Hemangiopoietin, a novel human growth factor for the primitive cells of both hematopoietic and endothelial cell lineages

Yong Jun Liu, Shi Hong Lu, Bin Xu, Ren Chi Yang, Qian Ren, Bin Liu, Bin Li, Min Lu, Feng Ying Yan, Zhi Bo Han, and Zhong Chao Han

The present study reports the identification of hemangiopoietin (HAPO), a growth factor capable of supporting the proliferation and survival of primitive hematopoietic and endothelial cells and protecting animals from death caused by radiation injury.

Materials and methods

Bacterial strains, plasmids, reagents, and cytokines

Vector pET22b (Novagen, Madison, WI) was used to construct plasmid for the expression of HAPO in Escherichia coli host BL21 (DE3). The intermediate plasmid construct was propagated in E. coli DH5α. Restriction enzymes were purchased from Takara Bio (Otsu, Shiga, Japan). The Pyrococcus furiosus (Pfu) DNA polymerase was purchased from Sangon (Shanghai, China). The primers of oligonucleotides were synthesized by BioAsia (Shanghai, China). Recombinant human SCF, EPO, granulocyte colony-stimulating factor (G-CSF), and IL-3 were kindly provided by Kirin Pharmaceuticals (Tokyo, Japan). Recombinant human granulocyte-macrophage CSF (GM-CSF) and VEGF were purchased from Sigma (St Louis, MO). Recombinant human EGF was purchased from Gibco BRL (Carlsbad, CA). The miniMACS immunomagnetic separation kits were purchased from the Miltenyi Biotech (Bergisch Gladbach, Germany).

Purification of HAPO

HAPO was initially purified from the urinary extracts of patients with aplastic anemia (AA) in our laboratory. Briefly, 55 L urine was collected...
from AA patients and subjected to a series of purification procedures, including ultrafiltration using an Amicon YM10 filter followed by a column of sephadex G50 to remove the salt, 3 M guanidine chloride treatment, alcohol precipitation (60% to 90% saturation), and chromatography by WGA–Sepharose 6MB (Pharmacia, Uppsala, Sweden), reverse high-performance liquid chromatography–C18 (HPLC–C18), HPLC–Superox 6, heparin-Sepharose CL-6B, and reverse-HPLC–nucleosil C4 columns.

**Purification of total RNA and RT-PCR amplification of HAPO cDNA clone**

Cellular total RNA was isolated from human fetal liver according to Chomczynski’s method. Reverse transcription–polymerase chain reaction (RT-PCR) was performed in accordance with the manufacturer’s instructions. Two oligonucleotide primers of sense and antisense orientations based on the HAPO sequence with forward and reverse sequences 5′-ATGCCATGGAAACACTTCTC-3′ and 5′-CGGGGTGTCGACACTTGTGAT-3′ were synthesized. The HAPO RT-PCR product was confirmed by sequencing.

**Molecular cloning and construction of expression plasmid pHAPO**

Synthetic oligonucleotides were designed to produce an 879 bp amplified DNA fragment. Primer 1, which introduced a 5′ NcoI site, was 5′CGGGTACCATGACACCTGCTGTTAG-3′. The sequence of primer 2 was 5′GGGGGATCTCGAGGTGTGGTGTAGCTTGA TGG-3′. An NcoI/XhoI fragment containing the N-terminal coding sequence of HAPO was constructed by ligating this fragment into plasmid pET22b. Expression plasmid pHAPO was constructed by ligating this fragment into plasmid pET22b-

**Expression and purification of recombinant HAPO**

To characterize the biologic properties of HAPO, recombinant human HAPO (rhHAPO) was produced in our laboratory. Briefly, 50 mL cultured E. coli BL21 (DE3)pHAPO cells were used to inoculate 1000 mL of 2 × YT medium (15 g/L bacto-tryptone, 10 g/L yeast extract, 5 g/L NaCl). The cell paste was resuspended in one-fourth vol 50 mM Tris-HCl (trishydroxymethyl)-amino-methane-HCl), pH 8.0, 20% sucrose. The supernatant was loaded onto a Fast Flow (Amersham, Arlington, IL) diethylaminoethyl (DEAE) Sepharose column. The column was washed and eluted by a salt gradient from 20 mM to 120 mM NaCl, and rhHAPO activity eluted at about 100 mM NaCl. The rhHAPO fractions were pooled, adjusted to 40 mM imidazole, and loaded onto a nickel (Ni) chelating column. The column was washed and eluted by a gradient from 60 mM to 200 mM imidazole. The rhHAPO fractions, eluted at 120 mM imidazole, were pooled and dialyzed to remove the imidazole. After dialysis, the fractions were applied to a Fast Flow sulphopropyl (SP) sepharose column. The rhHAPO was eluted, and the resulting protein solution was stored at −80 °C until used.

