Effects of human umbilical cord-derived mesenchymal stem cells on hematologic malignancies

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Abstract. Mesenchymal stem cells (MSCs) have been used in hematopoietic stem cell transplantation for years. However, the safety of MSCs applied in various types of hematologic malignancy has not been comprehensively explored. In the present study, the effects of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) on six representative hematologic malignancy cell lines were explored, including leukemia, multiple myeloma and lymphoma cells. Direct and indirect co-culture models were established, and cell proliferation was assessed by carboxyfluorescein diacetate succinimidyl ester staining. A cytometric bead array cytokine kit was used to quantify cytokines. The expression of interleukin (IL) -6 receptor elements on tumor cells was detected by reverse transcription-polymerase chain reaction and flow cytometry, and the effects of exogenous IL-6 on cell proliferation were determined using a Cell Counting kit-8 assay. The results demonstrated that hUC-MSCs inhibited the proliferation of most of the cell lines examined (THP-1, HL-60, K562 and RPMI-8226), but promoted the proliferation of Raji cells. In addition, hUC-MSCs secreted abundant IL-6, promoted the secretion of IL-10 by RPMI-8226 and Raji cells, and inhibited the secretion of tumor necrosis factor-α by THP-1 cells. These data indicate a varied effect of hUC-MSCs on various types of hematologic malignancy, including distinct mechanisms of cell-to-cell contact and cytokines. Researchers applying hUC-MSCs in lymphoma should be aware of a potential tumor growth-promoting effect.

Introduction

Mesenchymal stem cells (MSCs) are a type of adult stem cell that exhibits self-renewal capacity and differentiation potential; they occur in the bone marrow (BM), muscle, adipose tissue, bone, placenta and umbilical cord (1). Compared with other sources, MSCs from the umbilical cord (hUC-MSCs) may be the most optimal source on account of their abundant supply, improved expandability, lower probability of microbial contamination and reduced ethical limitations relative to MSCs from other sources (2).

MSCs have potential applicability in tissue damage, autoimmune diseases, neurodegenerative diseases, diabetes and cancer therapy (3-6). Furthermore, as they may support hematopoiesis and immune regulation, MSCs have been applied in hematopoietic stem cell (HSC) transplantation for years (7). A stem cell treatment used in the treatment of severe graft-vs.-host disease (GVHD) in children, Prochymal, was approved in Canada and New Zealand in 2012 (8). However, there is controversy regarding the application of MSCs in patients with a malignancy due to their potential cancer-promoting effects. Different groups have arrived at opposite conclusions concerning the effect of MSCs on tumors in vivo and in vitro (9-11). Our previous study indicated that the co-transplantation of HSCs and MSCs may prevent GVHD, but may simultaneously increase the relapse rate in patients with hematologic malignancy relative to the transplantation of HSCs alone (12). Accumulating evidence suggests that the interaction between MSCs and tumors is regulated by multiple factors, particularly in vivo: MSCs may migrate to the tumor sites and promote or inhibit the growth, invasion and metastasis of tumors (9,10) through cell-to-cell contact (13) or paracrine secretion (14). They not only regulate the cell cycle and apoptosis of tumor cells directly (15), but also induce immune tolerance by secreting immune regulatory factors, including indoleamine-2,3-dioxigenase, nitrogen monoxide, and interleukin (IL)-6, which may facilitate the immune escape of a tumor (16,17).

Prior studies on the effects of MSCs on tumors have predominantly focused on solid tumors; the effect of MSCs on hematologic malignancies has yet to be characterized. In the

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present study, the effects of hUC-MSCs on the proliferation of six cell lines representative of various types of hematologic malignancy, including leukemia (THP-1, HL-60, K562 and Jurkat), multiple myeloma (RPMI-8226) and lymphoma (Raji), were explored, including through direct and indirect co-culture models; the potential mechanisms of cell-to-cell contact and cytokines were then discussed.

