RESEARCH ARTICLE

CXCL12-CXCR4 Promotes Proliferation and Invasion of Pancreatic Cancer Cells

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Abstract

Objective: CXCL12 exerts a wide variety of chemotactic effects on cells. Evidence indicates that CXCL12, in conjunction with its receptor, CXCR4, promotes invasion and metastasis of tumor cells. Our objective was to explore whether the CXCL12-CXCR4 biological axis might influence biological behavior of pancreatic cancer cells. Methods: Miapaca-2 human pancreatic cancer cells were cultured under three different conditions: normal medium (control), medium + recombinant CXCL12 (CXCL12 group), or medium + CXCR4-inhibitor AMD3100 (AMD3100 group). RT-PCR was applied to detect mRNA expression levels of CXCL12, CXCR4, matrix metalloproteinase 2 (MMP-2), MMP-9, and human urokinase plasminogen activator (uPA). Additionally, cell proliferation and invasion were performed using CCK-8 colorimetry and transwell invasion assays, respectively. Results: CXCL12 was not expressed in Miapaca-2 cells, but CXCR4 was detected, indicating that these cells are capable of receiving signals from CXCL12. Expression of extracellular matrix-degrading enzymes MMP-2, MMP-9, and uPA was upregulated in cells exposed to exogenous CXCL12 ($P<0.05$). Additionally, both proliferation and invasion of pancreatic cancer cells were enhanced in the presence of exogenous CXCL12, but AMD3100 intervention effectively inhibited these processes ($P<0.05$). Conclusions: The CXCL12-CXCR4 biological axis plays an important role in promoting proliferation and invasion of pancreatic cancer cells.

Keywords: CXCL12 - CXCR4 - AMD3100 - pancreatic cancer - proliferation - invasion

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Introduction

Pancreatic cancer is highly malignant and often displays early metastasis and poor prognosis. A lack of specific symptoms makes early diagnosis difficult. For most patients with pancreatic cancer, at the time of diagnosis the local tumor is associated with wide invasion and distant metastasis; this advanced disease at diagnosis means the best window for treatment has already been missed (Stathis and Moore, 2010). Indeed, despite active treatment measures, e.g., surgery, radiotherapy, and chemotherapy, five-year survival rate of patients with pancreatic cancer remains less than 5% (Hidalgo, 2010). Therefore, further studies on the mechanisms of invasion and metastasis should be encouraged to explore early diagnosis and treatment of pancreatic cancer, and ultimately, to prolong survival time.

CXCL12, also known as stromal derived factor-1 (SDF-1), is a chemokine expressed in many tissues, including brain, heart, spleen, lung, liver, kidney, bone marrow, and thymus (Tashiro et al.,1993). CXCL12 exerts strong chemotactic effects on lymphocytes and plays important roles in immunologic system. The CXCL12 receptor is CXCR4, a G protein-coupled receptor expressed in most tissues (Nagasawa et al., 1996; Scotton et al., 2002). CXCR4 is detectable on the surface of lymphocytes, endothelial, and many cancer cells (Moll and Ransohoff, 2010). The interaction of CXCL12 and CXCR4 constitutes a molecular pair that is closely associated with inter-cellular signaling, cell invasion, and activation of downstream signaling (Salvatore et al., 2010; Terasaki et al., 2011).

Previous hypotheses proposed that the CXCL12-CXCR4 biological axis was involved in the generation, migration, and homing of lymphocytes and in mediating formation of vascular endothelium and heart during embryonic development (Ghosh et al., 2006; Fernadis et al., 2003). However, more recently, this axis has been found to not only contribute to immunoregulation and mediatiation of the normal inflammatory response, but also play an important role in human immunological diseases, especially in mediating HIV infection (Pitcher et al., 2010; Patrussi and Baldari, 2011). Increasingly, studies have been devoted to uncovering the effects of CXCL12-CXCR4 on behavior of tumor cells, especially on proliferation, differentiation, directional migration,

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infiltration, and invasion of malignant cells (Horuk, 2001; Dömötör et al., 2005).

Because pancreatic cancer has a high rate of lymph node metastasis, strong invasiveness, a high degree of malignancy, and significant biological characteristics, recent efforts have focused on identifying potential effects of CXCL12-CXCR4 in these tumor cells (Demetter et al., 2012). One study found low CXCL12 expression but high CXCR4 expression in pancreatic tumors, and expression of these proteins in nearby lymph nodes was an indicator of more advanced disease (Zhong et al., 2012). Further research into the CXCL12-CXCR4 axis may provide a better understanding of the progression of this disease, which is critical to improving detection and treatment measures.