**Cell isolation**

Human fetal bone marrow cells were obtained from aborted fetuses of 17 to 22 weeks’ gestation with informed consent. Adult bone marrow cells were obtained from consenting healthy donors. Umbilical cords were obtained from healthy, full-term placenta. Mononuclear cells (MNCs) were isolated by density gradient centrifugation on Ficoll-Hypaque. Human bone marrow CD34+, KDR+, or AC133+ cells were isolated with the use of miniMACS immunomagnetic separation kits in accordance with the manufacturer’s instructions. To improve the purity, the cells in the purified fraction were applied to a second column, and the purification steps repeated. The final purity obtained was 98%. The CD34+ KDR+ subpopulation was sorted from human fetal bone marrow MNCs on FACScalibur (Becton Dickinson, San Jose, CA). Human umbilical vascular endothelial cells (HUVECs) were isolated by treating the umbilical cords with trypsin/EDTA (trypsin/ethylenediaminetetraacetic acid) and were cultured on flasks in M199 medium containing 20% fetal calf serum (FCS). On reaching 80% confluence, the HUVECs were digested and collected for further studies. Mouse bone marrow cells were obtained from the femurs of 6 to 8 weeks of age and from male BALB/c mice and performed as described previously.21

**Cell culture**

Clonogenic culture. Three different culture systems were applied for bioassay of rhHAPO: culture system A, hematopoietic cells; culture system B, endothelial cells; and culture system C, hematopoietic and endothelial cells. In culture system A, bone marrow MNCs were cultured in a semi-solid culture consisting of 1% methylcellulose in Iscove modified Dulbecco medium (IMDM), 30% FCS, 1% bovine serum albumin (BSA), 10−4 M-mercaptoethanol, and 2 mM l-glutamine, with rhHAPO alone or rhHAPO plus a cocktail of cytokines including rhIL-3 (10 ng/mL), rhGM-CSF (10 ng/mL), and rhSCF (50 ng/mL), with or without rhEPO (3 U/mL). The cultures were incubated 37 °C in a humidified atmosphere with 5% CO2, Granulocyte, monocyte, megakaryocyte, and erythroblast colony-forming units (CFU-Mix), erythroblast burst-forming units (BFU-Ex), and CFU-GMs were identified on day 14 under an inverted microscope as described previously.22 In culture system B, the CD34+ KDR+ cells were cultured in a fibronectin-collagen–coated 96-well plate at concentration of 1 cell per well for 2 weeks. The M199 medium supplemented with 10 ng/mL VEGF, 10 ng/mL EGF, 100 U Penicillin, 100 U streptomycin, and 5% FCS was used for bioassay of bone marrow endothelial cells, with the addition of rhHAPO (100 ng/mL) on day 1 and day 8. In culture system C for both hematopoietic and endothelial progenitor cells, the CD34+ KDR+ cells were cultured in 96-well plates at 1 cell per well in a low-glucose MEM medium supplemented with 10 ng/mL IL-3, GM-CSF, VEGF, and EGF with or without the addition of rhHAPO on day 1 and day 8.

Liquid culture. The basic medium of liquid culture system was the IMDM supplemented with 10% FCS, 100 U/mL penicillin G, 100 μg/mL streptomycin, and 2 mM l-glutamine. The cells were cultured at various concentrations in a 24-well plate or a 96-well plate with or without rhHAPO (100 ng/mL). The cultured cells were trypsinized and counted. Viability of the cultured cells was measured by 0.4% trypan blue exclusion method at different time points.

For assay of HUVECs, the cells were seeded in 96-well plates at 5 × 103 cells per well in 100 μL RPMI 1640 medium with various concentrations of rhHAPO. The MTT (3(4, 5-di-methyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide) (Sigma) assay, as described previously,19 was used to test the effect of rhHAPO on the growth of HUVECs.

