

Original Article

Estrogen increases secretion of stromal cell derived factor-1 in human breast cancer cells

Fengliang Zhang¹, Hua Kang², Qing Xu¹

¹Department of General Surgery, Capital Medical University School of Rehabilitation, Beijing Charity Hospital, China Rehabilitation Research Center, Beijing 100068, P. R. China; ²Department of General Surgery, Xuanwu Hospital, Capital Medical University, Beijing 100068, P. R. China

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Abstract: Objective: Stromal cell derived factor-1 (SDF-1) is closely related to the biological characteristics of breast cancer. The aim of this article is to investigate if estrogen affects the biological characteristics of breast cancer via affecting secretion of SDF-1. Methods: The breast cancer cell lines MCF-7 and MDA-MB-231 used in this study were divided into control group, estrogen group and estrogen plus estrogen receptor (ER) antagonist group. These groups were treated with different concentrations of 17- β estradiol or the same concentration of 17- β estradiol for different times, respectively. Enzyme-linked immunosorbent assay and semi-quantitative reverse transcriptase polymerase chain reaction were performed. Results: Secretion of SDF-1 was detected in the cell basal medium of MCF-7. When adding a high physiological concentrations of 17- β estradiol (10⁻⁷ mol/L), the levels of SDF-1 secretion achieved a peak at 2 h and it was 6 times of control group (1823.16 \pm 325.18 pg/ml comparing to 308.23 \pm 9.23 pg/ml, $P < 0.01$). However, this effect could be eliminated by the pure estrogen antagonist ICI182 or ICI780. The SDF-1 mRNA levels were consistent with the determined SDF-1 protein levels. At the time point of 2 h, for the 10⁻⁷ mol/L group, the SDF-1 mRNA expression levels were higher than the antagonist group, with statistically significant differences ($P < 0.05$). Conclusions: It was found that secretion of SDF-1 can be increased by the physiological concentrations of estrogen mainly through regulation of estrogen receptor.

Keywords: Breast cancer, 17- β estradiol, MCF-7, MDA-MB-231, SDF-1

Introduction

Breast cancer is one of the hormone-dependent tumors. Estrogen has been found to play a key role in occurrence and progress of breast cancer. In addition to regulation of the gene expression of breast cancer through genomic or non-genomic pathways involved in estrogen binding to its receptor [1], the metabolites of estrogen itself also play an important role in breast cancer [2].

Stromal cell derived factor-1 (SDF-1) is one of main growth factors in the development of breast cancer. CXCR4 is the only receptor that is known as SDF-1 receptor. A number of studies have shown that SDF-1/CXCR4 signaling pathways are closely associated with cell growth, migration and invasion of breast cancer.

Estrogen increases the SDF-1 secretion levels of breast cancer cells and acts on the stromal

fibroblasts to produce SDF-1, performing cross-talk with other chemokines, stimulating the proliferation of breast cancer cells. This effect can be antagonized by pure ER antagonist ICI182 or ICI780 [3, 4].

This study selected estrogen receptor- α (ER- α)-positive and ER- α -negative breast cancer cell lines as the models to investigate the effect of estrogen on SDF-1 secretion to further affect the progression of breast cancer. It thus may provide new options for breast cancer treatment.

Materials and methods

Reagent and cells

Estrogen (17- β -estradiol) and estrogen receptor antagonist (ICI182 and ICI780) were purchased from Sigma, USA. RPMI-1640 containing 10% fetal calf serum, penicillin at a final concentra-

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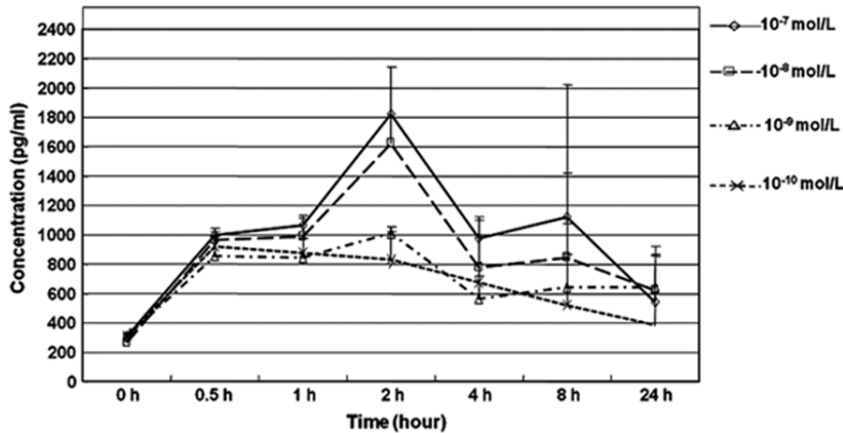


