Acta Biochim Biophys Sin, 2016, 48(3), 282–289
doi: 10.1093/abbs/gmv132
Advance Access Publication Date: 26 January 2016
Original Article

Original Article

Low-dose cisplatin-induced CXCR4 expression promotes proliferation of ovarian cancer stem-like cells

Yifei Chen†, Shuying Wang†, Shixia Bu, Minhua Xu, and Dongmei Lai*

The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200030, China

†These authors contributed equally to this work.
*Correspondence address. Tel/Fax: +86-21-64070434-27701; E-mail: laidongmei@hotmail.com

Received 25 September 2015; Accepted 12 November 2015

Abstract

Chemoresistance blocks the efficient treatment of epithelial ovarian cancer, which is the most lethal of all gynecological cancers. Cancer stem cells are believed to be at least partially responsible for the development of chemoresistance. In this study, the effect of cisplatin (CDP) on the enrichment and proliferation of cancer stem-like cells (CSLCs) was investigated, and the underlying mechanisms of action were elucidated. An *in vitro* anchor-free system was employed to enrich CSLCs from the SKOV3 human epithelial ovarian cancer cell line. Our results showed that treatment with low concentrations of CDP resulted in better-enriched CSLCs, with higher proliferative activities. Low dose of CDP was found to induce the expression of chemokine (C-X-C motif) receptor 4 (CXCR4), which is an important stemness marker in cancer stem cells as well as a promising therapeutic target for ovarian cancer treatment. Results also showed that overexpressed CXCR4 generated chemoresistance. Based on these results, it may be concluded that, at low concentrations, CDP itself contributes to the development of drug resistance. This finding provides novel insight into the mechanisms underlying chemoresistance and has significant therapeutic implications for epithelial ovarian cancer treatment.

Key words: human epithelial ovarian cancer, chemoresistance, cancer stem cell, CXCR4

Introduction

As the most lethal gynecological malignancy, epithelial ovarian cancer has become a leading threat to women's health [1,2]. At present, the standard therapeutic strategy against ovarian cancer involves aggressive surgical debulking, followed by platinum-based chemotherapy. Although the initial treatment is usually effective, drug resistance develops easily and most patients will relapse within 5 years [3–5]. Over 70% of the patients with advanced epithelial ovarian cancer experience disease recurrence and over 80% of them die, ultimately as a result of treatment inadequacies [4–6]. To establish effective therapeutic strategies, a comprehensive understanding of the basis of drug resistance development, in particular the molecular mechanisms involved in the early chemotherapeutic response, is essential.

Drug inactivity, drug target alteration, enhanced efflux, DNA repair, apoptosis inhibition, and cancer cell heterogeneity [7–9] have all been proposed as contributors to drug resistance development. However, the cancer stem cell model has also attracted much attention in the past decade, and cancer stem cells are believed to be a key factor in drug resistance development [10–14]. Cancer stem cells that are capable of self-renewing are believed to be the progenitors of cancer cells, and thought to contribute to tumor pathogenesis and recurrence [5,10–12]. Due to their quiescent state, elevated drug membrane transporter expression and immense DNA repairing capabilities, cancer stem cells are highly drug resistant. Consequently, cancer stem cells are immune to the effects of chemotherapy treatment, and can differentiate into new cancer cells that tend to present higher resistance than the original cancer cells.

Cancer stem cells have been successfully isolated from several cancer models [12]. Indeed, cancer stem cells with high tumorigenic abilities, enhanced cisplatin (CDP) chemoresistance and upregulated stem cell markers have been isolated from human primary ovarian tumor tissues [15–18]. Additionally, cancer stem cells also exist in long-term cancer cell cultures and immortalized cell lines [19,20]. Under stem cell-selective conditions, surviving cells can form anchorage-independent clusters, and then coalesce into larger, self-renewing spheroids [16,21].

In our previous study, cancer stem-like cells (CSLCs) were successfully enriched from ovarian cancer cell lines in a serum-free suspension culturing system [22,23]. In this system, CSLCs form nonadherent spheroids and display higher drug resistance and tumorigenic efficiency than the adherent differentiated parent cells. In CSLCs, the enhanced expression of a group of stemness marker genes, including chemokine (C-X-C motif) receptor 4 (*CXCR4*), was detected. CXCR4 appears to be associated with epithelial ovarian cancer development and metastasis, and poor overall patient survival, and has been proposed as an innovative therapeutic target [24–26].

Interestingly, it was found that CDP, at low concentrations, could be used to enrich CSLCs [22]. However, whether CDP acts as a stress-inducing selector for drug-resistant CSLCs, or provokes the development of CSLC features, is unknown. Answer this question is essential to elucidate the mechanisms underlying chemoresistance in cancer cells. Therefore, in this study, the effects of low doses of CDP on ovarian cancer cells and CSLCs *in vitro* were investigated.

Materials and Methods

Cell culture

The human ovarian epithelial cancer cell lines SKOV3, HO8910, and HO8910pm were purchased from the Shanghai Cell Bank of the Chinese Academy of Science (Shanghai, China). SKOV3 cells were cultured in McCoy's 5A medium (Sigma-Aldrich, St Louis, USA), supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. HO8910 and HO8910pm cells were cultured in RPMI-1640 medium (Gibco, Grand Island, USA), supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Adherent cells were cultured in regular plates at 37°C, in a humidified environment containing 5% CO_2.

