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C-Terminal Pentapeptide of Osteogenic Growth Peptide Regulates Hematopoiesis in Early Stage

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Abstract Osteogenic growth peptide (OGP) was characterized in regenerating bone marrow, which can increase osteogenesis and hematopoiesis. The carboxy-terminal pentapeptide is a naturally occurring human and murine mitogen equipotent to OGP. In this study, we evaluated the potential role of OGP10–14 in regulation of hematopoiesis in human hematopoietic stem cells and animal model. Our results showed CD34+ stem cells from umbilical cord blood (UBC) were significantly increased in OGP10–14 treated samples, which is nearly equivalent to the results obtained from the combinations of IL3, IL11, G-CSF, and EPO group. OGP10–14 can also stimulate the differentiation of stem cells from bone marrow at the level of noncommitted progenitor stem cells, thus increasing the number of reconstituted red and white cells as well as platelets after injected i.m. everyday continuing for 5 days in hematopoietic function damage mice comparing with the OGP-untreated group. These data implicate that the role of OGP10–14 regulating hematopoiesis is in the early stage of the whole hematopoietic growth factors (HGFs) regulating network, just like the position of interleukin 13 in the hematopoiesis network. J. Cell. Biochem. 101: 1423–1429, 2007. © 2007 Wiley-Liss, Inc.

Key words: osteogenic growth peptide; pentapeptide; hematopoiesis

Regulation of hematopoiesis remains an important goal of both clinical and experimental studies. It is particularly relevant for the enhancement of hematopoietic reconstruction to reduce morbidity and mortality in patients subjected to radiotherapy and chemotherapy, as well as bone marrow transplantation (BMT). The currently available experimental clinical treatment for stimulating post BMT reconstruction consists mainly of the administration of recombinant human granulocyte colony stimulating factor (rhG-CSF) and/or recombinant human granulocyte-macrophage colony stimulating factor (rhG-CSF), EPO et al. These cytokines affect directly on the proliferation of transplanted pluripotent cells already committed to the white cell or red cell lineages. Osteogenic growth peptide (OGP) consists of 15 residues identical to the C-terminal tail of histone H4, which can offer a balanced reconstruction of hematopoiesis after high dose radiotherapy and chemotherapy and after BMT. Just like many other factors regulating the activity of osteogenic cells, OGP in high abundance is present physiologically in the serum mainly in an inactive form of OGP–OGP binding protein complex [Bab, 1993; Robinson et al., 1995]. It is reported that C-terminal truncated pentapeptide of OGP, H-Tyr-Gly-Phe-Gly-Gly-OH (10–14) is a naturally occurring human and murine mitogen equipotent to OGP. In addition, data suggests that OGP (10–14) is generated from OGP by proteolysis cleavage upon dissociation of the OGP-OGPBP complexes [Chen et al., 2002; Rita 2002; Gurevitch et al., 1996], while OGP had been demonstrated to stimulate hematopoiesis reconstruction. Its effect and role as a hematopoietic factor in the whole hematopoiesis network has not yet been fully understood. Accordingly, the present experiments were designed to test the effect of the OGP on hematopoietic stem cells in vitro and

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in vivo [Bab and Chorev, 2002; Fazzi et al., 2002a,b, 2003, 2004].

MATERIALS AND METHODS

Animals

Female BALB/C mice weighing 20–25 g were purchased from Laboratory Animal Center of Academy of Military Medical Sciences, PRC.

Human Growth Factors and Cytokines

All the cytokines like IL3, IL11, G-CSF, and EPO were purchased from Peprotech Company, England. Cytokine combination usage dose is equal with single cytokine usage dose.

Administration of OGP-5

Chemical synthetic OGP-5 was supplied by SBS Genetech Ltd., purified according to the standard solid phase peptide synthesis methodology. For injection, sOGP was dissolved in phosphate-buffered saline (PBS) and administered intramuscularly everyday in different concentration continuing for 5 days.

Hematopoietic Function Damage Animal Model

Cyclophosphamide (100 mg/kg) and phenylhy-drazioe (60 mg/kg) i.p. injection everyday to BALB/C mice continuing for 3 days.

Effect of OGP10–14 on Peripheral Blood Cell Numbers

Blood was taken every 3 days after OGP injection from tail, every 0.5 ml blood add 10 ul 7.5% EDTA as anticoagulant, these blood sample was subjected to differential WBS counts in a coulter counter as well as RBC and platelet counts.

