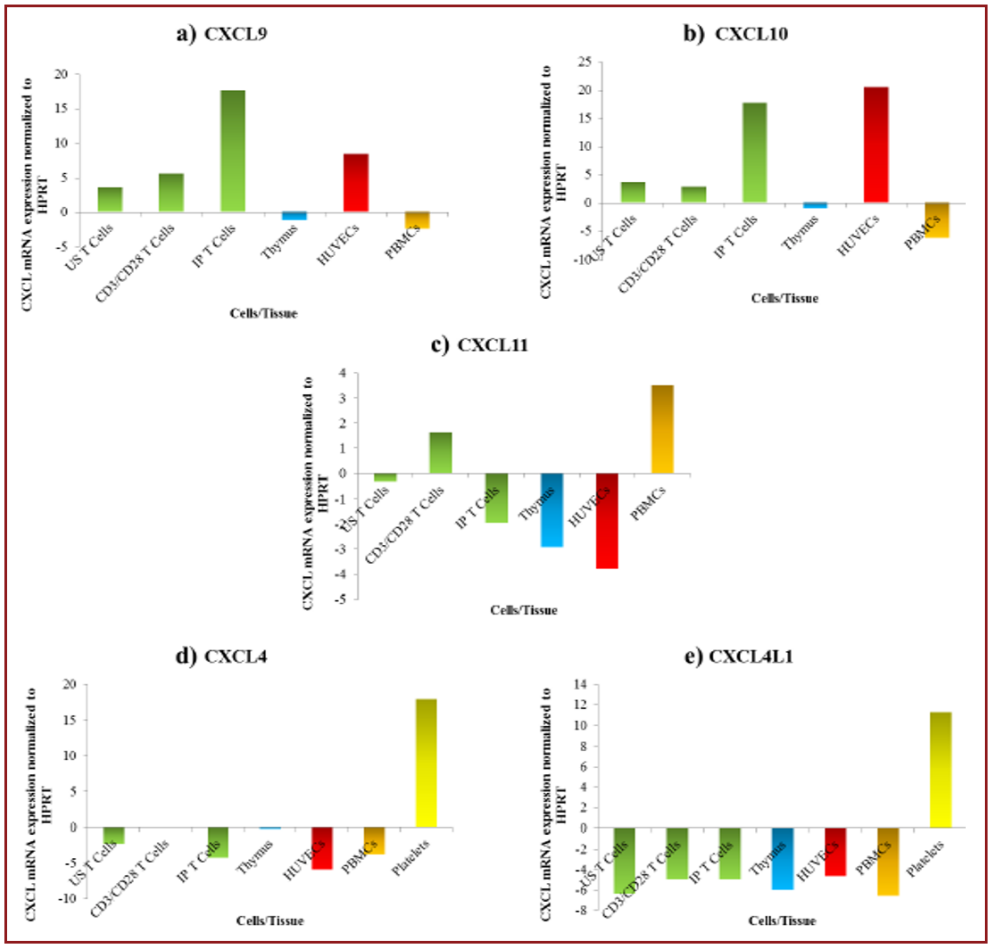

Figure 2. Expression of CXC chemokines in different cell types. **(a)** CXCL9, **(b)** CXCL10, **(c)** CXCL11, **(d)** CXCL4, **(e)** CXCL4L1 expression levels. Data show ΔCt values normalized to HPRT. Bars represent mean±standard deviation (n=3).

statistically significant (Fig. 3b, p>0.05).

These findings indicate that the expression balance of CXCR3 receptor variants is disrupted in endometrioid endometrial adenocarcinoma. The increase in CXCR3A and CXCR3-alt may enhance the proliferation and migration capacity of tumor cells. On the other hand, the decrease in CXCR3B, which has angiostatic effects, may promote tumor angiogenesis[13-16].

CXCL Expression in Normal Endometrium and Endometrioid Endometrial Adenocarcinoma

In the third stage of our study, we compared the expression of CXC chemokines in normal endometrium and endometrioid endometrial adenocarcinoma tissues. This analysis helped us understand how the chemokine expression profile changes in tumor tissue.

The expression of CXCL9, CXCL10, CXCL11, CXCL4, and CXCL4L1 was measured in triplicate by qRT-PCR in 50 normal endometrium and 50 endometrioid adenocarcinoma tissue samples and analyzed using the ΔCt method.

