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Expressions of IL-8 and CXCL5 in uterine endometrioid carcinomas which have frequent neutrophil infiltration and comparison to colorectal adenocarcinoma

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Summary. In endometrioid carcinomas (ECs) of the uterine corpus, neutrophil accumulation within the carcinoma cell clusters is a representative microscopic finding. Because this accumulation is active, some sort of transmitter ought to exist between the EC cells and neutrophils. Interleukin-8 (IL-8) and C-X-C motif chemokine ligand 5 (CXCL5) is a cytokine that attracts neutrophils *in vivo*. In this study, we investigated IL-8, CXCL5 and C-X-C motif chemokine receptor 2 (CXCR2) (their chemokine receptor) expressions in ECs by immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR). There are few reports on the relationship between these chemokines and ECs. For comparison, we enrolled samples of colorectal adenocarcinoma (CRAC), it is another representative tumor with neutrophil infiltration. We analyzed 30 ECs and 30 CRACs. We confirmed IL-8 expression (H-score ≥50 points) in 40% of EC and 7% of CRAC samples; CXCL5 expression in 7% of EC and 10% of CRAC samples; CXCR2 expression in 83% of EC and 53% of CRAC samples by immunohistochemistry. We examined each mRNA (*IL-8* and *CXCL5*) expression of 3 representative EC and 3 CRAC samples. Finding IL-8 expression might indicate that this cytokine is important for the process of neutrophil accumulation, particularly within ECs. The participation of CXCL5 regarding neutrophil accumulation within their carcinoma cell clusters might be restrictive compared to IL-8.

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Introduction

Endometrial carcinoma is generally divided into two categories, type I and type II, based primitively on whether or not it is estrogenic, the distinction between these two categories is based on clinicopathologic factors such as age, obesity, para-gravidity, presence/absence of hyperplasia, histological type and molecular disorders; the conceptions of type I as a low grade cancer represented by G1/2 endometrioid carcinoma (EC) and type II as high-grade cancer represented by serous carcinoma (SC) and clear cell carcinoma (CCC) have generally been accepted; type I frequently shows abnormalities of *PTEN*, microsatellite instability attributed to defects in DNA mismatch repair, mutations in *β-catenin* and *K-Ras*, and type II is not associated with hormonal risk factors represented by estrogen receptor and progesterone receptor expression status (Yasuda, 2014). Furthermore, 4 groups were described based on integrated genomic architecture rather than single genetic mutations, which are (1) ultramutated/polymerase ε mutated, (2) hypermutated/

Abbreviations. EC, endometrioid carcinoma; SC, serous carcinoma; CCC, clear cell carcinoma; IL, interleukin; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; HCC, hepatocellular carcinoma; RT-PCR, reverse transcription polymerase chain reaction; CRAC, colorectal adenocarcinoma; HE, hematoxylin and eosin; FFPE, formalin-fixed, paraffin-embedded; PDAC, pancreatic ductal adenocarcinoma; EMT, epithelial-to-mesenchymal transition.

microsatellite instability, (3) low-copy number abnormalities and (4) high-copy number abnormalities; high-somatic-copy number abnormalities were seen in serous-like tumors and correspond broadly to type II, while the low-somatic-copy number tumors correspond to type I (Goebel et al., 2018). The majority of endometrial carcinomas are ECs accounting for more than 80% of uterine corpus cancers, and the populations of SC and CCC of the uterine corpus are minor compared to EC (Yasuda, 2014).

Neutrophil accumulation within carcinoma cell clusters in ECs of the uterine corpus is a representative microscopic finding. Because this accumulation is active, some sort of transmitter ought to exist between the EC cells and neutrophils. Interleukin-8 (IL-8) is a member of the CXC chemokine family and is an influential cytokine causing neutrophils to accumulate *in vivo*. The basic biological effect is the attraction and activation of neutrophils (Wang et al., 2015). IL-8 is also expressed by various human tumor cells, including breast cancer, colon cancer, ovarian cancer, cervical cancer, gastric cancer, lung cancer, prostate cancer, renal cell carcinoma, thyroid cancer and pancreatic cancer (Yasumoto et al., 1992; Green et al., 1997; König et al., 1999; Tjiong et al., 1999; Veltri et al., 1999; Brew et al., 2000; Ivarsson et al., 2000; Hussain et al., 2010; Chen et al., 2012). Meanwhile, C-X-C motif chemokine ligand 5 (CXCL5) is initially identified to recruit neutrophils by binding to C-X-C motif receptor 2 (CXCR2), and CXCL5, previously named as neutrophil-activating protein 78, consists of 114 amino acids and belongs to the CXC chemokine subfamily (Cui et al., 2018). Abnormal elevation of CXCL5 and/or CXCR2 has been observed in the cancer tissues, such as colorectal cancer (Kawamura et al., 2012; Zhao et al., 2017a,b), pancreatic adenocarcinoma (Li et al., 2011) and hepatocellular carcinoma (HCC) (Zhou et al., 2015).