Materials and Methods

Materials

Miapaca-2 human pancreatic cancer cell line was purchased from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences; high-glucose DMEM medium and standard calf serum were purchased from Gibco (USA). Recombinant human chemokine 12 (CXCL12), CCK-8 reagent kit, and AMD3100 were purchased from Sigma (USA). Total RNA extraction kits (RNAfast200) were purchased from Fastagen Biotechnology (Shanghai); reverse transcription kits were purchased from TaKaRa (Japan). PCR primers were synthesized by Shanghai Bioengineering & Technology Services. Millicell small chambers were purchased from Millipore (USA); Matrigel and MTS kits were purchased from BD Biosciences (USA). PCR amplification apparatus was produced by Gene Company; all primers in RT-PCR were designed and synthesized by Beijing Aoke.

Cell culture

Miapaca-2 cells were cultured in high-glucose DMEM medium containing 100 mL/L calf serum in a 37ºC, 5% CO_2 incubator. Cells in logarithmic growth phase were used for the experiment, and randomly divided into three treatment groups: blank control group (i.e., serum-free medium group containing 5mg/L BSA), CXCL12 group (i.e., serum-free medium containing 5mg/L BSA and 100 ng/mL recombinant CXCL12), and AMD3100 group (i.e., serum-free medium containing 5mg/L BSA and 2 μg/mL CXCR4 receptor-specific blocker AMD3100). Cells in each group were cultured 24 hours. Each well was in triplicate; the experiment was repeated three times.

RT-PCR

Total RNA extraction kits were used according to manufacturer's instructions. cDNA was synthesized using 1 μg RNA and reverse transcription kits. Reverse transcription product (1 μL) was used as PCR template in 12.5 μL PCR Mix with 1 μL of each primer and 9.5 μL ddH2O (25 μL reaction volume). PCR primer sequences for the following genes are listed in Table 1: β-actin, CXCL12, CXCR4, MMP-2, MMP-9, uPA. PCR conditions were as follows: 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, followed by one cycle at 72°C for 5 minutes. Amplification products were separated on 2% agarose gels and photographed with a UV gel imaging system. Chemi Image 5500 automated electrophoresis gel image analyzer was used to determine the relative mean gray values (A) of the target product and ß-actin internal control; expression index (I) of the target product mRNA was calculated using the formula: I=Aproduct/A β-actin.

Cell proliferation, CCK-8 method

Following intervention, Miapaca-2 cells were digested by trypsin, prepared into 2×10^4 cells/mL suspensions with serum-free medium, then inoculated to a 96-well culture plate, 100 μL suspension per well. Cultures were placed in a 37°C, 5% CO_2 incubator for 24 h. Supernatant was discarded from the original wells, 20 μL CCK-8 solution and 200 μL mixture composed of culture medium were added. Plate was returned to 37°C, 5% CO_2 incubator with saturation humidity for 1 h. Finally, the plate was placed in the Enzyme-Linked Immunoassay Analyzer and absorbance value (OD) of each well was measured at 450 nm.

Detecting in vitro invasiveness of cells with Transwell method

Millicell chambers were placed into a 24-well plate. Matrigel matrix was dissolved at 4°C overnight then diluted; 100 μL of the diluted solution were added into the upper chamber of the plate, which was then placed at 37°C for 4 h to the solidify the Matrigel matrix. To the lower chamber 600 μL 100 mL/L DMEM medium containing fetal bovine serum were added. Following intervention, Miapaca-2 cells were digested by pancreatin and prepared into 1×10^5 cells/mL cell suspension with serum-free medium; 200 μL of this cell suspension were inoculated into the upper chamber of the plate, which was then incubated in a 37°C, 5% CO_2 incubator for 24 h. The unpenetrated cells were gently wiped from the surface with a cotton swab and MTS assay was used to detect the number of the cells penetrating the membrane, in strict accordance with kit instructions. The chamber was then removed and inverted until air-dried. Cells were stained by adding 500 μL 10 g/L crystal violet to the 24-well plate and incubating at 37°C for 30 min. Cells were washed 3 times for 5 min with 0.01 M PBS buffer. Five visual fields (200× magnification) were selected for each chamber to