Our results revealed that CXCL9 expression showed an increase in endometrioid endometrial adenocarcinoma compared to normal endometrium, but this increase was not statistically significant (Fig. 4a, p>0.05). CXCL10 expression was significantly decreased in tumor tissue (Fig. 4b, p<0.001). CXCL11 expression showed a significant increase in endometrioid endometrial adenocarcinoma (Fig. 4c, p<0.01).

CXCL4 expression showed a decreasing trend in endometrioid endometrial adenocarcinoma, but this difference was not statistically significant (Fig. 5a, p>0.05). CXCL4L1 expression was significantly decreased in tumor tissue (Fig. 5b, p<0.01).

These findings demonstrate that the CXC chemokine expression profile changes significantly in endometrioid endometrial adenocarcinoma. The increase in CXCL9 and CXCL11 may promote the recruitment of CXCR3+ immune cells to the tumor microenvironment[9, 14].

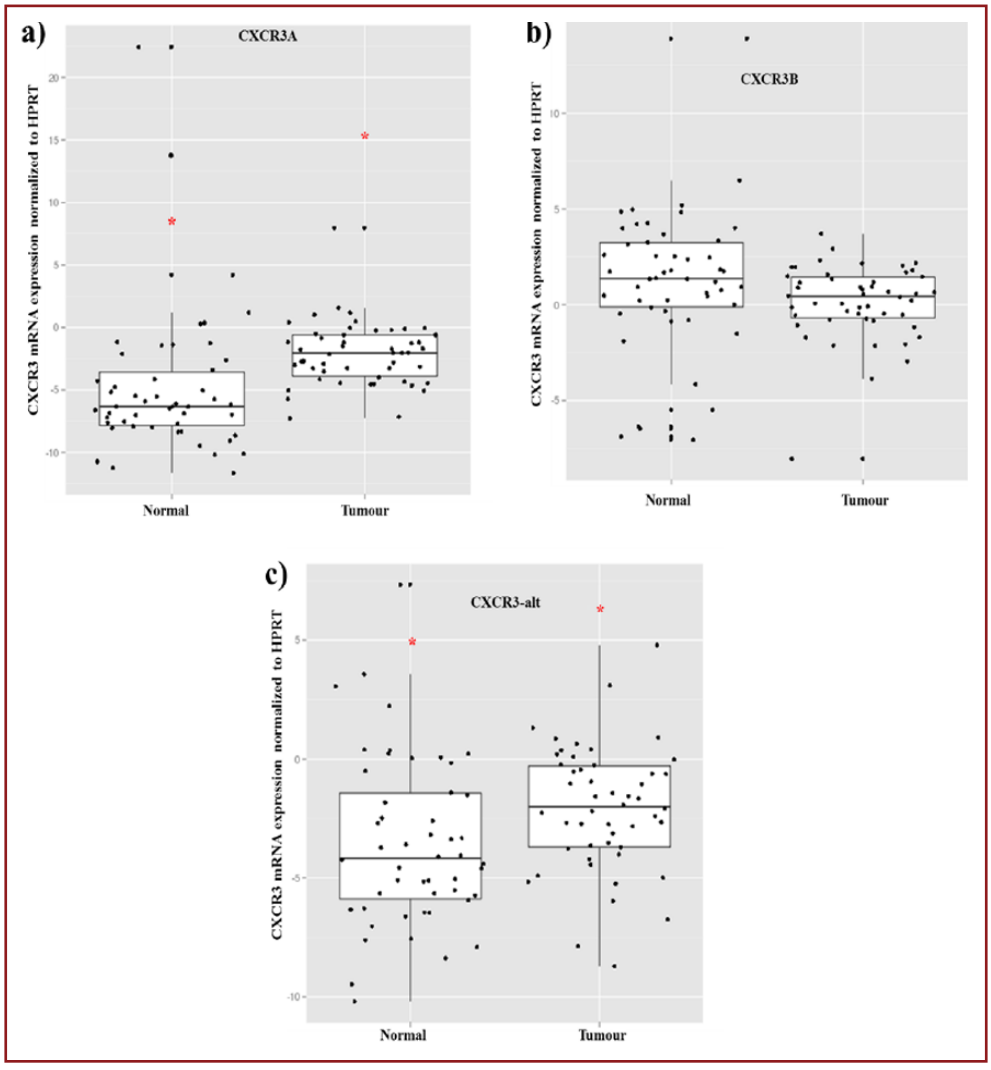

Figure 3. CXCR3 expression in normal endometrium and endometrioid endometrial adenocarcinoma. **(a)** CXCR3A, **(b)** CXCR3B, **(c)** CXCR3-alt expression levels. Data show ΔCt values normalized to HPRT. Boxes represent the interquartile range (IQR). Lines indicate the median value, and dots show outliers. Statistical analyses were performed using unpaired t-test. *p<0.05, **p<0.01, ***p<0.001.