Figure 1. The changes in SDF-1 secretion of MCF-7 cells in the presence of estrogen with different concentrations. MCF-7 cells were treated with different concentrations of 17- β estradiol (10^{-7} , 10^{-8} , 10^{-9} , and 10^{-10} mol/L). The expression levels of SDF-1 protein were represented by the concentration values that were determined by ELISA. The changes in SDF-1 expression levels for different time courses at various concentrations of 17- β estradiol were shown.

tion of 1×10^5 U/L, streptomycin at a final concentration of 100 mg/L were purchased from Gino biological pharmaceutical Co., Ltd. ER- α -positive cell lines MCF-7 and ER- α -negative cell lines MDA-MB-231 were purchased from European Collection of animal cells Corporation.

Screening of the SDF-1 protein secretion

When the cells were grown to 80%, equal volume of original culture medium and 17- β estradiol with different physiological concentrations containing 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} mol/L were added into the medium, respectively. They were cultured for 0, 0.5, 1, 2, 4, 8, and 24 hours, and then the cell culture medium were collected at different points of time to obtain the supernatant by centrifuging the cells for 20 minutes.

Experimental grouping

10^{-7} mol/L was selected as the physiological concentration for 17- β estradiol used in the further experiments. The experimental models were divided into control group, estrogen group and estrogen plus ER antagonist group. Equal volume of 10^{-6} mol/L ER antagonists (ICI182 and 780) were added into the last group before adding 17- β estradiol. The time points for the three experimental groups were 0, 0.5, 1, 2, 4, 8 and 24 hours. The cell culture medium was collected at different time points and RNA was extracted simultaneously. Then the samples were aliquoted for the cryopreservation at -20°C . These experiments were tripled.

Enzyme linked immunosorbent assay (ELISA)

When the cells were grown to 80% confluency, 100 μl of supernatant of cell culture medium were collected. ELISA was performed to determine the concentrations of SDF-1. The samples were diluted into a series of concentrations including 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml, 0.156 ng/ml and they were added into the control groups. The last group was set as a blank group

for comparing the results. The absorbance value for each well was read in a microplate reader. Therefore, the standard curves were plotted for the concentrations to the absorbance values. In this way, the SDF-1 concentrations could be determined on the basis of different absorbance values.

RT-PCR

SDF-1 primers were designed based on the SDF-1 gene open reading frame sequence (Gene bank NO: NM198120), and were synthesized by Shanghai Biological Engineering Company. The sequences of SDF-1 and β -actin primers were as follows: SDF-1_F, 5'-CCGCGCTCTGCCTCATGCGACGGAAG-3'; SDF-1_R, 5'-CTGTTTAAAGCTTTCTCCAGGTA-3'; β -actin_F, 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3'; β -actin_R, 5'-CTAGAAGCATTGCGGTGACGATGAGGG-3'.

Statistical methods

The experimental data were represented as mean values plus standard deviation ($\bar{X} \pm S$). The data between groups and within the groups were analyzed through *t* test and variance test by using SPSS 11.5 statistical software. The homogeneity and normality of variance test were carried prior to the analysis of data, whose test standard was $\alpha = 0.05$ (bilateral). It is considered as a statistical difference if *p* value was less than 0.05, while it would be considered to be a statistically significant difference if *P* value

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Table 1. The effect of 10^{-7} mol/L 17- β estradiol on MCF-7 SDF-1 protein

Reaction time(hours)	SDF-1 levels (n = 6, pg/ml)				F	P
	Control group	Estrogen group	ER blocking group			
0	297.44 \pm 23.21	308.20 \pm 9.23	306.45 \pm 39.87		0.63	0.481
0.5	303.53 \pm 10.43	1001.28 \pm 18.63	489.28 \pm 205.77		125.56	0.021
1.0	301.45 \pm 90.25	1067.77 \pm 62.29	381.24 \pm 246.99		161.71	0.002
2.0	307.23 \pm 5.42	1823.17 \pm 325.18	547.97 \pm 157.96		201.02	0.000
4.0	297.67 \pm 28.23	980.30 \pm 143.41	469.45 \pm 338.74		6.19	0.025
8.0	302.66 \pm 74.01	1121.15 \pm 904.08	508.44 \pm 230.56		172.93	0.001
24.0	289.04 \pm 61.29	546.45 \pm 377.52	330.48 \pm 223.32		0.85	0.440