Table 1. Primer Sequences Used in RT-PCR and the Expected Size of PCR Products

Gene	Primer sequence	Product size (bp)
β-actin	Upstream 5'-GACTTAGTTGCGTTACACCCTTTCT-3'	162
	Downstream 5'-GAACGGTGAAGGTGACAGCAGT -3'	
CXCL12	Upstream 5'-GTGGTCGTGCTGGTCCTC -3'	197
	Downstream 5'-CACACTTGTCTGTTGTTGTTCTTC-3'	
CXCR4	Upstream 5'- TCTGTGACCGCTTCTACC-3'	184
	Downstream 5'- AGGATGAGGATGACTGTGG-3'	
MMP-2	Upstream 5'- GATGATGCCTTTGCTCGTGC-3'	133
	Downstream 5'- CAAAGGGGTATCCATCGCCA-3'	
MMP-9	Upstream 5'- CAAAGGGGTATCCATCGCCA-3'	127
	Downstream 5'- TCGTAGTTGGCGGTCGTG--3'	
uPA	Upstream 5'- TAAGAGCTGGTGTCTGATTG-3'	248
	Downstream 5'- TTGGATGAACTAGGCTAAAA-3'	

Figure 1. mRNA Expression of CXCL12 and CXCR4 in Pancreatic Cancer Cells. RT-PCR was used to determine semi-quantitative expression of CXCL12 and CXCR4 in Miapaca-2 cells

calculate the number of cells that had passed through the membrane of the chamber.

Statistical methods and Research experience

SPSS17.0 software was used for data processing. Measurement data were expressed as mean ± standard deviation ($\bar{\chi}$±s). The single-factor analysis of variance was used to compare mRNA expression, proliferation, and invasion of pancreatic cancer cells between different groups. The pair-wise comparisons between groups were performed using SNK method. The above hypothesis test was a two-sided test; P<0.05 was considered as statistically significant. We have enough experience in conducting medical researches, and have published some results elsewhere (Huang et al., 2011; Li et al., 2011; Li et al., 2011; Li et al., 2011; Xu et al., 2011; Xu et al., 2011; Xu et al., 2011; Yan et al., 2011; Zhang et al., 2011; Gong et al., 2012; Liu et al., 2012; Gu et al., 2013; Li et al., 2012; Shu et al., 2012; Zhan et al., 2012; Zhan et al., 2012; Xu et al., 2012; Xu et al., 2012; Yu et al., 2012; Zhang, et al., 2012; Zhang et al., 2012; Chen et al., 2013; Dai et al., 2013; Deng et al., 2013; Huang et al., 2013; Liu et al., 2013; Liu et al., 2013; Liu et al., 2013; Lu et al., 2013; Sun et al., 2013; Wei et al., 2013; Wu et al., 2013; Yang et al., 2013; Yin et al., 2013; Yin et al., 2013).

Results

CXCL12 and CXCR4 mRNA expression in pancreatic cells

CXCL12 mRNA was not expressed in Miapaca-2 pancreatic cells, but CXCR4 mRNA was expressed, as shown in Figure 1.

MMP-2, MMP-9, and uPA mRNA expression

Matrix metalloproteinases, which can degrade extracellular matrix, play a critical role in tumor invasion and metastasis (Yamamoto et al., 2001). Similarly, pancreatic cells can secrete uPA, a protein that dissolves tissue matrix and promotes angiogenesis, tumor cell exfoliation, stromal invasion, etc. (Gorantla et al., 2011). RT-PCR was used to assess expression of these genes that are believed to promote invasiveness of pancreatic cancer cells, and comparisons were made between groups of cells receiving different treatments. Control cells received no intervention in culture, but one group of cells received exogenous recombinant human CXCL12 in culture to stimulate the CXCR4 receptor. A third group of cells were treated in vitro with AMD3100, a CXCR4 inhibitor, to block the CXCL12-CXCR4 axis. mRNA expression levels in Miapaca-2 cells of two matrix metalloproteinases,

Table 2. Relative mRNA Expression Levels of MMP-2, MMP-9, and uPA in Pancreatic Cancer Cells Following Culture in Normal Medium, Medium with Recombinant CXCL12 (CXCL12), or Medium with CXCR4 Inhibitor (AMD3100)

Culture treatment	*MMP-2*	*MMP-9*	*uPA*
Control	0.92±0.05	0.94±0.02	1.11±0.24
CXCL12	1.43±0.02*	1.15±0.03*	1.54±0.26*
AMD3100	0.66±0.11*#	0.91±0.02#	0.45±0.04*#
F	96.391	115.927	21.257
P	0.001	0.001	0.002