We performed this study to clarify IL-8 and CXCL5 expressions in ECs of the uterine corpus using immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR). There are few reports on the relationship between these chemokines and EC. For comparison, we enrolled colorectal adenocarcinoma (CRAC) as a representative type of tumor with neutrophil infiltration (Rao et al., 2012; Wikberg et al., 2017). Furthermore, it has been reported in relation to IL-8 and CXCL5 expressions (Brew et al., 2000; Kawamura et al., 2012; Zhao et al., 2017b). Meanwhile, because CXCR2 (their chemokine receptor) expression in CRACs has been reported by some researchers, we performed CXCR2 expression as well by immunohistochemistry (Lee et al., 2012; Desurmont et al., 2015; Zhao et al., 2017a).

Materials and methods

Materials

We collected 30 ECs of the uterine corpus and 30 CRACs obtained surgically at the University of Yamanashi Hospital. Two pathologists (K.M. and T.K.) independently reviewed hematoxylin and eosin (HE) stained slides blinded to the original pathological diagnosis. The Research Ethics Committee of the Faculty of Medicine, University of Yamanashi approved this study (approval number: 2186).

Neutrophil accumulation

We evaluated neutrophil infiltration by a representative HE stained slide in each individual case. The HE stained slides were scanned at low magnification (100×). Then 2 pathologists (K.M. and T.K.) together selected carcinoma cell clusters having the highest density of distinctly highlighted neutrophil infiltration (‘hot spot’) within each section. They counted the neutrophils in each ‘hot spot’ within a 400× microscopic field of an Olympus BX53 (Tokyo, Japan) microscope. We defined more than 10 neutrophils as neutrophil accumulation.

Immunohistochemistry

Sections 4-µm thick were cut from formalin-fixed, paraffin-embedded (FFPE) tissue blocks that were dewaxed and rehydrated. This was followed by immunohistochemical staining performed on representative slides. IL-8 (Polyclonal, Invitrogen, Carlsbad, USA, dilution 1:200) was used as the primary antibody. After inhibiting endogenous peroxidase, we used a positive control (lung cancer) to perform the primary antibody reaction. CXCL5 (Polyclonal, Abcam plc, Cambridge, UK, dilution 1:200) was used as the primary antibody. We performed antigen retrieval through heat treatment: autoclaving at 121°C for 10 min in citrate buffer pH 6. After inhibiting endogenous peroxidase, we used a positive control (pancreas cancer) to perform the primary antibody reaction. CXCR2 (Polyclonal, Abcam plc, Cambridge, UK, dilution 1:200) was used as the primary antibody. After inhibiting endogenous peroxidase, we used a positive control (rectum cancer) to perform the primary antibody reaction. We used the N-Histofine Simple Stain MAX PO (MULTI) (Nichirei Biosciences, Tokyo, Japan) with diaminobenzidine as a chromogen and a light counterstain with hematoxylin to perform immuno-histochemistry. Two pathologists (K.M. and T.K.) simultaneously reviewed immuno-stained sections using a double-headed light microscope.

We used the H-score as immunohistochemical evaluation system, which is calculated by adding the multiplication of the different staining intensities in four gradations with each percentage of positive cells; the H-score was classified as 0=0 to 49 points, 1=50 to 99 points, 2=100 to 199 points, and 3=200 to 300 points, we also defined 1, 2 or 3 classified specimens as positive and sections classified 0 as negative (Specht et al., 2015).

Microdissection and extraction of RNA from paraffin embedded tissue

Two 10 μm thick serial sections were cut from routinely processed, FFPE tissue blocks. The tumor tissue (immunostained area) was microdissected with a disposable syringe needle and the nucleic acids extracted by standard procedures. To avoid sampling problems, we selected non-necrotic tumor tissue with a considerable number of tumor cells. We used the RNeasy FFPE Kit (QIAGEN, Hilden, German) to extract RNA from the microdissected tissue samples.

RT-PCR

Total RNA was reverse transcripted using iScript gDNA Clear cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). All RT reactions were performed in the iCycler Thermal Cycler (Bio-Rad). After the RT reaction, we amplified the cDNA corresponding to *IL-8* (primers: 5'-GGTGCAGTTTTGCCAAGGAG-3' and 5'-TTCCTTGGGGTCCAGACAGA-3'; product size: 183bp) and *CXCL5* (primers: 5'- GAGAGAGCT GCGTTGCGTTT -3' and 5'- TTCAGGGAGGCTA CCACTTC -3'; product size: 123bp) using HotStarTaq DNA Polymerase (QIAGEN, Hilden, German). Samples were denatured at 95°C for 15 min followed by 40 three-step cycles (95°C for 30 s, 58°C for 30 s and 72°C for 1 min), and then at 72°C for 10 min in the iCycler Thermal Cycler (Bio-Rad). We used the amplification of glyceraldehyde-3-phosphate dehydrogenase as a quality control for RNA integrity (primers: 5'-GATGACAT CAAGAAGGTGGTGA-3' and 5'-TTCGTTGTCATA CCAGGAAATG-3'; product size: 186bp). Amplified fragments were separated on an agarose gel and visualized by Midori Green Advance staining (NIPPON Genetics, Tokyo, Japan).