Materials and methods

Isolation and culture of hUC-MSCs. The present study was approved by the Institutional Review Board of the Affiliated Hospital of the Academy of Military Medical Sciences (Beijing, China; protocol #2010-05-60). Written informed consent forms were obtained from the healthy umbilical cord donors. The isolation and identification of hUC-MSCs were performed as described previously (18); flow cytometry was used to identify hUC-MSCs, which were positive for cluster of differentiation (CD) 73, 90, 105 and 166, and negative for hematopoietic markers, including CD45, 14 and 34, and major histocompatibility complex class II-DR. Resuspended cells were plated at a density of 5x10^4/ml and maintained in DMEM/F12 (Hyclone; GE Healthcare, Chicago, IL, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in a humidified atmosphere with 5% CO_2 at 37°C. The ability of hUC-MSCs to differentiate into osteoblasts and adipocytes was confirmed prior to use, as previously described (18). Cells were used in the present study subsequent to 5-6 passages.

Culture and carboxyfluorescein diacetate succinimidyl ester (CFSE) staining of tumor cell lines. THP-1 and HL-60 acute myeloid leukemia (AML) cells, K562 chronic myeloid leukemia (CML) cells, Jurkat acute lymphoblastic leukemia (ALL) cells, RPMI-8226 multiple myeloma (MM) cells and Raji Burkitt's lymphoma cells were obtained from the China Infrastructure of Cell Line Resources (Beijing, China). All cell types were initially maintained in RPMI-1640 medium (Hyclone; GE Healthcare) supplemented with 10% FBS at 37°C in a humidified atmosphere of 5% CO_2. The medium was changed every three days. To be stained with CFSE (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), tumor cells were washed three times and resuspended in RPMI-1640 medium, then mixed with an equal volume of 5 µmol/l CFSE and incubated for 10 min at 37°C. The reaction was terminated by adding an equal volume of FBS for 10 min at 4°C. Finally, cells were washed and resuspended in DMEM/F12 medium supplemented with 10% FBS for experiments.

Direct and indirect co-culture models and assessment of cell proliferation. hUC-MSCs and CFSE-stained tumor cells were co-cultured in 6-well plates at the ratios of 2:1, 1:1, 1:4 and 1:16, or in the lower and upper compartments, respectively, of transwell plates at 2:1, 1:1 and 1:4. Tumor cells alone served as controls. Cells were cultured in DMEM/F12 medium supplemented with 10% FBS for 72 h at 37°C, and then tumor cells were harvested; 10^5 CFSE-positive cells from each sample were acquired and the FL1 fluorescence intensity was detected with an Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Data were analyzed using FCS Express 4.0 software (De Novo Software, Glendale, CA, USA). The experiment was repeated three times.

Measurement of cytokines by cytomteric bead array (CBA). hUC-MSCs alone, tumor cells alone or both at an equal concentration were cultured in 6-well plates for 72 h at 37°C. The supernatants were harvested and centrifuged (2,210 x g for 5 min at room temperature) to remove precipitation. Cell-free supernatants were collected and frozen at -80°C. The IL-2, -4, -6 and -10, tumor necrosis factor (TNF)-α and interferon (IFN)-γ content was measured according to the protocol for the CBA cytokine kit (BD Biosciences). Briefly, cytokine standards were prepared and cytokine capture beads were mixed; 50 µl of mixed capture beads, phycoerythrin detection reagent and samples or standards were then added to all assay tubes. The assay tubes were incubated for 3 h at room temperature in the dark, then washed and centrifuged. The supernatant was carefully aspirated and discarded, and bead pellets were resuspended with 300 µl wash buffer. Data were obtained and analyzed using CBA analysis with CellQuest software (version 3.3) and CBC software (version 3.0) (BD Biosciences). The experiment was repeated three times.

Detection of the expression of IL-6, IL-6Ra and IL-6 signal transducer (gp130) mRNAs by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted using the Pure RNA rapid extraction kit (Biomed, Beijing, China) according to the manufacturer's protocol and reverse transcribed using the First-Strand Synthesis system (Thermo Fisher Scientific, Inc.). Reverse transcription was performed at 42°C for 1 h then 25°C for 5 min. PCR was performed using GAPDH as internal control; primer sequences and thermocycling conditions are listed in Table I. The primers and Taq PCR Master MIX were supplied by Biomed (Beijing, China). All products were evaluated by electrophoresis on 1% agarose gel. The experiment was repeated three times.

