p38 MAPK Contributes to the Growth Inhibition of Leukemic Tumor Cells Mediated by Human Umbilical Cord Mesenchymal Stem Cells

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Key Words

Mesenchymal stromal cells • Leukemic tumor cells • Growth inhibition • Cell cycle arrest • p38 MAPK • SB203580 • siRNA

Abstract

Background/Aims: Mesenchymal stem cells (MSCs) have been implicated in antitumor therapy for hematopoietic and non-hematopoietic tumors. Cell-contact and soluble factors are demonstrated to play a role in the growth inhibition of tumor cells mediated by MSCs *in vitro*, while there is little clue about signaling pathways involved in the process. P38 MAPK has been implicated as a suppressor of cell proliferation and tumorigenesis. We here investigate whether p38 MAPK is involved in MSC-induced growth inhibition of leukemic tumor cells. **Methods:** We characterized the effect of human umbilical cord mesenchymal stem cells (UC-MSCs) on proliferation, cell cycle and phosphorylation pattern of p38 MAPK in HL60 and K562 cells. SB203580, a specific inhibitor of p38 MAPK, or p38 MAPK-small interfering RNA (siRNA), were used to identify the role of p38 in growth suppression by UC-MSCs. We also investigated the expression of cell cycle regulators. **Results:** Treatment with UC-MSCs led to potent proliferation-inhibition of

HL60 and K562 cells without inducing apoptosis. Growth inhibition by UC-MSCs was due to G0/G1 arrest. UC-MSCs increased phosphorylation of p38 MAPK in HL60 and K562 cells. Pharmacological inhibition or genetic silencing (through siRNA) of p38 MAPK partially abrogated the proliferation-suppression and cell cycle arrest caused by UC-MSCs. UC-MSCs also modulated the expression of cell cycle regulatory proteins in HL60 and K562 cells while SB203580 reversed the effect. **Conclusion:** Taken together, our findings indicate that p38 MAPK is critical for the growth inhibitory effect of UC-MSCs on leukemic tumor cells.

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Introduction

Mesenchymal stem cells derived from bone marrow (BM-MSCs) exhibit a potent anti-proliferative effect on tumor cells of hematopoietic and non hematopoietic origin *in vitro* [1]. The growth inhibition was caused by the arrest of tumor cells in the G1 phase of the cell cycle,

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accompanied by decreased expression of cyclin D2. Recently, Khakoo et al. [2] showed that MSCs exert potent antitumorigenic effects in a model of kaposi's sarcoma. The *in vivo* tumor-suppressing effect of MSCs correlates with their ability to inhibit the AKT activity in target cells in a contact-dependent manner. Yet *in vitro* MSCs can inhibit the activation of the Akt protein kinase within some but not all tumor and primary cell lines. Zhu et al. [3] further showed that MSCs can inhibit tumor-cell proliferation in a contact-independent manner and identified DKK-1 (dickkopf-1), secreted by MSCs and acting as a negative regulator of WNT signaling pathway, to be one of the molecules responsible for the inhibitory effect. However, the growth rate of tumor cells was not fully restored to the normal level by RNA interference of DKK-1 or by neutralizing antibodies against DKK-1. This indicates that other pathways may be involved in MSC-mediated inhibition of tumor growth.

The mitogen-activated protein kinases (MAPKs) are members of intracellular kinases that serve as focal points for diverse extracellular stimuli and function to regulate various cellular processes. The p38 MAPK is known to be activated by various cytokines and stressful stimuli and thus plays an essential role in cellular responses including cell proliferation, differentiation and apoptosis [4]. For example, p38 MAPK is a key negative regulator of cell proliferation in several cell types, including cardiomyocytes [5], corneal epitheliums [6] and umbilical vascular endothelial cells [7]. One remarkable role for the p38 MAPK pathway is the regulatory effect on G1/S as well as G2/M cell cycle checkpoint in response to cellular stress [8]. Negative regulation of cyclin D at both the transcription and post-transcriptional levels was proposed as a potential target for p38 MAPK in the regulation of G1/S progression. Besides, p38 MAPK has recently been revealed as a suppressor of tumor development [9]. Inactivation of this pathway supports cellular transformation *in vitro* and promotes cancer development in mouse cancer models [10]. Also, downregulation of p38 MAPK were reported to trigger proliferative responses in quiescent leukemia cells [11]. We therefore investigated whether activation of p38 MAPK is involved in the suppression of leukemia cells mediated by MSCs.

