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Exosomes from mesenchymal stromal cells enhance imatinib-induced apoptosis in human leukemia cells via activation of caspase signaling pathway

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Abstract

Background aims. Imatinib (IM), a tyrosine kinase inhibitor targeting the BCR-ABL oncoprotein, remains a major therapeutic strategy for patients with chronic myelogenous leukemia (CML). However, IM resistance is still a challenge in the treatment of CML. Recently, it was reported that exosomes (Exo) were involved in drug resistance. Therefore, the present study investigated whether Exo secreted by human umbilical cord mesenchymal stromal cells (hUC-MSC-Exo) affected the sensitivity of K562 cells to IM. *Methods.* hUC-MSC-Exo were isolated and identified. K562 cells were then treated or not with IM (1 μmol/L) in combination with hUC-MSC-Exo (50 μg/mL). Cell viability and apoptosis were determined by cell counting kit 8 (CCK-8) and annexin V/propidium iodide (PI) double staining, respectively. Apoptotic proteins, caspase and their cleaved forms were detected by Western blot. *Results.* It was shown that hUC-MSC-Exo alone had no effect on cell viability and apoptosis of K562 cells. However, hUC-MSC-Exo promoted IM-induced cell viability inhibition and apoptosis. Moreover, hUC-MSC-Exo enhanced the increased Bax expression and the decreased Bcl-2 expression that were induced by IM. Compared with IM alone, caspase-9 and caspase-3 were further activated by combination of hUC-MSC-Exo with IM. Finally, the effects of hUC-MSC-Exo on K562 cells could be reversed by pretreatment of K562 cells with caspase inhibitor Z-VAD-FMK (30 μmol/L) *Discussion.* These results indicate that hUC-MSC-Exo enhanced the sensitivity of K562 cells to IM via activation of caspase signaling pathway. Therefore, combining IM with hUC-MSC-Exo could be a promising approach to improve the efficacy of CML treatment.

Key Words: *apoptosis, caspase, exosome, imatinib, mesenchymal stromal cells*

Introduction

Imatinib (IM) is the front-line drug for patients at early stage of chronic myelogenous leukemia (CML) [1,2]. However, IM resistance and side effects occur in 22% patients who generally went through an unfavorable long-term survival [3,4]. Thus, it is urgent to develop a novel strategy to overcome the resistance.

Mesenchymal stromal cells (MSCs) are multipotent adult stem cells that are considered a promising tools for regenerative medicine [5]. The effects of MSCs on solid tumors are controversial. Some studies have shown that tumor progression and metastasis were promoted by MSCs [6,7]. In contrast, other studies have shown that MSCs suppressed tumor growth [8,9]. For leukemia, research in our and other laboratories found that MSCs inhibited leukemia cell proliferation and induced their differentiation [10–12]. However, MSCs were shown to protect leukemia cells from IM-induced apoptosis [13,14]. The varied effects of MSCs on leukemia cells might be attributed to releasing different kinds of cytokines and microvesicles [6,10,12,14].

Exosomes (Exo) are microvesicles with diameters of 40–100 nm that are released by most cell types [15]. An increasing number of studies have shown that Exo plays key roles in intercellular communication because it contains a variety of RNAs, proteins and lipids [16]. Recent evidence indicates that Exo derived from MSCs (MSCs-Exo) exerts antitumor effects

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[17,18]. Furthermore, MSCs-Exo play important roles in drug resistance in gastric cancer and multiple myeloma [19,20]. However, whether MSCs-Exo have any effects on biological characteristics of leukemia cells, especially the sensitivity of leukemia cells to IM, has not been investigated.

It has been reported that functions of Exo are similar to those of their parental cells. The therapeutic application of Exo is even more promising than cells since Exo have no risk of aneuploidy, lower possibility of immune rejection and lung barrier following in vivo allogeneic administration [15,16]. Therefore, in the present study, we aim to investigate whether hUC-MSC-Exo have any effects on biological characteristics of K562 cells, especially the sensitivity of K562 cells to IM. Moreover, we will try to clarify the possible mechanisms.

