Effect of lung extract growth factors on the differentiation of umbilical cord stem cells into blood cells \textit{in vitro} in adult mouse

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Abstract

There are many diseases related to disorganized blood cells, especially erythrocytes and granulocytes. Therefore, it is important to find some methods for producing them. In this research work, we used extract of mouse lung to investigate the effects of its hematopoietic growth factors on stem cell differentiation \textit{in vitro}. Stem cells were isolated from umbilical cord by enzyme digestion and cultured in an appropriate culture medium. In this study, four test groups were considered including experimental groups 1 & 2 (E1 & E2), which were exposed to 50\% and 70\% concentration of lung extract for 7 days, respectively, the sham (Sh) group, which was not exposed to lung extract, and the control (C) group, which were the same volume of mouse blood. E and Sh groups were incubated for 7 days. Mentioned groups were marked with alkaline phosphatase detection kit, their cells were counted and hematopoietic factors were measured. Morphological, cytological and differential examinations showed significant changes in E2 group as compared to the Sh group. E2 cells were differentiated into erythroid and myeloid lineages. These findings suggest that growth factors in lung extract (especially in E2) have progressive effects on umbilical cord stem cell differentiation into blood cells.

Keywords: umbilical cord, stem cell, lung extract, differentiation, erythroid, myeloid lineages

1 Introduction

Stem cells have a great therapeutic potential due to their capacity of self-renewal and multilineage differentiation [1-5]. Two broad types of mammalian stem cells include embryonic stem cells that are found in blastocysts, and the adult stem cells that exist in adult tissues. In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In adult organisms, stem cells act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin or intestinal tissues [6].

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Highly plastic stem cells are umbilical cord cells [7]. The umbilical cord contains two arteries and one vein, which are surrounded by Wharton’s jelly. The cord is covered by an epithelium which is derived from the developing amnion. Recent data show that sections of umbilical cord contain stem cells such as hematopoietic stem cells (HSCs) [8, 45]. HSCs have the potential for self renewal and differentiation into all lineages of blood cells [9].

There are many diseases related to disorganized blood cells [10], so it is important to find a way for producing them. Currently, bone marrow (BM) is the major source of stem cell for cell therapy. However; aspiration of BM involves invasive procedures, and differentiation potential of BM stem cells decrease significantly with age [11]. Therefore, the search for alternative source of stem cell has an important value. Cord blood and umbilical cord cells may be an ideal source due to their accessibility, painless procedures to donors, promising source for autologous cell therapy and lower risk of viral contamination [12, 13]. Thus, we focused on umbilical cord as an alternative source of stem cells especially HSCs. Research has shown that lung extract has several factors that are important in hematopoiesis. Among these factors are granulocyte-macrophage colony-stimulating factor (GM-CSF) [14], interleukin-8, interleukin-1a and monocyte chemoattractant protein-1 [15]. These factors in lung extract probably can stimulate HSCs to differentiate into blood cells. Thus, in this research we used the extract of mouse lung to induce production of blood cells from umbilical cord stem cells.

2 Materials and methods

2.1. Mouse strains
In this work, 50 NMRI mice weighting 2.5×10^2 ± 2×10^3 kg were used. Monogamous copulation was set up, and day one of vaginal plug observation was determined as zero day of gestation. All mice were allowed adlibitum access to food and water. The mice were housed in animal room kept at a 24 ± 2 ºC with a 12-h light/dark cycle.

2.2. Umbilical cord preparation
Pregnant mice were sacrificed by cervical dislocation on day 15 of development. After removing fetuses from the uterus and amniotic membrane, umbilical cords were carefully amputated, rinsed with HBSS (Hanks’ balanced salt solution), minced into 1-2 mm^3 segments using sterile scalpel, and prepared for incubation upon tissue disaggregation in collagenase technique [16].

2.3. Incubation condition
Cord cells were incubated in culture medium of Dulbecco’s modification of Eagle’s medium (DMEM, Sigma-Aldrich, St Louis, USA), supplemented with 15% fetal calf serum (FCS, Sigma Aldrich, St. Louis, USA). To incubate cord cells, they were transferred into culture plates. The incubation period was 7 days at 37 ºC in a humidified atmosphere consisting of 5% CO_2 and 95% air. During the experiment the various environmental parameters of the culture were kept constant.

