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Hemangiopoietin inhibits apoptosis of MO7e leukemia cells through phosphatidylinositol 3-kinase–AKT pathway ☆

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Abstract

Hemangiopoietin (HAPO) is a growth factor that significantly stimulates proliferation and survival of the primitive cells of hematopoietic and endothelial lineages. To determine the mechanism of action of HAPO, the anti-apoptotic activity and signal transduction pathway of HAPO were investigated using a factor-dependent leukemia cell line, the MO7e cells. Recombinant human HAPO (rhHAPO) was produced in *Escherichia coli* and purified by a series of column chromatography with a purity of more than 95%. rhHAPO significantly supported the survival of MO7e cells after deprivation of granulocyte–macrophage colony stimulating factor and activated phosphatidylinositol 3-kinase (PI3K). When the MO7e cells were treated with two specific inhibitors to PI3K (LY294002 or wortmannin), a significant loss of cell viability with evidence of apoptosis was observed. Moreover, the protein kinase B (Akt), one of the downstream effectors of PI3K-dependent survival signaling, was activated in HAPO-stimulated MO7e cells. Phosphorylation of Akt at serine 473 and its downstream molecular Bad at serine 136 was induced by HAPO, but was blocked by two PI3K inhibitors, LY294002 and wortmannin. In addition, HAPO inhibited caspase-3 activities and poly(ADP-ribose) polymerase degradation. Such an effect of HAPO was also significantly blocked by either LY294002 or wortmannin. These results indicate that HAPO protects MO7e cells from apoptotic death through a PI3K–Akt pathway.

Keywords: Hemangiopoietin; Phosphoinositide-3 kinase; Apoptosis; Megakaryoblastic cell

We have previously reported a novel growth factor, the hemangiopoietin (HAPO), which was originally identified and purified from the urine of patients with aplastic anemia [1]. Recently, a human recombinant HAPO (rhHAPO) has been produced based on the N-terminal amino acid sequence of purified native HAPO. The rhHAPO supports survival and proliferation of the primitive cells of both hematopoietic and endothelial cell lineages, although the specific receptor is still unknown. Most interestingly, this growth factor efficiently protects mice from radiation damage. To determine the mechanism of action of HAPO, we studied the signal transduction pathway through which rhHAPO acts on cell survival in the MO7e cells, a human growth factor-dependent leukemia cell line undergoing rapid apoptotic death in the absence of cytokines [2,3].

Phosphatidylinositol 3-kinase (PI3K) is recruited and activated during the intracellular signal transduction of many growth factor receptors, and is implicated in the signaling of survival factors [4]. PI3K has several targets, including the serine–threonine kinase Akt, an oncogene that can be activated by serum and a variety of growth factors with the ability to activate PI3K [5,6]. Activation of Akt delivers a survival signal that inhibits apoptosis induced by growth factor withdrawal [7]. Akt phosphorylates Bad and thereby inactivates Bcl-2 and

☆ *Abbreviations:* HAPO, hemangiopoietin; PAGE, polyacrylamide gel electrophoresis; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PARP, poly(ADP-ribose) polymerase; PI, propidium iodide; IMDM, Iscove's modified Dulbecco's medium; WST-1, 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate; SH2, Src homology 2; GM-CSF, granulocyte–macrophage colony stimulating factor; FITC, fluorescein isothiocyanate; FCS, fetal calf serum; PBS, phosphate-buffered saline.

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Bcl-XL, two anti-apoptotic Bcl-2 family members [8]. Ultimately, activation of Akt leads to the inhibition of caspase activity and protection against apoptotic cell death [9].

The present study provides evidence showing that HAPO acts as an anti-apoptosis factor through the PI3K–Akt pathway.

Materials and methods

Materials. Recombinant human HAPO (rhHAPO) was produced in the GMP laboratory of our institute. Briefly, based on the N-terminal amino acid sequence of purified native HAPO, degenerated nucleic acid and the probes were designed to screen the cDNA of HAPO from a human fetal liver cDNA library. A special HAPO cDNA fragment with 879 bp was obtained from the cDNA library. Recombinant human HAPO (rhHAPO) with a predicted molecular weight of 31.8 kDa was produced by expressing this cDNA fragment in *Escherichia coli* and purified by a series of column chromatography with a purity of more than 95% (will be published in detail elsewhere).