**Flow cytometric assay**

The expression of CD34, AC133, KDR/Flik-1, c-kit, and stem cell antigen–1 (Sca-1) molecules on purified or cultured bone marrow cell populations was analyzed by means of flow cytometry. Fluorescein isothiocyanate (FITC) or phycoerythrin (PE)–conjugated anti-CD34, PE-conjugated anti-KDR (Flik-1), FITC-conjugated anti-Sca-1, and PE-conjugated anti–c-kit were provided by Becton Dickinson/PharMingen (San Diego, CA) and PE-conjugated anti-AC133 was provided by Miltenyi Biotech. Negative controls were stained with an isotype-matched FITC- or PE-conjugated immunoglobulin G (IgG), and compensation was adjusted with the use of the single-stained cell samples. Dual-color antibody marker analysis was performed by means of a FACScalibur and CellQuest software (Becton Dickinson/PharMingen).

**In vitro and in vivo study in mice**

BALB/c male mice, 6 to 8 weeks of age, were used for study. Bone marrow cells were collected from femurs in vitro colony assay of long-term culture-initiating cells (LTC-ICs) was performed as previously described.21 Briefly, the confluent marrow stroma of BALB/c mice seeded in 24-well plates developed within 3 to 4 weeks and was then irradiated at 15 Gy. These confluent layers recharged with murine bone marrow MNCs at a cell density of 1 × 109/mL. Then, rhHAPO was added to the culture and incubated with cells at 37 °C for 5 weeks. The liquid medium was then removed, and a methylcellulose semi-solid medium, supplemented with

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EPO (3 U/mL), GM-CSF (10 ng/mL), G-CSF (20 ng/mL), IL-3 (20 ng/mL), and SCF (50 ng/mL), was plated directly in situ. The number of colonies was scored after 14 days of culture. Evaluation of the effect of rhHAPO in vivo in mice was performed as previously described.21

**Radiation**

Male BALB/c mice were exposed to gamma radiation from an experimental 137Cs irradiator (GammaCell 40; Atomic Energy of Canada, Kanata, ON, Canada) at 1.32 Gy/min. Exposure time was adjusted so that each animal received a 6 Gy dose.

**Statistical analysis**

Data are expressed as mean ± standard deviation (SD) of at least 3 independent experiments. Student t test for paired samples was applied to determine the difference. P values less than .05 were considered statistically significant.

**Results**

**Identification, cloning, and expression of HAPO**

To identify possible growth factors for hemangioblasts, we screened proteins capable of stimulating proliferation of human hematopoietic stem/progenitor cells (HSPCs) and HUVECs in vitro from the urine of patients with aplastic anemia. A polypeptide named hemangiopoietin (HAPO) was searched for on the basis of its biologic activity in stem/progenitor cells (HSPCs) and HUVECs in vitro from the urine of patients with aplastic anemia. Recombinant human HAPO is expressed by pET-22b vector as secreted protein in periplasm. Recombinant HAPO was purified from the urine of patients with aplastic anemia by using a series of chromatographic columns including DEAE sepharose, Ni chelation, and SP sepharose Fast Flow columns. Purified HAPO has a predicted molecular mass of 31 800 Da (Figure 1C).

**Table 1. Effect of rhHAPO on colony formation of human fetal bone marrow progenitor cells in response to cytokine stimulation**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Colony types, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU-M</td>
</tr>
<tr>
<td>PBS</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>HAPO</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>CC</td>
<td>10 ± 1.41</td>
</tr>
<tr>
<td>CC + HAPO</td>
<td>13 ± 1.29</td>
</tr>
</tbody>
</table>

CC, the appropriate cocktail of cytokines, included rhIL-3 (10 ng/mL), rhGM-CSF (10 ng/mL), rhSCF (50 ng/mL), and rhEPO (3 U/mL). The rhHAPO was added at 100 ng/mL final concentration. Data are pooled from 3 separate experiments and expressed as mean ± SD. Bone marrow cells were plated a cell density of 1 × 10^5 cells mm dish in a methylcellulose system as described in “Materials and methods.”

CFU indicates colony-forming unit; M, macrophage; G, granulocyte; BFU-E, erythroblast burst-forming unit; Mix, mixed colony; PBS, phosphate-buffered saline.