2.4. Test groups
In this research, four test groups were considered including experimental 1 & 2 (E1 and E2) which were exposed to 50% and 70% concentration of lung extract (prepared according to Tetsuro and colleagues' method in 1982) [17] for 7 days, respectively, sham (Sh) group, which was not exposed to lung extract, and the control (C), which was the same volume of mouse blood. E and Sh groups were incubated for 7 days.
2.5. Recognition of stem cells
Stem cells of sham, E1 and E2 groups were marked with alkaline phosphatase detection kit (CHEMICON, International Inc., USA).

2.6. Morphological and differential methods
In day 7 of incubation, the amount of hematocrit and differentiated blood cells including red blood cells (RBCs), white blood cells (WBCs), segmented (seg.) neutrophils, band neutrophils, eosinophils, basophils, lymphocytes and monocytes in all groups were determined with automatical method using Sysmex apparatus. The amount of hemoglobin was determined with a hemoglobin kit, and the mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated.

2.7. Statistical analysis
The mean values were recorded for E1, E2, Sh and C groups. All data were analyzed with SPSS software by calculating means and standard errors of means (mean ± SEM). The significance of differences among 4 groups were compared using one-way analysis of variance (ANOVA). A significant difference of P≤0.05 was considered as statistically significant in all experiments.

3 Results

3.1. Determination of stem cells
Cultured cells marked with alkaline phosphatase detection kit represented the presence of stem cells, which appeared in red colour (Fig. 1) in all samples of sham, E1 and E2 groups.

3.2. Morphological examination
Comparision of differentiated blood cells in sham, E1 and E2 showed no morphological changes as compared with the control group (figure5).

3.3. Cell differentiation and statistical analysis
Statistical analysis of the amount of hematocrit, hemoglobin, MCV, MCH, MCHC, RBC, WBC, seg. neutrophils, band neutrophils, eosinophils, basophils, lymphocytes and monocytes in control, sham, E1 and E2 have been shown in figures 2, 3 and 4.

Percentage of hematocrit showed a significant decrease in both sham (P<0.001) and E1 (P<0.01) groups compared with control, however, no significant change between E2 and the control groups were detected (Fig. 2).

Sham and E1 groups showed a significant decrease (P<0.001) in the amounts of hemoglobin, RBC and WBC, compared to the control, while these factors in E2 groups were in a normal range (Figs. 2 and 4). MCV, MCH and MCHC in E2 group did not change significantly, compared to control groups, while a significant increase of MCV (P<0.05) in E2, a significant increase of MCHC (P<0.05) in sham, and a significant decrease of MCHC (P<0.001) in E1 were revealed, compared to the control groups (Fig. 3).

Statistical analysis revealed a significant decrease in seg. neutrophil counts in sham (P<0.01) and E1 (P<0.001), while no change was detected in E2, compared to the control samples. Band neutrophil counts in both E1 and E2 samples had a significant increase (P<0.01) in comparision with the control samples (Fig. 4). Eosinophil, basophil, lymphocyte and monocyte counts, in spite of increasing in E1 and E2 groups, showed no significant statistical changes in comparision with control groups (Fig. 4).
Figure 1: Photomicrographs of umbilical cord cells in vitro on day one of incubation marked with alkaline phosphatase detection kit (magnification ×720). (a) Sham sample; (b) experimental sample exposed to 50% lung extract (E1); (c) experimental sample exposed to 70% lung extract (E2) (stem cells appear in red).

Figure 2: Histograms of hematocrit (a), hemoglobin (b) and RBC (c) changes in control, sham, experimental 50% (E1) and experimental 70% (E2) samples (mean ± SEM) (*P<0.05, **P<0.01, ***P<0.001).
Figure 3: Histograms of MCH (a), MCHC (b) and MCV (c) changes in control, sham, experimental 50% samples (E1) and experimental 70% (E2) samples (mean ± SEM) (*P<0.05, **P<0.01, ***P<0.001).
Figure 4: Histograms of WBC (a), seg. neutrophils (b), band neutrophils (c), eosinophils (d), basophils (e), lymphocytes (f) and monocytes (g) changes in control, sham, experimental 50% (E1) and experimental 70% (E2) samples (mean ± SEM) (*P<0.05, **P<0.01, ***P<0.001).
Figure 5: photomicrographs of blood cells Gimsa staining in control (a), sham (b), experimental 1 (c) and experimental 2 (d) groups. (Sn: segmented neutrophil, l: lymphocyte, m: monocyte)

4 Discussion

Knowledge about stem cell science and its potential applications began in the aftermath of the bombings in Hiroshima and Nagasaki in 1945 [18]. Twenty years ago, the first embryonic stem cells were isolated from mouse preimplantation blastocyst-stage embryos [19, 20].