LY294002 and wortmannin were purchased from Biomol (Plymouth Meeting, PA). Fetal calf serum was purchased from Hyclone Laboratories (Logan, UT). Rabbit anti-Bad, anti-phospho-Bad, and anti-caspase-3 polyclonal antibodies were obtained from Santa Cruz Biotechnology (CA). Monoclonal anti-p85 antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Polyclonal antibodies against Akt and phosphospecific (Ser-473) Akt were all purchased from New England BioLabs (Beverly, MA). Secondary antibodies conjugated to horseradish peroxidase were purchased from Dako (Cambridge, UK) and ECL system (Amersham–Pharmacia Biotech). Protease inhibitors and other reagents were purchased from Calbiochem (San Diego, CA) or Sigma (St. Louis, MO). The ApoAlert caspase fluorescent assay kit was purchased from Clontech Laboratories (Palo Alto, CA) and apoptosis detection kit was from R&D Systems (Minneapolis, MN).

Cell culture. MO7e is a human megakaryocytic leukemia cell line requiring GM-CSF for proliferation [10]; cells were cultured in IMDM with 10% (v/v) FCS, 1% penicillin and streptomycin (Sigma, St. Louis, MO), and 20 ng/ml GM-CSF at 37 °C and 5% CO_2. In some experiments, MO7e cells were deprived of growth factors for 18 h in IMDM containing 1% (w/v) FCS before stimulation with rhHAPO, GM-CSF or PBS for indicated time. When indicated, the cells were incubated for 1 h with a PI3K inhibitor wortmannin (50 nM) or LY294002 (25 μM) before growth factor stimulation.

Viability and apoptosis assays. Cell viability was assessed by both trypan blue dye exclusion and the cleavage of the sulfonated tetrazolium salt WST-1 to formazan (Boehringer–Mannheim, Mannheim, Germany) as described previously [11]. The cells were treated with or without GM-CSF or HAPO for 24–120 h. Each well was incubated with a 1:10 dilution of WST-1 for 30 min and the optical density (OD) was determined at 450 nm in a multiwell spectrophotometer (ELISA reader, Dynatech MR 5000). Cell morphology was observed with a light microscope at 60× magnification.

For sub-G1 apoptotic population analysis, the cells were deprived of GM-CSF for 18 h, washed twice with PBS, and then incubated with rhHAPO at different concentrations for 96 h. After incubation with rhHAPO, the cells were harvested and fixed with 70% ethanol overnight. As a negative control, cells were incubated without any cytokines for the same time duration. All fixed cells were stained with PI/RNase solution (3.2 mM sodium citrate, 50 μg/ml propidium iodide, 50 μg/ml RNase A, and 0.1% Triton X-100) for 30 min at room temperature. After staining, the cells were resuspended and analyzed by a FACScan.

Apoptosis was assessed using an AnnexinV/PI binding assay. Flow cytometric analysis of AnnexinV-FITC and PI-stained cells was performed using an apoptosis detection kit in accordance with the manufacturer's instruction. Briefly, MO7e cells were washed with PBS and resuspended in 1× binding buffer. Ten microliters AnnexinV and 10 μl of PI were added to the cells for 15 min at room temperature. After incubation, 400 μl of 1× binding buffer was added and the cells were analyzed for AnnexinV binding within 1 h using a FACScan. Ten thousand events were acquired on a FACSort (Becton–Dickinson) flow cytometry and analyzed with CellQuest (Becton–Dickinson) software.

DNA fragmentation analysis. The cells were incubated for the indicated times in the medium supplemented with or without rhHAPO (50 ng/ml), washed in PBS, and then incubated in cell lysis buffer (10 mM EDTA, 50 mM Tris–HCl, pH 8, 0.5% SDS, and 0.5 mg/ml proteinase K) for 14 h at 50 °C. After an additional 3 h incubation with the addition of 0.25 mg/ml RNase, the genomic DNA was extracted with phenol–chloroform and precipitated with ethanol. DNA fragments were visualized after 1.8% agarose gel electrophoresis by ethidium bromide staining.