*Vs Control group, P<0.05; #Vs CXCL12 group, *P*<0.05

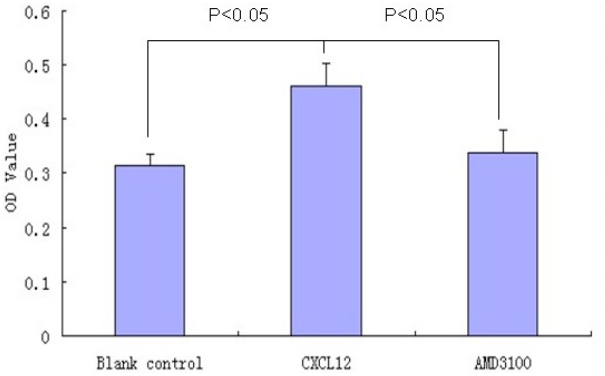

Figure 2. Proliferation of Pancreatic Cancer Cells Differs among Treatment Groups. CCK-8 assay was used to determine cell viability/proliferation by colorimetry. Culture of cells in medium containing recombinant CXCL12 increased their proliferation

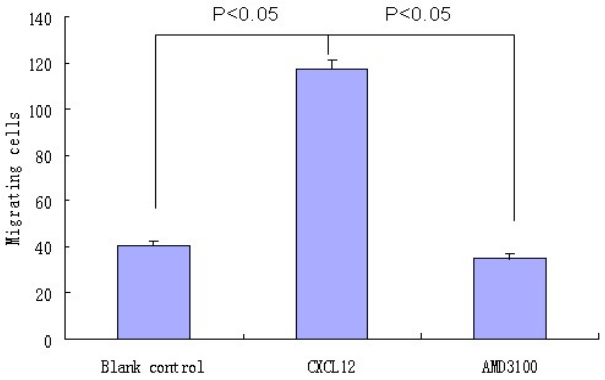

Figure 3. Invasive Ability of Miapaca-2 Cells Following Exposure to Recombinant CXCL12 or CXCR4 Inhibitor. Transwell invasion assay was used to determine the mean number of migrating cells

MMP-2 and MMP-9, as well as uPA were lowest in the AMD3100 and control groups and significantly higher in the CXCLl2 group (P <0.05, Table 2).

Proliferation of pancreatic cancer cells

A CCK-8 colorimetry assay was used to determine cell viability and is useful in assessing cell proliferation. We used the assay to compare proliferation of Miapaca-2 cells following culture with normal medium, medium containing recombinant CXCL12, or CXCR4 inhibitor. Compared to the control group, proliferation of Miapaca-2 cells exposed to culture medium with CXCL12 was significantly higher (P <0.05); AMD3100 can effectively suppress this proliferative capacity of pancreatic cancer cells (Figure 2).

Changes in invasiveness of pancreatic cancer cells

A transwell invasion experiment can determine the capacity of cells to migrate and invade the local area. This experiment demonstrated that adding recombinant CXCL12 to the culture medium promoted invasion and metastasis of pancreatic cancer cells (Figure 3); the number (122.33±4.16) of cells penetrating the chamber membrane in this group was significantly larger compared to the control group (41.33±1.16; P <0.05). Further, following AMD3100 treatment significantly fewer cells (37.00±1.73) penetrated the membrane compared to the CXCL12 group (P<0.05).

Discussion

Chemokines use chemical signals to attract other cells, such as immune cells that must reach a site of infection. The interaction between the chemokine CXCL12 and its receptor CXCR4 forms the CXCL12-CXCR4 biological axis, which plays roles in proliferation, adhesion, invasion, and organ-specific metastasis of a variety of malignant cell types (Balkwill, 2004; Cheng et al., 2009; Wu et al., 2009). A growing number of studies have confirmed that CXCR4 is expressed on the surface of a variety of tumor cells (such as salivary adenoid cystic carcinoma, breast cancer, and oral squamous carcinoma cells). It interacts with its corresponding chemokines, including CXCL12, and the molecular pair then influences the biological behavior of tumor cells (Muller et al., 2006; Kato et al., 2003; Almofti et al., 2004). We found here that, although pancreatic cancer cells do not themselves express CXCL12, they do express CXCR4 which was confirmed by immunoblotting mehhod (Grzesiak et al. 2007); therefore, these cells are capable of responding to chemotactic signals from CXCL12. Interestingly, the CXCR4 receptor can be blocked by the specific inhibitor AMD3100. Through its blockade on CXCR4, AMD3100 can inhibit invasion and metastasis of malignant cells (Blanco et al., 2000) and has fewer side effects in clinical application (Lukacs et al., 2002).

