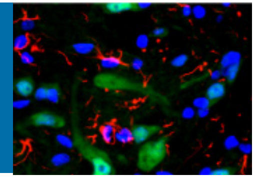
IDRAGON
Management System

Imedicine
Journal homepage: www.imedicine.org.cn



Original article

Culture method of rat hematopoietic stem cell (HSC)

Gui-qin Huang*

Department of anatomy, kunming medical university, Kunming 650500

Abstract: Objective: To isolate and culture rat hematopoietic stem cells in vitro. **Methods:** Bone marrows of 6-8 week-old SD rats were harvested. Cell suspension was isolated and hematopoietic stem cells were extracted for primary culture. The growth of primary cells was observed under inverted phase contrast microscope. **Results:** After cell inoculation, cells began to adhere to the wall at 24 hours, and the cells grew adherently at 3 days, with small and round volume and smooth cell boundary. The number and volume of suspended cells increased and the cell boundary became rough at 4 ~ 8 days. Most cells grew in suspension at 9 ~ 12 days, and the number and volume of cells increased further. The number of round cells in bone marrow suspension cells cultured for 2-3 weeks was over 90%, but the number of cells was reduced. **Conclusion:** The successful primary culture of hematopoietic stem cells provides a guarantee for the subsequent autologous transplantation of hematopoietic stem cells.

Key words: HSC; Rat; Cell culture

Introduction

Stem cells are cells with both self-renewal ability and multi-differentiation potential, which can produce offspring stem cells with identical phenotype and genotype as well as themselves and can differentiate into various functional cells at the same time. This phenomenon is called horizontal differentiation of stem cells (Qian Y, et al 2018). According to their developmental stages, stem cells differentiate into embryonic stem cells and adult stem cells. According to the traditional concept, embryonic stem cells are totipotent and can differentiate into almost all tissues and organs of the body. The stem cells in adult tissues or organs, i.e., adult stem cells, are generally considered to be tissue-specific and can only differentiate into specific cells or tissues (Frascoli, M, et al 2018). However, the latest research indicates that adult stem cells also have the potential to transversely differentiate into other types of cells or tissues in specific environments (Yao. J., et al 2008).

Hematopoietic stem cell (HSC) has the ability to self-renew and differentiate into various mature cells in the blood and immune system, making it an ideal target cell for gene therapy. At the same time, hematopoietic stem cells are heterogeneous cell populations, lacking direct morphological identification features and with low contents in bone marrow, umbilical cord and peripheral blood. In recent years, surface-specific antigens of various HSCs and other types of blood cells have been isolated, making the identification and isolation of HSCs and other specific types of cells possible.

Materials and methods

Materials

Laboratory Animals

A number of SD rats with 6–8 weeks of age were provided by the Experimental Animal Center of Kunming Medical University.

Main reagents and instruments

1640 culture medium (Gibco), fetal bovine serum (Gibco), PBS solution, inverted microscope (leica), 5% CO incubator (37°C) (Sanyo), laminar flow clean bench (Suzhou Sujing Instruments Co., Ltd.), percoll cell separation (sigma subpackaging), etc.

Article history: Received: 28 January 2021 Revised: 15 February 2021 Accepted: 12 Mar 2021 Published: 28 Mar 2021

*Corresponding author: Gui-qin Huang
E-mail: huangguiqin@163.com

Experimental method

Preparation of percoll cell isolate

The stock solution of percoll cell separation solution was mixed with 9% NaCl (prepared with triple distilled water) in a ratio of 9: 1, diluted to 60% (density 1.077) with 0.9% normal saline, filtered, and placed in a 4 C refrigerator for later use.

Sampling

For SD rats aged 6–8 weeks, they were narcotized to death with 3.6% chloral hydrate, and the hind limbs were immersed in 75% alcohol for 3–5 min. The skin, muscles and soft tissues of the hind limbs were separated with large scissors, the hip joint was opened sharply, and the joint capsule was separated. The whole hind limb was removed from the femoral head (be careful not to cut the femoral head). The muscles above the femur and tibia were carefully removed with small scissors, and they were removed as clean as possible. Detach the ankle and knee joint as above, and pay attention to protect the diaphysis to keep the bone marrow cavity closed. The femur and tibia were immersed in 75% alcohol for 5-8min again. After the femur and tibia were rinsed in the ultra-clean bench with sterile PBS, the metaphysis at both ends was cut with sterile large scissors. The bone marrow in the bone marrow cavity was rinsed by sucking 1640 using a 1ml syringe until the effluent was clear. The cell suspension was collected into a sterile 50ml centrifuge tube, and allowed to stand for 5–8 min at room temperature to allow the residual muscle tissue and bone slag to settle. The supernatant was subpackaged into a sterile 15ml centrifuge tube for 1000r/min, and gradient centrifugation was performed with percoll cell separation solution (2000r/min, 30min).