*P < .05 versus PBS.
†P < .05 versus CC group.
Therefore, we used the concentration of 100 ng/mL to investigate the effect on hematopoietic progenitor cells (HPCs) with the use of human fetal bone marrow mononuclear cells. Puriﬁed CD34+ cells were cultured at 5 × 10^5/mL in QBSF-60 (Quality Biological, Gaithersburg, MD) serum-free medium containing a cocktail of cytokines including rhIL-3 (10 ng/mL), rhSCF (50 ng/mL), TPO (20 ng/mL), and 100 ng/mL of rhHAPO (the rhHAPO was not added in the control). Data are pooled from 3 separate experiments and expressed as mean ± SD.

<table>
<thead>
<tr>
<th>Cell subset</th>
<th>Control</th>
<th>rhHAPO</th>
<th>Control</th>
<th>rhHAPO</th>
<th>Control</th>
<th>rhHAPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+</td>
<td>10</td>
<td>10</td>
<td>426 ± 4</td>
<td>526 ± 22*</td>
<td>62 ± 5</td>
<td>159 ± 42*</td>
</tr>
<tr>
<td>AC133</td>
<td>7 ± 0.1</td>
<td>7 ± 0.1</td>
<td>179 ± 2</td>
<td>288 ± 12*</td>
<td>61 ± 17</td>
<td>244 ± 17*</td>
</tr>
<tr>
<td>KDR+</td>
<td>0.4 ± 0.02</td>
<td>0.4 ± 0.02</td>
<td>0.2 ± 0.03</td>
<td>0.4 ± 0.02*</td>
<td>0.11 ± 0.02</td>
<td>0.39 ± 0.01*</td>
</tr>
</tbody>
</table>

Puriﬁed adult bone marrow CD34+ cells were cultured at 1 × 10^6/mL in QBSF-60 (Quality Biological, Gaithersburg, MD) serum-free medium containing a cocktail of cytokines including rhIL-3 (10 ng/mL), rhSCF (50 ng/mL), TPO (20 ng/mL), and 100 ng/mL of rhHAPO (the rhHAPO was not added in the control). Data are pooled from 3 separate experiments and expressed as mean ± SD.

*P < .05 versus control.
rhHAPO significantly stimulated the proliferation of HUVECs. Human fetal bone marrow MNCs were seeded in culture system C for 72 hours, and the adherent cells were isolated and seeded in culture system B for 7 days. FACS analysis revealed that the percentage of KDR<sup>+</sup> and VWF<sup>+</sup> cells was increased from 11.89% to 15.82% and from 11.74% to 21.67%, respectively (Figure 4B). When the adherent cells were further incubated in a culture system containing SCF, IL-3, TPO, and rhHAPO (Figure 4C), the formation of VWF<sup>+</sup> endothelial cell colonies (CFU-EPCs) was significantly increased compared with control culture containing no added rhHAPO. The mean (± SD) number of colonies per culture well in rhHAPO group was 35 (± 4) colonies, compared with 21 (± 3) colonies in control group (P < .05).

Further studies were performed with the use of purified KDR<sup>+</sup> human fetal bone marrow cells. In 2 representative experiments, purified KDR<sup>+</sup> cells were plated at a cell density of 1 × 10<sup>3</sup>/mL in culture system B containing a cocktail of cytokines as described, with or without rhHAPO. All cultures were performed in quadruplicate and scored after 21 days of culture with the use of an inverted microscope. The rhHAPO significantly stimulated the formation of hematopoietic (CFU-Mix) and endothelial colonies (CFU-EPCs) (Figure 4C).

**Effect of rhHAPO on the growth of murine bone marrow in vitro**

The biologic activity of rhHAPO on hematopoiesis was also studied in mice with the use of a long-term bone marrow culture system. It was found that rhHAPO stimulated the proliferation of murine bone marrow cells after 4 weeks of culture. FACS analysis revealed an increase in the numbers of various stem cell subpopulations, including CD34<sup>+</sup>, Sca-1<sup>+</sup>, and Flk-1<sup>+</sup> cells, in response to rhHAPO stimulation. Particularly, the stimulatory effect of rhHAPO on the expansion of the CD34<sup>+</sup> cell subset was statistically significant, as compared with control culture (Figure 5A).

