Kaohsiung Journal of Medical Sciences (2016) 32, 1–9

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.kjms-online.com

ORIGINAL ARTICLE

Gonadotropins facilitate potential differentiation of human bone marrow mesenchymal stem cells into Leydig cells *in vitro*

Lin Hou [a], Qiang Dong [b,*], Yun-Jian Wu [b], Yuan-Xing Sun [a], Yan-Yu Guo [c], Yue-Hong Huo [c]

[a] *Department of Urology, Number 5 Hospital of Datong, Datong, China*
[b] *Department of Urology, West China Hospital, Sichuan University, Chengdu, China*
[c] *Department of Rheumatology, Number 5 Hospital of Datong, Datong, China*

Received 30 June 2015; accepted 16 September 2015
Available online 30 November 2015

KEYWORDS
Gonadotropins;
Human bone marrow mesenchymal stem cells;
Leydig cells;
Testosterone

Abstract Infertility due to low testosterone levels has increased in recent years. This has impacted the social well-being of the patients. This study was undertaken to investigate the potential of gonadotropins in facilitating differentiation of human bone marrow mesenchymal stem cells (BMSCs) into Leydig cells *in vitro*. BMSCs were isolated, cultured, and their biological characteristics were observed. BMSCs were induced with gonadotropins *in vitro* and their ability to differentiate into Leydig cells was studied. The level of expression of 3-beta hydroxysteroid dehydrogenase (3β-HSD) and secretion of testosterone were determined using flow cytometry and enzyme-linked immunosorbent assay, respectively, and the results were compared between the experimental and control groups. The cultured BMSCs showed a typical morphology of the fibroblast-like colony. The growth curve of cells formed an S-shape. After inducing the cells for 8–13 days, the cells in the experimental group increased in size and showed typical characteristics of Leydig cells, and the growth occurred in spindle or stellate shapes. Cells from the experimental group highly expressed 3β-HSD, and there was a gradual increase in the number of Leydig cells. The control group did not express 3β-HSD. The level of testosterone in the experimental group was higher than the control group ($p < 0.05$). Additionally, the cells in the experimental group secreted higher levels of testosterone with increased culture time. The expression of Leydig cell-specific markers in the experimental group was significantly higher ($p < 0.05$). With these findings, BMSCs can be considered a new approach for the treatment of patients with low androgen levels.

Conflicts of interest: All authors declare no conflicts of interest.

* Corresponding author. Department of Urology, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang, Chengdu, Sichuan, 610041, China.
E-mail address: dqiang@gmail.com (Q. Dong).

http://dx.doi.org/10.1016/j.kjms.2015.10.008

Introduction

In recent years, with the rising industrial and social development, environmental pollution is also on the rise. A lot of stressful conditions that young men face in their life, along with indulging in bad habits, such as drinking, smoking, and staying up late, have led to an increase in the number of patients with infertility. Although the extent of infertility varies considerably among countries, infertility has been recognized as a public health issue worldwide by the World Health Organization (WHO), and has the potential to threaten the stability of individuals, relationships, and communities [1]. Infertility has been associated in patients with low testosterone levels, and has serious implications on their mental and social well-being. It has become the focus of research in the past decade, and is now considered an andrological disease.

Transplantation of Leydig cells has become a main treatment modality in patients with low testosterone levels [2,3]. When transplanted, Leydig cells are involved in regulating the secretion of androgens under the body's normal physiological laws [4]. However, this method is limited by the nonavailability of cell sources, immune rejection, and a relatively short duration of action [5,6], and therefore, is not conducive to clinical applications.