Enrichment of CSLCs

The CSLCs were enriched as reported previously [23,27]. Briefly, SKOV3 cells, in the logarithmic phase, were dissociated by 0.25% trypsin–ethylenediaminetetraacetic acid (Life Technologies, Carlsbad, USA) for 1–2 min at 37°C, and single cells were suspended in Dulbecco's modified Eagle's medium/F12 medium (Invitrogen, Carlsbad, USA) supplemented with 10 ng/ml basic fibroblast growth factor (Invitrogen) and 10% knockout serum (Gibco) in low-attachment plates. CDP was added when indicated. Dead cell debris was removed every 2 days by centrifugation at 300 *g* for 5 min. Spheroids were then dispersed in fresh medium. After being incubated for 1 week, CSLC spheroids were selected for further treatment or examination.

Cell viability assay

Cell viability was detected by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Spheroid cells were isolated by centrifugation (300 *g* for 5 min), and adherent cells were dissociated by trypsinization. Approximately 1×10^4 cells/well were seeded in fresh culture medium in 96-well regular plates for adherent cells, and in low-attachment plates for CSLCs. Then 10 μl of MTT solution (5 mg/ml; Sigma-Aldrich) was added, and the mixture was incubated for 4 h at 37°C. The medium was carefully removed and the converted dye was solubilized with 150 μl dimethyl sulfoxide (DMSO; Sigma-Aldrich). Absorbance was measured at 490 nm with a microplate reader (Bio-Rad, Hercules, USA). Cell counting kit-8 (Dojindo, Kumamoto, Japan) was employed to evaluate the viability of CSLCs according to the manufacturer's protocol. Six wells were run for each condition, and the experiment was repeated three times.

Real-time quantitative polymerase chain reaction

Cells were harvested and rinsed with phosphate buffered saline (PBS), and RNA was extracted using Trizol reagent (Life Technologies) according to the manufacturer's protocol. Genomic DNA contamination was excluded by DNase I (Fermentas, Hanover, USA) treatment. Reverse transcription was performed with a ReverTra Ace-α kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. SYBR-Green Real-time PCR Master Mix Plus (Toyobo) was used to perform the quantitative polymerase chain reaction (qPCR) on a Mastercycler ep realplex (Eppendorf, Hamburg, Germany). Primers used in this study were the same as those reported previously [27].

Flow cytometric analysis

SKOV3 cells were dissociated by trypsin as described above before being rinsed with PBS, and then blocked in blocking buffer (1% bovine serum albumin in PBS) for 30 min. Dissociated cells were then incubated for 30 min at 4°C with phycoerythrin (PE)-conjugated mouse antibodies against human CXCR4 (eBioscience, San Diego, USA). After being washed twice with blocking buffer, cells were detected on a Cytomics FC500 flow cytometer (Beckman Coulter, Pasadena, USA). A PE-conjugated mouse IgG control (eBioscience) was used as a nonspecific control. 7-Aminoactinomycin D (7-AAD; BD Pharmingen, Franklin Lakes, USA) was added to each sample to exclude dead cells.

Cell migration assay

Cell migration assays were performed in trans-well invasion chambers (8.0 μm; Corning Inc., Coring, USA) according to the manufacturer's instructions. Complete culture medium (600 μl) was added to the lower chambers and 5×10^4 cells in 200 μl culture medium without FBS were seeded into the top chambers, and were allowed to migrate overnight. Cells were fixed with methanol and visualized by crystal violet staining. Cells that did not migrate to the lower chamber were removed by scraping with a cotton swab. At least three fields of view were observed by microscopy.

Cell transfection

The CXCR4 overexpressing vector, GV142-CXCR4, was purchased from Shanghai Genechem Co. Ltd (Shanghai, China). The expression of CXCR4 was driven by a cytomegalovirus promoter. Plasmids were transfected into SKOV3 cells with Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen).

Statistical analysis

Data are shown as the mean ± standard deviations (SD). Student's *t*-test (two tailed) was used to compare two groups. $P < 0.05$ was considered statistically significant.

Results

CDP promoted the enrichment of CSLCs

To investigate the effect of CDP upon epithelial ovarian CSLCs, its action on the formation of CSLC spheroids from an immortalized epithelial ovarian cancer cell line (SKOV3) was determined. **Figure 1A** shows that, in the serum-free culture system, surviving SKOV3 cells appeared to have aggregated to form anchor-free spheroids. Consistent with our previous results [23,27], these spheroids displayed the classic characteristics of CSLCs, as shown by the upregulated expression of a group of cancer stem cell marker genes, including *ALDH1*, *ALDH2*, *CXCR4*, *MyD88*, and *LIN28* [27] (**Fig. 1B**).

As shown in **Fig. 1C**, SKOV3 CSLCs demonstrated superior spheroid accumulation in the presence of 5 mg/l of CDP, with fewer dispersed single cells, when compared with mock-treated controls. The spheroid formation efficiency under CDP treatment was also higher than that of controls (**Fig. 1D**). Enhanced CSLC proliferative activity, in the presence of CDP, was confirmed by CCK-8 assay (**Fig. 1E**). Results indicated that CDP treatment, at low concentrations, assisted the enrichment and proliferation of CSLCs.