Effect of OGP10–14 on Hematopoietic Stem Cells From Bone Marrow and Umbilical Cord Blood

Colony-forming unit-granulocyte macrophage (CFU-GM): add 100 ng/ml GM-CSF to culture system (2×10^5 ml^{-1} PBMNC) as positive control, add different concentration of OGP to the culture system, 5% CO_2 incubator at 37°C for 6 days, over 50 cells equals one granulocyte-macrophage colony forming unit.

Colony-Forming Unit-Erythrocyte Megakaryocyte (CFU-E CFU-M)

EPO (4 IU) was added to the culture system as erythrocyte progenitor stem cell colony forming control, 100 ng IL11 was added as megakaryocyte progenitor stem cell colony forming control, IL3 was added as mix colony forming control, and different concentrations of OGP was added to the culture system in a 5% CO_2 incubator at 37°C for 3 days; >8 cells equals one erythrocyte colony forming unit in a 5% CO2 incubator at 37°C for 7 days and >2 cells equals one megakaryocyte colony forming unit.

CD34+ Cells Purification

Ten microliters of umbilical cord blood was taken and transferred into RPMI 1640 medium containing heparin, mononuclear cells were separated by ficoll density gradient centrifugation. CD34+ cells were separated by Dynabeadsl®, according to the manufacturer's recommendations (DYNAL), briefly, add CD34+ antibody to label the wanted cells and then add the Dynabeads to mononuclear cells from cord blood and incubate at 2–8°C on a Dynall® sample mixer. Place the tube in the magnet for 10 min, discard the supernatant, and wash the bead-bound cells 3–5 times to obtain a high purity. Yielding is about 60% and purity is about 90%.

RESULTS

Effect of OGP10–14 on Hematopoietic Stem Cells From Bone Marrow of Normal Mice

To observe the effect of OGP on the CFU-GM, CFU-E, CFU-Meg growth of mice marrow cell cultured. OGP (10–6 M to 10–10 M), respectively, were added to MNC in vitro and the colony number of CFU-GM, CFU-E, CFU-Meg were counted. Results showed that the colony number of CFU-GM, CFU-E, CFU-Meg was increased by the stimulation of OGP (Fig. 1) dose dependently between 0.1 nmol and 1,000 nmol per day per mouse comparing with untreated samples. The maximal effect was triggered by the 1 and 10 nmol per day dose ($P < 0.01$) and is totally due to OGP addition. The increasing efficacy effect of optimally OGP-treated group is about 50% comparing with positive cytokine control group.

Effect of OGP10–14 on Hematopoietic Stem Cells From Bone Marrow of Cyclophosphamide Treated Mice

We would like to see the stimulation effect of OGP10–14 on radiochemotherapy damaged hematopoietic system since the above results