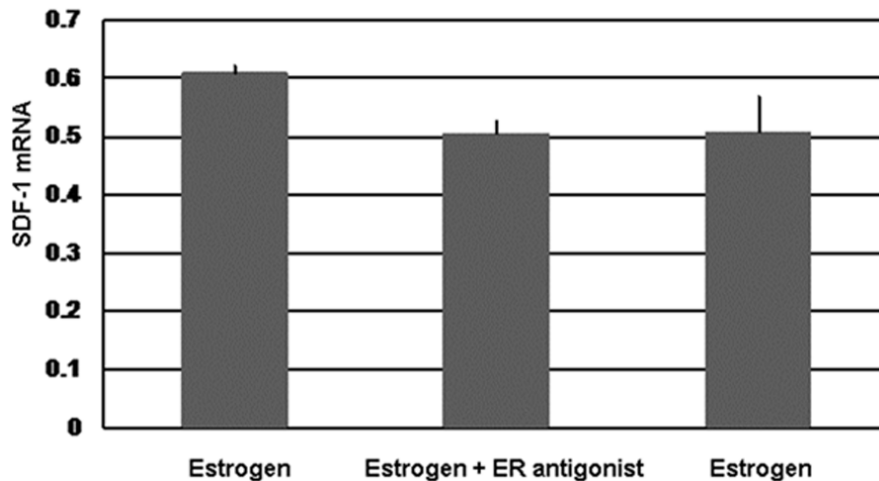


Figure 2. The SDF-1 mRNA levels in estrogen group, ER antagonist group, and control group. MCF-7 cells in the estrogen group, ER antagonist group, and control group were treated with 10^{-7} mol/L 17- β estradiol for 2 hours. The SDF-1 mRNA levels were determined by using RT-PCR.

was less than 0.01. Excel software was used for the image analysis.

Results

Effects of 17- β estradiol on levels of SDF-1 protein in MCF-7 cells

To determine the secretion of SDF-1 in MCF-7, ELISA was performed. It was found that the basic level of SDF-1 secretion was 308.20 ± 97.23 pg/ml, while no SDF-1 secretion could be detected in MDA-MB-231 cells. It was found that no SDF-1 protein could be detected either in the basic state MDA-MB-231 or when the cells were treated by estrogen. However, the secretion of SDF-1 could be detected in MCF-7 cells even only in their basic state and the secretion was found to be increased by estrogen. Among the physiological concentrations of 17- β estradiol range from 10^{-7} to 10^{-10} mol/L,

10^{-7} mol/L was found to be the most effective. In addition, the role of estrogen represented a bimodal change and achieved the peak after 2 hours. Another small peak appeared after 8 hours and the SDF-1 levels were gradually restored to the basic state after 24 hours as they were shown in **Figure 1**.

At the time point of 0 h, the average levels of SDF-1 for all the groups were 308.20 ± 97.23 pg/ml, and 811.28 ± 275.16 pg/ml at 0.5 h; 817.06 ± 289.87 pg/ml at 1 h; 1118.93 ± 617.01 pg/ml at 2 h; 663.87 ± 302.44 pg/ml at 4 h; 687.97 ± 542.63 pg/ml at 8 h; 562.59 ± 245.31 pg/ml at 24 h. Each difference between 0 h time point and others including 0.5 h, 1 h, 2 h, 4 h, 8 h was statistically significant ($P < 0.05$), but the difference between 0 h and 24 h was not statistically significant ($P > 0.05$).

Effect of 17- β estradiol on protein and mRNA levels of SDF-1 in MCF-7 cells

After 17- β estradiol treatment of breast cancer cells for different times, the SDF-1 secretion levels were compared and shown in **Table 1**. The expression levels of SDF-1 mRNA in control group, estrogen group, and antagonist group were 0.506 ± 0.063 , 0.608 ± 0.013 and 0.503 ± 0.024 , respectively. The P values between antagonist group and estrogen group and control group were 0.029 and 0.026, respectively, which were considered to be significant (**Figure 2**). At the time point of 2 h, for the 10^{-7} mol/L group, the SDF-1 mRNA expression levels were higher than the antagonist group, with statisti-

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cally significant differences ($P < 0.05$). But SDF-1 secretion levels were less affected by the lower concentration of estrogen. These results suggest that higher concentration of estrogen could increase the SDF-1 secretion levels of MCF-7 cells.

Discussion

In this study, the ER-positive cell line MCF-7 and the ER-negative cell line MDA-MB-231 were selected as the experimental cells. The results demonstrated that the secretion levels of SDF-1 were observed to be increased in estrogen-treated MCF-7 cell line and achieved a peak in 10^{-7} mol/L within 2 hours, about 6 folds of the basic level of secretion. Compared to the control group, the difference was significant, indicating estrogen can promote the secretion of SDF-1. However, no SDF-1 secretion was detected in ER-negative MDA-MB-231 cell line either when they were treated with or without estrogen. It suggests that MDA-MB-231 cells do not express SDF-1 under the above physiological concentrations of estrogen, which is consistent with the previous data [5]. The results confirmed that estrogen plays its roles in ER-positive cells.