Statistical analysis

We used the Pearson's chi-square test. A p-value of less than 0.05 indicates statistical significance. Statistical analysis was carried out using the IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA).

Results

Neutrophil accumulation

Results of neutrophil accumulation are summarized in Table 1. It was confirmed in 63% of ECs of the uterine corpus and 87% of CRACs.

IL-8 immunostaining in ECs of the uterine corpus and CRACs

Results of immunohistochemical studies are summarized in Table 2. ECs of the uterine corpus showed the following immunostaining patterns: 60% classified 0, 37% classified 1, 3% classified 2, and 0% classified 3 (Fig. 1C). CRAC showed the following immunostaining patterns: 93% classified 0, 3% classified 1, 3% classified 2, and 0% classified 3 (Fig. 2C). Using the two-tailed Pearson's chi-square test, EC of the uterine corpus and CRAC cases were significantly different in IL-8 immunostaining expression (p=0.005).

CXCL5 immunostaining in ECs of the uterine corpus and CRACs

Results of immunohistochemical studies are summarized in Table 2. ECs of the uterine corpus showed the following immunostaining patterns: 93% classified 0, 3% classified 1, 3% classified 2, and 0% classified 3 (Fig. 1D). CRACs showed the following immunostaining patterns: 90% classified 0, 7% classified 1, 3% classified 2, and 0% classified 3 (Fig. 2D). Using the two-tailed Pearson's chi-square test, EC of the uterine corpus and CRAC cases were not significantly different in CXCL5 immunostaining expression.

CXCR2 immunostaining in ECs of the uterine corpus and CRACs

Results of immunohistochemical studies are summarized in Table 2. ECs of the uterine corpus

Table 1. Neutrophil accumulation of in 30 ECs of the uterine corpus and 30 CRACs.

Tumor type	Neutrophil accumulation (%)
EC (n=30)	19 (63%)
CRAC (n=30)	26 (87%)

EC, endometrioid carcinoma; CRAC, colorectal adenocarcinoma.

Table 2. Expressions of IL-8, CXCL5 and CXCR2 in 30 ECs of the uterine corpus and 30 CRACs.

Tumor type		H-score (Classification)*				p-value**
		0	1	2	3	
IL-8	EC (n=30)	18	11	1	0	0.005
	CRAC (n=30)	28	1	1	0	
CXCL5	EC (n=30)	28	1	1	0	0.839
	CRAC (n=30)	27	2	1	0	
CXCR2	EC (n=30)	5	15	10	0	0.017
	CRAC (n=30)	14	13	3	0	

IL-8, interleukin-8; CXCL5, C-X-C motif chemokine ligand 5; CXCR2, C-X-C chemokine receptor 2; EC, endometrioid carcinoma; CRAC, colorectal adenocarcinoma. *0=0 to 49 points; 1=50 to 99 points; 2=100 to 199 points; 3=200 to 300 points. **Pearson's chi-square test.