Matrix metalloproteinases (MMPs), which are zinc-dependent endopeptidases, can degrade most protein components in vascular endothelial cells and extracellular matrix. These proteins play a critical role in tumor invasion and metastasis. In pancreatic cancer, two members of this family, MMP-2 and MMP-9, are more highly expressed than they are in normal tissues (Durlik and Gardian, 2012). MMP-2 and MMP-9 can degrade components of the intercellular matrix and basement membrane to promote dissolution, breaking through matrix, and local or distant metastasis of tumor cells (Yamamoto et al., 2001). Pancreatic cancer cells also secrete uPA, or urokinase, which binds plasminogen and activates degradation of extracellular matrix (Gorantla et al., 2011). Here, we confirmed that MMP-2 and MMP-9, as well as uPA, are expressed in pancreatic cancer cells. However, their expression significantly increased when cells were cultured in the presence of CXCL12, suggesting that the chemotactic signal from the CXCL12-CXCR4 axis triggers increased expression of these genes to promote invasion.

A key feature of cancer is overproliferation; dysregulation of the cell cycle can cause infinite expansion, division, and growth of cells. The proliferation rate and growth patterns of tumors have significant impacts on disease development and prognosis of the patients; indeed, the proliferation rate of tumor cells can indirectly reflect the tumor malignancy (Ravi et al., 1998). Proliferation of malignant cells can be affected by changes in many different proteins, but evidence indicates that the CXCL12-CXCR4 biological axis is important to this process (Chen et al., 2006; Li et al., 2008). We found that, in fact, the proliferation rate of pancreatic cancer cells was significantly increased in the presence of exogenous CXCL12. Importantly, AMD3100 inhibition on CXCR4 can effectively suppress this proliferative capacity of pancreatic cancer cells.

The effects of the CXCL12-CXCR4 biological axis also extend to growth and metastasis of ovarian cancer and breast cancer cells (Guo et al., 2011). Metastasis of tumor cells, however, has been inhibited by treatment with AMD3100 (Lapteva et al., 2005). We confirmed these features in pancreatic cancer using a transwell invasion assay. Exposure to exogenous CXCL12 promoted invasion of pancreatic cancer cells, while AMD3100 administration significantly reduced this capacity. This finding indicates that the ability of CXCL12 to induce invasion and metastasis of pancreatic cancer cells can be inhibited by blocking the CXCR4 receptor. Additionally, these findings demonstrate that the effect of CXCL12 on invasive capacity is achieved by through the CXCR4 receptor.

In summary, the CXCL12-CXCR4 biological axis plays an important role in promoting in vitro proliferation and invasion of tumor cells. These effects may be related to the observed up-regulation of MMP-2, MMP-9, and uPA expression. However, all effects of CXCL12 exposure on pancreatic cancer could be reversed by administration of the inhibitor of CXCR4 receptor, AMD3100. Therefore, AMD3100 warrants further investigation as an anti-metastatic drug.

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References

Almofti A, uchida D, Begum NM, et al (2004). The clinicopathological significance of the expression of CXCR4 protein in oral squamous cell carcinoma. *Int J Oncol*, **25**, 65-71.

Balkwill F (2004). The significance of cancer cell expression of the chemokine receptor CXCR4. *Semin Cancer Biol*, **14**, 171-9.

Blanco J, Barretina J, Henson G, et al (2000). The CXCR4 antagonistAMD3100 efficiently inhibits cell-surface-expressed human immunodeficiency virus type 1 envelope-