However, the decrease in CXCL10, CXCL4, and CXCL4L1, which have angiostatic properties, may support tumor angiogenesis[10,11,17].

CXCL and CXCR3 Correlations

In the fourth stage of our study, we examined the correlations between CXC chemokines and CXCR3 receptor variants in normal endometrium and endometrioid endometrial adenocarcinoma (Figs. 6 and 7 accordingly). This analysis helped us understand how the chemokine network is reorganized.

ΔCt data were used to determine CXCL-CXCL, CXCL-CXCR3, and CXCR3-CXCR3 correlations in normal endometrium and endometrioid endometrial adenocarcinoma. Correlations were calculated using Pearson's correlation coefficient (r).

In normal endometrium, a strong positive correlation was observed between CXCL4 and CXCL4L1 (r=0.708, p<0.05). Additionally, a moderate positive correlation was detected between CXCL11 and CXCR3B (r=0.534, p<0.05).

In endometrioid endometrial adenocarcinoma, a strong positive correlation was observed between CXCL9 and CXCL10 (r=0.759, p<0.05). Additionally, a moderate positive correlation was detected between CXCL9 and CXCR3A (r=0.431, p<0.05).

These correlation analyses demonstrate that the chemokine network is regulated differently in normal endometrium and endometrioid endometrial adenocarcinoma. In tumor tissue, it was observed that correlations between pro-inflammatory and pro-angiogenic chemokines were strengthened[18,24].