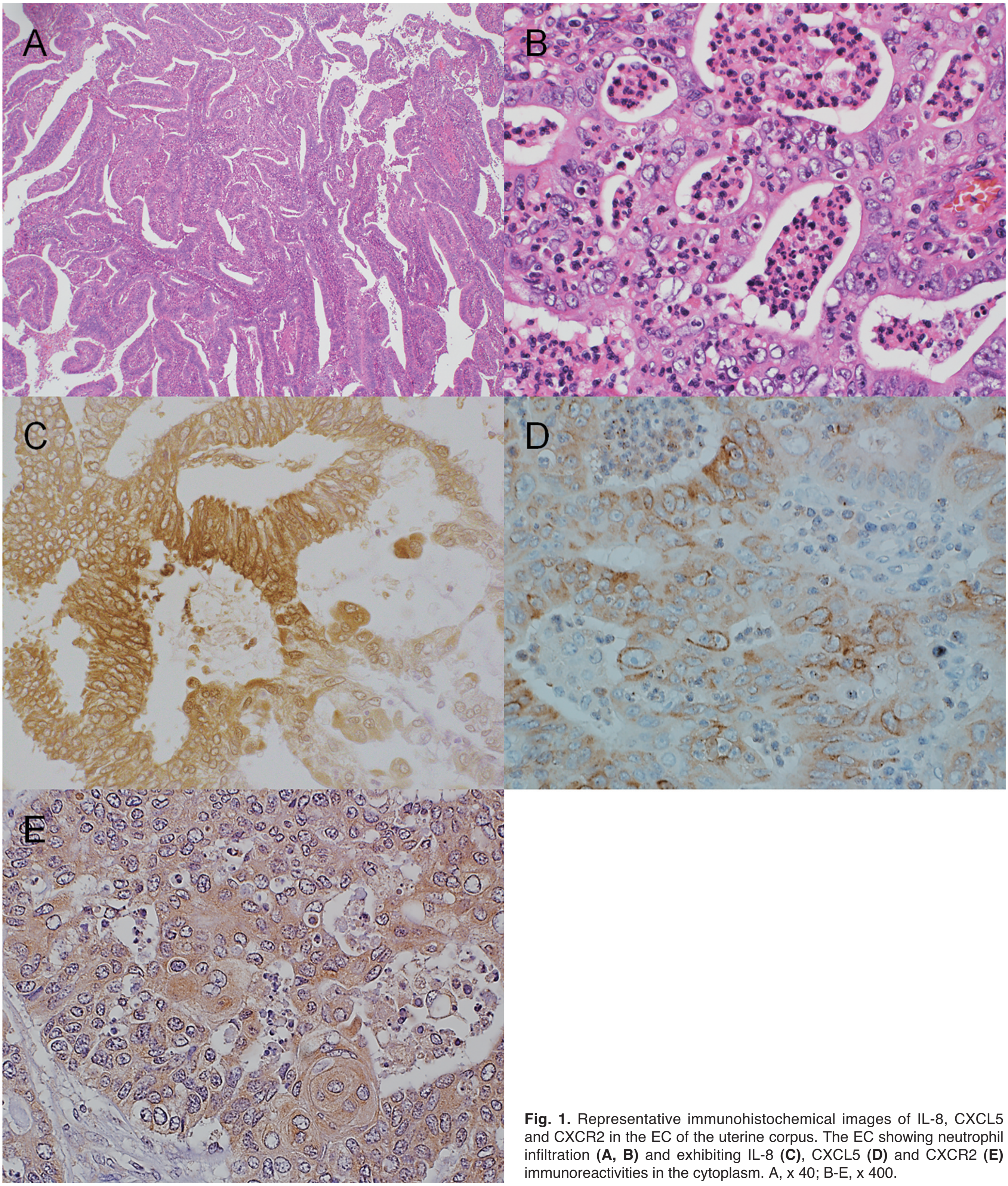

Fig. 1. Representative immunohistochemical images of IL-8, CXCL5 and CXCR2 in the EC of the uterine corpus. The EC showing neutrophil infiltration **(A, B)** and exhibiting IL-8 **(C)**, CXCL5 **(D)** and CXCR2 **(E)** immunoreactivities in the cytoplasm. A, x 40; B-E, x 400.