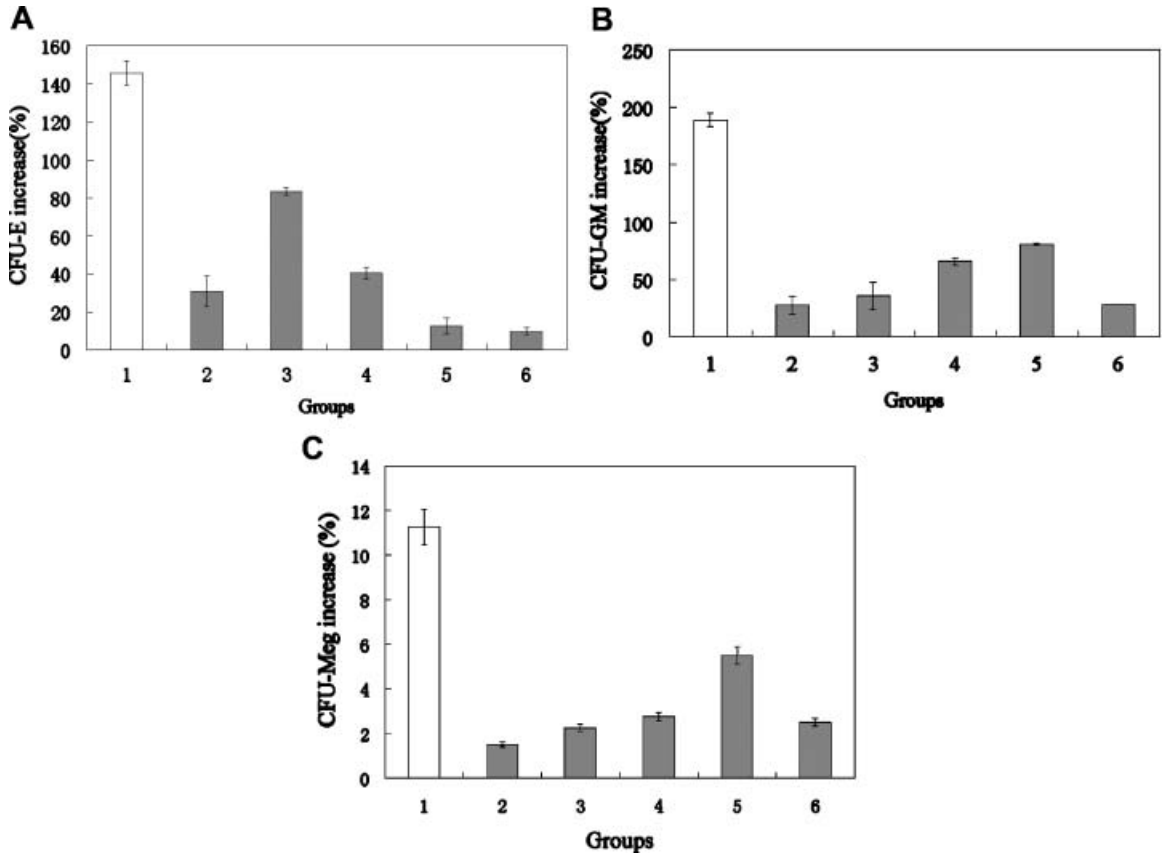

Fig. 1. Colony forming unit (CFU) test on bone marrow-derived cells. Bone marrow derived MNC was separated into different groups below: Group1.Positive control group **A**: EPO **B**: GM-CSF **C**: IL11 2–6. OGP5 treatment group: 10–6 M, 10–7 M, 10–8 M, 10–9 M, 10–10 M CFU increase(%) = colonies in sample-colonies in untreated group/colonies in untreated group group.

showed its effect on normal marrow stem cells proliferation. Cy-treated group was used as hematopoietic dysfunction model. Two days after OGP10–14 injection (control group mice were injected with PBS, positive control group were injected with either EPO, GM-CSF, or IL11), mice were injected with cyclophosphamide and phenylhy-drazioe i.p. everyday continuing for 3 days at the same time, 5 days after drug injection, hematopoietic stem cells were separated from mice bone marrow and the effect of OGP on proliferation and differentiation of hematopoietic stem cells was assessed in vitro (Fig. 2). The numbers of erythrocyte, granulocyte, and megakaryocyte cloning forming unit in optimal OGP10–14 therapy group are two- to fourfold higher than the Cy-treated only group ($P < 0.01$), and equal to the numbers of cytokine positive control group. The present result demonstrated that OGP10–14 treatment can stimulate the proliferation and differentiation of hematopoietic stem cells in compromised hematopoietic system as effective as their cytokine control.

Effects of OGP10–14 on Proliferation and Differentiation of Hematopoietic Stem Cells From Umbilical Cord Blood

Umbilical cord blood is another source of hematopoietic stem cells. Our results showed that OGP10–14 alone could induce noncommittal differentiation of progenitor hematopoietic stem cells from umbilical cord blood, and the numbers of CFU of erythrocyte, granulocyte, and megakaryocyte in optimally OGP-treated group was equal to the numbers comparing with positive control group (Fig. 3), suggesting that OGP works much better (as effective as cytokine control) on umbilical cord blood stem cells than bone marrow stem cells for unknown reason. Total number of CD34+ cells increased 13.4-fold in the presence of middle dose OGP10–14 alone for 5 days, the greatest increase in cell number of 15.6-fold was obtained with the