induced apoptosis. *Antimicrob Agents Chemother*, **44**, 51-56.
Chen G, Shen ZL, Wang L, et al (2013). Hsa-miR-181a-5p expression and effects on cell proliferation in gastric cancer. *Asian Pac J Cancer Prev*, **14**, 3871-5.
Chen GS, Yu HS, Lan CC, et al (2006). CXC chemokine receptor CXCR4 expression enhances tumorigenesis and angiogenesis of basal cell carcinoma. *Br J Dermatol*, **154**, 910-8.
Cheng Z, Zhou S, Wang X, et al (2009). Characterization and application of two novel monoclonal antibodies against human CXCR4: cell proliferation and migration regulation for glioma cell line in vitro by CXCR4/SDF-1 alpha signal. *Hybridoma (Larchmt)*, **28**, 33-41.
Dai XZ, Yin HT, Sun LF, et al (2013). Potential therapeutic efficacy of curcumin in liver cancer. *Asian Pac J Cancer Prev*, **14**, 3855-9.
Demetter P, Maréchal R, Verset L, et al (2012). Molecular changes in pancreatic cancer: implications for molecular targeting therapy. *Acta Gastroenterol Belg*, **75**, 210-4.
Deng QQ, Huang XE, Ye LH, et al (2013).Phase II trial of Loubo® (Lobaplatin) and pemetrexed for patients with metastatic breast cancer not responding to anthracycline or taxanes. *Asian Pac J Cancer Prev*, **14**, 413-7.
Dömötör A, Peidl Z, Vincze A, et al (2005). Immunohistochemical distribution of vanilloid receptor, calcitonin-gene related peptide and substance P in gastrointestinal mucosa of patients with different gastrointestinal disorders. *Inflammopharmacology*, **13**, 161-77.
Durlik M, Gardian K (2012). Metalloproteinase 2 and 9 activity in the development of pancreatic cancer. *Pol Przegl Chir,* **84**, 377-82.
Fernadis AZ, Cherla RP, Ganju RK (2003). Differential regulation of CXCR4-mediated T-cell chemotaxis and mitogen-activated protein kinase activation by the membrane tyrosine phosphatase, CD45. *J Biol Chem*, **278**, 9536-43.
Gao LL, Huang XE, Zhang Q, et al (2011). 14.A Cisplatin and vinorelbine (NP) regimen as a postoperative adjuvant chemotherapy for completely resected breast cancers in China: final results of a phase II clinical trial. *Asian Pac J Cancer Prev*, **12**, 77-80.
Ghosh S, Preet A, Groopman JE, Ganju RK (2006). Cannabinoid receptor CB2 modulates the CXCL12-CXCR4-mediated chemotaxis of T lymphocytes. *Mol Immunol*, **43**, 2169-9.
Gong P, Huang XE, Chen CY, et al (2012). Comparison on Complications of Peripherally Inserted Central Catheters by Ultrasound Guide or Conventional Method in Cancer Patients. *Asian Pac J Cancer Prev*, **13**, 1873-5.
Gorantla B, Asuthkar S, Rao JS, Patel J, Gondi CS (2011). Suppression of the uPAR-uPA system retards angiogenesis, invasion, and in vivo tumor development in pancreatic cancer cells. *Mol Cancer Res*, **9**, 377-89.
Grzesiak JJ, Smith KC, Burtom DW, Deftos LJ, Michael bouvet (2007). Integrin- mediated laminin-1 adhesion upregulates CXCR4 and IL-8 expression in pancreatic cancer cells. *Surgery*, **141**, 804-14.
Gu M, Li SY, Huang XE, et al (2013). A phase II study on continuous infusional paclitaxel and 5-Fu as first-line chemotherapy for patients with advanced esophageal cancer. *Asian Pac J Cancer Prev*, **13**, 5587-91.
Guo L, Cui ZM, Zhang J, Huang Y (2011). Chemokine axes CXCL12-CXCR4 and CXCL16/CXCR6 correlation with lympnode metastasis in epithelial ovarian carcinoma. *Chin J Cancer*, **30**, 336-343.
Hidalgo M (2010). Pancreatic cancer. *N Engl J Med*, **362**, 1605-17.
Horuk R (2001). Chemokine receptors. *Cytokine Growth Factor Rev*, **12**, 313-35.
