

·Review·

Recent advances in andrology-related stem cell research

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Abstract

Stem cells hold great promise for regenerative medicine because of their ability to self-renew and to differentiate into various cell types. Although embryonic stem cells (ESC) have greater differentiation potential than adult stem cells, the former is lagging in reaching clinical applications because of ethical concerns and governmental restrictions. Bone marrow stem cells (BMSC) are the best-studied adult stem cells (ASC) and have the potential to treat a wide variety of diseases, including erectile dysfunction (ED) and male infertility. More recently discovered adipose tissue-derived stem cells (ADSC) are virtually identical to bone marrow stem cells in differentiation and therapeutic potential, but are easier and safer to obtain, can be harvested in larger quantities, and have the associated benefit of reducing obesity. Therefore, ADSC appear to be a better choice for future clinical applications. We have previously shown that ESC could restore the erectile function of neurogenic ED in rats, and we now have evidence that ADSC could do so as well. We are also investigating whether ADSC can differentiate into Leydig, Sertoli and male germ cells. The eventual goal is to use ADSC to treat male infertility and testosterone deficiency. (*Asian J Androl* 2008 Mar; 10: 171–175)

Keywords: stem cells; bone marrow stem cells; adipose tissue-derived stem cells; erectile dysfunction; male infertility

1 Introduction

Stem cells are endowed with the capacity to self-renew and to differentiate into various cell types, depending on the stimuli (signals) that they received. For

ease of discussion, stem cells shall be classified into embryonic stem cells (ESC) and adult stem cells (ASC). Whereas ESC are derived from the inner cell mass of a blastocyst, ASC usually originate from various tissues of a developed individual (adult). Because ASC can also be isolated from a developing individual (fetus, infant or child), they are alternatively called somatic stem cells.

The differentiation potential of stem cells is hierarchized into totipotent, pluripotent and multipotent. A fertilized egg is totipotent and can differentiate into any cell type. An ESC is pluripotent and can differentiate into any cell type, except a fertilized egg. An ASC is multipotent

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and can differentiate into most cell types of its tissue origin. However, numerous studies have shown that ASC can differentiate into cell types beyond their tissue origin (e.g. bone marrow stem cells [BMSC] differentiating into cardiomyocytes); therefore, ASC appear to possess a certain degree of pluripotency [1–3]. In any case, although ESC is undoubtedly superior to ASC in differentiation potential, its research has been hampered by ethical concerns and governmental restrictions. ASC research, in contrast, is moving at a faster pace and has reached clinical trials ahead of ESC research.

Various types of ASC have been discovered in various tissues, the largest class being the mesenchymal stem cells (MSC), which reside in virtually all post-natal organs and tissues [4]. Among various types of MSC, BMSC and hematopoietic stem cells (HSC) were discovered the earliest and have been investigated most thoroughly. In a normal individual, HSC eventually differentiate into various hematopoietic cells, such as RBC, WBC and platelets. BMSC, in contrast, are not as clearly understood in terms of what cell types they are destined to become. Available evidence points to their possible role as replacement cells for the routine maintenance of normal tissues, such as in the kidney [5], and for the repair of damaged tissues, such as in an infarcted heart [6].

Hundreds of reports have collectively shown that BMSC can differentiate into various cell types including adipocytes, endothelial cells, epithelial cells, glial cells, hepatocytes, neurons, cardiac muscle cells, skeletal muscle cells and smooth muscle cells [1–3]. Furthermore, many of these reports have used animal models to demonstrate the feasibility of using BMSC for treatment of degenerative and inflammatory diseases.

2 Using stem cells to treat andrological diseases

Compared with other fields, andrology has been relatively late to embrace stem cells as potential therapeutic agents, with researches being concentrated in two areas: erectile dysfunction (ED) and male infertility. In 2003, Deng *et al.* [7] showed that BMSC transduced with eNOS were able to improve the erectile function of aged rats. In 2004, our research team at the University of California San Francisco (UCSF) showed that ESC transfected with brain-derived neurotrophic factor (BDNF) could restore the erectile function of rats whose cavernous nerves were experimentally damaged [8]. In 2007, three other ED-related papers were published.