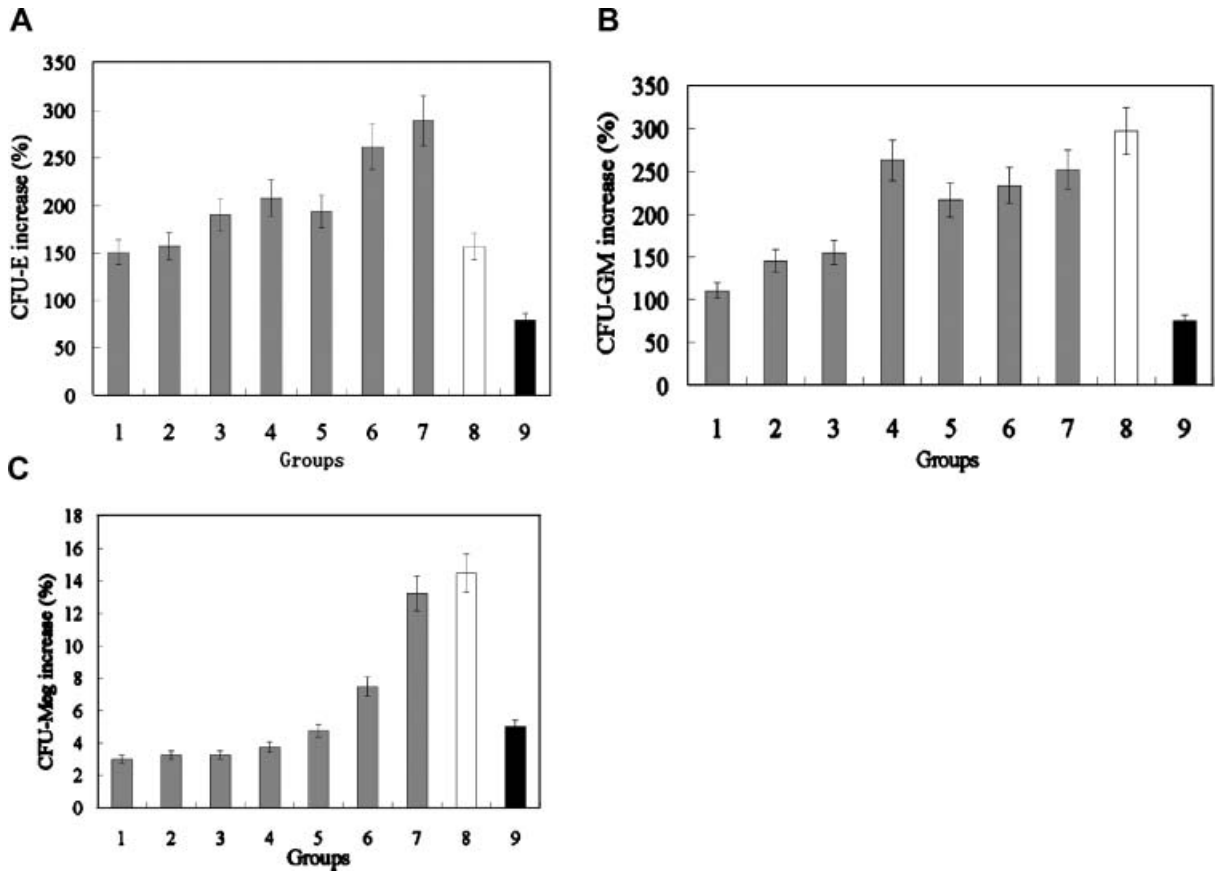

Fig. 2. Colony forming unit (CFU) test on bone marrow-derived cells from Cy-treated mice. OGP was injected everyday continuing for 5 days, cy was injected 2 days after OGP onset, hematopoietic stem cells were separated from mice bone marrow 5 days after and groups are: Group 1–7. OGP5 treatment group: 10–11 to 10–5 M (OGP + Cy) 8 Positive control group **A**: EPO **B**: GM-CSF **C**: IL11(cytokines + Cy) 9 untreated group (no drug addition, no cy treatment).

combinations of IL3, IL11, G-CSF, and EPO in vitro, while IL3 and G-CSF alone also stimulate an increase CD34+ cell number 5.5-fold and 8.6-fold (Fig. 4).