Huang XE, Li CG, Li Y, et al (2011). Weekly TP regimen as a postoperative adjuvant chemotherapy for completely resected breast cancer in China: final result of a Phase II trial. *Asian Pac J Cancer Prev*, **12**, 2797-800.
Huang XE, Wei GL, Huo JG, et al (2013). Intrapleural or intraperitoneal lobaplatin for treatment of patients with malignant pleural effusion or ascites. *Asian Pac J Cancer Prev*, **14**, 2611-4.
Kato M, Kitayama J, Kazama S, Nagawa H (2003). Expression pattern of CXC chemokine receptor-4 is correlated with lymph node metastasis in human invasive ductal carcinoma. *Breast Cancer Res,* **5**, R144-50.
Lapteva N, Yang AG, Sanders DE, Strube RW, Chen SY (2005). CXCR4 knockdown by small interfering RNA abrogates breast tumor growth in vivo. *Cancer Gene Ther* , **12**, 84-9.
Li CG, Huang XE, Li Y, et al (2011). Clinical observations on safety and efficacy of OxyContin® administered by rectal route in treating cancer related pain. *Asian Pac J Cancer Prev,* **12**, 2477-8.
Li CG, Huang XE, Li Y, et al (2011). Phase II trial of Irinotecan plus Nedaplatin (INP) in treating patients with extensive stage small cell lung cancer. *Asian Pac J Cancer Prev*, **12**, 487-90.
Li CG, Huang XE, Xu L, et al (2012). Clinical Application of Serum Tumor Associated Material (TAM) from Non-small Cell Lung Cancer Patients. *Asian Pac J Cancer Prev*, **13**, 301-4.
Li JK, Yu L, Shen Y, et al (2008). Inhibition of CXCR4 activity with AMD3100 decreases invasion of human colorectal cancer cells in vitro. *World J Gastroenterol*, **14**, 2308-13.
Li Y, Yan PW, Huang XE, et al (2011). MDR1 gene C3435T polymorphism is associated with clinical outcomes in gastric cancer patients treated with postoperative adjuvant chemotherapy. *Asian Pac J Cancer Prev*, **12**, 2405-9.
Liu J, Huang XE, Tian GY, et al (2013). Phase II Study on Safety and Efficacy of Yadanzi® (Javanica oil emulsion injection) Combined with Chemotherapy for Patients with Gastric Cancer. *Asian Pac J Cancer Prev*, **14**, 2009-12.
Liu W, Li SY, Huang XE, et al (2012). Inhibition of tumor growth in vitro by a combination of extracts from rosa roxburghii tratt and fagopyrum cymosum. *Asian Pac J Cancer Prev*, **13**, 2409-14.
Liu YC, Zhou SB, Gao F, et al (2013). Phase II study on breast conservative surgery plus chemo- and radiotherapy in treating Chinese patients with early staged breast cancer. *Asian Pac J Cancer Prev*, **14**, 3747-50.
Liu YC, Zhou SB, Gao F, et al (2013).Chemotherapy and late course three dimensional conformal radiotherapy for treatment of patients with stage III non- small cell lung cancer. *Asian Pac J Cancer Prev*, **14**, 2663-5.
Lu YY, Huang XE, Xu L, et al (2013). Potential predictors of sensitivity to pemetrexed as first-line chemotherapy for patients with advanced non-squamous NSCLCs. *Asian Pac J Cancer Prev*, **14**, 2005-8.
Lukacs NW, Berlin A, Schols D, Skerlj RT, Brideger GJ (2002). AMD3100, a CXCR4 antagonist, attenuates allergic lung inflammation and airway hyperreactivity. *Am J Pathol*, **160**, 1353-60.
Moll NM, Ransohoff RM (2010). CXCL12 and CXCR4 in bone marrow physiology. *Expert Rev Hematol*, **3**, 315-22.
Muller A, Sonkoly E, Eulert C, et al (2006). Chemokine receptors in head and neck cancer: association with metastatic spread and regulation during chemotherapy. *Int J Cancer*, **118**, 2147-57.
Nagasawa T, Hirota S, Tachibana K, et al (1996). Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature,*