Preparation of cell lysates. For each assay, 4×10^6 cells were incubated with various concentrations of rhHAPO for the designed time. The cells were harvested and centrifuged at 500*g* for 10 min at 4 °C. The pellets were resuspended in 200 μl lysate buffer containing 1% NP-40, 50 mmol/L Tris–HCl, 0.25% sodium deoxycholate, 150 mmol/L NaCl, 1 mmol/L EGTA, 1 mmol/L PMSF, 1 mg/mL leupeptin, 1 mmol/L Na_3VO_4, and 1 mmol/L NaF. The cellular materials were left on ice for 30 min and then sonicated for 10 s at a 10% pulse with a Heart System ultrasonic processor XL. The lysates were centrifuged at 9000*g* for 7 min at 4 °C. The supernatants were frozen at −70 °C. Protein concentration of the supernatants was quantified with the Bradford (Bio-Rad) protein assay. For inhibitor studies, 4×10^6 cells were preincubated with LY294002 (25 μM) or wortmannin (50 nM) for 1 h before the addition of rhHAPO.

Immunoprecipitation and Western blot analysis. The cell lysates were incubated with the indicated primary antibody overnight at 4 °C. The antigen–antibody complexes were then precipitated with protein A–Sepharose beads for 2 h at 4 °C, washed three times with lysis buffer, and then boiled for 5 min with sodium dodecyl sulfate (SDS) sample buffer. The boiled supernatants were electrophoresed on a SDS–polyacrylamide gel electrophoresis (PAGE) and then electrotransferred to the immobilon polyvinylidene difluoride membranes (Millipore). The membranes were blocked for 2 h in 2% bovine serum albumin–TBST (20 mM Tris–HCl, pH 7.6, 0.15 M sodium chloride, and 0.1% Tween 20), incubated with primary antibodies in TBST for 1 h, washed three times with TBST, and incubated for 1 h with a horseradish peroxidase-conjugated anti-mouse or rabbit immunoglobulin (Amersham) diluted 1:10,000 in TBST. After three washes in TBST, the specific protein complexes were identified using the 'SuperSignal'-enhanced chemiluminescence (ECL) reagent (Pierce, Rockford, IL).

Analysis of PI3K and Akt activities. PI3K activity associated with anti-p85 antibody immunoprecipitation was assayed as described previously [12]. Briefly, 1.5×10^7 cells were lysed and immunoprecipitated with anti-p85 antibody. The precipitates were subjected to a lipid kinase assay using phosphatidylinositol as a substrate. The products were extracted with $CHCl_3$:MeOH (2:1, vol/vol) and separated by oxalate-treated TLC plates (Silica Gel 60; Merck, Darmstadt, Germany) using a solvent system of $CHCl_3$:MeOH:H_2O:25% NH_4OH (90:65:8:12, vol/vol/vol/vol). The kinase products generated were visualized by an autoradiography, and the ^{32}P incorporation was quantified using a Fuji image analyzer (model BAS-2000). Activity of Akt was analyzed by Western blotting of total cell lysates using an anti-phosphorylated-Akt antibody.

Caspase-3 assay. The caspase-3 activity was determined using the ApoAlert Caspase fluorescent assay kit according to the manufacturer's protocol. Briefly, protein extracts (150 μg) were incubated for

60 min at 37 °C in 100 mM Hepes containing 10% sucrose, 0.1% CHAPS, 1 mM EDTA, 10 mM DTT, pepstatin (10 μg/ml), and leupeptin (10 μg/ml) with the fluorogenic substrate DEVD-AFC (25 mM) in a total volume of 500 μl. Cleavage of the substrate emitted a fluorescence signal quantified in a Perkin–Elmer LS3 fluorescence spectrophotometer (excitation, 400 nm; emission, 505 nm).