Methods

Ethical statement

This study was approved by the ethical committee of the Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College. Human umbilical cords (n = 12) were obtained from healthy donors with written informed consent.

Reagents and antibodies

IM and polyethylene glycol (PEG) were purchased from Sigma. Z-VAD-FMK was purchased from Selleck. Exosome-human CD63 isolation/detection kit was from Invitrogen. The CCK-8 kit, annexin V/PI apoptosis detection kit, CD9 and CD63 antibodies were purchased from BD Biosciences. The following antibodies were from Cell Signaling Technology: anti-β-Actin anti-Bax, anti-Bcl-2, anti-caspase-9, anti-cleaved-caspase-9, anti-caspase-3, anti-cleaved-caspase-3, anti–poly ADP-ribose polymerase (PARP) and anti-cleaved-PARP.

Cell culture

hUC-MSCs were isolated and identified as previously described [6,10]. They were maintained in DMEM/F-12 (Gibco) supplemented with 10% FBS or Exo-free FBS (Gibco), 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L glutamine and 10 ng/mL epidermal growth factor (PeproTech). Cells were cultured in humidified atmosphere with 5% CO_2 at 37°C. hUC-MSCs at passage 3–6 were used for the following experiments.

Human leukemia cell line K562 was purchased from pathological cell bank in Institute of Hematology, Chinese Academy of Medical Sciences. Cells were cultured in the RPMI-1640 medium (Gibco), supplemented with 10% FBS, 2 mmol/L L-glutamine, 100 IU/mL penicillin, and 100 mg/mL streptomycin in humidified atmosphere with 5% CO_2 at 37°C.

Exosome isolation and identification

Exo was isolated as previous report and a patent (US9005888) [21,22]. PEG with molecular weight 8000 was dissolved in PBS at concentration of 500 mg/mL. hUC-MSCs culture media were harvested and centrifuged at 5000*g* for 40 min at 4°C to remove cells and debris. PEG solution was added to the supernatant at the volume proportion of 1:5. The mixture was incubated at 4°C overnight. After incubation, the supernatant was centrifuged at 5000*g* for 40 min at 4°C. After centrifugation, supernatant was aspirated, and the pellet at the bottom was resuspended with phosphate-buffered saline at room temperature for 20 min. Then Exo solution was stored at –80°C.

Exo was dropped onto the copper grid. After absorbing excessive liquid, Exo was negatively stained with 3% (w/V) sodium phosphotungstate solution (pH 6.8) for 5 min and washed gently with double distilled water. Exo were observed and photographed by transmission electron microscopy (TEM). Exo was selected randomly to measure the mean diameter.

For surface markers detection, $CD63^+$ Exo was first isolated from pre-enriched Exo solution using human CD63 isolation/detection kit. Exo bound beads were then stained with Phycoerythrin-labeled CD9 and CD63 antibodies. Finally, CD9 and CD63 expression was detected by flow cytometry (LSRII, BD Biosciences).

Cell viability assay

Cell viability was determined by CCK-8. Cells were seeded in 96-well plates (5000 cells/well) in 200 μL medium per well and incubated at 37°C for 60 h with or without IM/hUC-MSC-Exo. After incubation, CCK8 was added (20 μL/well) and incubated for 2 h at 37°C. The absorbance at 450 nm was determined with microplate reader.

Apoptosis assay

Annexin V/PI apoptosis detection kit (BD Biosciences) was used to evaluate apoptosis according to the manufacturer's instructions. K562 cells cultured with hUC-MSC-Exo (50 μg/mL), IM (1 μmol/L) or both for 60 h were harvested. Cells were incubated with 5 μL annexin V–fluorescein isothiocyanate (FITC) for 15 min. Subsequently, PI staining was performed. Cell apoptosis was detected by flow cytometry (LSRII, BD Biosciences).