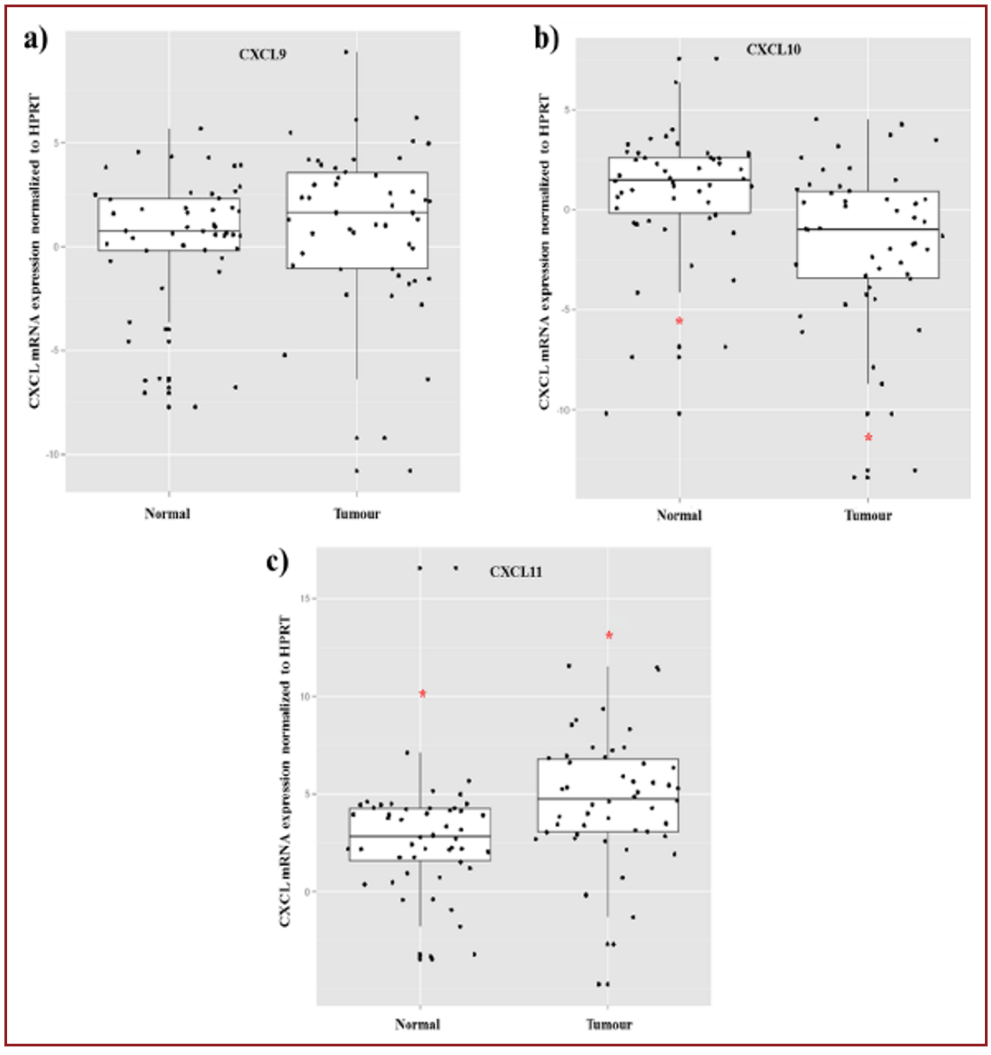

Figure 4. CXCL9/10/11 expression in normal endometrium and endometrioid endometrial adenocarcinoma. **(a)** CXCL9, **(b)** CXCL10, **(c)** CXCL11 expression levels. Data show ΔCt values normalized to HPRT. Boxes represent the interquartile range (IQR). Lines indicate the median value, and dots show outliers. Statistical analyses were performed using unpaired t-test. *p<0.05, **p<0.01, ***p<0.001.

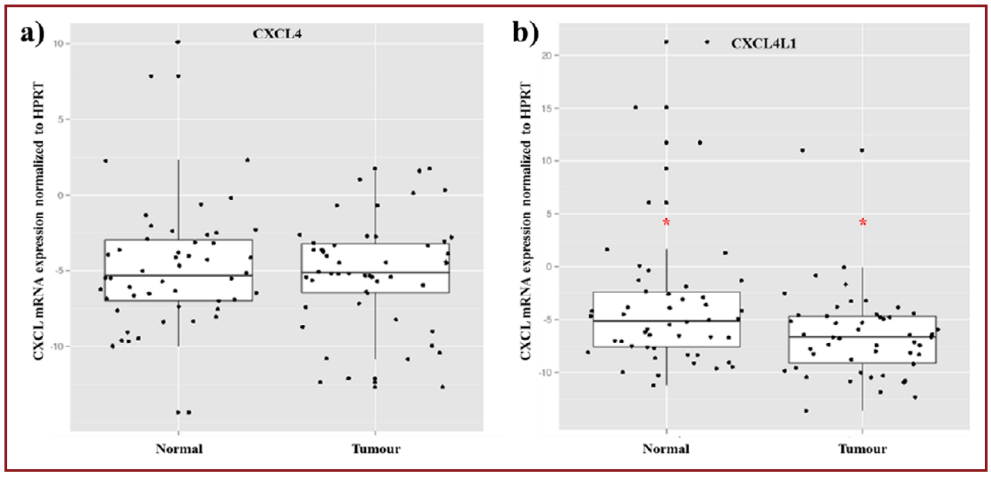

Figure 5. CXCL4/4L1 expression in normal endometrium and endometrioid endometrial adenocarcinoma. **(a)** CXCL4, **(b)** CXCL4L1 expression levels. Data show ΔCt values normalized to HPRT. Boxes represent the interquartile range (IQR). Lines indicate the median value, and dots show outliers. Statistical analyses were performed using unpaired t-test. *p<0.05, **p<0.01, ***p<0.001.