Detection of the expression of IL-6Ra and gp130 proteins by flow cytometry. Flow cytometry was used to identify the expression of IL-6Ra and gp130 proteins on the membranes of tumor cells, which were labeled with monoclonal antibodies against IL-6Ra conjugated to allophycocyanin (APC; APC Mouse Anti-Human CD126, catalog no. 562090, BD Biosciences) and gp130 conjugated to phycoerythrin (PE; PE Mouse Anti-Human CD130, catalog no. 555757, BD Biosciences). Tumor cells were incubated with the 1:100 diluted antibodies for 15 min at room temperature in the dark. At least 10,000 events were acquired on the BD Accuri C6 and data were analyzed using the Accuri C6 software (version 1.0, BD Biosciences). The experiment was repeated three times.

Assessment of effects of IL-6 on cell proliferation. Tumor cells were seeded in 96-well plates at 1x10^4 cells/well with increasing concentrations of recombinant human IL-6 (0, 10, 20, 50 and 100 ng/ml; R&D Systems, Inc., Minneapolis, MN, USA) and incubated at 37°C. At 72 h, 10 µl Cell Counting kit-8 (CCK-8) reagent (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to the wells for 2-5 h at 37°C, and the absorbance at 450 nm was measured with an iMark...
microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The experiment was repeated three times.

Statistics. The experimental data are presented as mean values, with bars representing the standard deviation. Data were analyzed using GraphPad Prism version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS version 19 (IBM Corp., Armonk, NY, USA) software. Determinations of statistical significance were performed using Student's t-test for comparisons of two groups or an analysis of variance (ANOVA) followed by Tukey's post-hoc test for comparisons of multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

hUC-MSCs affect cells from different types of hematologic malignancy differently in co-culture. hUC-MSCs and tumor cells were co-cultured in 6-well plates for 72 h. The mean fluorescence intensity (MFI) of cells stained with CFSE is gradually attenuated in the process of proliferation. Increases or decreases in MFI relative to a control indicated the inhibition or promotion of tumor cell proliferation by hUC-MSCs, respectively.

For the AML cell lines THP-1 (Fig. 1A) and HL-60 (Fig. 1B), and the CML cell line K562 (Fig. 1C), MFI in the experimental groups was higher than in the control groups, and increased with the concentration of hUC-MSCs, indicating that the hUC-MSCs inhibited proliferation in a dose-dependent manner. hUC-MSCs only promoted proliferation when added at a 1:1 concentration to the ALL Jurkat cells (Fig. 1D). In the MM cell line RPMI-8226 (Fig. 1E), hUC-MSCs inhibited proliferation at all doses. hUC-MSCs significantly promoted the proliferation of the Burkitt's lymphoma cell line Raji (Fig. 1F). These data indicated that hUC-MSCs have various effects on cancer cell proliferation. In summary, hUC-MSCs inhibited the proliferation of THP-1, HL-60, K562 and RPMI-8226 cells, but promoted Raji cell proliferation (Table II).

hUC-MSCs affect cells from different types of hematologic malignancy differently in transwells. To explore the roles of cell-to-cell contact and cytokines secreted by hUC-MSCs, CFSE-stained tumor cells were separated from hUC-MSCs by transwells and cultured for 72 h. Cell proliferation was assessed as previously described.

For the leukemia cell lines THP-1 (Fig. 2A), HL-60 (Fig. 2B) and K562 (Fig. 2C), hUC-MSCs inhibited proliferation, consistent with the previous results, suggesting that inhibitory cytokines were involved in the effect. However, for the ALL cell line Jurkat (Fig. 2D), hUC-MSCs inhibited cell proliferation in transwells, whereas there had been almost no effect on proliferation observed when co-cultured directly; we speculated that the growth promotion caused by cell-to-cell contact may have been related to the inhibitory cytokines secreted by hUC-MSCs. For the Burkitt's lymphoma cell line Raji (Fig. 2F), although hUC-MSCs significantly promoted cell proliferation in direct co-culture, they had no effect in transwells, implying that cell-to-cell contact may have been a dominant mechanism for growth promotion in Raji cells.