In this study, we examined the growth inhibitory effects of human umbilical cord mesenchymal stem cells (UC-MSCs) on leukemic tumor cells, and we further investigated whether p38 MAPK signaling pathway was involved in the process. We found that p38 MAPK contributed to the proliferation inhibition, cell cycle arrest

and modulation of cell cycle regulators in leukemic tumor cells caused by UC-MSCs. These results suggest that p38 MAPK might be a potential therapeutic target for treatment of leukemic tumors.

Materials and Methods

Reagents

The antibodies against phospho-p38MAPK, p38MAPK, p27^{Kip1}, and cyclin D2 were purchased from Cell Signaling Technology (Beverly, MD, USA), Actin was from Santa Cruz. SB203580 was from Sigma.

Tumor cell culture

The human leukemic tumor cell lines HL60 and K562 cells were maintained in RPMI 1640 medium (Gibco Life Technologies, Paisley, UK) supplemented with 10% fetal calf serum (FCS) in a humidified atmosphere with 5% CO₂ at 37° C. Synchronized leukemic tumor cells, when indicated, was achieved by incubation in RPMI 1640 medium with 0.1% FCS for 24 h.

Isolation and culture of UC-MSCs

UC-MSCs were isolated from umbilical cords obtained from local maternity hospitals with donors' informed consent. Human tissue collection for research was approved by the institutional review board of the Chinese Academy of Medical Science and Peking Union Medical College. The details of isolation, *ex vivo* expansion and identification of UC-MSCs were essentially as described previously [12]. Cells of passage four to six were used in this study. UC-MSCs (30 Gy irradiated) were preplated and allowed to adhere overnight at 37° C before co-culture with tumor cells.

Proliferation assays

Tumor cells (10⁴ per well) were seeded in flat-bottom 96-well plates, and treated with indicated doses of UC-MSCs for 72h. The proliferation of tumor cells was measured using the cell proliferation BrdU ELISA kit (Roche Diagnostics, Mannheim, Germany) according to manufacturer's instructions. BrdU was added for the last 18 h of incubation. After fixation, the mouse monoclonal anti-BrdU-POD antibody was added. The immune complexes were revealed by the subsequent substrate reaction, and quantified by measuring the absorbance at 405 nm with a reference wavelength at 490 nm in a microliter plate reader.

Cell cycle analysis

Tumor cells were plated into 6-well culture plates at a density of 1×10⁶/well and were cultured with or without UC-MSCs (10⁵ per well) for 48h before harvested and quantified. 10⁶ tumor cells were fixed with 70% cold ethanol at 4° C for more than 12 h, washed with phosphate-buffered saline twice. The cells were then incubated with 50μg/ml RNase A (Sigma) for 20 min at 37° C and stained with 50 mg/ml propidium iodide

Fig. 1. UC-MSCs inhibit proliferation of HL60 and K562 cells by G0/G1 arrest without induction of apoptosis. (A) Proliferation inhibitory effect of UC-MSCs on tumor cells. Tumor cells in 96-well plates (10^4 per well) were treated with grading doses of UC-MSCs for 72h and the cell proliferation was measured by BrdU ELISA kit. The ability of UC-MSCs to inhibit cell proliferation was determined by comparing with those cells without UC-MSCs treatment (% of control) and each bar represents the mean $\pm$ SD of triplicate determinations. The data are representative of three independent experiments. * $P < 0.05$ versus untreated cells. (B) UC-MSCs protect tumor cells from apoptosis. Tumor cells in 6-well plates (10^6 per well) were maintained in RPMI 1640 with 1% FCS for 48 h with or without UC-MSCs (10^5 per well) and cell apoptosis was evaluated by annexin V staining and PI incorporation. (C) UC-MSCs block HL60 and K562 cells at G0/G1 phase. Synchronized tumor cells (10^6) cultured with or without UC-MSCs (10^5) for 48h were analyzed for cell-cycle progression as described in Materials and methods. Percentages of cells in each phase are shown in the upper corner of each cell-cycle graph. The results are representative of three independent experiments.

(PI) (Sigma) at room temperature for 5min. DNA content was analyzed in a FACScalibur flow cytometer (BD) using MODFIT software (Verity).