CDP induced the expression of CXCR4

To gain insights into the underlying mechanisms of action of CDP on CSLCs, the expression patterns of stemness marker genes, including

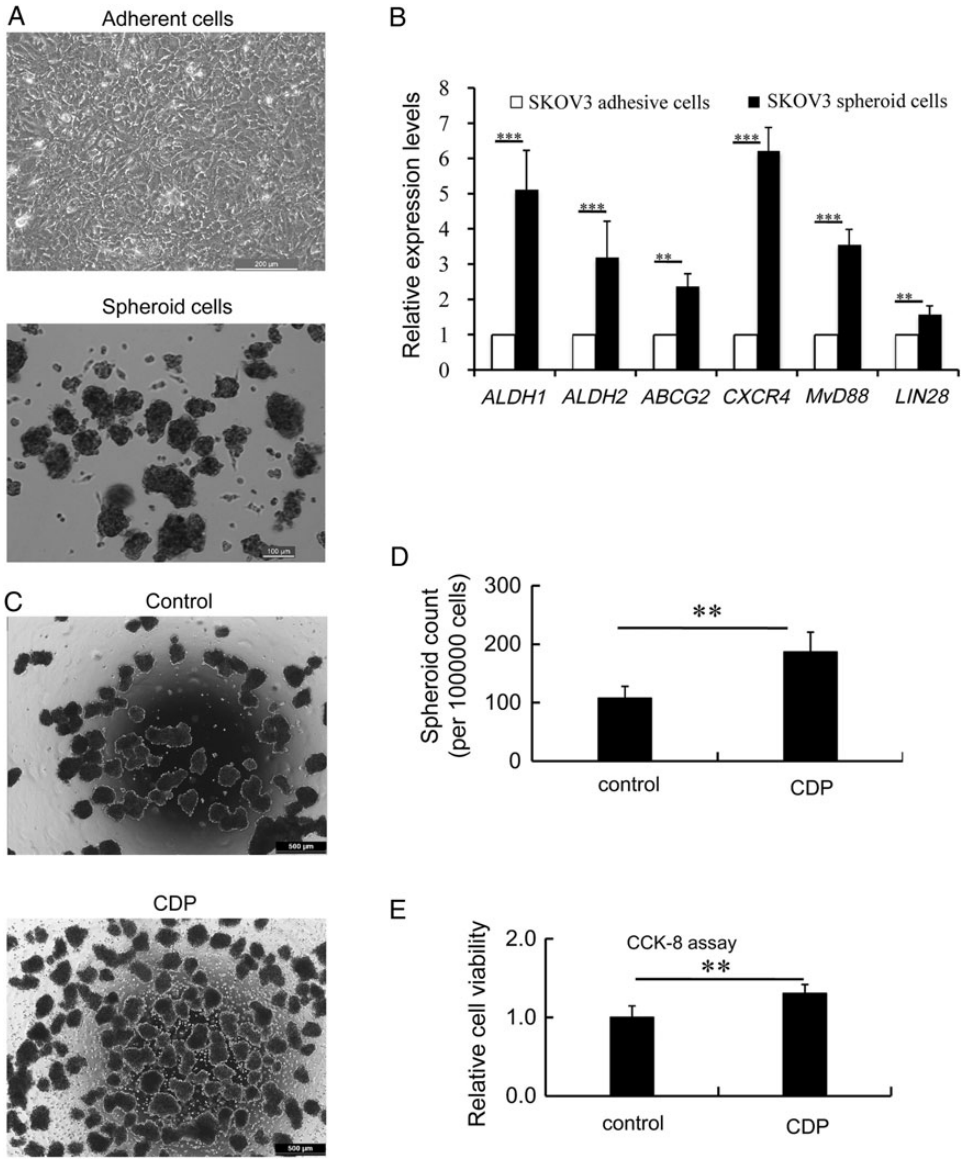

Figure 1. Low-dose CDP promoted the enrichment and proliferation of CSLCs (A) SKOV3 parent cells were adherent epithelial cells in a normal culture system. When transferred to serum-free medium in low-attachment dishes, CSLCs accumulated as spheroids. (B) The relative expression levels of cancer stem cell marker genes (*ALDH1, ALDH2, CXCR4, MyD88,* and *LIN28*) were quantified by real-time qPCR. 18s rRNA was used as an internal standard control. Experiment was performed in triplicate, and data are shown as the mean ± SD (***$P < 0.001$; **$P < 0.01$). (C) In the presence of 5 mg/l of CDP, CSLC spheroids were observed (C) and counted (D) using microscopy. Experiment was performed in triplicate, and data are shown as the mean ± SD (**$P < 0.01$). (E) Cell viability of CSLCs was assessed using CCK-8 assay. Result presents the average of at least three independent experiments, and the error bars indicated the standard derivations (**$P < 0.01$).

ALDH1, *ALDH2*, *CXCR4*, *MyD88*, and *LIN28*, after CDP treatment were examined in both adherent and spheroid CSLCs. **Figure 2A,B** shows that after treatment with 5 mg/l of CDP for 24 h, the expression of CXCR4 was significantly enhanced, while the expressions of other genes remained unchanged or were just moderately altered. These findings suggested that CXCR4 might play an