To determine if rhHAPO acts on the growth of early murine stem cells, we tested the effect of rhHAPO on the proliferation of murine bone marrow long-term culture-initiating cells (LTC-ICs) as described previously. As seen in Figure 5Bii, rhHAPO stimulated the growth of LTC-IC–derived colonies as compared with control culture (Figure 5Bi). In 2 representative experiments, the number of LTC-IC–derived colonies in the presence of rhHAPO (10 ± 2.3/10<sup>3</sup> plated cells) was 5-fold higher than in the absence of rhHAPO (2 ± 0.8/10<sup>3</sup> plated cells).

**In vivo effect of rhHAPO in normal mice**

To confirm the stimulatory effect of HAPO in vivo, normal BALB/c mice received a daily subcutaneous injection of rhHAPO at 10 μg for 7 consecutive days. Figure 6A shows that on day 14 after injection of rhHAPO, the number of bone marrow nucleated cells per femur was 1.6-fold higher than in the control mice. A significant increase in the numbers of bone marrow CD34<sup>+</sup>, c-kit<sup>+</sup>, and Sca-1<sup>+</sup> cells was observed in the mice receiving rhHAPO compared with the control mice (Figure 6B). In addition, injection of rhHAPO also resulted in an increase in circulating white blood cells (Figure 6C) and platelets (Figure 6D) in rhHAPO-treated mice. The number of circulating red blood cells had no significant change after administration of rhHAPO (Figure 6E). However, the percentage of reticulocytes slightly increased in rhHAPO-treated mice as compared with control mice (Figure 6F).
Radioprotective effect of rhHAPO in mice

On the basis of the observation that rhHAPO stimulated normal hematopoiesis in vivo, the effect of rhHAPO on the recovery of radiation injury was further studied with the use of irradiated mice. The mice were treated with total body irradiation of 6 Gy and then received a daily subcutaneous injection of rhHAPO for 7 consecutive days. The irradiated mice receiving no rhHAPO started to die on day 6, and only 7% of mice survived for as long as 20 days after irradiation. In contrast, the survival rate of the irradiated mice receiving rhHAPO was significantly increased. Interestingly, the radioprotection of rhHAPO was found to be dose dependent. A high dose of rhHAPO (15 μg) completely protected animals from death caused by irradiation (Figure 7A).

Further study on the recovery of hematopoiesis in irradiated mice injected with rhHAPO found a significant increase in the degree of bone marrow nucleated cells on days 14 and 21 after irradiation (Figure 7Bi). The mice injected with rhHAPO also had an increased number of white blood cells (Figure 7Bii) and platelets (Figure 7iii) on days 14 and 21 after irradiation as compared with control mice.
CD34+ point, 3 mice in each group were randomly sampled and were subjected to a daily subcutaneous injection of rhHAPO (10 μg) or PBS. At each time point, 3 mice in each group were randomly sampled and were subjected to examination for the content of the bone marrow CD34+ cells from the murine femurs (i) and the quantitative changes in the number of white blood cells (ii), platelets (iii), and red blood cells (iv) in the blood of the mice after irradiation. Data are represented as the mean ± SD. *P < .05 versus control.

Discussion

Aplastic anemia is an elusive disease, not only having a cytopenia and marrow hypoplasia but also containing key information on hematopoietic stem cell regeneration and microenvironmental evolution.26-27 Therefore, we initiated a search for a novel growth factor from the urine of patients with aplastic anemia. In the present work, we report the successful identification and purification of HAPO, a growth factor acting on the primitive cells of both hematopoietic and endothelial cell lineages. Sequence analysis indicates that the cDNA clone of HAPO, isolated from human fetal liver cDNA library, is an alternative splicing variant lacking exon 5 of PRG 4.23-25 To characterize the biologic activities of HAPO, a partial cDNA fragment of HAPO was expressed in E. coli BL21, and highly purified rhHAPO was obtained for various in vitro and in vivo biologic assays.

We first demonstrated a stimulatory effect of rhHAPO on human hematopoiesis using different human culture systems. The rhHAPO stimulated the proliferation of human hematopoietic progenitor cells. Cloning efficacy analysis indicates that rhHAPO had a stimulatory effect on CFU-Mix but not on CFU-GM, CFU-G, CFU-M, and BFU-E, suggesting a predominant action of rhHAPO on human primitive hematopoietic stem/progenitor cells.