Results

HAPO inhibits apoptosis of MO7e induced by withdrawal of GM-CSF

The MO7e cell line is strictly growth factor-dependent. Previous studies have shown that when growing cells are deprived of cytokine they die by apoptosis and their nuclear DNA is degraded to oligonucleosome size fragments [2,3]. The effect of rhHAPO at different concentrations on MO7e cell survival was determined by sub-G1 apoptotic population analysis. Nearly all the cells without HAPO were dead ($85.55 \pm 5.07\%$) after 96 h. Treatment of the cells with 5 or 10 ng/ml HAPO slightly alleviated this apoptotic death ($80.30 \pm 6.06\%$ and $74.63 \pm 5.80\%$, respectively). It was observed that 50 ng/ml of HAPO or above had a significant survival effect on MO7e cells as compared with control culture cells in the absence of HAPO (apoptosis rate down to $40.82 \pm 4.21\%$, $p < 0.01$; $n = 3$, Fig. 1A). Therefore, a concentration of 50 ng/ml was used for all subsequent experiments.

Fig. 1B shows viability assay of MO7e cells stimulated with GM-CSF, rhHAPO or PBS (control) over a period of 120 h. We observed that at 72 h of cytokine deprivation, only 40–50% of MO7e cells remained viable in control group. By contrast, 80–90% of MO7e cells remained viable in the presence of rhHAPO (50 ng/ml). After 120 h of GM-CSF deprivation, all cells died in control culture, while 40% cells remain viable in rhHAPO-stimulated culture. Although rhHAPO potentially inhibited apoptosis of MO7e induced by withdrawal of GM-CSF, the ability seems weaker when compared with GM-CSF.

At 72 h after cytokine deprivation, most rhHAPO-stimulated cells appeared morphologically intact and viable under a phase-contrast microscope, whereas the control MO7e cells were completely apoptotic. Conditioned medium from rhHAPO-stimulated MO7e cells did not affect the survival of wild-type MO7e cells, suggesting a direct effect of rhHAPO on cell survival. HAPO serves predominantly as a survival factor for MO7e cell and does not induce MO7e cell differentiation. The MO7e cell line did not undergo megakaryocytic differentiation in response to HAPO stimulation. The expression of megakaryocytic cell surface marker CD41 was not increased in HAPO-treated cells compared with those untreated cells. Furthermore, measurement of DNA contents with immunophenotyping did not demonstrate significant increase in polyploidization in the $CD41^+$ cell fraction when cultured with HAPO (data not shown). It is different with the case of SCF, which not only inhibited premature senescence but also enhanced differentiation and maturation of the megakaryocytes [13].

HAPO activates PI3K–Akt pathways

We next studied the possible involvement of PI3K and Akt pathway in HAPO-dependent anti-apoptosis activity. Activation of PI3K was examined by kinase assay for PI3K and that of Akt was analyzed by Western blotting using anti-phospho-Akt antibody. For these experiments, MO7e cells were depleted of GM-CSF for 18 h, stimulated with rhHAPO (50 ng/ml), and immunoprecipitated using an anti-p85 antibody. The precipitates obtained were then subjected to a kinase assay of PI3K using phosphatidylinositol as a substrate.