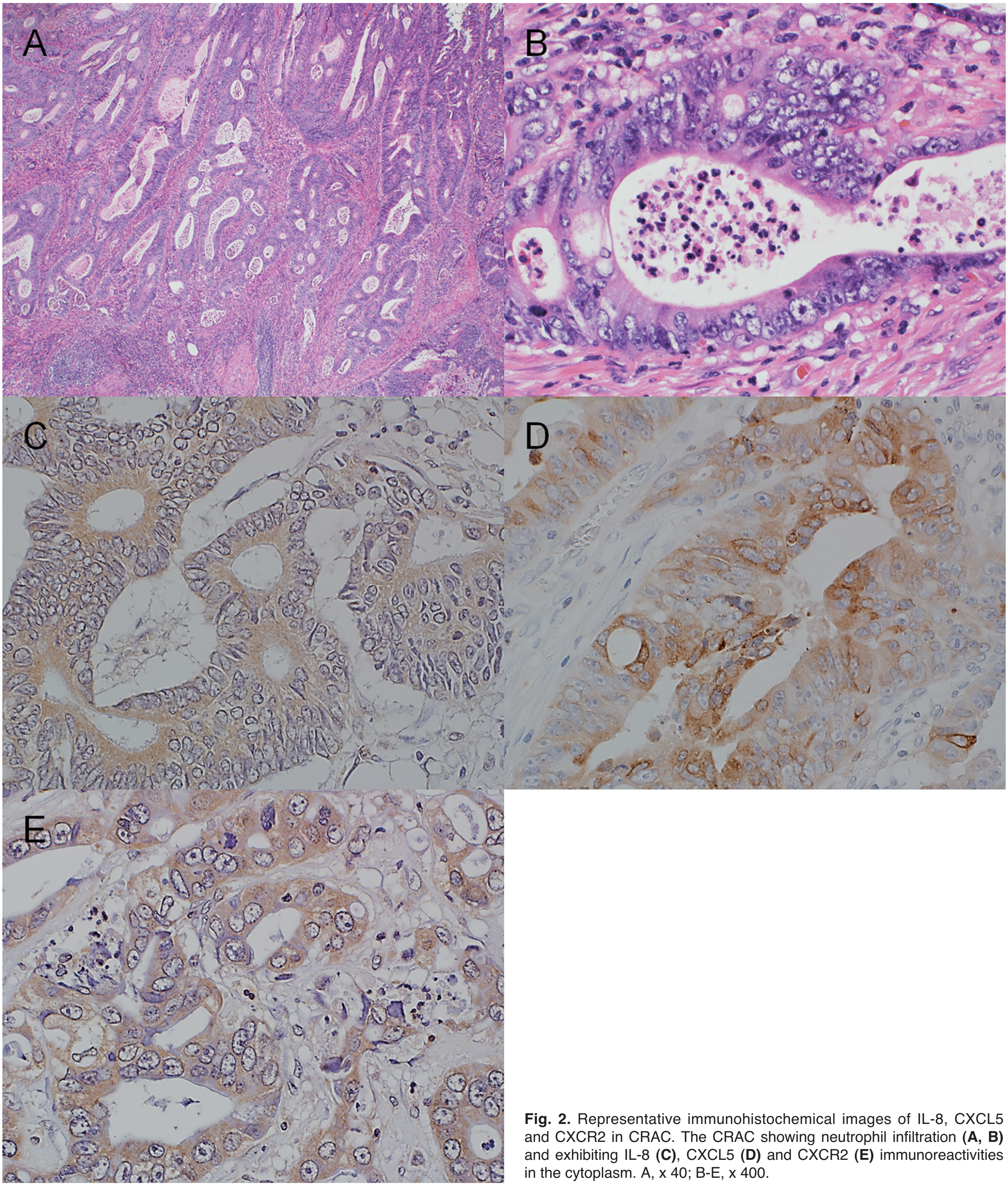

Fig. 2. Representative immunohistochemical images of IL-8, CXCL5 and CXCR2 in CRAC. The CRAC showing neutrophil infiltration **(A, B)** and exhibiting IL-8 **(C)**, CXCL5 **(D)** and CXCR2 **(E)** immunoreactivities in the cytoplasm. A, x 40; B-E, x 400.

showed the following immunostaining patterns: 17% classified 0, 50% classified 1, 33% classified 2, and 0% classified 3 (Fig. 1E). CRACs showed the following immunostaining patterns: 47% classified 0, 43% classified 1, 10% classified 2, and 0% classified 3 (Fig. 2E). Using the two-tailed Pearson's chi-square test, EC of the uterine corpus and CRAC cases were significantly different in CXCR2 immunostaining expression (p=0.017).

Relation to between neutrophil accumulation and IL-8/CXCR2 or CXCL5/CXCR2 immunostaining in ECs of the uterine corpus and CRACs

Results are summarized in Table 3. Using the two-tailed Pearson's chi-square test, the presence of neutrophil accumulation was not significantly different in IL-8/CXCR2 or CXCL5/CXCR2 immunostaining expression.

Table 3. The relation between NA and immunoexpression of IL-8/CXCR2 or CXCL5/CXCR2 in 30 ECs of the uterine corpus and 30 CRACs.

	EC (n=30)			CRAC (n=30)		
	NA (+)	NA (-)	p-value*	NA (+)	NA (-)	p-value*
IL-8 (+)/CXCR2 (+)	8	4	0.560	2	0	0.457
IL-8 (+)/CXCR2 (-)	0	0		0	0	
IL-8 (-)/CXCR2 (+)	7	6		11	3	
IL-8 (-)/CXCR2 (-)	4	1		13	1	
CXCL5 (+)/CXCR2 (+)	1	0	0.648	1	1	0.415
CXCL5 (+)/CXCR2 (-)	1	0		1	0	
CXCL5 (-)/CXCR2 (+)	14	10		12	2	
CXCL5 (-)/CXCR2 (-)	3	1		12	1	

NA, neutrophil accumulation; IL-8, interleukin-8; CXCR2, C-X-C chemokine receptor 2; CXCL5, C-X-C motif chemokine ligand 5; EC, endometrioid carcinoma; CRAC, colorectal adenocarcinoma. *Pearson's chi-square test.

IL-8 and CXCL5 mRNA expressions in ECs of the uterine corpus and CRACs by RT-PCR

We examined *IL-8* and *CXCL5* mRNA expressions in 3 representative EC of the uterine corpus and 3 CRAC samples each having immunostained areas (Fig. 3).

Discussion

Hijacking the immune system is a very common strategy that cancers utilize to benefit their long-term growth and survival against locally limited resources such as decreased oxygen in solid tumor tissues. To overcome this shortage of resources, cancer cells express various cytokines, growth factors, and receptors for cytokines and growth factors to become independent of the mitogens that are supplied other than themselves (Liou, 2017).

We confirmed IL-8 expression (H-score ≥50 points) in 40% of EC of the uterine corpus and 7% of CRAC samples. The IL-8 immunostaining was significantly different between the ECs and CRACs (p=0.005), which might show that the ECs have IL-8 productivity exceeding that of CRACs. Browne et al. showed a low intensity expression of IL-8 in 60% of 10 ovarian EC cases by immunohistochemistry (Browne et al., 2013). These findings taken together might indicate that IL-8 plays an important role in neutrophil accumulation, particularly within ECs of the uterine corpus. Meanwhile, Shi et al. showed that elevated expression of IL-8 in pancreatic ductal adenocarcinoma (PDAC) tumors under hypoxic conditions was associated with the cancer's metastatic ability in xenograft mice (Shi et al., 1999). Ning et al. used cell lines and animal models to show that overexpression of IL-8 is associated with progression, angiogenesis and chemoresistance in colon cancer (Ning et al., 2011). Cui et al. showed a higher tissue *IL-8* mRNA in colorectal cancers compared to colorectal adenomas. These levels were related to Dukes' stages suggesting that increased IL-8 may be an