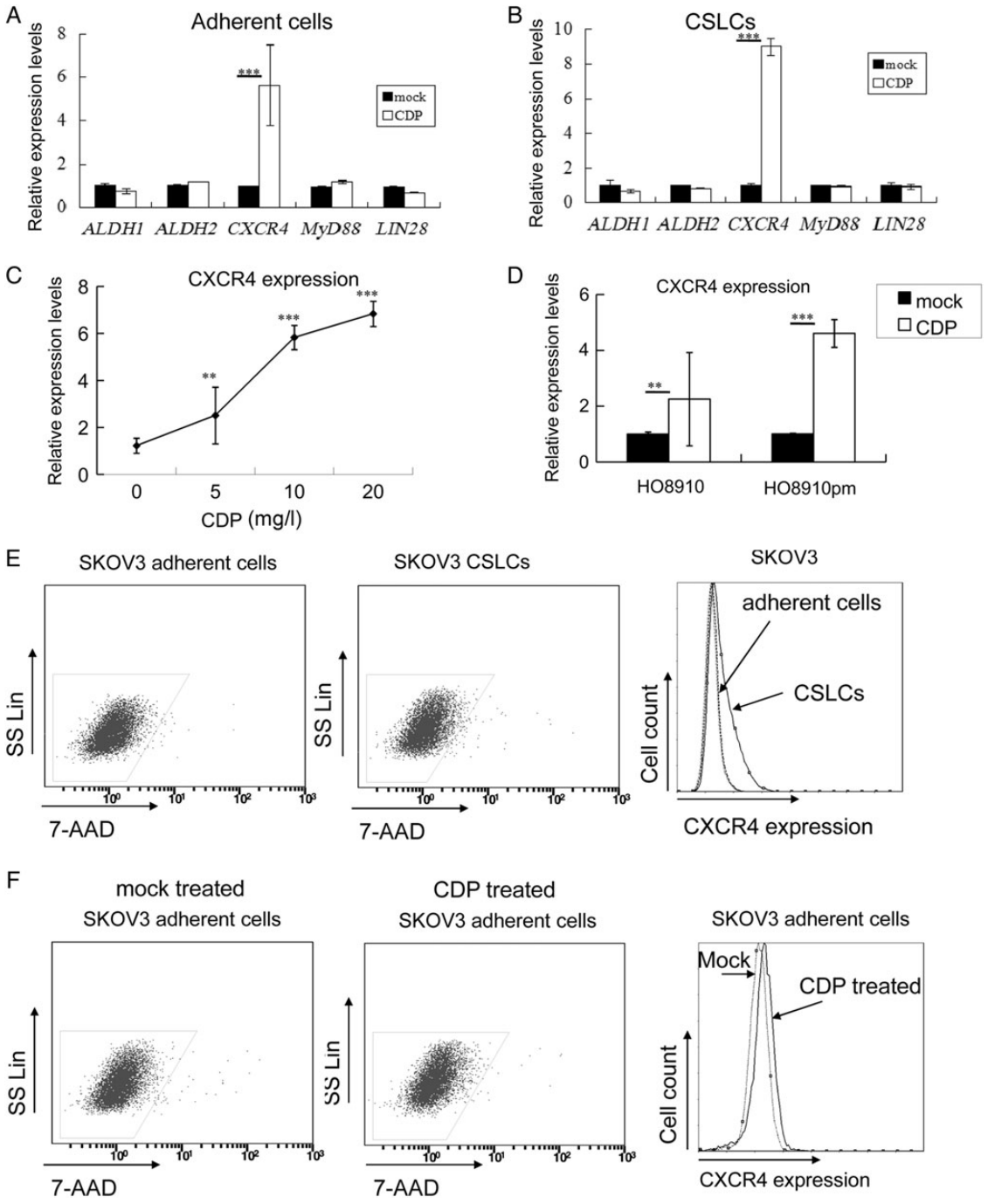

Figure 2. CDP treatment induced the expression of CXCR4 (A) The expression of stemness marker genes (*ALDH1*, *ALDH2*, *CXCR4*, *MyD88*, and *LIN28*) was quantified in SKOV3 adherent cells. Mock (DMSO) treated cells were employed as controls, and 18s rRNA was used as an internal standard control. Result was presented as the average of at least three independent experiments, and the error bars indicated the standard derivations (***$P < 0.001$). (B) The relative expressions of stemness marker genes in SKOV3 CSLC spheroids, which were treated by DMSO (mock) or CDP, were quantified. Result was presented as the average of at least three independent experiments, and the error bars indicated the standard derivations (***$P < 0.001$). (C) CXCR4 expressions in SKOV3 cells, which were treated with CDP at different concentrations, were quantified by qPCR (***$P < 0.001$; **$P < 0.01$). (D) The CXCR4 expressions with (CDP) or without (mock) CDP were quantified in HO8910 and HO8910pm, respectively. Each experiment was performed in triplicate, and data are shown as the mean ± SD (***$P < 0.001$; *$P < 0.05$). (E) Both adherent cells and CSLC spheroids were dissociated into single cells by trypsinization and incubated with PE-conjugated antibodies against CXCR4. CXCR4 protein was detected by flow cytometric analysis, and positive cells were counted. PE-conjugated mouse IgG was used as the nonspecific control, and 7-AAD was used to stain the dead cells. 7-AAD negative cells were gated for the CXCR4 analysis. (F) Adherent SKOV3 cells that were treated by DMSO (mock) or CDP were incubated with PE-conjugated antibodies against CXCR4, and then examined by flow cytometry. PE-conjugated mouse IgG was used as the nonspecific control, and 7-AAD was used to stain the dead cells. 7-AAD negative cells were gated for the CXCR4 analysis.

important role in the CDP-induced enrichment and proliferation of CSLCs. Thus, the effect of different concentrations of CDP (0, 5, 10, and 20 mg/l) on CXCR4 gene expression was further determined, and results demonstrated that CXCR4 expression was positively correlated with CDP concentration (**Fig. 2C**). Similar results observed in another two epithelial ovarian cancer cell lines (HO8910 and HO8910pm) further confirmed the stimulating effect of CDP on CXCR4 expression (**Fig. 2D**).

Additionally, CXCR4 transcription and protein expression levels in both SKOV3 adherent cells and CSLCs were also evaluated. Using flow cytometry, it was found that few adherent cells were CXCR4 positive (0.07%), while ~5.15% CXCR4-positive cells were detected among CSLC spheroids (**Fig. 2E**). Treatment with 5 mg/l of CDP for 48 h led to significantly elevated CXCR4 expression levels and CXCR4 cell positivity among SKOV3 adherent cells (from 0.07% to 1.74%) (**Fig. 2F**). This finding further confirmed the cancer stem cell characteristics of CSLCs in our serum-free suspension culture system, and the CXCR4-inducing activity of CDP in epithelial ovarian cancer cells.