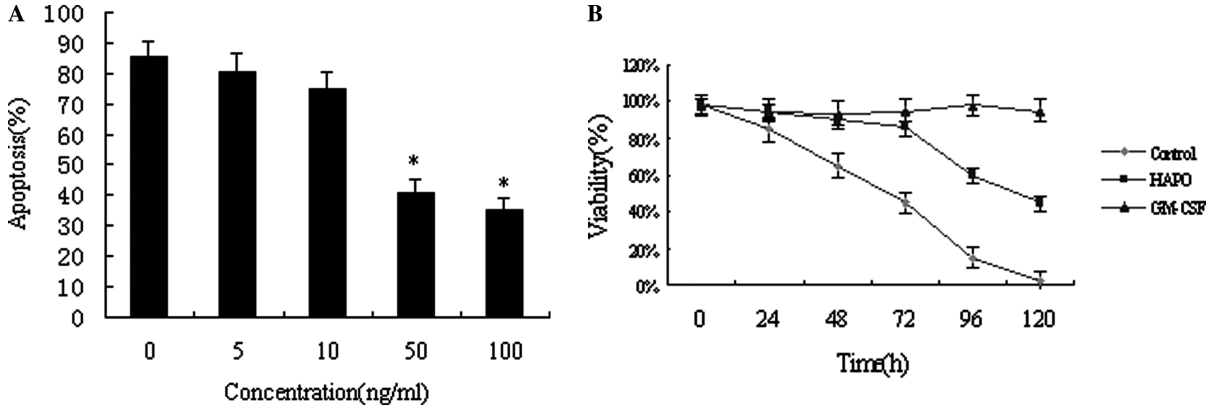

Fig. 1. rhHAPO inhibits apoptosis of MO7e induced by withdrawal of GM-CSF. (A) After cytokine starvation for 18 h, MO7e cells were treated with rhHAPO at different concentrations for 96 h. The parallel control cells were treated with PBS at the same volume as rhHAPO. Apoptosis of cells was evaluated by sub-G1 apoptotic population analysis. Data are means ± SD of triplicate cultures. Results are representative of three independent experiments. $^*P < 0.01$ versus control cells. (B) MO7e cells were cultured in serum containing medium with or without GM-CSF (20 ng/ml) or rhHAPO (50 ng/ml). Viable cells were measured with time by WST-1 assay as described under Materials and methods.

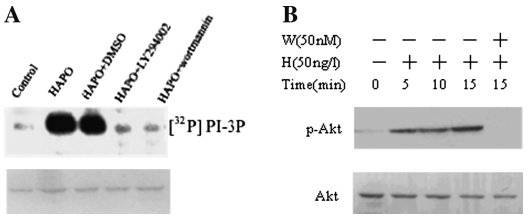

Fig. 2. Activation of PI3K and Akt upon rhHAPO stimulation. (A) MO7e cells were depleted of GM-CSF for 18 h and then incubated with rhHAPO for 5 min. Cell lysates with equal amounts of protein were subjected to an immunoprecipitation with an anti-p85 antibody. The immunocomplexes were used for PI3K assays as mentioned under Materials and methods. A β-actin Western blot showing the equal loading. (B) Akt activity was analyzed by Western blotting of total cell lysates using an anti-phospho-Akt (ser473) antibody. The upper panel shows the blotting pattern of anti phospho-Akt (p-Akt) antibody, and the lower panel shows the reblotting pattern of the same filter using the anti-Akt antibody, indicating the same loading of each lane. H, rhHAPO (50 ng/ml); W, wortmannin (50 nM).

Fig. 2A shows an activation of PI3K after rhHAPO addition. The rhHAPO induced a significant increase in PI3K activity. Pretreatment of cells with LY294002 (25 μM) or wortmannin (50 nM) for 1 h potently inhibited PI3K activity induced by rhHAPO and reduced lipid phosphorylation. As shown in Fig. 2B, Akt phosphorylation was induced by adding rhHAPO to cells. This activity was inhibited by a PI3K-specific inhibitor wortmannin, indicating that Akt functions at the downstream of PI3K in HAPO signaling.

To determine whether HAPO-stimulated PI3K activity is critical for cell survival, MO7e cells were incubated with or without rhHAPO in the presence or absence of wortmannin (50 nM) or LY294002 (25 μM). The apoptotic population was measured by flow cytometric analysis. A time-dependent increase of apoptotic cells was observed after withdrawal of GM-CSF, but the cells treated with rhHAPO exerted lower staining with AnnexinV$^+$/PI$^-$ than the control cells. As expected, although rhHAPO significantly reduced the apoptotic population from $27.29 \pm 4.40\%$ to $10.41 \pm 2.50\%$ at 36 h, the effect of HAPO was potently blocked by PI3K inhibitors. Pre-treated cells before addition of HAPO with PI3K inhibitor LY294002 or wortmannin significantly increased the population of apoptotic cells to $29.15 \pm 3.82\%$ and $22.14 \pm 4.10\%$, respectively ($p < 0.01$; $n = 3$, Fig. 3A). DNA ladder further showed a participation of the PI3K–Akt pathway in the survival function of rhHAPO (Fig. 3B).