Bivalacqua *et al.* [9] showed that BMSC alone or transduced with eNOS were able to reverse age-associated ED. Song *et al.* [10] showed that magnetic resonance could be used to non-invasively evaluate human BMSC in corpus cavernosa of rats and rabbits. Finally, Song *et al.* [11] showed that immortalized human BMSC (by v-myc transfection) transplanted into rat corpus cavernosum could differentiate into endothelial and smooth muscle cells.

In regard to male infertility, a 2003 paper by Toyooka *et al.* [12] showed that ESC could form male germ cells *in vitro*. Specifically, they showed that the differentiation of ESC into male germ cells depended on embryoid body formation and was greatly enhanced by the inductive effects of bone morphogenetic protein 4-producing cells. They further showed that the induced germ cells could participate in spermatogenesis when transplanted into reconstituted testicular tubules, demonstrating that ESC can produce functional germ cells *in vitro*. In 2004, another paper by Geijsen *et al.* [13] showed that ESC-derived embryoid bodies supported maturation of primordial germ cells into haploid male gametes, which, when injected into oocytes, restored the somatic diploid chromosome complement and developed into blastocysts. Also in 2004, Nayernia *et al.* [14] reported the *in vitro* generation of a germ cell line (SSC1) from the pluripotent teratocarcinoma cells and showed that the SSC1 cell line formed mature seminiferous tubule structures and supported spermatogenesis after transplantation into recipient testes. In 2006, West *et al.* [15] published a detailed protocol for the *in vitro* generation of germ cells from murine ESC. Finally, and most significantly, Nayernia *et al.* [16] demonstrated for the first time that ESC-derived germ cells were able to generate offspring mice.

Another landmark was reached in 2006 when Nayernia *et al.* [17] for the first time showed that murine BMSC could differentiate into male germ cells. The human version of this line of research was published the following year by Drusenheimer *et al.* [18]. Finally, Lue *et al.* [19] showed that BMSC transplanted into the testis of a busulfan-treated infertility mouse model appeared to differentiate into germ cells, Sertoli cells and Leydig cells. This finding raises the possibility of using BMSC to treat male infertility and testosterone deficiency.

3 The emergence of adipose tissue-derived stem cells

In a 1999 paper, Stashower *et al.* [20] showed that the stromal fraction of adipose tissue contained a large population (> 75%) of cells that expressed the CD34 antigen, a well-known HSC marker that is also expressed in BMSC. Although acknowledging that CD34 is expressed in endothelial cells that are part of the stromal fraction, the authors nevertheless proclaimed the existence of progenitor cells in the adipose tissue. In a 2000 paper, Halvorsen *et al.* [21] reported that, depending on the culture condition, adipose tissue-derived stromal cells could be induced to express adipocyte-specific or osteoblast-specific proteins. In 2001, six additional papers further reported the multipotent nature of cultured adipose tissue-derived stromal cells. Five of these papers continued to demonstrate these cells' adipogenic and osteogenic potential [22–26], while the sixth paper showed for the first time that these cells could also differentiate into chondrocytes and myocytes [27]. As of today, near the end of 2007, there are more than 220 ADSC-related articles and the list of ADSC-differentiated cell types now includes endothelial, epithelial, muscle (cardiac, skeletal, and smooth), Schwann cells, hepatocytes and neurons.

As a new comer in the stem cell circle, ADSC is naturally subjected to comparison with BMSC, the prototype of ASC that also resides in an adipose-rich environment. Although there are the expected inconsistencies and variations among different studies, the general consensus is that ADSC and BMSC are virtually identical in cell surface marker profile, gene expression profile and differentiation potential [28]. This consensus has been reaffirmed in a recent preclinical study in which ADSC and BMSC were found equally effective in treating a porcine model of cardiac infarction [29]. However, clonogenic studies have established that the number of BMSC in bone marrow is approximately 1 in 25 000 to 1 in 100 000, whereas the average frequency of ADSC in processed lipoaspirate is approximately 2% of nucleated cells [28]. Therefore, the yield of ADSC from 1 g of fat is approximately 5 000 cells, whereas the yield of BMSC is 100–1 000 cells per milliliter of marrow. Furthermore, although bone marrow can only be obtained in limited quantity, the adipose tissue is usually obtainable in abundance, especially in our increasingly obese society. The safety of the tissue isolation procedure is another advantage of ADSC over BMSC, as it has been shown that between 1994 and 2000 there were zero deaths in 66 570 liposuction procedures and a serious adverse event rate of only 0.068% [30]. Therefore, although

ADSC and BMSC are virtually identical in their usefulness as regenerative cell sources, the difference in their clinical application potential is quite obvious.