The range of estrogen concentration in normal female is 10^{-7} ~ 10^{-10} mol/L. In this study, the SDF-1 secretion level of MCF-7 in the group with high concentration of 10^{-7} mol/L was 6 folds of control group. It was obvious changes in SDF-1 secretion levels, suggesting the improvement of estrogen could promote the proliferation of ER-positive breast cancer cells. At the molecular level, SDF-1 mRNA of MCF-7 cells was determined by using RT-PCR, showing that SDF-1 mRNA levels in the high concentration estrogen of 10^{-7} mol/L group has a significant difference compared to control group. Therefore, the effect of estrogen may be dose-dependent. An *in vitro* study carried in SKBR-3 reported that when estrogen concentration is less than 10 nM (10^{-8} mol/L), the ER signal pathways on cell membrane cannot be induced [6]. Most of the *in vitro* experiments suggested that only when the estrogen concentration is higher than physiological concentrations (10^{-3} mol/L); estrogen can play a role [7].

In this experiment the role of estrogen in MCF-7 cell line achieved to a peak after 2 hours, and continued for 4 hours. Then the effect was weakened after the 4 hour and there is no difference in each group after 24 hours. RT-PCR

was used for determining SDF-1 mRNA levels, in which 10^{-7} mol/L estrogen group for 2 hours were significantly higher than it in control group. The difference between the two groups was significant that can further verify the effect of estrogen at the molecular level. While some other studies reported that the period of estrogen role is after 16-24 h. This may be due to different estrogen has different pharmacological effects, half-life and effect channel of estrogen receptors. Because estrogen dose not bind to ER directly but through binding to the growth factor including SDF-1, IGF-1R, EGFR etc. to pass through the cell membrane [8, 9]. However, there is a small number of protein-mediated rapid estrogen signaling channels on the cell membrane [10, 11]. This may be involved in the rapid role of estrogen.

Estrogen receptor antagonist ICI182 and 780 were used to block ER, and the role of estrogen was observed to be significantly inhibited and the expression levels of SDF-1 were decreased. The difference between estrogen group and antagonist group was statistically significant ($P < 0.05$), showing that ICI182 and ICI780 have obvious antagonism functions on estrogen. In addition, when MCF-7 cells were pre-incubated with ICI182 and ICI780, estrogen did not increase SDF-1 mRNA and protein expression significantly. It suggests that the regulation of estrogen was achieved through the ER pathway. The results of this study showed that estrogen may increase the expression and secretion of SDF-1 via a dose- and time-dependent manner. Thus, it was speculated that the mechanism of action of estrogen used in hormone therapy for breast cancer patients may be related to the increased expression of SDF-1. As a target gene of estrogen, SDF-1 plays its role through binding to receptor CXCR4 and can be combined with ER- α to regulate the transcriptional activity and the responsiveness to estrogen. High concentrations of estrogen in some breast cancer cells could increase human SDF-1 levels, acting on the stromal fibroblasts to produce SDF-1. Previous studies have found that stromal fibroblasts express ER- β and MCF-7 cells express ER- α . It means that estrogen plays its role mainly through its ER- α and ER- β binding. In breast cancer, it mainly was dominated by ER- α and the excessive activation of ER- α is one of important factors in the occurrence and development of estrogen-dependent breast cancer. Recent studies have shown that estrogen can cause serine phosphorylation of some

sites in ER- α serine and generate anti-phosphotyrosine antibody [12] to act on breast cancer. However, the results are quite different, for example, some studies reported that phosphorylation of serine could improve overall survival and disease-free survival for breast cancer patients [13], but the role of phosphorylation could induce drug resistance to tamoxifen in premenopausal breast cancer patients [14]. Moreover, SDF-1 can promote the growth of breast cancer cells and inhibit apoptosis of breast cancer cells through autocrine and paracrine [15], simultaneously attracting the endothelial cells to the tumor to promote tumor angiogenesis [16]. The findings in this study suggest that in the development of breast cancer, estrogen could regulate the expression of SDF-1 through the ER signal transduction pathway, thereby promoting tumor progression. Additionally, these results also showed that estrogen receptor antagonist ICI182 and ICI780 could inhibit estrogen promotion of the proliferation of human breast cancer cells.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qing Xu, Department of General Surgery, Capital Medical University School of Rehabilitation, Beijing Charity Hospital, China Rehabilitation Research Center, Beijing 100068, P. R. China. Tel: +86 13501158095; E-mail: lala0518@sina.com

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