Effects of OGP10–14 on Numbers of Peripheral Blood Cells

It was showed that OGP10–14 treatment could induce a balanced multi-lineage enhancement of progenitor stem cells, we would like to see its effect on numbers of peripheral blood cell, 2 days after OGP10–14 injection, mice were injected cyclophosphamide and phenylhy-drazioe i.p. everyday continuing for 3 days at the same time, the animals were killed 18 days after the onset of the OGP10–14 treatment, blood was taken from tail and sent to the general hospital for assay, control group injected cyclophosphamide and phenylhy-drazioe for 3 days without OGP10–14 injection. Results showed that numbers of peripheral blood cells in cy-treated group has incline to revert back to normal levels itself even without any drug treatment, but OGP10–14-drug addition group could shorten the recovery period, increase the number of blood cells greatly comparing with the numbers in cy-treated control group (Fig. 5) to the extend that equal or even higher than the numbers in normal mice peripheral blood (data not shown), suggesting its potential role in cancer patients whose blood cells dropped sharply after chemotherapy or radiotherapy.

DISCUSSION

We could see that the differentiation effect of OGP10–14 on hematopoietic stem cells from human umbilical cord blood was better than the differentiation effect of OGP10–14 on hematopoietic stem cells from mice marrow, this result indicated that OGP10–14 treatment would be especially useful in human clinical treatment

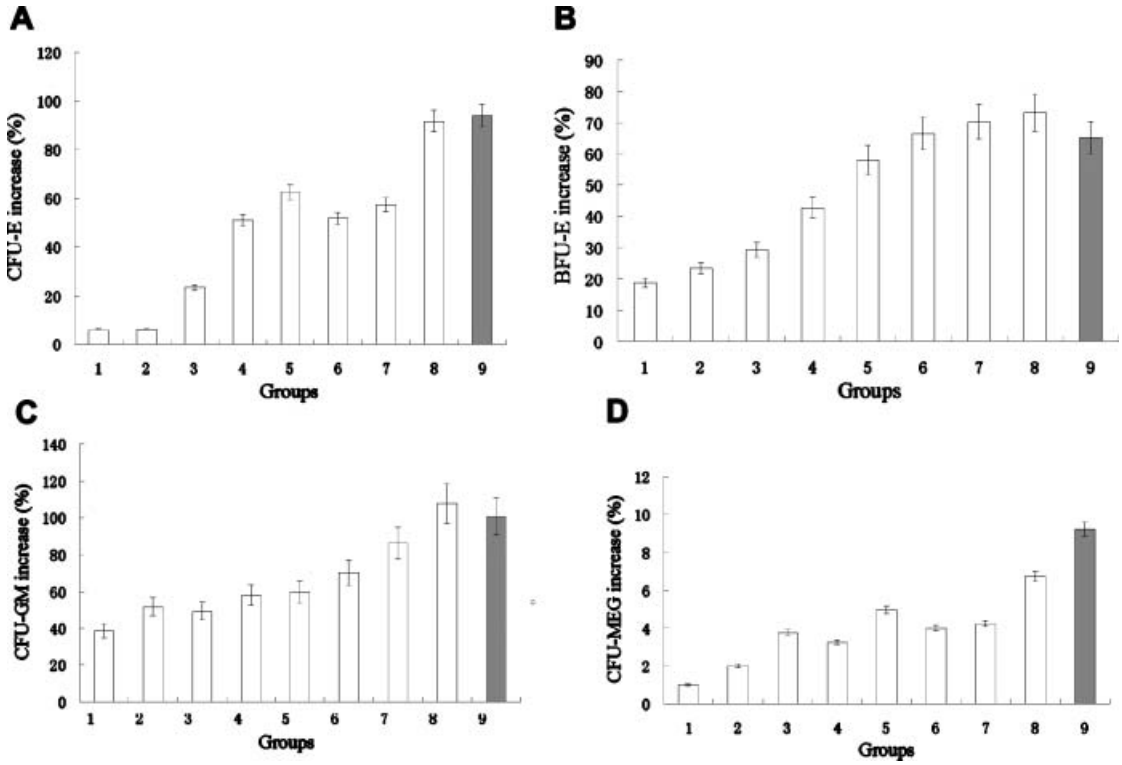

Fig. 3. Colony forming unit (CFU) test on HSC from umbilical cord blood. Umbilical cord blood derived MNC was separated into different groups below: 1–8. OGP treatment group, concentration from 10–12 to 10–5 mol/L 9. Positive control group **A**, **B**: EPO **C**: GM-CSF **D**: IL11 CFU increase = colonies in sample-colonies in untreated group/colonies in untreated group.