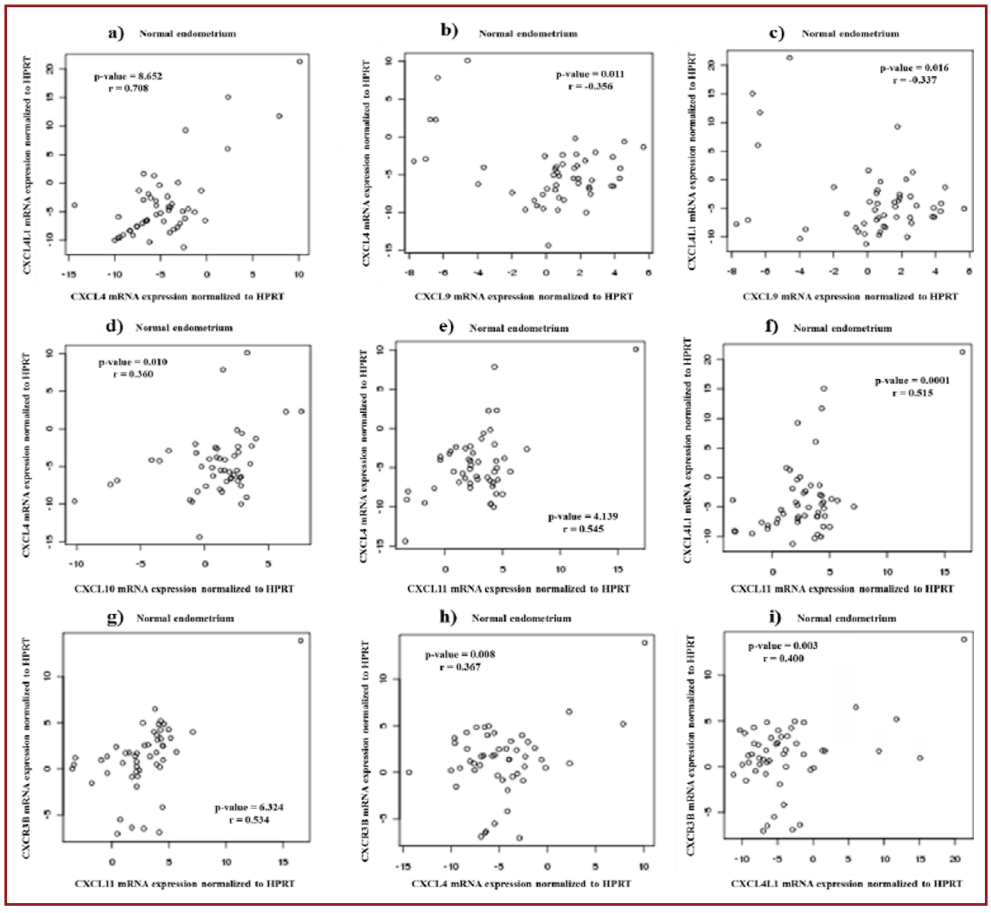

Figure 6. Chemokine correlations in normal endometrium. Each graph shows the correlation between two genes. r: Pearson correlation coefficient, p: significance value.

One-way ANOVA Analysis

Finally, we performed a one-way ANOVA analysis to examine the expression changes of CXC chemokines and CXCR3 receptor variants in more detail (Fig. 8).

2-ΔΔCt was calculated for 50 normal endometrium and 50 endometrioid endometrial adenocarcinoma samples, and fold changes in chemokine ligand or receptor expression were determined.

ANOVA analysis confirmed that CXCR3A and CXCR3-alt expression significantly increased in endometrioid endometrial adenocarcinoma ($p<0.05$). Among CXC chemokines, the expression of CXCL9 and CXCL11 was markedly increased in tumor tissue ($p<0.05$).

These results demonstrate that the expression of pro-inflammatory and potentially pro-tumorigenic CXC chemokines and CXCR3 receptor variants is increased in endometrioid endometrial adenocarcinoma. These changes may play an important role in reshaping the tumor microenvironment and disease progression[16,17,20].

These comprehensive analyses reveal that the CXC chemokine network is complexly reorganized in endometrioid endometrial adenocarcinoma. These findings may contribute to identifying potential therapeutic targets and better understanding the molecular mechanisms of the disease.

Conclusion

This study presents a comprehensive profile of the CXC chemokine network in normal endometrium and endometrioid endometrial adenocarcinoma. Our findings show that the expression balance of CXC chemokines and CXCR3 receptor variants changes significantly during the process of endometrial carcinogenesis.