Apoptosis analysis

Tumor cells (10^6 per well) were plated into 6-well culture plates and were cultured with 1% FCS in the presence or absence of UC-MSCs (10^5 per well) for 48h. The AnnexinV/ PI apoptosis detection kit from BD was used for apoptosis assay according to manufacturer's instructions. Briefly, at least 10^6 tumor cells were washed and resuspended in binding buffer (1:10 diluted in phosphate-buffered saline). Then the cells were incubated with AnnexinV (5 μ L) and PI (5 μ L) for 15 min. For every condition, 15,000 events were collected and analyzed by flow cytometer.

Small interfering RNA transfection

Specific small interfering RNAs (siRNAs) targeting p38 MAPK (sip38, SignalSilence® p38 MAPK siRNA) and negative control siRNAs (siCtrl, SignalSilence® Control siRNA) were purchased from Cell Signaling Technology (Beverly, MD, USA). HL60 and K562 cells were transfected with double-stranded siRNA using the Hiperfect Transfection Reagent (Qiagen) according to the manufacturer's instructions. In brief, 6 μ L of Hiperfect Transfection Reagent and finally 100 nM of siRNA were diluted with serum-free RPMI 1640 medium, preincubated at room temperature for 10 min and then added to the tumor cells maintained in RPMI 1640 medium containing 10% FCS. Tumor cells were incubated with transfection complexes at 37° C for 72 h and then the p38 MAPK protein level was detected by western blot to assess silence efficiency.

Fig. 2. UC-MSCs increase phosphorylation of p38 MAPK in HL60 and K562 cells in a time-dependent manner. Synchronized tumor cells (5×10^6) were treated with UC-MSCs (5×10^5) for the indicated time. Protein extracts were then prepared and both phosphorylated and total p38 MAPK in the extracts were determined by Western blot analysis. Each lane contained 50 μ g protein. Actin was shown as a loading control. The results are representative of three independent experiments.

Fig. 3. Pharmacological inhibition of p38 MAPK partially abrogates MSC-induced proliferation suppression in HL60 and K562 cells. 10^4 tumor cells were treated with 10^3 UC-MSCs, 10 μ M of SB203580 (SB), or in combinations (SB 30 min before MSC) as indicated for 72 h and the cell proliferation was determined by BrdU incorporation assay. Values are expressed as percentage of untreated cells (% of control) and each bar represents the mean $\pm$ SD of triplicate determinations. * $P < 0.05$ versus tumor cells with MSCs treatment only. The results are representative of three independent experiments.

48 h post-transfection, tumor cells were treated with UC-MSCs. Cell proliferation of tumor cells was assayed 72 h after treatment with UC-MSCs, and cell cycle progression was analyzed 48 h after coculture with UC-MSCs.

Western blot analysis

After indicated treatment, tumor cells were harvested and lysed in RIPA buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP40, 0.25% deoxycholic acid, 1 mM EGTA, 1 mM EDTA, 1 mM NaF, 1 mM Na_3VO_4) with protease inhibitor cocktail (Sigma). Protein concentration of the lysates was quantified by the Bradford protein assay (Pierce Rockford, IL). Equal

amounts of protein (50 μ g) were electrophoresed in 5% or 15% sodium dodecyl sulfate-polyacrylamide gel and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked for 1 h with 5% bovine serum albumin (BSA) in Phosphate Buffered Saline (PBS), and incubated with primary antibodies diluted 1: 1000 in 5% BSA at 4°C overnight. After washing, membranes were incubated with the corresponding horseradish peroxidase-conjugated secondary antibody (Pierce) diluted 1: 4000 in 5% BSA. The immunocomplexes were visualized with an enhanced chemiluminescence detection kit according to the manufacturer's instructions (Pierce). When necessary,

Fig. 4. Pharmacological inhibition of p38 MAPK partially reverses MSC-induced G0/G1 phase arrest in HL60 and K562 cells. Synchronized tumor cells (10^6) were treated with 10^5 UC-MSCs, 10 μ M SB203580 (SB), or in combinations (SB 30 min before MSC) as indicated and cell-cycle progression was analyzed after 48h. Untreated tumor cells were kept as controls. Percentages of cells in each phase are shown in the upper corner of each cell-cycle graph. The results are representative of three independent experiments.

membranes were stripped by incubation in 62.5 mM Tris-HCl (pH 6.8), 2% SDS, and 100 mM 2-mercaptoethanol for 30 min at 50 °C. After washing, membranes were reprobed with other antibodies.