C-X-C motif chemokine 12 (CXCL12) is the primary heterodimeric ligand of CXCR4. To further demonstrate the effect of CDP on CXCR4/CXCL12 axis in ovarian cancer cells, the expression of CXCL12 was examined in SKOV3 (**Fig. 3A**), HO8910, and HO8910pm (**Fig. 3B**) cells. Results indicated that CDP also promotes the expression of CXCL12 in ovarian cancer cells. In addition to CXCR4, CXCR7 is also the receptor of CXCL12, and has been shown to be expressed in cancer stem cells. Accordingly, it was also found that the CXCR7 was highly expressed in SKOV3 CSLCs (**Fig. 3C**). **Figure 3D,E** shows that CDP slightly elevates the expression of CXCR7 in epithelial ovarian cancer cells. However, the promoting activity of low-dose CDP on CXCR7 was not as effective as on CXCR4.

Overexpressed CXCR4 elevated the expression of stemness markers

As shown above, CDP treatment at low concentrations promoted the formation of CSLC spheroids and enhanced the expression of CXCR4.

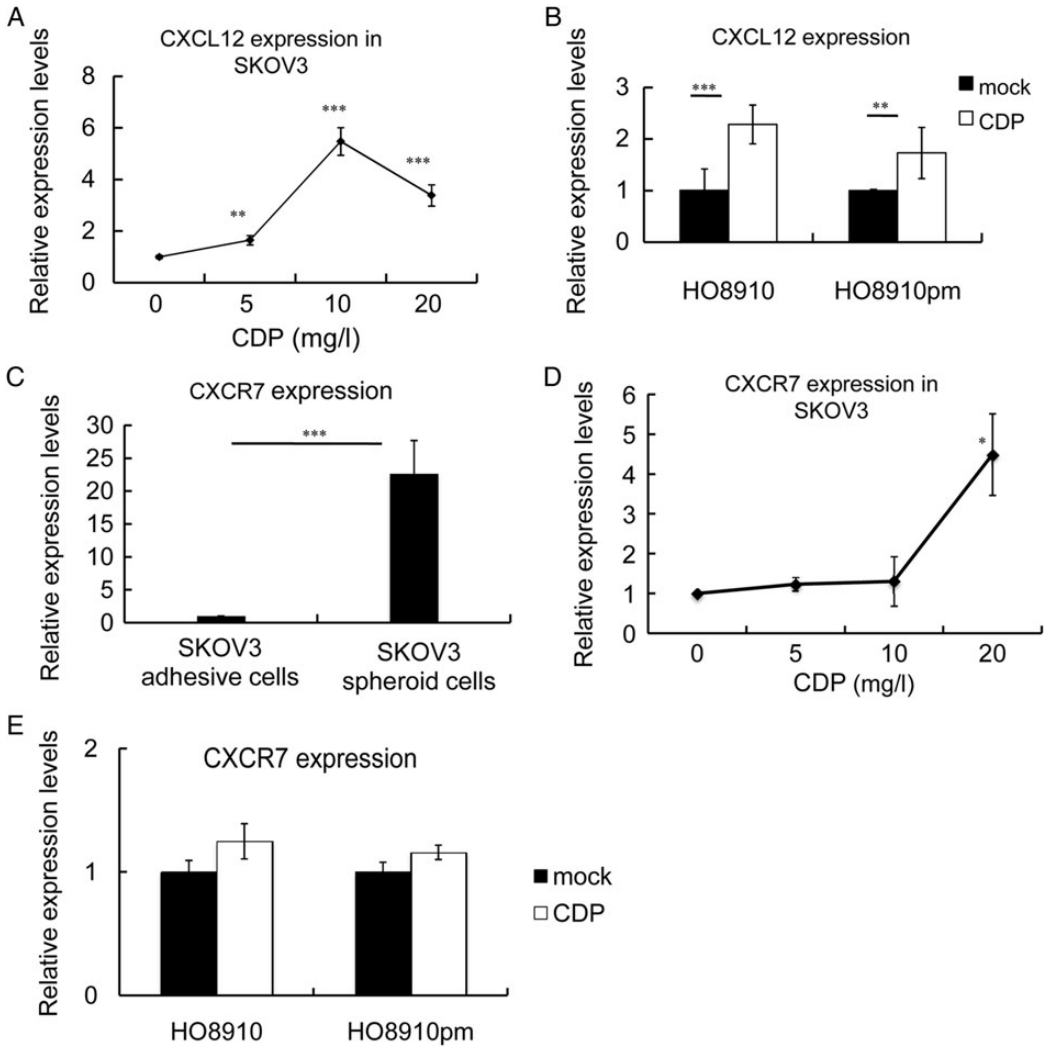

Figure 3. The expression of CXCL12 and CXCR7 was stimulated by CDP (A) CXCL12 expression was quantified by qPCR in SKOV3 cells that were treated with 0, 5, 10, or 20 mg/l of CDP. (B) The CXCL12 expression was examined in HO8910 and HO8910pm cells that were mock or CDP treated. (C) The expression of CXCR7 was compared in SKOV3 adhesive cells and spheroid cells by qPCR. (D) The effect of CDP on the expression of CXCR7 in SKOV3 cells was examined by qPCR. (E) The CXCR7 expression level was evaluated by qPCR in HO8910 and HO8910pm cells. 18s rRNA was used as an internal standard control. Experiments were performed in triplicate, and data are shown as the mean ± SD (***$P<0.001$; **$P<0.01$; *$P<0.05$).