4 Clinical application of adipose tissue-derived stem cells

The therapeutic potential of ADSC has been tested in several medical disciplines, particularly orthopedics, cardiology and neurology. Although most of these studies took place in pre-clinical settings (i.e. using animal models), a few clinical trials involving human patients have in fact been conducted. In 2004, Lendeckel *et al.* [31] reported the successful application of ADSC in repairing the cranial defects of a 7-year-old girl who suffered severe head injuries due to an accidental fall. In 2006 and 2007, two separate papers reported the successful application of ADSC for cosmetic surgeries, primarily breast augmentation, on more than 70 patients [32, 33]. Three other papers have reported the use of ADSC in circumventing graft-versus-host reactions in human patients [34–36].

5 Potential andrological application of adipose tissue-derived stem cells

To our knowledge, our research team at UCSF (with our collaborators at Peking University and Sun Yat-Sen University) is the only group conducting research on ADSC to treat andrological diseases, specifically, ED and infertility. Our research is both basic science and clinical. On the basic side, we have performed various experiments to characterize ADSC isolated from mice, rats, pigs and humans, resulting in the publication of 2 papers and the submission of 2 manuscripts. In the first published paper we showed that ADSC could be induced by isobutylmethylxanthine (IBMX) to differentiate into neuron-like cells [37]. In the second paper we showed that the IBMX-induced neuronal differentiation was mediated by the IGF-I signaling pathway [38]. The significance of these two studies is that ADSC has the potential to treat degenerative neurological diseases, including neurogenic ED, which frequently occurs to patients who have undergone pelvic floor surgeries or radiation.

We have recently submitted for publication a study titled "Characterization of stem and progenitor cells in adipose tissue". This study was motivated by the fact that, despite having been investigated in more than 220

studies, ADSC remain unidentified in adipose tissue. In this study we used immunohistochemistry, immunofluorescence, flow cytometry and western blot analysis to look for cells expressing vascular and stem cell markers. The results showed that ADSC are located in or near blood vessels, especially capillaries. In regard to our other studies we have obtained evidence of ADSC differentiating into endothelial, skeletal and smooth muscle cells. The significance of these studies is that ADSC undoubtedly have the potential to treat urological and andrological diseases. In the meanwhile, our medical researchers have applied ADSC to treat neurogenic ED in rats and obtained promising results. More recently, we have been investigating whether ADSC can differentiate into sperm, Leydig and Sertoli cells.

6 Concluding remarks

Because of their regenerative potential, stem cells are ideal therapeutic agents for degenerative diseases such as ED and defective conditions such as male infertility. In animal studies both ESC and BMSC have shown promise for treating these two andrological diseases. However, the challenges facing the use of ESC as a therapeutic regime are ethical concerns and the expected legal battles that could postpone its clinical application indefinitely. Although recent advances have shown that ESC could be generated through “reprogramming” of somatic cells [39–41], the clinical application of this type of ESC is still questionable because of the need to use viral vectors and the complicated procedure. However, the use of BMSC for regenerative medicine appears to be more acceptable to the public and the procedure much less complicated. Therefore, it can reasonably be expected that BMSC will reach the bedside ahead of ESC. Nevertheless, given the evidence that BMSC and ADSC are virtually identical in therapeutic potential, who would choose the former and not the latter for treating his ED or infertility?

Although the number of patients who have been treated with ADSC is still too small to make ADSC the obvious choice among the various types of stem cells, one additional factor to be considered is that ADSC has been used commercially to treat more than 2 500 horses with an approximate success rate of 75% (www.vet-stem.com). Therefore, the evidence for ADSC as a regenerative medicine is solid, and our own research at UCSF clearly shows that ADSC is a promising therapeutic

entity for treating andrological diseases.

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