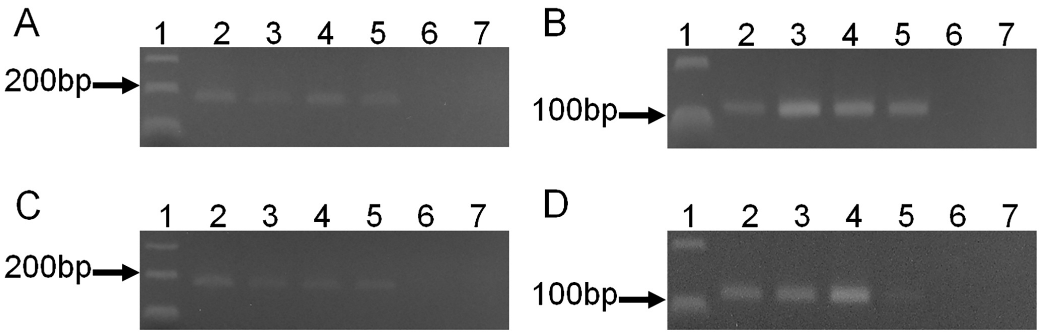

Fig. 3. Representative results of IL-8 **(A)** and CXCL5 **(B)** mRNA detected by RT-PCR analysis in 3 EC samples having their respective immunostained areas. Representative results of IL-8 **(C)** and CXCL5 **(D)** mRNA detected by RT-PCR analysis in 3 CRAC samples having their respective immunostained areas. Lane 1 is DNA size markers (100 bp ladder). Lane 2 is a positive control. Positive bands are shown in all cases (Lanes 3-5). Lane 6 is a negative control (water). Lane 7 is a negative control without reverse transcriptase (tissue of Lane 5).

important contributor to cancer progression (Cui et al., 2009). We showed that neutrophil accumulation was frequently confirmed in the ECs (63%) and CRACs (87%). Although these reports seem to indicate that EC and CRAC cells might promote neutrophil accumulation as a secondary effect rather than as a primary purpose, it should be noted that neutrophils can actively promote cancer growth. Fang et al. summarized the functions of neutrophils, which induce mutation of cancer suppressive genes, secrete cytokines and enzymes that promote the growth, metastasis and vascular infiltration of malignant cells and can produce reactive oxygen species, nitric oxide and arginase and suppress cytotoxic activity of lymphocytes, natural killer cells and activated T cells (Fang et al., 2018). Furthermore, Grosse-Steffen et al. showed that neutrophil-derived elastase cleaves E-cadherin in PDAC cell lines, and infiltrating neutrophils correlated with tumor cell expression of the epithelial-to-mesenchymal transition (EMT) marker ZEB1 (Grosse-Steffen et al., 2012). Galdiero et al. in a review article reported that epidemiological evidence suggests neutrophil infiltration within human cancers may be associated with a poor clinical outcome as observed in patients with metastatic and localized clear cell renal cell carcinomas, bronchioloalveolar carcinoma, HCC, colorectal carcinoma and head and neck squamous cell carcinoma. In addition, neutrophil infiltration has been correlated with tumor grade in human gliomas and with more aggressive types of pancreatic tumors (Galdiero et al., 2013). These reports indicate that carcinoma cells might initiate and attract neutrophils for their own tumor progression. Furthermore, Fu et al. indicated that combination therapy targeting IL-6 and IL-8 signaling activity can inhibit cell viability, colony-forming activity and cell migration of PDAC cells (Fu and Lin, 2018). This suggests IL targeting might be a future therapy for malignant tumors.

We confirmed CXCL5 expression (H-score ≥50 points) in 7% of EC of the uterine corpus and 10% of CRAC samples, they were a low rate compared to IL-8 expression, the CXCL5 immunostaining was not significantly different between the ECs and CRACs. This may indicate that the participation of CXCL5 regarding neutrophil accumulation within carcinoma cell clusters in their carcinomas is restrictive. Nevertheless, recent studies have indicated that CXCL5 also contributed to carcinogenesis, proliferation, migration and invasion of cancer cells, Cui et al. demonstrated that the activation of β-catenin signaling contributed to the invasion- and EMT-promoting effects of CXCL5-CXCR2 axis in papillary thyroid carcinoma cells (Zhou et al., 2015; Zhao et al., 2017b; Cui et al., 2018).

On the other hand, we confirmed a high rate of CXCR2 expression (H-score ≥50 points) in 83% of EC of the uterine corpus and 53% of CRAC samples by immunohistochemistry, which might show that the autocrine system of IL-8 and CXCL5 produced by these tumor cells exists. Meanwhile, because the CXCR2 immunostaining was significantly different between the ECs and CRACs (p=0.017), this might show that the dependency on the system in the ECs is higher compared to CRACs. Nevertheless, because CXCR2 ligands include CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and IL-8 (CXCL8), further studies would be needed in order to understand correctly the system (Desurmont et al., 2015).