Statistical Analysis

Data are presented as mean $\pm$ SD. One-way ANOVA analysis of variance was used to make comparisons of parameters among groups. P less than 0.05 was considered significant. The SPSS 11.5 software package was used for the statistical tests.

Results

UC-MSCs inhibit tumor-cell proliferation by G0/G1 phase arrest in the absence of apoptosis

To determine whether UC-MSC inhibit growth of leukemic tumor cells, we examined its effect on proliferation of HL60 and K562 cell lines. These cells were treated with grading doses of UC-MSCs for 72h. We demonstrated that UC-MSCs inhibited tumor cell growth in a dose-dependently manner, with the maximum effect observed at a ratio of 1:1 (MSCs: tumor cells) (Fig. 1A). In order to determine whether UC-MSCs inhibit cell proliferation by inducing cell apoptosis, we stained the cells maintained in 1% FCS medium with Annexin V/PI

and found that treatment of UC-MSCs (1:10) for 48 h did not induce cell apoptosis (Fig. 1B). To further explore the mechanism by which UC-MSCs inhibited tumor cell growth, We analyzed the cell-cycle progression of HL60 and K562 cells treated with UC-MSCs (1:10) for 48 h by flow cytometry. The fraction of HL60 in the G0/G1 phase was 44% in control, whereas, was 51% in the presence of UC-MSCs. Similarly, the fraction of K562 cells in the G0/G1 phase was greatly increased in response to MSCs treatment (Fig. 1C). These results indicate that UC-MSCs inhibit growth of tumor cells by G0/G1 arrest, not apoptosis induction.

UC-MSCs increase phosphorylation of p38 MAPK in tumor cells

It has been demonstrated that BM-MSCs modified the phosphorylation pattern of the p38 pathways in B-lymphocytes, accompanied with the proliferation inhibition of B-lymphocytes [13]. We thus examined whether MSCs had an effect on phosphorylation of p38 MAPK in tumor cells. Synchronized tumor cells were treated with MSCs for indicated time and the level of total and phosphorylated p38 MAPK were analyzed by Western blotting using the antibodies against these kinases. As shown in Fig. 2, UC-MSCs induced the phosphorylation of p38 MAPK in a time-dependent

Fig. 5. siRNA down-regulation of p38 MAPK partially abrogates MSC-induced proliferation inhibition (B) and cell cycle arrest (C) in HL60 and K562 cells. (A) p38 MAPK expression is suppressed when the targeting siRNA was introduced. Tumor cells were transfected with negative control siRNA (siCtrl) or siRNA targeting p38 MAPK (sip38). Untreated tumor cells were kept as control. 72 h post-transfection, cells were harvested and lysed. Immunoblotting was performed using antibodies against p38-MAPK. Actin was used as a loading control. The results are representative of three independent experiments. (B) Tumor cells were treated with siCtrl or sip38 for 48 h before harvested and quantified. Transfected tumor cells in 96-well plates (10^4 per well) were cultured with or without UC-MSCs (10^3 per well) for 72 h and then cell proliferation of tumor cells was determined by BrdU incorporation assay. Values are expressed as percentage of siCtrl cells without MSCs treatment (% of control) and each bar represents the mean $\pm$ SD of triplicate determinations. * $P < 0.05$ versus siCtrl cells with MSCs treatment. The results are representative of three independent experiments. (C) Tumor cells were treated with siCtrl or sip38 for 48 h before harvested and quantified. 10^6 transfected tumor cells were cultured with or without 10^5 UC-MSCs for 48 h and then the cell-cycle progression of tumor cells was analyzed by flow cytometry. Percentages of cells in each phase are shown in the upper corner of each cell-cycle graph. The results are representative of three independent experiments.

manner. The phosphorylation peaked at 20 min and declined thereafter.

Pharmacological inhibition of p38 MAPK partially attenuates MSC-induced anti-proliferation effect

To test whether p38 MAPK is critical for MSC-induced anti-proliferative effect, we treated HL60 and K562 cells with 10 μ M of SB203580 (a special inhibitor of p38 MAPK) for 30 min prior to the UC-MSCs treatment, then examined the growth of these cells. As

shown in Fig. 3, the growth-inhibitory effect of UC-MSCs was distinctly blocked by the pretreatment with SB203580.