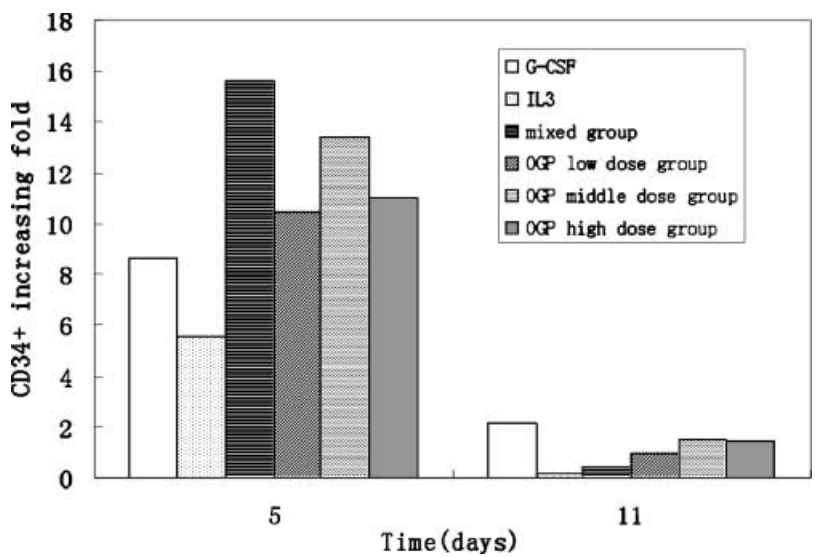

Fig. 4. Cord blood derived CD34+ cells proliferation after OGP treatment. CD34+ cells were separated and add different amount of OGP: Mixed group: IL3 + IL11 + G-CSF + EPO. OGP low dose group: 10–11 mol/L. OGP middle dose group: 10–8 mol/L. OGP high dose group: 10–5 mol/L. CD34 increasing fold = numbers in sample – numbers in untreated group/numbers in untreated group.