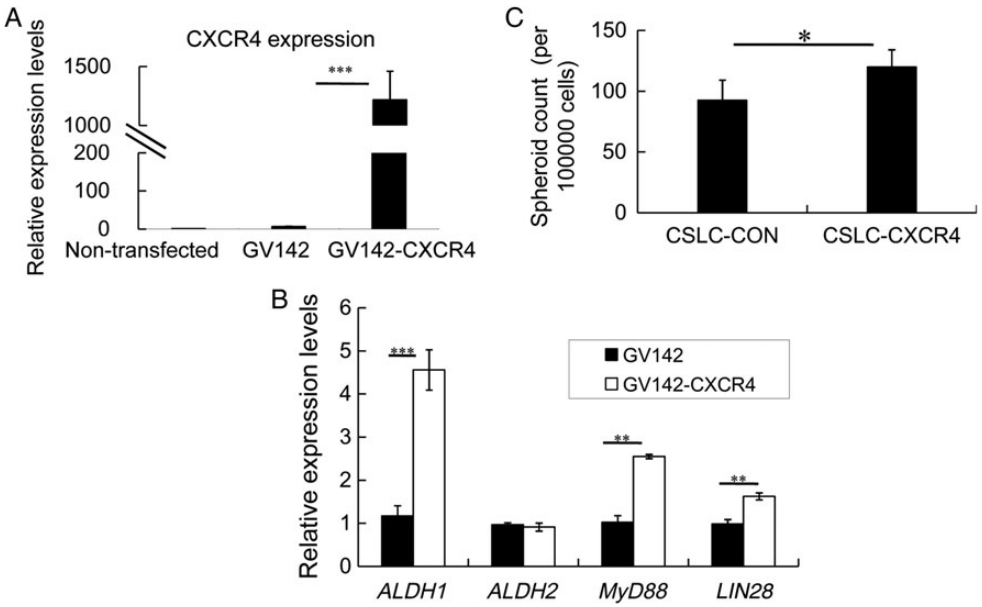

Figure 4. Overexpressed CXCR4 stimulated the formation of CSLC spheroids (A) CXCR4-overexpressing plasmid (GV142-CXCR4) was transfected into SKOV3 cells and the expression of CXCR4 was examined by qPCR. Result was presented as the average of at least three independent experiments, and the error bars indicated the standard derivations (***$P < 0.001$). (B) CXCR4 overexpression led to elevated expression of cancer stem cell marker genes that were quantified by qPCR. Experiment was performed in triplicate, and data are shown as the mean ± SD (***$P < 0.001$; **$P < 0.01$). (C) The CSLC spheroid formation efficiency of SKOV3 cells transfected with control (CSLC-CON) or CXCR4-overexpressing plasmids (CSLC-CXCR4) was quantified. Experiment was performed in triplicate, and data are shown as the mean ± SD (*$P < 0.05$).

Therefore, the effect of CXCR4 overexpression on ovarian cancer cell properties was examined. To this end, the CXCR4-overexpressing plasmid, GV142-CXCR4, was constructed and transfected into SKOV3 adherent cells (**Fig. 4A**). Gene expression analysis by qPCR revealed the enhanced expressions of a group of cancer stem cell marker genes (*ALDH1*, *ALDH2*, *MyD88*, and *LIN28*) upon CXCR4 overexpression (**Fig. 4B**). The elevated expression of CXCR4, thus, appeared to be associated with the augmented cancer stem cell features of SKOV3 cells.

To further investigate the function of CXCR4 in CSLC enrichment and proliferation, the CSLC spheroid formation efficiency after CXCR4 transfection was analyzed. The rate of spheroid formation was elevated after CXCR4 overexpression, demonstrating the stimulating effect of CXCR4 on CSLC formation (**Fig. 4C**).

CXCR4 promoted CDP resistance and cell migration

As discussed above, CDP-induced CXCR4 expression promoted the enrichment and development of cancer stem cell features in CSLCs. One of the most important characteristics of cancer stem cells is chemoresistance. Therefore, the relationship between CXCR4 expression and drug resistance in SKOV3 cells was then investigated. After being transfected with CXCR4 overexpression vectors (GV142-CXCR4), SKOV3 cells were incubated with 0, 10, 20, or 30 mg/l of CDP. MTT assays revealed a consistently higher viability of SKOV3 cells transfected with CXCR4-overexpressing vectors than those transfected with control vectors (**Fig. 5A**). Our results also showed that AMD3100, a small-molecule antagonist of CXCR4, efficiently sensitized SKOV3 cells to CDP treatment (**Fig. 5B**), thus confirming the contribution of overexpressed CXCR4 toward elevated chemoresistance.

CXCR4 is a chemokine receptor involved in regulating the migration of cancer cells [24,26]. Because CDP treatment could elevate CXCR4 expression, whether it consequently promoted cell migration at low concentrations was investigated. SKOV3 cells were firstly treated with 5 mg/l of CDP for 36 h, and then allowed to migrate from serum-free medium to normal culturing medium overnight. Crystal violet staining permitted the visualization of the migrated cells using microscopy. Results showed that CDP treatment promoted the motility of SKOV3 cells (**Fig. 5C**). SKOV3 cells, which were transfected with GV142-CXCR4, presented higher motility than that of SKOV3 cells transfected with control plasmids (GV142) (**Fig. 5D**). These results suggested that CDP incubation led to enhanced cell migration capabilities by elevating the expression of CXCR4.