The concept of mesenchymal stem cells (MSCs) was proposed in 1968 by Friedenstein et al. [7], when they found that MSCs are a kind of adult stem cells, derived from autologous cells. MSCs display a high degree of proliferation, self-renewal capacity, and a potential for multidirectional differentiation of pluripotent stem cells [8,9], and the MSCs in bone marrow mesenchymal stem cells (BMSCs) and fetal cord blood show a greater level of these properties [10]. Also, there exists a difference in quality among the cell sources of placenta, amniotic fluid, umbilical vein endothelial layer, peripheral blood, liver, muscle, fat, skin, and other tissues [11—13]. MSCs derived from early mesoderm can assure neuroectodermal and endoderm differentiation in exceptional conditions, with a variety of features added to the cell differentiation potential [14]. Kale et al. [15] reported that in a mouse model with acute renal failure, transplanted MSCs could differentiate into renal tubular epithelial cells, thus restoring the structure and function of the kidneys. In their study, Wang et al. [16] observed that BMSCs transplanted into the seminiferous tubules within the seminiferous epithelium significantly restored the damaged rat spermatogenic cells. If the direct differentiation of BMSCs into Leydig cells can be induced, the issues of cell origin and immune rejection can be addressed simultaneously. There are several advantages of using BMSCs, which include: (1) their multi-directional differentiation potential; (2) their ease of acceptance and growth in the testis, thereby overcoming the shortcomings of the immune rejection in transplanted cells; (3) the important role they play in improving the testicular environment, and in promoting rapid recovery of androgen levels; and (4) the ease of collecting BMSCs from autologous, allogeneic Leydig cells, thus avoiding ethical issues of transplantation [17]. Mäkelä et al. [18] reported that various testicular cell growth microenvironments direct differentiation of BMSCs. Additionally, they described BMSCs as cells with strong plasticity [18]. Transplantation of BMSCs through the multi-use injection method may result in a large number of cells in different positions and therefore, may not efficiently lead to androgen production. Furthermore, the recipient animals need more stringent requirements, thereby still leaving a gap between research and clinical applications.

Leydig cells are distributed in the seminiferous tubules of loose connective tissues, and account for 2—4% of the total testicular cell number [19]. Leydig cells are the main source of testosterone in males [20]. At present, several studies report that BMSCs induce germ cell differentiation and *in vivo* transplantation. Kale et al [15] transplanted BMSCs into the testes of rats; the transplanted cells successfully survived and differentiated into steroid hormone secreting Leydig cells. Furthermore, MSCs were found to induce cAMP production *in vitro* that in turn showed the ability to differentiate into Leydig cells to produce testosterone. Lue et al. [3] injected the BMSCs into the developmental stages of rat testis. After 2 weeks of culturing *in vitro*, these cells exhibited the characteristics of Leydig cells and showed the ability to secrete testosterone. In a previous study [21], we injected mouse BMSCs labeled with Hoechest33342 into the testis. Interestingly, the cells did not show immune rejection after transplantation in the short term. In two separate studies, Ge et al. [22,23] observed that the mature Leydig cells are terminal cells, which do not have the ability to undergo mitosis. Therefore, it becomes necessary for the stem Leydig cells (SLC) and progenitor Leydig cells (PLC) to maintain the number of cells in the body. Such research confirms that MSCs have far-reaching applications in the male reproductive system.

In the present study, our aim was to isolate and culture BMSCs *in vitro*; to use hormone-induced methods to cause differentiation of Leydig cells; to study the testicular growth in a microsimulation environment in order to promote the differentiation of BMSCs into Leydig cells; to verify the pluripotent characteristics and plasticity of BMSCs; and to discuss *in vitro* differentiation in drug-induced conditions. An indepth research on these cells and their differentiation phenomena will lay a foundation for clinical applications in the future.

Methods

Source of bone marrow specimens

Volunteers were selected from among the medical inpatients from the West China Hospital, Sichuan University, Chengdu, China. Out of nine cases selected from January 2011 to October, 2011, five were males and four were females, with an average age of 37.2 $\pm$ 9.4 years. These patients were checked to rule out any disease history before the onset of the new hematopoietic system disorder or serious infectious disease. The bone marrow was transplanted through bone marrow puncture. At all times, the volunteers signed informed consent accepted by the Ethics Committee of West China Hospital of Sichuan University.

Isolation and culture of BMSCs

Density gradient centrifugation was used to obtain BMSCs. Anticoagulated bone marrow fluid was centrifuged (200*g* for