HAPO phosphorylates Bad and activates PI3K–Akt–Bad pathway

Bad is known as a key regulator of apoptosis in growth factor-dependent hematopoietic cell lines [14]. Phosphorylation of Bad at serine 112 and 136 residues can be stimulated by many growth factors [15,16]. The roles of

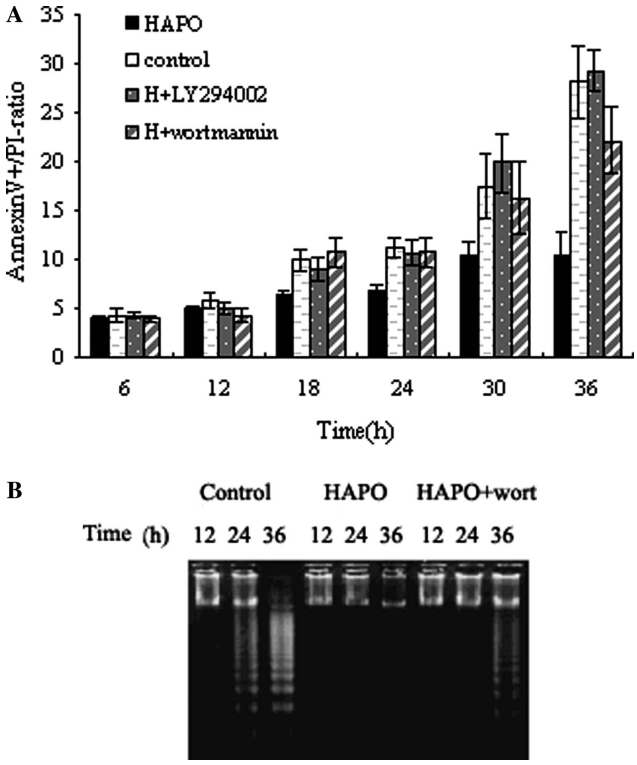

Fig. 3. Involvement of PI3K and Akt in the antiapoptotic signaling of rhHAPO. (A) MO7e cells grew without GM-CSF for 18 h and then treated with rhHAPO, LY294002 (25 μM), wortmannin (50 nM), or an identical amount of vehicle DMSO (control). At the times indicated, the cells were stained with AnnexinV/PI and quantitated by flow cytometry. The data are obtained from a representative experiment. Each point indicates the mean of triplicate determinations, and the error bars represent the range. (B) The DNA ladder also shows that PI3K–Akt pathway participates in the survival function of rhHAPO. The concentrations of rhHAPO and wortmannin used were 50 ng/ml and 50 nM, respectively. Control, cells cultured without cytokines; Wort, wortmannin.

PI3K, Akt, and the Bad pathway have been characterized in several systems, including IL-3 signaling [17].

Fig. 4A shows a time-related phosphorylation of Bad when MO7e cells were treated with rhHAPO. Interestingly, the PI3K inhibitors, either LY294002 or wortmannin, completely suppressed the phosphorylation of Bad induced by rhHAPO (Fig. 4B).

HAPO inhibits activation of caspase-3 and prevents the cleavage of PARP

Caspase-3 is known to play an important role in growth factor withdrawal-induced apoptosis. Caspase-3 is activated by multiple proteolytic cleavages of its 32 kDa precursor form to generate an active p12/p17 complex, which has been used to monitor the activation of caspase-3 [18–21]. When MO7e cells were incubated with rhHAPO for 24 and 36 h, and then analyzed by Western blotting for proteolytic processing of caspase-3, the cleaved fragment was weaker or undetectable