Discussion

In spite of the development of novel diagnostic and treatment strategies, the survival rate of epithelial ovarian cancer patients remains quite low, chiefly due to elevated chemoresistance and disease recurrence. The presence of cancer stem cells could at least partially explain the origin of chemoresistance. Increasing studies have focused on this cell group, in order to understand chemoresistance development and to find potentially novel therapeutic strategies [5,10–12]. Certain marker proteins, such as CXCR4, have been detected in cancer stem cells and identified as possible treatment targets [24–26]. Overexpression of CXCR4 appears to be associated with chemoresistance, metastasis, and reduced survival rates in ovarian cancer patients [24].

Some studies have successfully isolated highly chemoresistant cancer stem cells from ovarian cancer tumor tissues and ascites [16–18]. Furthermore, CSLCs have also been extracted from long-term cultured immortalized ovarian cancer cell lines [19–21]. These CSLCs display the typical features of cancer stem cells, and provide a valuable model for investigating the molecular mechanisms underlying chemoresistance in cancer cells.

According to our and other groups' previous studies, anticancer drug treatment can promote the enrichment of CSLCs [6,22,28].

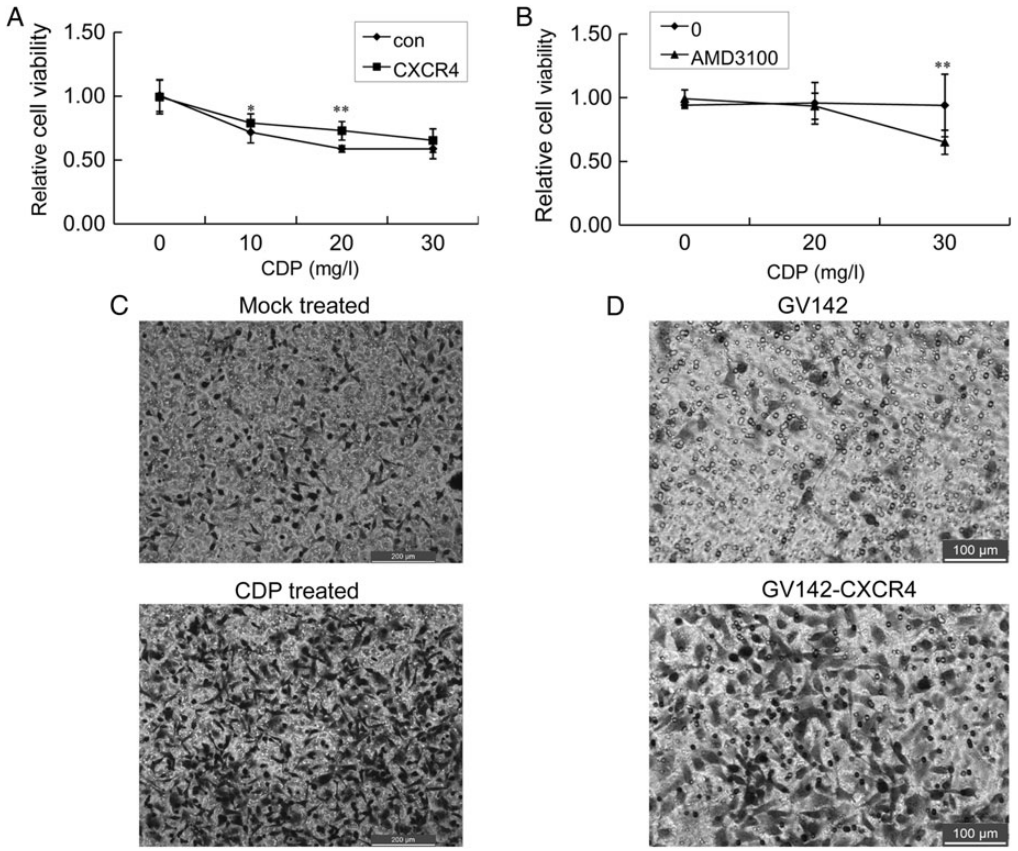

Figure 5. Elevated CXCR4 expression promoted drug resistance and cell migration (A) MTT assay was used to detect the cell viability of SKOV3 cells, which were transfected with control (con) or CXCR4-overexpressing plasmids (CXCR4). Result was presented as the average of at least three experiments, and data are shown as the mean ± SD (**P < 0.01; *P < 0.05). (B) AMD3100, which is the antagonist of CXCR4, sensitized the SKOV3 cells to CDP treatment. MTT assay was used to detect cell viability. Result was presented as the average of at least three experiments, and the error bars indicated the standard derivations (**P < 0.01). (C) Cell motility was examined by cell migration assays. Cells were treated with DMSO (mock) or 5 mg/l of CDP for 36 h and allowed to migrate overnight. (D) Cells were transfected with control (GV142) or CXCR4-overexpressing plasmids (GV142-CXCR4), and allowed to migrate overnight. Migrated cells were fixed with methanol, visualized with crystal violet staining, and observed with microscopy.

However, it is not clear if these anticancer drugs can promote the formation of CSLCs or more efficiently select surviving cells. Addressing this question is essential for understanding the mechanisms governing chemoresistance development, and for optimizing therapeutic strategies against ovarian cancer.

In this study, we found that incubation with low concentrations of CDP led to elevated expression of CXCR4, which was much more abundant in CSLCs than in parent adherent cells. Our results also revealed that artificially overexpressing exogenous CXCR4 resulted in increased efficient CSLC spheroid formation, cell motility, and resistance. Additionally, CXCR4 overexpression also contributed to elevated expression of cancer stem cell markers. Furthermore, our results demonstrated that the combination of CDP with a CXCR4 antagonist displayed elevated antiproliferative activities when compared with CDP alone. Based on these findings, we concluded that treatment with low concentrations of CDP promoted the formation of CSLC spheroids through induction of CXCR4 expression.