The results revealed that hUC-MSCs have distinct effects on different types of hematologic malignancies, likely due to diverse mechanisms. In summary, growth inhibition caused by hUC-MSCs in THP-1, HL-60, K562 and RPMI-8226 cells is likely to be associated with inhibitory cytokines, whereas the growth promotion of Raji is likely to be caused by cell-to-cell contact. For Jurkat cells, the effect varied depending on the concentration of hUC-MSCs (Table II).

**Table I. Primers for PCR.**

| Gene          | Primer sequences (5'-3')                      | Reaction condition                        | Size (bp) |
|---------------|-----------------------------------------------|-------------------------------------------|-----------|
| IL-6 receptor α chain | F: CATTGCCATTTTCTCTAGGTTTC 94˚C for 30 sec, 60˚C for 30 sec, | 280       |
| R: GTGCCACCCAGCCAGCTATC 72˚C for 60 sec, 40 cycles |                           |           |
| IL-6 signal transducer | F: CATAGTCGTC GCTTGTGCTTAG 94˚C for 30 sec, 62˚C for 30 sec, | 527       |
| R: GATCTCTGGCGCTCCTC 72˚C for 60 sec, 40 cycles |                           |           |
| IL-6 | F: CCCCGATCCCCAGGAGAAGA 94˚C for 30 sec, 57˚C for 30 sec, | 349       |
| R: GCTGGAGGATGAGATGTTGTG 72˚C for 60 sec, 40 cycles |                           |           |
| GAPDH | F: GAGTCAACGATTTGGGCTG 94˚C for 30 sec, 57˚C for 30 sec, | 238       |
| R: TTGATTTTGAGGGATCTCG 72˚C for 60 sec, 40 cycles |                           |           |

IL-6, interleukin-6; F, forward; R, reverse.

**HUC-MSCs secrete abundant IL-6 and affect the secretion of tumor cells.** In order to investigate the specific cytokines involved in the effects described in the previous results, the levels of IL-2, -4, -6 and -10, and TNF-α and IFN-γ were detected by CBA; it was observed that the hUC-MSCs secreted abundant IL-6, whereas the secretion of IL-2, IL-4, IL-10, TNF-α and IFN-γ was limited (Fig. 3A).

These cytokines were then detected in the supernatants of the tumor cells cultured alone, and co-cultured 1:1 with hUC-MSCs, for 72 h. It was confirmed that hUC-MSCs secreted a high level of IL-6 in the co-culture supernatants. In addition, it was observed that hUC-MSCs affected the secretion by tumor cells. As presented in Fig. 3B, THP-1 cells secreted a small amount of IL-6 and TNF-α, and the secretion of TNF-α was inhibited when co-cultured with hUC-MSCs;
K562 cells secreted a small amount of IL-6; RPMI-8226 and Raji cells secreted IL-10, which was promoted by hUC-MSCs. It was inferred that hUC-MSCs may promote the secretion of IL-10 and inhibit the secretion of TNF-α by tumor cells.

The expression of IL-6R mRNA and protein varies between cells from different types of hematologic malignancy. IL-6 must bind with the receptor composed of IL-6Rα and gp130 to activate the downstream signaling pathway, which may affect the development of multiple types of tumor (19). In order to determine whether tumor cell lines were affected by IL-6, the expression of IL-6, IL-6Rα and gp130 mRNA in tumor cell lines was assessed using RT-PCR; it was observed that HL-60, RPMI-8226 and THP-1 cells expressed IL-6Rα and gp130 mRNA, K562 and Jurkat cells expressed gp130 mRNA, whereas Raji cells expressed neither (Fig. 4A; Table II). In addition, the expression of IL-6 mRNA by THP-1 and K562 cells was identified, consistent with the conclusion from CBA that they could secrete a small amount of IL-6.

Subsequently, the expression of IL-6Rα and gp130 protein on the membrane of the tumor cells was detected by flow cytometry. The results were consistent with the RT-PCR results: THP-1, HL-60 and RPMI-8226 cells expressed IL-6Rα and gp130 protein, and K562 and Jurkat cells expressed gp130 protein, whereas Raji cells expressed neither (Fig. 4B; Table II).