Firstly, we observed significant changes in the expression patterns of CXCR3 receptor variants. We found that CXCR3A and CXCR3-alt expression significantly increased in endometrioid endometrial adenocarcinoma, while CXCR3B expression tended to decrease. These findings suggest that the pro-proliferative CXCR3A/CXCR3-alt axis becomes hyperactive in tumor tissue, while the angiostatic CXCR3B axis is suppressed.

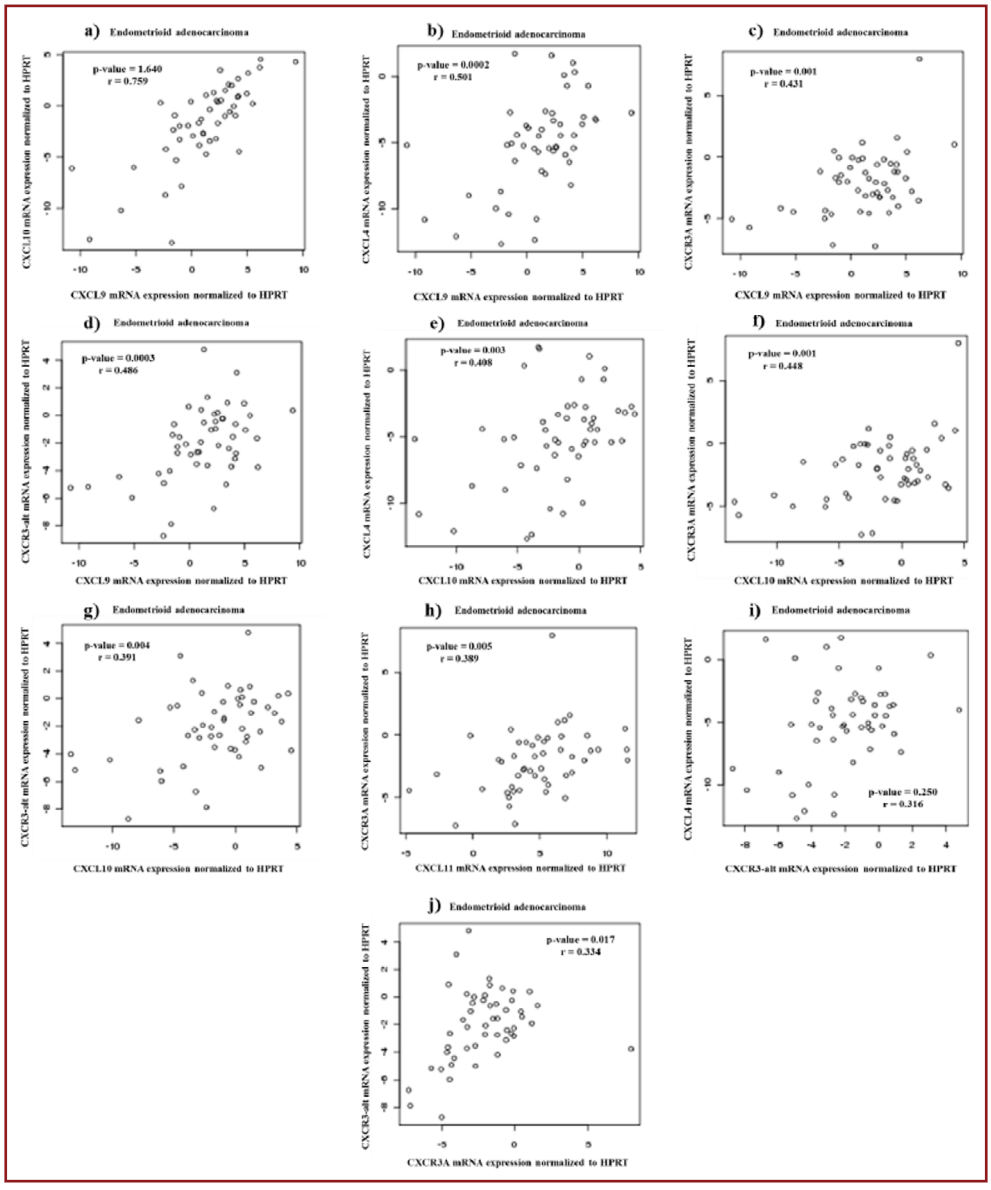

Figure 7. Chemokine correlations in endometrioid endometrial adenocarcinoma. Each graph shows the correlation between two genes. r: Pearson correlation coefficient, p: significance value.