Pharmacological inhibition of p38 MAPK partially reverses the G0/G1 arrest of tumor cells mediated by UC-MSCs

To examine whether p38 MAPK activation is involved in MSC-induced cell cycle arrest of tumor cells, synchronized HL60 and K562 cells were preincubated with SB203580 10 μ M for 30 min before treatment with

Fig. 6. UC-MSCs decrease the expression of cyclin D2 and increase the expression of p27^{kip1} in tumor cells, while SB203580 reverses the effect. Tumor cells (5×10^6) were treated with UC-MSCs (5×10^5) for the indicated time, or pretreated with 10 μ M of SB203580 for 30 minutes and then exposed to UC-MSCs (5×10^5) for 48 h. Protein extracts were then prepared and the expression of cyclin D2 and p27^{kip1} in the extracts were determined by Western blot analysis. Each lane contained 50 μ g protein. Actin was shown as a loading control. The results are representative of three independent experiments.

UC-MSCs. Cell cycle analysis showed that G0/G1 phase arrest in HL60 and K562 cells mediated by UC-MSC was also partially reversed by the pretreatment with SB203580 (Fig. 4). The results suggest that activation of p38 MAPK by UC-MSCs is important for G0/G1 arrest of tumor cells.

Knockout of p38 MAPK by siRNA partially reverses MSC-mediated growth inhibition of tumor cells

Pharmacological inhibitors are usually associated with nonspecific inhibitory effect, therefore we used specific p38 MAPK siRNA to knockout its expression. As shown in Fig. 5A, treatment of tumor cells with p38 siRNA (sip38) effectively reduced the expression of p38 MAPK as determined with specific antibody of p38. We then examined MSC-induced cell proliferation of tumor cells treated with siRNA. Similar to the effect of SB203580 (Fig. 3), knockout of p38 attenuated MSC-induced proliferation inhibition (Fig. 5B). We further analyzed the effects of p38 knockout on MSC-induced cell cycle arrest by flow cytometry. Fig. 5C shows that knockout of p38 significantly reversed the G0/G1 arrest caused by UC-MSCs. These results confirm that p38 MAPK is required for the growth inhibition of tumor cells mediated by UC-MSCs.

UC-MSCs modulate the expression of cell cycle regulators through p38 MAPK

To better understand the molecular mechanisms involved in MSC-induced growth inhibition of tumor cells, we investigated the expression level of cell cycle regulators which have been implicated in the growth inhibition of tumor cells caused by BM-MSCs [1]. As shown in Fig. 6, UC-MSCs markedly decreased the expression of cyclin D2 and increased the expression of p27^{kip1} in a time-course manner. Notably, the p38 MAPK inhibitor, SB203580, potentially reversed the effect of UC-MSCs. These data suggest that MSC-induced modulation of cell cycle regulators requires p38 MAPK activation.

Discussion

MSCs are known to inhibit tumor growth of hematopoietic and non-hematopoietic origin *in vitro*. Cell-contact, DKK-1 and WNT signaling pathway have been described as modulating tumor-cell proliferation [1, 3]. Here, we demonstrate for the first time that UC-MSCs is able to suppression the proliferation of leukemic tumor cells in a dose-dependent manner *in vitro* and p38 MAPK contributes to the inhibitory effect. Although p38 MAPK

was implicated to be a tumor suppressor [10]. p38 MAPK has not been reported to mediate tumor-cell suppression by MSCs. Our hypothesis that p38 MAPK is involved in the growth-suppression of leukemic tumor cells by UC-MSCs *in vitro* was supported by the following facts: (1) UC-MSCs were able to induce the phosphorylation of p38 in a time-dependent manner; (2) Pharmacological inhibition or genetic silencing of the p38 MAPK partially abrogated the growth inhibitory effect of UC-MSCs; (3) In addition, UC-MSCs modulated the expression of cell cycle regulator through p38 MAPK pathway.

It has been reported that BM-MSCs mediated growth inhibition of immune cells and tumor cells by cell cycle arrest without induction of apoptosis [14-17]. Our results indicate that UC-MSCs treatment led to proliferation inhibition and G0/G1 arrest of leukemic tumor cells, similar to the effect of BM-MSCs.