Exogenous IL-6 promotes the proliferation of tumor cells expressing IL-6R. To determine whether IL-6 is a mediator of the effects of hUC-MSCs on tumor cells, the effects of exogenous IL-6 on the proliferation of tumor cells were examined. Cells were cultured in medium supplemented with increasing concentrations of recombinant human IL-6 for 72 h, and the proliferation was assessed by a CCK-8 assay. As presented in Fig. 5, exogenous IL-6 significantly promoted the proliferation of THP-1 and HL-60 cells (P<0.05), but exhibited no significant effect on K562, Jurkat, RPMI-8226 and Raji cells (Table II). Combined with the previous results, it can be concluded that IL-6 is not a key mediator of the effects of hUC-MSCs; the stimulation of IL-6 on THP-1 and HL-60 cells may be offset by other inhibitory cytokines when co-cultured with hUC-MSCs.

Discussion

In the past, MSCs were most commonly isolated from BM. However, the aspiration of BM involves invasive procedures, limiting its availability. Therefore, an alternative source of MSCs is clinically valuable. It has been demonstrated that hUC-MSCs also exhibit potential applicability for allogeneic HSC transplantation similar to that of BM-MSCs (20); hUC-MSCs have subsequently become a new focus for stem cell research due to their abundant supply. However, considering
the emerging evidence that BM-MSCs may increase the risk of cancer relapse (10-12), it remains necessary to evaluate the safety of hUC-MSCs prior to their clinical application.

At present, there is no definite conclusion about the pro- or anti-tumorigenic effect of MSCs, which may be influenced by the MSC source, tumor type and experimental conditions. In the present study, the effects of hUC-MSCs on leukemia, MM and lymphoma cells were investigated simultaneously in order to exclude the factors of different MSC sources and experimental conditions; the whole results are summarized in Table II. It was revealed that hUC-MSCs predominantly exerted an inhibitory effect on leukemia cells; previous research has also demonstrated that MSCs from other sources, including BM and umbilical cord blood, may inhibit the development of leukemia by affecting the cell cycle and apoptosis (21-23). It may be inferred that this inhibition is associated with specific

![Figure 2](image_url)

Figure 2. hUC-MSCs affect the proliferation of tumor cells in transwells. hUC-MSCs and carboxyfluorescein diacetate succinimidyl ester-stained tumor cells, including (A) THP-1, (B) HL-60, (C) K562, (D) Jurkat, (E) RPMI-8226 and (F) Raji cells, were separately cultured in the lower and upper compartments of transwells for 72 h, and the MFI of tumor cells was assayed by flow cytometry. Left: Flow cytometry profiles of one representative experiment. Right: MFI of tumor cells from 3 independent experiments. Data are expressed as the mean ± standard deviation. *P<0.05, **P<0.01 compared with the control group.

hUC-MSCs, human umbilical cord-derived mesenchymal stem cells; MFI, mean fluorescence intensity.

| Tumor type                        | Cell line | IL-6Rα | gp130 | Exogenous IL-6 | Co-culture | Transwell |
|-----------------------------------|-----------|--------|-------|----------------|------------|-----------|
| Acute myeloid leukemia            | THP-1     | +      | +     | U              | D          | D         |
| Acute promyeloid leukemia         | HL-60     | +      | +     | U              | D          | D         |
| Chronic myeloid leukemia          | K562      | -      | +     | N              | D          | D         |
| Acute lymphoblastic leukemia      | Jurkat    | -      | +     | N              | N          | D         |
| Multiple myeloma                  | RPMI-8226 | +      | +     | N              | D          | D         |
| Burkitt’s lymphoma                | Raji      | -      | -     | N              | U          | N         |

IL-6, interleukin-6; IL-6R, interleukin-6 receptor; IL-6Rα, interleukin-6 receptor α chain; gp130, interleukin 6 signal transducer; hUC-MSCs, human umbilical cord-derived mesenchymal stem cells; +, cells express the corresponding protein; -, cells do not express the corresponding protein; U, proliferation of tumor cells promoted; D, proliferation of tumor cells inhibited; N, proliferation of tumor cells unaffected.
inhibitory cytokines secreted by hUC-MSCs, as cytokine receptors are expressed on the majority of leukemia cells. However, a specific cytokine was not confirmed in the present study by the subsequent experiments, although the effect was likely to have been independent of IL-6, as it promoted or did not affect cell proliferation in the present study. Zhu et al (24) previously demonstrated that MSCs inhibited the proliferation of K562 by secreting Dickkopf Wnt signaling pathway inhibitor 1 (DKK-1) to negatively regulate the Wnt signaling pathway. Whether DKK-1 is also associated with other types of leukemia requires further investigation.