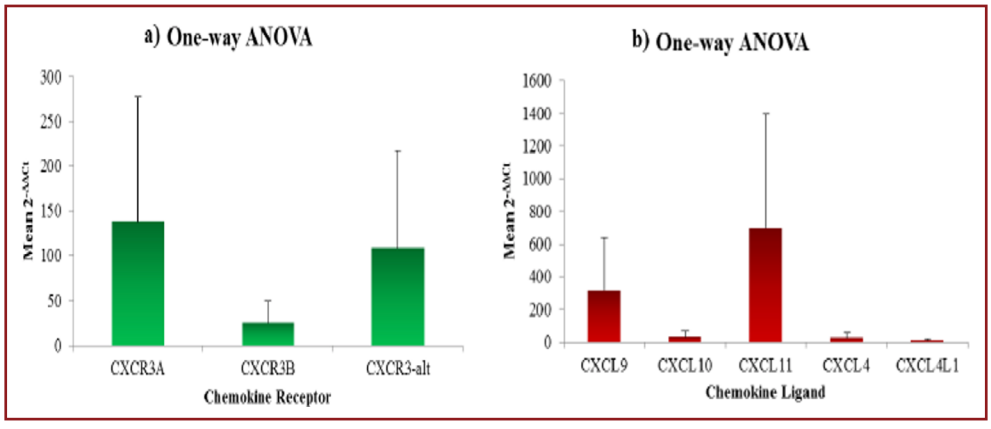

Figure 8. One-way ANOVA analysis for normal endometrium and endometrioid endometrial adenocarcinoma. **(a)** Fold change expressions for CXCR3 receptor variants, **(b)** Fold change expressions for CXCL chemokines. Bars represent mean±standard error. *p<0.05, **p<0.01, ***p<0.001.

We also observed significant changes in the expression profiles of CXC chemokines. We found that CXCL9 and CXCL11 increased in endometrioid endometrial adenocarcinoma, while CXCL10, CXCL4, and CXCL4L1 decreased. These results indicate that immune cell recruitment and angiogenesis processes are complexly regulated in tumor tissue.

Our correlation analyses revealed that the chemokine network is reorganized in normal endometrium and endometrioid endometrial adenocarcinoma. In particular, correlations between pro-inflammatory chemokines were observed to be strengthened in tumor tissue. This suggests that inflammatory processes may be increased in the tumor microenvironment.

Our ANOVA analysis confirmed the increase in CXCL9, CXCL11, CXCR3A, and CXCR3-alt expression in endometrioid endometrial adenocarcinoma. This finding indicates that pro-proliferative and pro-inflammatory processes become dominant in tumor tissue.

When these findings are evaluated together, we can conclude that the CXC chemokine network in endometrioid endometrial adenocarcinoma is reorganized to support tumor growth and spread. While the increase in CXCR3A and CXCR3-alt may enhance the proliferation and migration capacity of tumor cells, the decrease in angiostatic factors (CXCR3B, CXCL10, CXCL4, CXCL4L1) may promote tumor angiogenesis.

The results of our study suggest that CXC chemokines and CXCR3 receptor variants can be evaluated as potential prognostic biomarkers and therapeutic targets for endometrial cancer. In particular, strategies targeting CXCR3A and CXCR3-alt or increasing CXCR3B activity may offer new approaches in the treatment of endometrial cancer.

Future studies should confirm these findings at the protein level and examine the functional role of the CXC chemokine network in endometrial carcinogenesis using in vitro and in vivo models. Additionally, long-term follow-up studies should be conducted to evaluate the relationship between chemokine expression patterns and patient prognosis.

In conclusion, this study presents a comprehensive profile of the CXC chemokine network in endometrioid endometrial adenocarcinoma, contributing to a better understanding of the molecular mechanisms of the disease and the development of potential new treatment strategies.

Ethics Committee Approval: This study was conducted in accordance with the ethical standards of the Helsinki Declaration.

Peer-review: Externally peer-reviewed.

Use of AI for Writing Assistance: Not declared.