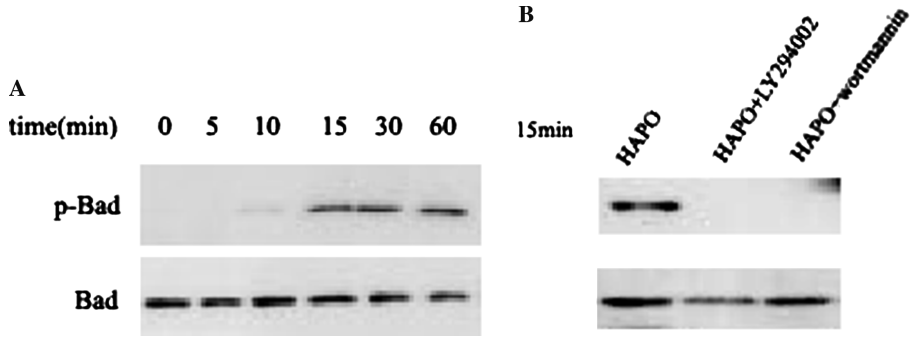

Fig. 4. rhHAPO phosphorylates Bad and activates PI3K–Akt–Bad pathway. (A) MO7e cells were cultured in GM-CSF-withdrawal medium for 18 h and treated with or without rhHAPO for indicated time. Lysates were then made and the immunoblots were performed using antibodies against phospho-Bad (ser136). (B) Pretreatment of MO7e cells with LY294002 (25 μM) or wortmannin (50 nM) before addition of rhHAPO completely suppressed the phosphorylation of Bad induced by rhHAPO.

compared with those untreated cells (Fig. 5A). Accordingly, caspase-3 protease activity was decreased in rhHAPO-treated cells as measured by fluorogenic assay, using DEVD-AFC as a fluorogenic substrate. When the cells were pre-incubated with LY294002 or wortmannin to block the activation of PI3K, abundant active caspase-3 was detectable and reached a level almost equal to that of the control cells (Fig. 5B).

The activation of caspase-3 during the apoptotic process after cytokine depletion may result in a proteolytic degradation of several cellular substrates and lead to propagation of apoptosis signaling and structural disintegration of cells. PARP is an endogenous caspase-3 substrate [22] and its degradation in MO7e cells was therefore analyzed by Western blotting using anti-cleaved-PARP polyclonal antibody. As seen in Fig. 5C, as the original 116 kDa protein depredated during the apoptotic process, the apoptotic fragment (approximately 89 kDa) could be found in the control cells. By contrast, rhHAPO potently delayed this process, alleviating the degree of cleavage over the period of 24 and 36 h.

Discussion

Growth-promoting and/or survival cytokines activate the signal transduction pathways responsible for cell survival. In turn, withdrawal of these cytokines can result in a rapid inactivation of these pathways and lead ultimately to cell apoptosis. A number of studies have suggested that PI3K is able to mediate signaling induced by a number of growth factors and survival cytokines [23,24]. The activation of PI3K leads to suppression of apoptosis in a variety of cells. Studies using dominant negative constructs of PI3K to inhibit PI3K signaling confirm that PI3K is essential for maintaining cell survival [25,26]. Furthermore, the treatment of PI3K with pharmacological inhibitors such as LY294002 and wortmannin can lead to cell apoptosis or loss of the anti-apoptotic effect of growth factors [27,28].

To determine the signal transduction pathway through which HAPO acts on cell survival, the effect of rhHAPO on PI3K was studied in MO7e cells. The present study showed that treatment of MO7e cells with rhHAPO after GM-CSF removal significantly inhibited apoptosis. The corresponding increased PI3K activity could be blocked during treatment with LY294002 or wortmannin, indicating an involvement of PI3K pathway in blocking apoptosis.

It has been known that Akt is one of the downstream targets of PI3K that mediates the anti-apoptotic action. Activation of Akt may induce the phosphorylation of Bad, inhibition of cytochrome *c* release from mitochondria [29], and phosphorylation and inactivation of human caspase-9 [30]. Bad, as a member of the Bcl-2 family proteins, is able to promote cell death at least in part through heterodimerization with the survival proteins Bcl-2 and Bcl-XL. Phosphorylation of Bad on serine residues 136 by Akt results in its cytosolic sequestration by 14-3-3 proteins and its inactivation [16].

We therefore investigated the effect of HAPO on Akt and Bad activation. rhHAPO was found to be able to activate Akt and phosphorylate Bad through PI3K. PI3K inhibitors block rhHAPO-induced activation and phosphorylation of Akt and Bad. These results further indicate that rhHAPO utilizes the PI3K–Akt–Bad survival pathway in MO7e cells.