IL-6 serves an important function in MM, as it may promote the differentiation of B cells into plasma cells and accelerate MM development by stimulating proliferation and inhibiting the apoptosis of malignant plasma cells (25,26). The application of IL-6 monoclonal antibody to treat MM
Figure 4. The expression of IL-6Rα and gp130 mRNA and protein in the tumor cells. (A) The expression of IL-6, IL-6Rα and gp130 mRNA in the tumor cells was detected by reverse transcription-polymerase chain reaction. THP-1 expressed all three, HL-60 and RPMI-8226 expressed IL-6Rα and gp130, K562 expressed gp130 and IL-6, Jurkat expressed gp130 only, and Raji expressed none. (B) The expression of IL-6Rα and gp130 proteins on tumor cells was detected by flow cytometry. The x-axis represents IL-6Rα and the y-axis represents gp130. THP-1, HL-60 and RPMI-8226 expressed both; K562 and Jurkat expressed gp130 only; Raji expressed neither. The experiments were repeated three times. IL-6Rα, interleukin-6 receptor α; gp130, interleukin 6 signal transducer; IL-6, interleukin 6.

Figure 5. Exogenous IL-6 promotes the proliferation of THP-1 and HL-60 cells. Tumor cells were cultured in medium supplemented with 0, 10, 20, 50 or 100 ng/ml of recombinant human IL-6 for 72 h; cell proliferation was assessed by a Cell Counting kit-8 assay. The experiments were repeated three times. The data are expressed as the means ± standard deviation. *P<0.05, increase vs. control. IL-6, interleukin-6.
has exhibited clinical efficacy (27,28). However, in the present study, the MM cell line RPMI-8226 exhibited no response to exogenous IL-6 despite expressing complete IL-6 receptors; proliferation was even inhibited by hUC-MSCs, which may be explained by a lack of signals downstream of IL-6, and by the expression of certain inhibitory cytokine receptors, as may also occur on leukemia cells.

Research on the effect of MSCs on lymphoma is controversial, possibly due to the different sources of MSCs and tumor cell types. Ahn et al (29) demonstrated that human adipose tissue derived-MSCs inhibited the growth of EL4 T-cell lymphoma cells by affecting the cell cycle and apoptosis. In contrast, in a study on mantle cell lymphoma, Medina et al (30) proposed that BM-MSCs may promote growth and migration, and inhibit apoptosis through the activation of the nuclear factor-xB pathway. In the present study, hUC-MSCs promoted the growth of Raji Burkitt’s lymphoma cells. Consistent with the study by Medina et al (30), growth promotion from cell-to-cell contact is likely to exhibit a notable effect on Raji cell growth, possibly due to a lack of cytokine receptor expression.

hUC-MSCs may also affect the secretion of tumor cells. A previous clinical study demonstrated that IL-6 and -10 levels were positively correlated in patients with lymphoma (31); exogenous IL-6 also increased the secretion of IL-10 by MM cells, and IL-10 promotes the development of MM in conjunction with IL-6 (32). The present study also revealed that hUC-MSCs promoted the secretion of IL-10 by MM and lymphoma cell lines, which may contribute to the secretion of IL-6.

Collectively, the results indicate a varying effect of hUC-MSCs on cells from various types of hematologic malignancy associated with cytokines and cell-to-cell contact depending on the expression of cytokine receptors on the cells. In particular, researchers applying hUC-MSCs in lymphoma should be aware of a potential tumor growth-promoting effect.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

QL and YP performed the cytology experiments, and QL was a major contributor in writing the manuscript. TL and JX performed the cytometric bead array and flow cytometry experiments. YT performed the reverse transcription polymerase chain reaction experiments. HC and BZ conceived and designed the experiments, and gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of the Affiliated Hospital of the Academy of Military Medical Sciences (Beijing, China; protocol no., 2010-05-60). Written informed consent was obtained from all participants.

Consent for publication

Written informed consent for publication was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

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