Our data suggested that chemotherapy itself could result in chemoresistance via inducing the expression of CXCR4, which promoted the proliferation of CSLCs. This is a significant discovery in our understanding of the molecular mechanisms underlying chemoresistance development during chemotherapy treatment. Our findings may also provide valuable indications for optimizing clinic therapeutic strategies against epithelial ovarian cancer, particularly in terms of determining appropriate CDP dosages for each individual patient.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 81401216 and 81200471), the Shanghai Municipal Health & Family Planning Commission (No. 20134Y128), the Shanghai Jiaotong University School of Medicine (No. 13XJ10067), and the Shanghai Committee of Science and Technology (Nos. 14YF1408200 and 14411961500).

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics. *CA Cancer J Clin* 2014, 64: 9–29.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011, 61: 69–90.
3. Cho KR, Shih IeM. Ovarian cancer. *Annu Rev Pathol* 2009, 4: 287–313.
4. Sudo T. Molecular-targeted therapies for ovarian cancer: prospects for the future. *Int J Clin Oncol* 2012, 17: 424–429.
5. Foster R, Buckanovich RJ, Rueda BR. Ovarian cancer stem cells: working towards the root of stemness. *Cancer Lett* 2013, 338: 147–157.

6. Abubaker K, Latifi A, Luwor R, Nazaretian S, Zhu H, Quinn MA, Thompson EW, *et al*. Short-term single treatment of chemotherapy results in the enrichment of ovarian cancer stem cell-like cells leading to an increased tumor burden. *Mol Cancer* 2013, 27: 12–24.
7. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, Sarkar S. Drug resistance in cancer: an overview. *Cancers (Basel)* 2014, 6: 1769–1792.
8. Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O, Castedo M, *et al*. Molecular mechanisms of cisplatin resistance. *Oncogene* 2012, 31: 1869–1883.
9. Galluzzi L, Vitale I, Michels J, Brenner C, Szabadkai G, Harel-Bellan A, Castedo M, *et al*. Systems biology of cisplatin resistance: past, present and future. *Cell Death Dis* 2014, 5: e1257.
10. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005, 5: 275–284.
11. Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med* 2007, 58: 267–284.
12. Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. *Nat Rev Cancer* 2012, 12: 133–143.
13. Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF. A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* 2012, 488: 522–526.
14. Driessens G, Beck B, Caauwe A, Simons BD, Blanpain C. Defining the mode of tumour growth by clonal analysis. *Nature* 2012, 488: 527–530.
15. Ahmed N, Abubaker K, Findlay JK. Ovarian cancer stem cells: molecular concepts and relevance as therapeutic targets. *Mol Aspects Med* 2014, 39: 110–125.
16. Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. *Cancer Res* 2005, 65: 3025–3029.
17. Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, Yan PS, *et al*. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res* 2008, 68: 4311–4320.
18. Liao J, Qian F, Tchabo N, Mhawech-Fauceglia P, Beck A, Qian Z, Wang X, *et al*. Ovarian cancer spheroid cells with stem cell-like properties contribute to tumor generation, metastasis and chemotherapy resistance through hypoxia-resistant metabolism. *PLoS One* 2014, 9: e84941.
19. Kondo T, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. *Proc Natl Acad Sci USA* 2004, 101: 781–786.
20. Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. *Cancer Res* 2005, 65: 6207–6219.
21. Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS. *In vitro* propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev* 2003, 17: 1253–1270.
22. Ma L, Lai D, Liu T, Cheng W, Guo L. Cancer stem-like cells can be isolated with drug selection in human ovarian cancer cell line SKOV3. *Acta Biochim Biophys Sin* 2010, 42: 593–602.
23. Luo X, Dong Z, Chen Y, Yang L, Lai D. Enrichment of ovarian cancer stem-like cells is associated with epithelial to mesenchymal transition through an miRNA-activated AKT pathway. *Cell Prolif* 2013, 46: 436–446.
24. Kajiyama H, Shibata K, Terauchi M, Ino K, Nawa A, Kikkawa F. Involvement of SDF-1alpha/CXCR4 axis in the enhanced peritoneal metastasis of epithelial ovarian carcinoma. *Int J Cancer* 2008, 122: 91–99.
25. Krohn A, Song YH, Muehlberg F, Droll L, Beckmann C, Alt E. CXCR4 receptor positive spheroid forming cells are responsible for tumor invasion *in vitro*. *Cancer Lett* 2009, 280: 65–71.
26. Yagi H, Tan W, Dillenburg-Pilla P, Armando S, Amornphimoltham P, Simaan M, Weigert R, *et al*. A synthetic biology approach reveals a CXCR4-G13-Rho signaling axis driving transendothelial migration of metastatic breast cancer cells. *Sci Signal* 2011, 4: ra60.
27. Chen YF, Wang SY, Shen H, Yao XF, Zhang FL, Lai D. The marine-derived fungal metabolite, terrein, inhibits cell proliferation and induces cell cycle arrest in human ovarian cancer cells. *Int J Mol Med* 2014, 34: 1591–1598.
28. Latifi A, Abubaker K, Castrechini N, Ward AC, Liongue C, Dobill F, Kumar J, *et al*. Cisplatin treatment of primary and metastatic epithelial ovarian carcinomas generates residual cells with mesenchymal stem cell-like profile. *J Cell Biochem* 2011, 112: 2850–2864.