We then wanted to know if rhHAPO acts on the level of hemangioblast. It has been suggested that hemangioblast is a transient cell stage during embryonic development.3,4 Therefore, it is difficult to obtain a sufficient number of purified hemangioblasts from embryonic tissue for cytokine bioassay. However, previous observations have indicated that adults maintain a reservoir of hemangioblasts. For example, AC133+ human bone marrow and peripheral blood cells have the capacity to differentiate into endothelial cells. In addition, postnatal CD34+ and CD34+KDR+ cells can also generate hematopoietic and endothelial progeny in different in vitro and in vivo assays.8-11,14 On the basis of these observations, we tested the effect of rhHAPO on these stem cell subsets using culture systems both for hematopoietic-endothelial progenitor cells and for endothelial cells. The rhHAPO was found to directly stimulate the proliferation and differentiation of purified CD34+, KDR+, or CD34+KDR+ cells into both hematopoietic and endothelial cells. The results obtained clearly show a predominantly stimulating action of rhHAPO at the level of the primitive cells of both hematopoietic and endothelial cells.

To determine if rhHAPO also acts in mice, similar experiments were conducted in the murine system. The rhHAPO was found to enhance proliferation of murine hematopoietic stem/progenitor cell populations including CD34+, Sca-1-, and Flk-1- cells. Moreover, rhHAPO stimulated the growth of murine bone marrow LTC-ICs in liquid culture. These results demonstrate once again a stimulating action of rhHAPO on an early stage of hematopoietic stem cells.

Subsequent experiments were conducted to evaluate the clinical potential of rhHAPO in vivo in normal and irradiated mice. Administration of rhHAPO in normal mice resulted in a significant increase in bone marrow hematopoietic stem/progenitor cells, together with a slight increase of white blood cells and platelets in blood. These data indicate a positive action of rhHAPO on hematopoiesis in vivo and imply a potential of this factor in hemoprotection. We therefore performed in vivo studies on radioprotection of rhHAPO. The results obtained demonstrate that rhHAPO significantly protected animals from death caused by radiation damage. The irradiated mice injected with rhHAPO had an enhanced survival rate. Such a radioprotective effect of rhHAPO was dose dependent, starting at a rhHAPO dose of 55 μg. An injection of a higher dose of rhHAPO (15 μg) resulted in a 100% survival rate of the irradiated mice. Comparative analysis of control and experimental groups of mice revealed that the mechanism of action of rhHAPO that protects mice from irradiation was related to an accelerated hematopoiesis in response to rhHAPO stimulation.

We have noted that, in both in vitro and in vivo studies, a higher dose was required for rhHAPO to be effective compared with that necessary for other cytokines, including SCF, TPO, G-CSF, and FLT-3 ligand.28-30 This phenomenon suggests that HAPO may act on a relatively rare cell population and that therefore a larger amount of HAPO is needed for activation of the targeted cells. Another possibility is that HAPO may be a unique growth factor because the normal serum level of HAPO (56.42 ± 14.26 ng/mL) measured in our laboratory (ranging from 18.3 ng/mL to 91.1 ng/mL; n = 46; data not shown), by means of a sandwich enzyme-linked immunosorbent assay (ELISA), was found to be relatively higher than that of SCF, TPO, G-CSF, or FLT-3 ligand in normal serum. In addition, we must point out that the rhHAPO used in the study was preserved in a buffer without any exogenous protein as protector. This preservation may not be the best one and may cause a loss of some activity of rhHAPO. Full characterization of biologic activities, pathophysiological significance, and clinical potential of HAPO will require
further studies in both animal and human systems, including preparation of highly pure and active rhHAPO and identification of HAPO receptor and signal transduction pathway as well as its modulating action on cell apoptosis and DNA repair.

In summary, the present study reports the identification and partial biologic characterization of HAPO and suggests that this cytokine possesses an important clinical potential for the management of various cytopenias and radiation injury and for the expansion of hematopoietic and endothelial stem/progenitor cells. The results obtained from in vitro studies suggest that HAPO is the growth factor acting on the primitive cells of both hematopoietic and endothelial cell lineages. In vivo in mice, although short-term radioprotection by rhHAPO has been demonstrated, further studies are needed to confirm if rhHAPO acts directly on primitive hematopoietic cells as observed in vivo, with the use of the competitive transplantation assay.

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References

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