The caspases are stored as proenzymes and can be cleaved into active fragments upon apoptotic induction. The process of cleavage is mediated by proteolysis and generates two subunits. These two subunits form heterodimers that eventually produce the enzymatic activities of caspases [31]. Caspase-3 plays a pivotal role in execution of apoptosis, which involves the activation of caspases and subsequent cleavage of several cellular substrates including PARP, gelsolin, actin, lamins, and fodrin [32–35]. In this manner, caspase-3 activation may lead to propagation of apoptosis signaling and structural disintegration of cells. The effect of HAPO on the activity of caspase 3 was therefore

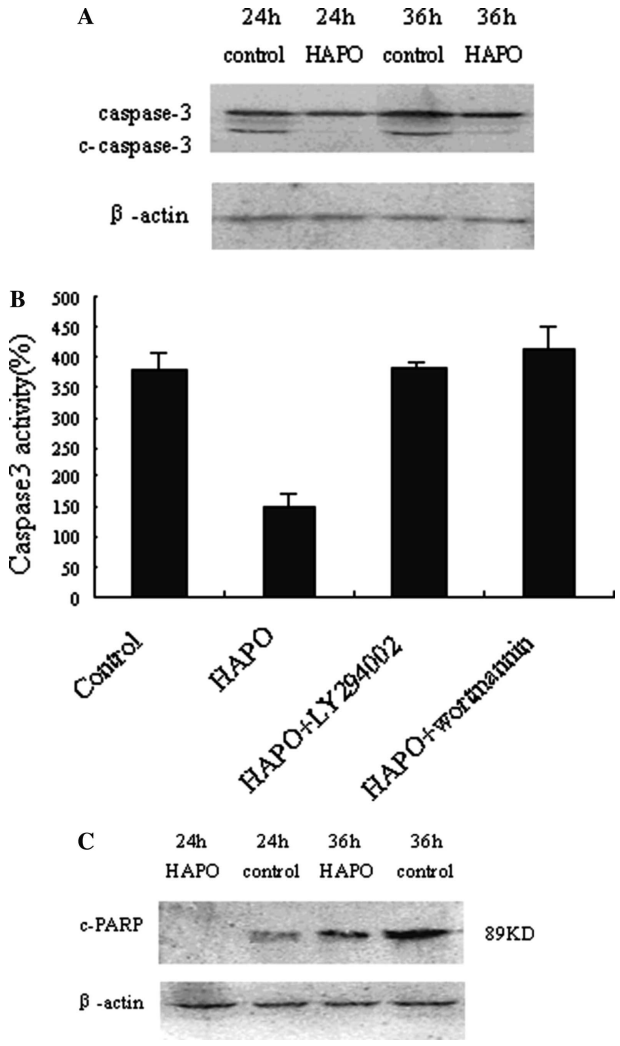

Fig. 5. Activation of PI3K by rhHAPO inhibits the activity of caspase-3 and prevents the cleavage of PARP. (A) Treatment of MO7e cells with rhHAPO suppressed the production of active caspase-3 fragment. Reprobing the same blot with anti-β-actin after stripping demonstrates equal amounts of protein in the extracts. (B) Activation of PI3K inhibited the enzymatic activity of caspase-3. The cells were starved for 18 h before treatment with or without rhHAPO and LY294002 (25μM) or wortmannin (50 nM). Cell lysates were collected at 36 h and assayed for caspase-3 activity. The data represent means ± SE from four independent experiments. Control, MO7e cells cultured in cytokine-free medium. (C) rhHAPO inhibited cleavage of PARP in MO7e cells. MO7e cells were treated with or without rhHAPO for the indicated time. The presence of 89-kDa proteolytic fragment of PARP was detected by immunoblotting using an anti-cleaved-PARP antibody.

investigated in this study. We observed that HAPO inhibited the activation of caspase-3. Such an inhibitory effect of HAPO on caspase 3 could be completely reversed by the addition of PI3K inhibitors. Moreover, we found that HAPO blocked caspase-3 activation and PARP cleavage during GM-CSF deprivation. Again, such an activity of HAPO could be abrogated by the addition of PI3K inhibitors, suggesting that activation of caspase-3 during growth factor-deprived induced apoptosis is attenuated by rhHAPO through the PI3K signaling pathway.

Taken together, our data demonstrate that HAPO protects MO7e cells from apoptotic death by inactivating caspase 3 and blocking the PARP degradation through a PI3K–Akt pathway.

Acknowledgments

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