Authorship Contributions: Concept: H.G., R.D.; Design: H.G.; Supervision: R.D.; Fundings: R.D.; Materials: H.G., R.D.; Data Collection or Processing: H.G.; Analysis or Interpretation: H.G., R.D.; Literature Search: H.G.; Writing: H.G.; Critical Review: R.D.

Conflict of Interest: None declared.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7–30. [CrossRef]
2. Lortet-Tieulent J, Ferlay J, Bray F, Jemal A. International patterns and trends in endometrial cancer incidence, 1978-2013. J Natl Cancer Inst 2018;110:354–61. [CrossRef]
3. Crosbie EJ, Kitson SJ, McAlpine JN, Mukhopadhyay A, Powell ME, Singh N. Endometrial cancer. Lancet 2022;399:1412–28. [CrossRef]
4. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. Lancet 2016;387:1094–108. [CrossRef]
5. Matei D, Filiaci V, Randall ME, Mutch D, Steinhoff MM, DiSilvestro PA, et al. Adjuvant chemotherapy plus radiation for locally advanced endometrial cancer. N Engl J Med 2019;380:2317–26. [CrossRef]
6. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: Diagnosis, treatment and follow-up. Int J Gynecol Cancer 2016;26:2–30. [CrossRef]
7. Sorosky JI. Endometrial cancer. Obstet Gynecol 2012;120:383–97. [CrossRef]
8. Chow MT, Luster AD. Chemokines in cancer. Cancer Immunol Res 2014;2:1125–31. [CrossRef]
9. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. Nat Rev Immunol 2017;17:559–72. [CrossRef]
10. Strieter RM, Polverini PJ, Kunkel SL, Arenberg DA, Burdick MD, Kasper J, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 1995;270:27348–57. [CrossRef]
11. Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, et al. CXC chemokines in angiogenesis. J Leukoc Biol 2000;68:1–8. [CrossRef]
12. Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. J Exp Med 2003;197:1537–49. [CrossRef]
13. Billottet C, Quemener C, Bikfalvi A. CXCR3, a double-edged sword in tumor progression and angiogenesis. Biochim Biophys Acta 2013;1836:287–95. [CrossRef]
14. Ehlert JE, Addison CA, Burdick MD, Kunkel SL, Strieter RM. Identification and partial characterization of a variant of human CXCR3 generated by posttranscriptional exon skipping. J Immunol 2004;173:6234–40. [CrossRef]

15. Korniejewska A, McKnight AJ, Johnson Z, Watson ML, Ward SG. Expression and agonist responsiveness of CXCR3 variants in human T lymphocytes. Immunology 2011;132:503–15. [CrossRef]
16. Fulton AM. The chemokine receptors CXCR4 and CXCR3 in cancer. Curr Oncol Rep 2009;11:125–31. [CrossRef]
17. Vandercappellen J, Van Damme J, Struyf S. The role of CXC chemokines and their receptors in cancer. Cancer Lett 2008;267:226–44. [CrossRef]
18. Balkwill F. Cancer and the chemokine network. Nat Rev Cancer 2004;4:540–50. [CrossRef]
19. Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–6. [CrossRef]
20. Furuya M, Yoneyama T, Miyagi E, Tanaka R, Nagahama K, Miyagi Y, et al. Differential expression patterns of CXCR3 variants and corresponding CXC chemokines in clear cell ovarian cancers and endometriosis. Gynecol Oncol 2011;122:648–55. [CrossRef]
21. Kawada K, Sonoshita M, Sakashita H, Takabayashi A, Yamaoka Y, Manabe T, et al. Pivotal role of CXCR3 in melanoma cell metastasis to lymph nodes. Cancer Res 2004;64:4010–7. [CrossRef]
22. Guo Q, Gao BL, Zhang XJ, Liu GC, Xu F, Fan QY, et al. CXCL12-CXCR4 axis promotes proliferation, migration, invasion, and metastasis of ovarian cancer. Oncol Res 2014;22:247–58. [CrossRef]
23. Westrich JA, Vermeer DW, Colbert PL, Spanos WC, Pyeon D. The multifarious roles of the chemokine CXCL14 in cancer progression and immune responses. Mol Carcinog 2020;59:794–806. [CrossRef]
24. Zlotnik A, Yoshie O. Chemokines: A new classification system and their role in immunity. Immunity 2000;12:121–7. [CrossRef]