Primary culture of hsc

First, the prepared percoll cell separation solution was added into a 15ml centrifuge tube, and the cell suspension was slowly injected along the tube wall, so that the percoll cell separation solution was in the lower layer and the cell suspension was in the upper layer, and the cells could pass through the separation solution to be layered during centrifugation (the volume ratio of the cell suspension to the percoll cell separation solution was 1:1); after centrifugation, the cells were layered in three layers to form an upper layer 1640 culture medium, with the separation solution layer in the middle and the red blood cell layer at the bottom, and the upper two layers. After washed with PBS twice, cell suspension was prepared with culture solution 1640 containing 15% fetal bovine serum. Two SD rats were added with 15ml of complete medium and inoculated into culture flasks (Corning). The cells were cultured in an incubator at 37 C and 5% CO2, and the growth of the cells was observed. The medium was changed two to three times a week. After 2-3 weeks, only the isolated suspended cells were present in the flask. The growth of the primary and passage cells was observed under an inverted phase contrast microscope day by day.

Experimental results

After the single cell suspension was made, it was inoculated in a culture flask. After 24 hours, the cells adhered to the wall, then grew well, and the cells were scattered and floating. In 3d, the cells grew adherent, small in size, round and smooth in cell boundary (Figure 1). After 4 ~ 8 days, the number and volume of suspended cells increased, and the cell boundary became rough. After 9 ~ 12 days, most of the cells grew in suspension, and the number and volume of cells further increased. At the 2nd-3rd week of culture, the number of round cells in bone marrow suspension cells can reach more than 90%, but the number of cells is small (Figure 2).

Discussion

The low content of hematopoietic stem cells in bone marrow is one of the difficulties in separation and purification. Hematopoietic stem cells account for about 0.5% of nucleated

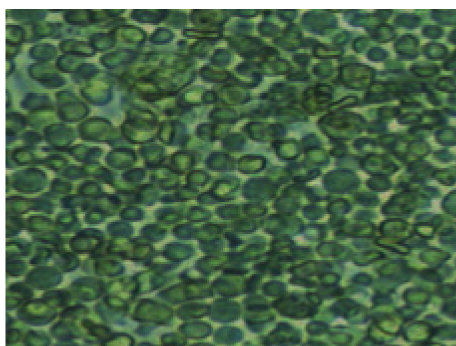


Figure 1. HSC - 3 d

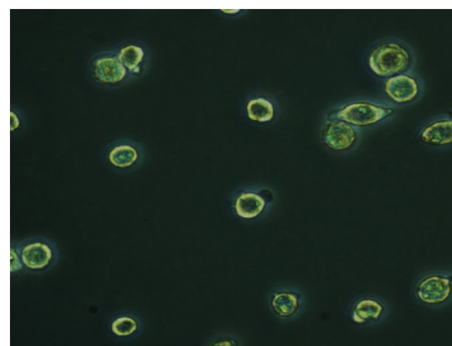


Figure 2. HSC-2W

cells in bone marrow (Zhang X., et al 2018), while the content in peripheral blood is even lower. The results show that the immunomagnetic beads separation method is simple and easy, and has little influence on the cell viability, by specifically binding monoclonal antibodies with antigen markers specially guided on the surface of hematopoietic stem cells, and then separating by magnetic beads on the antibodies in the magnetic field (Ariunjargal, G., et al 2018). The obtained stem cells have high purity and are widely used in gene therapy. To further prove the originality and pluripotency of the isolated hematopoietic stem cells depends on the long-term detection of hematopoietic reconstitution ability in vivo, which requires the NOD/SCID mouse transplantation experiment (Lois and C.J, et al 2002).