Although the number of cases was small, the presence of neutrophil accumulation was not significantly different in IL-8/CXCR2 or CXCL5/CXCR2 immunostaining expression in ECs and CRACs. Further studies using more cases would be needed in order to examine these exact relevancies.

In conclusion, our results showed that IL-8 expressed at a relatively high rate particularly in ECs of the uterine corpus. This might suggest that IL-8 is an important cytokine for attracting neutrophils into this tumor. The participation of CXCL5 regarding neutrophil accumulation within their carcinoma cell clusters might be restrictive compared to IL-8.

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References

Brew R., Erikson J.S., West D.C., Kinsella A.R., Slavin J. and Christmas S.E. (2000). Interleukin-8 as an autocrine growth factor for human colon carcinoma cells *in vitro.* Cytokine 12, 78-85.

Browne A., Sriraksa R., Guney T., Rama N., Van Noorden S., Curry E., Gabra H., Stronach E. and El-Bahrawy M. (2013). Differential expression of IL-8 and IL-8 receptors in benign, borderline and malignant ovarian epithelial tumours. Cytokine 64, 413-421.

Chen Y., Shi M., Yu G.Z., Qin X.R., Jin G., Chen P. and Zhu M.H. (2012). Interleukin-8, a promising predictor for prognosis of pancreatic cancer. World J. Gastroenterol. 18, 1123-1129.

Cui D., Zhao Y. and Xu J. (2018). Activated CXCL5-CXCR2 axis promotes the migration, invasion and EMT of papillary thyroid carcinoma cells viamodulation of β-catenin pathway. Biochimie 148, 1-11.

Cui G., Yuan A., Goll R., Vonen B. and Florholmen J. (2009). Dynamic changes of interleukin-network along the colorectal adenoma-carcinoma sequence. Cancer Immunol. Immunother. 58, 1897-1905.

Desurmont T., Skrypek N., Duhamel A., Jonckheere N., Millet G., Leteurtre E., Gosset P., Duchene B., Ramdane N., Hebbar M., Van Seuningen I., Pruvot F.R., Huet G. and Truant S. (2015). Overexpression of chemokine receptor CXCR2 and ligand CXCL7 in liver metastases from colon cancer is correlated to shorter disease-free and overall survival. Cancer Sci. 106, 262-269.

Fang L.P., Xu X.Y., Ji Y. and Huang P.W. (2018). The prognostic value of preoperative neutrophil-to-lymphocyte ratio in resected patients with pancreatic adenocarcinoma. World J. Surg. 42, 3736-3745.

Fu S. and Lin J. (2018). Blocking interleukin-6 and interleukin-8 signaling inhibits cell viability, colony-forming activity, and cell migration in human triple-negative breast cancer and pancreatic