The p38 MAPKs affect a variety of intracellular responses, with well-recognized roles in inflammation, cell-cycle regulation, cell death, development, differentiation, senescence and tumorigenesis [9]. The involvement of p38 MAPK in cell growth inhibition is associated with the induction of G0/G1 arrest in various cell types depending on the stimulating signal [18-21]. The tumour-suppressive activity of p38 MAPK can largely be attributed to its modulatory effects on G1/S and G2/M cell cycle checkpoint [22], negative regulation of proliferation and induction of apoptosis [23-25]. However, the role of p38 MAPK in tumor-suppression by MSCs remains unclear. The findings herein showed that UC-MSCs activated the p38 MAPK pathway in leukemic tumor cells, similar to the previous observation that MSCs modified the activation pattern of p38 MAPK in B lymphocytes [13]. Furthermore, we demonstrated that the p38 MAPK pathway was required for the growth inhibition of leukemic tumor cells induced by UC-MSCs because pharmacological inhibition or genetic silencing of p38 MAPK dramatically reversed the effect of UC-MSCs.

Accumulating evidence is defining a critical role of p38 MAPK in cell cycle checkpoint. Actually, p38 MAPK may regulate cell cycle progression through multiple mechanisms. One means by which p38 blocks cell cycle may be to inhibit the expression of cyclin D [26, 27]. Cyclin D proteins are positive cell cycle regulators. They bind to and active cyclin dependent kinase 4 and 6 (cdk4/6) and this active complex is essential for the transition to S phase and further cell proliferation. Unlike other cyclins that are periodically induced during the cell cycle

progression, the levels of cyclin D are regulated by the extracellular mitogenic environment. Thus, cyclin D proteins are believed to serve as “links” between the extracellular environment and the core cell cycle apparatus [28]. p38 MAPK has been shown to reduce the levels of cyclin D by transcription downregulation [29] and proteosomal degradation [30]. Moreover it has been reported that MSC dramatically downregulated the expression of cyclin D2 in tumor cells [1]. Therefore the association between cyclin D2 and p38 MAPK in the growth suppression of tumor cells by UC-MSCs was determined in this study. We observed that the p38 MAPK chemical inhibitor, SB203580, partially reversed the downregulation of cyclin D2 mediated by UC-MSCs, suggesting that UC-MSCs decrease the expression of cyclin D2 through p38 pathway. However, the exact level at which cyclin D2 is regulated, and the potential components that p38 phosphorylates to decrease cyclin D2 expression remain unclear, which will require further investigation.

Another important mechanism by which p38 MAPK blocks cell cycle is to upregulate the expression of CDK inhibitor p27^{kip1}. It has been reported that p38 is involved in G1 cell-cycle arrest of iTregs controlled by cdk inhibitor p27^{kip1} [31]. Sustained expression of p27^{kip1} which coincidences with activation of p38 prevents phosphorylation of retinoblastoma protein (Rb) by cdk4 or 6. Hypophosphorylation of Rb allows for binding of the transcription factor E2F. Hence E2F-dependent gene-expression, which are necessary for entry into S-phase, is prevented.

Therefore we investigated the effect of UC-MSCs on p27^{kip1} expression in tumor cells. We observed that UC-MSCs treatment led to a marked increase of p27^{kip1} expression in tumor cells. Notably, SB203580, significantly abrogated the upregulation of p27^{kip1}, suggesting that the p38 MAPK pathway is implicated in MSCs-induced growth inhibition, possibly through the upregulation of p27^{kip1}.

The p38 MAPK is known to be activated by pro-inflammatory and stressful stimuli, while MSCs could secrete several pro-inflammatory cytokines, such as hepatocyte growth factor (HGF). It has been reported that treatment with HGF inhibits the proliferation of a number of tumor cell lines [32, 33], and p38 is required for HGF's inhibitory effect on MSC proliferation [34]. Further studies are needed to identify the cytokines responsible for tumor suppression by MSCs and HGF may be one of the candidates.

In conclusion, our results provide new insight into how UC-MSCs modulate leukemic tumor growth *in vitro*. Although it is known that p38 MAPK is a suppressor of tumor development, this is the first report demonstrating that the p38 MAPK is required for leukemic tumor suppression by UC-MSCs. Our data bring new insights into clinical therapeutic trials using UC-MSC infusion for tumor-suppression purposes.

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