Hematopoietic cell is a cell type that is easy to differentiate in vitro. Therefore, it is still impossible to simulate the whole process of hematopoiesis in vivo in vitro. In vitro culture system, suspension culture of tissue mass, single-layer or double-layer liquid culture, co-culture of hematopoietic cells and thymocytes and diffusion box culture have been used (Wu. J, et al 1982). The time for hematopoietic cells to maintain proliferation in vitro is relatively short, while T-lymphocytes disappear quickly during the process of culture (Dong, Q, et al 1999). Therefore, the significance of hematopoietic cell culture in vitro is not only to study in vitro hematopoiesis, that is, to maintain the conditions of sustained proliferation and differentiation of hematopoietic cells in vitro culture, but also to separate and remove immunocompetent cells through in vitro culture (Dong, Q, et al 1999).

Hematopoietic stem cells are suspension growth cells, and the whole bone marrow cell suspension collected during culture must be subjected to gradient centrifugation with percoll cell separation solution to remove most of red blood cells. There is no horizontal centrifuge in the laboratory, so percoll cell separation liquid can't see the white membrane layer after gradient centrifugation, so it is necessary to collect all liquid except red blood cells and centrifuge again to collect HSC. Because percoll cell separation solution has a certain toxic effect on cells, it is best to rinse with PBS before inoculation. Change the fluid 2-3 times a week, and ensure the cell density for each passage. Sparse cells are not conducive to the proliferation of HSC. The proliferation ability of HSC in serum-only medium is not strong, so we can consider adding some growth factors, such as 50ng/ml stem cell factor and 20ng/ml IL-3.

Acknowledgements

We appreciate fruitful revision from professor Xiao Zhang

Conflict Interests

No conflict of interest

Reference

Ariunjargal, G., Isolation and biological characterization of hematopoietic stem cells from mice at different stages of hematopoietic development at multiple anatomical sites. Inner Mongolia University.

Chinese Journal of Experimental Hematology, Tian. J., Phenotype and purification of mouse hematopoietic stem cells. 2012. 20(1): p. 196-199.

Dong, Q., et al., Reconstruction of hematopoietic function in mice after high-dose chemotherapy using bone marrow hematopoietic stem cells amplified by microvascular endothelial cells in vitro. 1999.

Frascoli, M., M. Proietti, and F. Grassi, Phenotypic analysis and isolation of murine hematopoietic stem cells and the promise of inheritance.

Infectious Disease Network Dynamics, Yao. J., Isolation and cloning of hematopoietic stem cells and liver stem cells. 2005. 000(008): p. 34-35.

Journal of Experimental Hematology, Shu. J., immunophenotype and functional analysis of mouse hematopoietic stem cells. 1997(4): p. 346-349.

Journal of Mudanjiang Medical College, Wu. J., Properties of hematopoietic stem cells and their application in the treatment of hematopoietic disorders. 1982(2).

Kurre, P. and H.P.J.C.O.i.M.T. Kiem, Progress towards hematopoietic stem cell gene therapy. 2000. 2(4): p. 400-411.

Lois and C.J. Science, Germline Transmission and Tissue-Specific Expression of Transgenes Delivered by Lentiviral Vectors. 2002. 295(5556): p. 868.

Qian Y, Et al., Effects of microvascular endothelial cells on hematopoietic stem cell proliferation in mice. 2018.

Xiaocui Zhang, et al., Isolation and purification of hematopoietic stem cells from mouse bone marrow and electron microscopic observation. 2018. V.40(04): p. 112-116.

大鼠造血干细胞（HSC）培养方法

黄桂琴 *

昆明医科大学解剖教研室，昆明 650500

[**摘要**]：**目的**：体外分离培养大鼠造血干细胞。**方法**：取 6-8 周 SD 大鼠骨髓，分离细胞悬液，提取造血干细胞进行原代培养。在倒置相差显微镜下观察原代细胞的生长情况。**结果**：细胞接种后，24h 开始有细胞贴壁，3d 细胞呈贴壁生长 , 体积小、圆形 , 细胞边界光滑，4 ～ 8 天悬浮细胞数量增多 , 体积增大 , 细胞边界渐趋粗糙，9 ～ 12 天大部分细胞呈悬浮生长 , 细胞数量进一步增多 , 体积进一步增大。培养至第 2-3 周的骨髓悬浮细胞中圆形细胞可达 90％以上，但是细胞数量较少。**结论**：造血干细胞原代培养成功为后续的造血干细胞自体移植实验提供了保证。

[**关键词**]：HSC；大鼠；细胞培养

[**通讯作者**]：黄桂琴

[**邮箱**]：huangguiqin@163.com