382, 635-8.
Patrussi L, Baldari CT (2011). The CXCL12-CXCR4 axis as a therapeutic target in cancer and HIV-1 infection. *Curr Med Chem*, **18**, 497-512.
Pitcher J, Shimizu S, Burbassi S, Meucci O (2010). Disruption of neuronal CXCR4 function by opioids: preliminary evidence of ferritin heavy chain as a potential etiological agent in neuroAIDS. *J Neuroimmunol*, **224**, 66-71.
Ravi D, Ramadas K, Mathew BS, et al (1998). Angiogenesis during tumor progression in the oral canity is related to reduced apoptosis and high tumor cell proliferation. *Oral Oncol*, **34**, 543-8.
Salvatore P, Pagliarulo C, Colicchio R, Napoli C (2010). CXCR4-CXCL12-dependent inflammatory network and endothelial progenitors. *Curr Med Chem*, **17**, 3019-29.
Scotton CJ, Wilson JL, Scott K, et al (2002). Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res*, **62**, 5930-8.
Shu J, Li CG, Liu YC, et al (2012). Comparison of serum tumor associated material (TAM) with conventional biomarkers in cancer patients. *Asian Pac J Cancer Prev*, **13**, 2399-403.
Stathis A, Moore MJ (2010). Advanced pancreatic carcinoma: current treatment and future challenges. *Nat Rev Clin Oncol*, **7**, 163-72.
Sun MQ, Meng AF, Huang XE, et al (2013). Comparison of psychological influence on breast cancer patients between breast-conserving surgery and modified radical mastectomy. *Asian Pac J Cancer Prev*, **14**, 149-52.
Tashiro K, Tada H, Heilker R, et al (1993). Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins. *Science*, **261**, 600-3.
Terasaki M, Sugita Y, Arakawa F, et al (2011). CXCL12-CXCR4 signaling in malignant brain tumors: a potential pharmacological therapeutic target. *Brain Tumor Pathol*, **28**, 89-97.
Wei GL, Huang XE, Huo JG, et al (2013). Phase II study on pemetrexed-based chemotherapy in treating patients with metastatic gastric cancer not responding to prior palliative chemotherapy. *Asian Pac J Cancer Prev*, **14**, 2703-6.
Wu X, Lee VC, Chevalier E, Hwang ST (2009). Chemokine receptors as targets for cancer therapy. *Curr Pharm Des*, **15**, 742-57.
Wu XY, Huang XE, You SX, et al (2013). Phase II study of pemetrexed as second or third line combined chemotherapy in patients with colorectal cancer. *Asian Pac J Cancer Prev*, **14**, 2019-22.
Xu HX, Huang XE, Li Y, et al (2011). A clinical study on safety and efficacy of Aidi injection combined with chemotherapy. *Asian Pac J Cancer Prev*, **12**, 2233-6.
Xu HX, Huang XE, Qian ZY, et al (2011). Clinical observation of Endostar® combined with chemotherapy in advanced colorectal cancer patients. *Asian Pac J Cancer Prev,* **12**, 3087-90.
Xu JW, Li CG, Huang XE, et al (2011). Ubenimex capsule improves general performance and chemotherapy related toxicity in advanced gastric cancer cases. *Asian Pac J Cancer Prev*, **12**, 985-7.
Xu T, Xu ZC, Zou Q, Yu B, Huang XE (2012). P53 Arg72Pro polymorphism and bladder cancer risk--meta-analysis evidence for a link in Asians but not Caucasians. *Asian Pac J Cancer Prev*, **13**, 2349-54.
Xu X, Wang L, Xu HQ, Huang XE, et al (2013). Clinical comparison between paclitaxel liposome (Lipusu®) and paclitaxel for treatment of patients with metastatic gastric cancer. *Asian Pac J Cancer Prev*, **14**, 2591-4.
Yamamoto H, Itoh F, Iku S, et al (2001). Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human pancreatic adenocarcinomas: clinicopathologic and prognostic significance of matrilysin expression. *J Clin Oncol,* **19**, 1118-27.
Yan PW, Huang XE, Yan F, et al (2011). Influence of MDR1 gene codon 3435 polymorphisms on outcome of platinum-based chemotherapy for advanced non small cell lung cancer. *Asian Pac J Cancer Prev*, **12**, 2291-4.
Yang L, Huang XE, Zhou JN (2013). Risk assessment on anastomotic leakage after rectal cancer surgery: an analysis of 753 patients. *Asian Pac J Cancer Prev*, **14**, 4447-53.
Yin HT, Tian QZ, Guan L (2013).In vitro and in vivo evaluation of the antitumor efficiency of resveratrol against lung cancer. *Asian Pac J Cancer Prev*, **14**, 1703-6.
Yin HT, Zhang DG, Wu XL(2013). In vivo evaluation of curcumin-loaded nanoparticles in a A549 xenograft mice model. *Asian Pac J Cancer Prev*, **14**, 409-12.
Yu DS, Huang XE, Zhou JN, et al (2012). A Comparative Study on the Value of Anal Preserving Surgery for Aged People with Low Rectal Carcinoma in Jiangsu, China. *Asian Pac J Cancer Prev*, **13**, 2339-40.
Zhan YP, Huang XE, Cao J et al (2012). Clinical study on safety and efficacy of Qinin® (cantharidin sodium) injection combined with chemotherapy in treating patients with gastric cancer. *Asian Pac J Cancer Prev*, **13**, 4773-6.
Zhan YP, Huang XE, Cao J, et al (2012). Clinical safety and efficacy of Kanglaite® (Coix Seed Oil) injection combined with chemotherapy in treating patients with gastric cancer. *Asian Pac J Cancer Prev*, **13**, 5319-21.
Zhang LQ, Huang XE, Wang J (2011). The cyclin D1 G870A polymorphism and colorectal cancer susceptibility: a meta-analysis of 20 populations. *Asian Pac J Cancer Prev*, **12**, 81-5.
Zhang XZ, Huang XE, Xu YL, et al (2012). A Phase II Study on Voriconazole in Treating Chinese Patients with Malignant Hematological Disorder and Invasive Aspergillosis. *Asian Pac J Cancer Prev*, **13**, 2415-8.
Zhong W, Chen W, Zhang D, et al (2012). CXCL12-CXCR4 axis plays pivotal roles in the organ-specific metastasis of pancreatic adenocarcinoma: A clinical study. *Exp Ther Med*, **4**, 363-369.