cancer cells. Anticancer Res. 38, 6271-6279.
Galdiero M.R., Bonavita E., Barajon I., Garlanda C., Mantovani A. and Jaillon S. (2013). Tumor associated macrophages and neutrophils in cancer. Immunobiology 218, 1402-1410.
Goebel E.A., Vidal A., Matias-Guiu X. and Blake Gilks C. (2018). The evolution of endometrial carcinoma classification through application of immunohistochemistry and molecular diagnostics: past, present and future. Virchows Arch. 472, 885-896.
Green A.R., Green V.L., White M.C. and Speirs V. (1997). Expression of cytokine messenger RNA in normal and neoplastic human breast tissue: identification of interleukin-8 as a potential regulatory factor in breast tumours. Int. J. Cancer 72, 937-941.
Grosse-Steffen T., Giese T., Giese N., Longerich T., Schirmacher P., Hänsch G.M. and Gaida M.M. (2012). Epithelial-to-mesenchymal transition in pancreatic ductal adenocarcinoma and pancreatic tumor cell lines: the role of neutrophils and neutrophil-derived elastase. Clin. Dev. Immunol. 2012, 720768.
Hussain F., Wang J., Ahmed R., Guest S.K., Lam E.W., Stamp G. and El-Bahrawy M. (2010). The expression of IL-8 and IL-8 receptors in pancreatic adenocarcinomas and pancreatic neuroendocrine tumours. Cytokine 49, 134-140.
Ivarsson K., Ekerydh A., Fyhr I.M., Janson P.O. and Brännström M. (2000). Upregulation of interleukin-8 and polarized epithelial expression of interleukin-8 receptor A in ovarian carcinomas. Acta Obstet. Gynecol. Scand. 79, 777-784.
Kawamura M., Toiyama Y., Tanaka K., Saigusa S., Okugawa Y., Hiro J., Uchida K., Mohri Y., Inoue Y. and Kusunoki M. (2012). CXCL5, a promoter of cell proliferation, migration and invasion, is a novel serum prognostic marker in patients with colorectal cancer. Eur. J. Cancer 48, 2244-2251.
König B., Steinbach F., Janocha B., Drynda A., Stumm M., Philipp C., Allhoff E.P. and König W. (1999). The differential expression of proinflammatory cytokines IL-6, IL-8 and TNF-alpha in renal cell carcinoma. Anticancer Res. 19, 1519-1524.
Lee Y.S., Choi I., Ning Y., Kim N.Y., Khatchadourian V., Yang D., Chung H.K., Choi D., LaBonte M.J., Ladner R.D., Nagulapalli Venkata K.C., Rosenberg D.O., Petasis N.A., Lenz H.J. and Hong Y.K. (2012). Interleukin-8 and its receptor CXCR2 in the tumour microenvironment promote colon cancer growth, progression and metastasis. Br. J. Cancer 106, 1833-1841.
Li A., King J., Moro A., Sugi M.D., Dawson D.W., Kaplan J., Li G., Lu X., Strieter R.M., Burdick M., Go V.L., Reber H.A., Eibl G. and Hines O.J. (2011). Overexpression of CXCL5 is associated with poor survival in patients with pancreatic cancer. Am. J. Pathol. 178, 1340-1349.
Liou G.Y. (2017). Inflammatory cytokine signaling during development of pancreatic and prostate cancers. J. Immunol. Res. 2017, 7979637.
Ning Y., Manegold P.C., Hong Y.K., Zhang W., Pohl A., Lurje G., Winder T., Yang D., LaBonte M.J., Wilson P.M., Ladner R.D. and Lenz H.J. (2011). Interleukin-8 is associated with proliferation, migration, angiogenesis and chemosensitivity *in vitro* and *in vivo* in colon cancer cell line models. Int. J. Cancer 128, 2038-2049.
Rao H.L., Chen J.W., Li M., Xiao Y.B., Fu J., Zeng Y.X., Cai M.Y. and Xie D. (2012). Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis. PLoS One 7, e30806.
Shi Q., Abbruzzese J.L., Huang S., Fidler I.J., Xiong Q. and Xie K. (1999). Constitutive and inducible interleukin 8 expression by hypoxia and acidosis renders human pancreatic cancer cells more tumorigenic and metastatic. Clin. Cancer Res. 5, 3711-3721.
Specht E., Kaemmerer D., Sänger J., Wirtz R.M., Schulz S. and Lupp A. (2015). Comparison of immunoreactive score, HER2/neu score and H score for the immunohistochemical evaluation of somatostatin receptors in bronchopulmonary neuroendocrine neoplasms. Histopathology 67, 368-377.
Tjiong M.Y., van der Vange N., ten Kate F.J., Tjong-A-Hung S.P., ter Schegget J., Burger M.P. and Out T.A. (1999). Increased IL-6 and IL-8 levels in cervicovaginal secretions of patients with cervical cancer. Gynecol. Oncol. 73, 285-291.
Veltri R.W., Miller M.C., Zhao G., Ng A., Marley G.M., Wright G.L. Jr., Vessella R.L. and Ralph D. (1999). Interleukin-8 serum levels in patients with benign prostatic hyperplasia and prostate cancer. Urology 53, 139-147.
Wang J., Wang Y., Wang S., Cai J., Shi J., Sui X., Cao Y., Huang W., Chen X., Cai Z., Li H., Bardeesi A.S., Zhang B., Liu M., Song W., Wang M. and Xiang A.P. (2015). Bone marrow-derived mesenchymal stem cell-secreted IL-8 promotes the angiogenesis and growth of colorectal cancer. Oncotarget 6, 42825-42837.
Wikberg M.L., Ling A., Li X., Öberg Å., Edin S. and Palmqvist R. (2017). Neutrophil infiltration is a favorable prognostic factor in early stages of colon cancer. Hum. Pathol. 68, 193-202.
Yasuda M. (2014). Immunohistochemical characterization of endometrial carcinomas: endometrioid, serous and clear cell adenocarcinomas in association with genetic analysis. J. Obstet. Gynaecol. Res. 40, 2167-2176.
Yasumoto K., Okamoto S., Mukaida N., Murakami S., Mai M. and Matsushima K. (1992). Tumor necrosis factor alpha and interferon gamma synergistically induce interleukin 8 production in a human gastric cancer cell line through acting concurrently on AP-1 and NF-kB-like binding sites of the interleukin 8 gene. J. Biol. Chem. 267, 22506-22511.
Zhao J., Ou B., Feng H., Wang P., Yin S., Zhu C., Wang S., Chen C., Zheng M., Zong Y., Sun J. and Lu A. (2017a). Overexpression of CXCR2 predicts poor prognosis in patients with colorectal cancer. Oncotarget 8, 28442-28454.
Zhao J., Ou B., Han D., Wang P., Zong Y., Zhu C., Liu D., Zheng M., Sun J., Feng H. and Lu A. (2017b). Tumor-derived CXCL5 promotes human colorectal cancer metastasis through activation of the ERK/Elk-1/Snail and AKT/GSK3β/β-catenin pathways. Mol. Cancer 16, 70.
Zhou S.L., Zhou Z.J., Hu Z.Q., Li X., Huang X.W., Wang Z., Fan J., Dai Z. and Zhou J. (2015). CXCR2/CXCL5 axis contributes to epithelial-mesenchymal transition of HCC cells through activating PI3K/Akt/GSK-3β/Snail signaling. Cancer Lett. 358, 124-135.

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