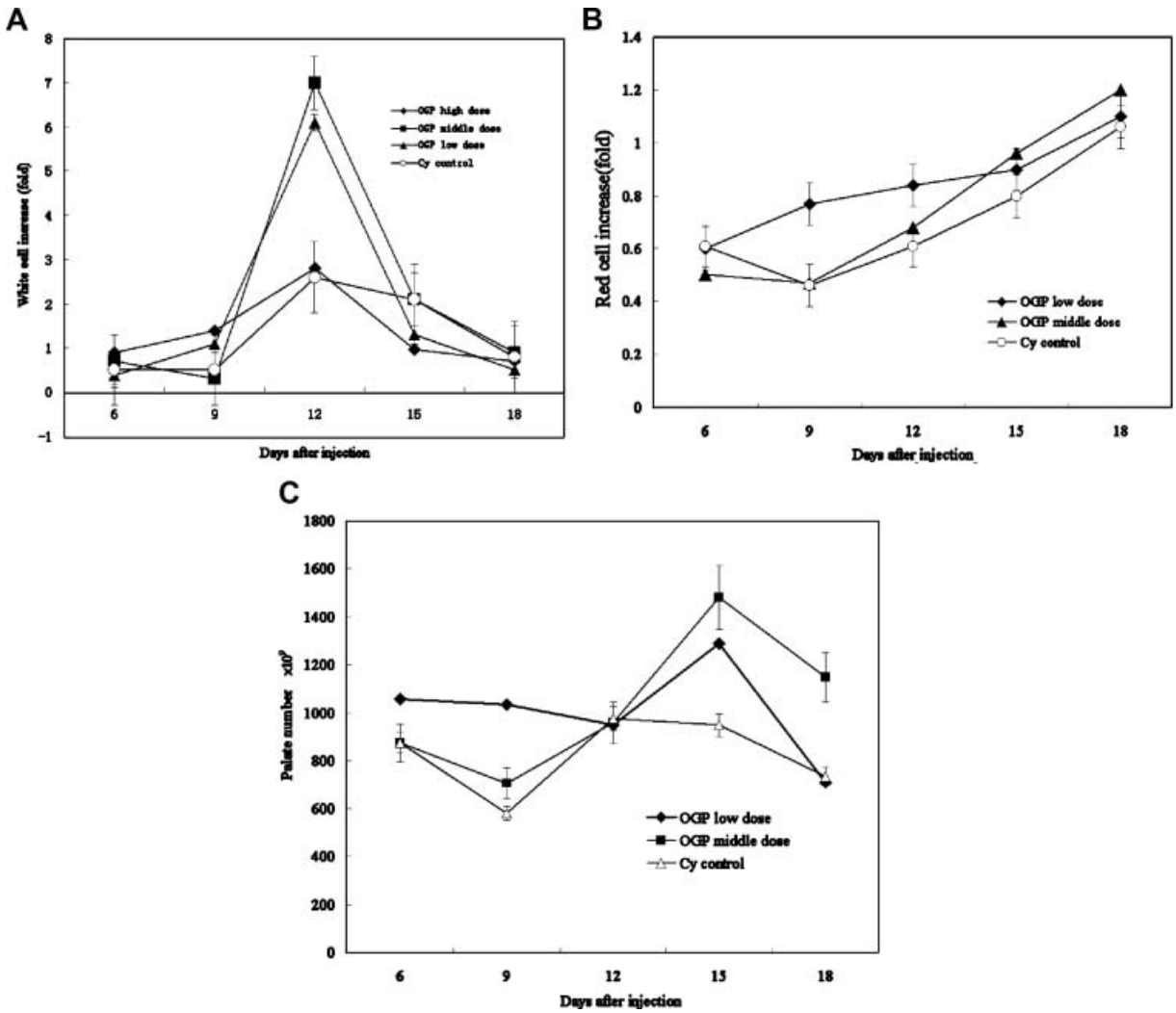

Fig. 5. The effect of OGP5 on peripheral blood cell numbers in Cy treated mice OGP was injected everyday continuing for 5 days, cy was injected 2 days after OGP onset: OGP low dose:10−10 g/mouse/day, OGP middle dose:10−8 g/mouse/day, OGP high dose:10−6 g/mouse/day. **A**: White cell increase in OGP-Cy and Cy control group comparing to normal mice. **B**: Red cell increase in OGP-Cy and Cy control group comparing to normal mice. **C**: Palates number.

for stimulating post BMT marrow reconstruction after radiochemotherapy [Bab and Chorev, 2002]. The hematopoietic microenvironment reconstruction can reduce morbidity and mortality in patients subjected to radiotherapy and chemotherapy by reducing infection incidence and other medical hurdles, shortening pancytopenic period, hemopoietic system dysfunction period, and hospitalization period. Hematopoietic stem cells from four different sources have been or are being used for the reconstitution of lymphohematopoietic function after myeloablative, near-myeloablative, or nonmyeloablative treatment. The various stem cell sources such as bone marrow, fetal liver, peripheral blood, and cord blood differ in their reconstitution and immuogenic characteristics, which are based on the proportion of early pluripotent and self renewing stem cells to lineage committed late progenitor cells, anyhow, hematopoietic stem cells expansion in vitro has now become an indispensable procedure before hematopoietic stem cell transplantation. There are several ways to expand hematopoietic stem cells in vitro, for example, the combined use of hematopoietic growth factors (HGFs) clinically which could induce higher stem cell proliferation than HGFs alone. Our results showed that OGP alone could induce a relatively higher CD34+ stem cells

proliferation comparing with IL3 or G-CSF alone, and nearly equivalent to the combination HGFs use, suggesting that OGP alone was as effective as combination use of several HGFs which is commonly used clinically. There is a serious shortcoming for the combination of HGFs, at the time they stimulate the proliferation of stem cells, they also give a differentiation pressure to the stem cells, we could see from Figure 4 that on Day 11 after injection, all the CD34+ stem cells disappeared and have differentiated, but we could still see CD34+ cells on OGP10–14 group alone, this characteristic of OGP10–14 may indicate an important potential role in myeloablative therapy, autologous or allogeneic BMT or umbilical cord blood transplantation. From all the experiments above, we concluded that OGP10–14 regulate hematopoiesis proliferation and differentiation in the early stage of the whole HGFs regulating network, just like the position of interleukin 13 in the hematopoiesis network, this could also be explained by the evolution theory of HGFs, from the theory we know that early HGFs in the hematopoiesis network has low evolution rate, while OGP is a high conservative protein from mice to human.

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