In 1983, Ogawa and his colleagues described the physiology of HSCs in Blood [21]. Other scientists showed that whole body irradiated mice could be rescued from fatal hematopoietic failure by injection of suspensions of cells from blood forming organs such as the bone marrow [22].

In mid 20th century, three laboratories demonstrated that the injected bone marrow cells can directly regenerate the blood forming system [23-25].

To date, the only known treatment for hematopoietic failure is transplantation of bone marrow cells or HSCs [26]. But blood from the placenta and umbilical cord is a rich source of HSCs, too [27]. In the late 1980s, umbilical cord blood was recognized as an important clinical source of HSCs [28, 29].

Recent data have shown that sections of umbilical cord, such as Wharton’s jelly [30] and perivascular tissue [31] contain stem cells such as HSCs, thus, a great amount of stem cells could be isolated from the whole umbilical cord without tissue dissection. Consequently, in this research we used whole umbilical cord to achieve more stem cells.

In the present study, we used mouse lung extract which contains hematopoietic factors. Robert and colleagues in 2001 showed that lung extract contains factors including colony stimulating factor (CSF) such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukins such as interleukin-8 and interleukin-1α, and monocyte chemoattractant protein-1 [15].

There is now a large amount of evidence indicating that GM-CSF plays a particular role in the development of lung [32-34]. There is also evidence that GM-CSF is important in regulating pulmonary surfactant homeostasis [35-38]. The GM-CSF-receptor is a member of the hematopoietin receptor
superfamily and is comprised of an alpha chain (GM-CSFR alpha) specific for GM-CSF, and a beta chain shared with interleukin-3 and interleukin-5 receptors [39, 40]. GM-CSF, which was originally identified because of its effects on hematopoietic progenitors, has proved to exert varying effects on differentiation of blood cells [41]. Thus, we used lung extract to induce hematopoiesis.

In 2001, Kaufman et al., co-cultured the embryonic HSCs with bone marrow cells. They could differentiate HSCs into erythrocytes and produce hemoglobin [42]. This research confirms our findings that amount of hematocrit, hemoglobin and erythroid lineages in E2 groups were in normal range as compared with control groups.

In 2003, Chadwick et al., showed that BMP4 and the cytokines such as SCF (stem cell factor), FLT3 (Fims-like tyrosine kinase3) ligand, IL3 (interleukin-3), IL6 (interleukin-6) and GCSF (granulocyte colony stimulating factor) have progressive effects on differentiation of erythroid and myeloid lineage [43]. These findings also agree with our results that hematopoietic factors in 70% concentration of lung extract can induce differentiation of erythroid and myeloid (especially granulocyte) lineage in E2 groups.

We also observed a significant increase in differentiation of WBC (especially neutrophils) in E2 samples. This may be due to the presence of GM-CSF in the lung extract. Others, such as Takahashi et al., [44] and Yamamoto et al., [46] have confirmed that GM-CSF can cause differentiation of HSCs into granulocyte. Furthermore, as shown in the present work, a significant increase in band neutrophils, which can differentiate into seg. neutrophils, is in agreement with these findings.

As our findings showed, hematopoiesis occurs at a low level in samples with no lung extract (sham) or with a low lung extract (E1), while a 70% concentration of lung extract (E2) hematopoietic signs, such as the amounts of hematocrit, hemoglobin, MCV, MCH, MCHC, RBC, WBC, seg. neutrophils, band neutrophils, eosinophils, basophils, lymphocytes and monocytes are similar to that of control groups (mouse blood). These results confirm that hematopoiesis in E2 groups is similar to normal blood formation.

In conclusion, we suggest that lung extract has hematopoietic potency on umbilical cord cells, which may be a useful method in clinics and medicine as a cheap and applicable source of blood cells.

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