

## Spermatogonial Stem Cells: A New Pluripotent Source for Repairment in Regenerative Medicine

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Received: 5 Oct 2014

Revised : 29 Oct 2014

Accepted: 15 Nov 2014

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### Abstract

Recently new reports have proved the pluripotency of spermatogonial stem cells (SSCs) derived from male gonad. This pluripotent stem cells resembled Embryonic stem cells recognized as Embryonic Stem like cells (ES like cells). ES like cells forms sharp edge colonies that are immunopositive to pluripotency markers and have differentiation capacity to Ectodermal, Mesodermal and Endodermal layers. ES like cells may have therapeutic application in tissue engineering and treatment for disease because of their ability to differentiation into various cell types. Embryonic stem like cells derived culturing of spermatogonial cells which has self-renewal and differentiation capacity to all three germ layers make them as a new and unlimited source for cell therapy strategies.

The perspective of pluripotency and differentiation ability of ES like cells obtained in mice has clinical application in other species particularly in humans that solves many problems of cell therapy in regenerative medicine.

These characteristics propose the therapeutic use of spermatogonial stem cells as a possible alternative source for generation of various cell types that are useful in treatment of degenerative diseases.

**Keywords:** Spermatogonial stem cells; Pluripotency; ES like cells; Regenerative medicine

*Please cite this article as:* Nazm Bojnordi M, Ghasemi Hamidabadi H. Spermatogonial Stem Cells: A New Pluripotent Source for Repairment in Regenerative Medicine. *Res Mol Med.* 2015; 3 (1): 1-5.

### Introduction

Different sources of pluripotent stem cells are recognized in developmental biology. These sources include Embryonic stem (ES) cells originated from blastocyst inner cell mass; Embryonic germ (EG) cells derived primordial germ cells (PGCs) and Embryonic carcinoma (EC) cells derived from testicular tumors (1-3). Recently researches have proved the generation of pluripotent stem cells from Spermatogonial stem cells (SSCs) under culture condition (4, 5). Although the main important role attributed to SSCs is spermatogenesis and producing gametes, but recent researches show their novel ability of transition into pluripotent cells that differentiate into all three germ layers (6). This ability is useful in studying the mechanisms that regulate the pluripotency or differentiation state of stem cells (7). SSCs convert to ES like cells during special culture. ES like cells have the capacity of embryoid bodies (EBs) formation. Almost every

three germ layer derivatives can be derived from these structures. (8, 9). ES like cell colonies show similar phenotypical appearance with ES cells (8, 9). Previous reports indicate that ES like cells from both neonatal and adult mouse testis have differentiation potential to generate all type of cells in the body. The interesting issue is the generation of these pluripotent stem cells occurred only under culture condition not required any cell transfection or genetic manipulation (10, 11). ES like cells derived from testis can be used as an alternative cell therapy source for human therapeutic strategies (7).

Some criteria such as unlimited propagation activity as well as differential capacity of ES like cells derived from spermatogonial stem cells proposed them as a desirable source usable in cell transplantation for repairing various diseases (10, 11). Also, no tumorigenic activity has been reported after transplantation of these cells to reception animals.

Furthermore, there are no ethical problems associated with other stem cells such as ES cells. Auto transplantation of these cells solves many immune rejection problems. Overall, these characteristics proposes ES like cells as appropriate source in treatment of degenerative diseases (10, 11). Derivation of pluripotent stem cells from patients suffered from genetic diseases provides models that facilitate the mechanisms involved in genetic disorders (4, 5). ES like cells derived from male gonad plays important role in treatment of specific diseases using cell lines formation that provide sufficient cells for transplantation strategies (11,12).

#### *Origin of Spermatogonial stem cells*

The origin of spermatogonial stem cells is from PGCs that migrate from extra embryonic sites to the early gonadal ridge during organogenesis (13). The first step of germ cell development is specification in which PGCs express specific markers and then migrate to developing gonad. The final step includes sex specific development (14, 15). SSCs are small population of cells reside on the basal membrane of the seminiferous tubule. They are able to establish stable spermatogenesis, which transforms genetic information from one generation to next generation (16, 17).

#### *Induction of ES like colonies from SSCs*

Previous reports indicate various protocols for derivation of ES like cells from male gonad; Kanatsu-Shinohara induced pluripotent stem cells from testis via culturing the mouse spermatogonial stem cells in special medium of ES (18). Guan applied different induction method for generation of ES like cells from adult mouse testis. He added GDNF only at the first step and then cultured cells in a simple medium supplemented with serum (19). It seems that the appearance time of ES like cells from SSCs is related to the properties of used culture system. Generation of ES like cells via Guan protocol which is independent from feeder layer, takes the longest time (19). Kanatsu-Shinohara showed failure in generation of ES like cells if ES medium was used at first for culturing SSCs (20, 21). Boulanger transplanted adult mouse SSCs into mammary gland and indicate that they differentiate into mammary epithelial cells (22). In total, transformation of SSCs into ES like cells needs no special defined protocol. Final destination of SSCs are determined by their surrounded environment; it means that they are committed to the gametogenic precursors only under the seminiferous tubule environment condition, but outside this condition, they transform to another lineage with different fate map (10). Following previous researches, nazm bojnordi isolated ES like cells from neonatal mouse testis under simple co-culture condition

with sertoli cells which was a novel method compared to other complex and time consuming methods (23).

#### *Molecular features in SSCs and ES like cells*

ES like cells express similar markers to ES cells in molecular level. Pluripotency markers are detectable in both of them indicating the same pluripotent characteristic of these cells (24). The transition from cultured SSCs to ES like cells is accompanied by extensive changes in gene expression. These changes are seen in the expression of pluripotent genes such as *Sox2*, *Oct4*, *Myc*, and *Klf4* (25, 26). Also, ES cell markers stage specific embryonic antigen-1 (SSEA-1) and high levels of alkaline phosphates were also found in ES like cells compared with SSCs (23, 27, 28). Also, our previous research confirmed that ES like cells show high levels of expression of the pluripotency genes Nanog and C-myc, similar to ES. Furthermore, germ cell-specific genes such as *Stra8*, *Mvh* and *Piwil2* down regulated which confirmed previous results (23) (Table 1).

**Table 1.** Pluripotency and spermatogonial gene expression in SSCs and SSC-derived ES like cells

Marker	SSCs	ES like cells
SSEA1	-	+
Oct4	+	+
Klf4	-	+
SOX2	-	+
Nanog	-	+
C-myc	-	+
ALP	+	+
Mvh	-	+
Piwil2	-	+
Stra8	-	+
Ckit	-	+
Dazl	-	+
Plzf	-	+

#### *Differentiation capacity of ES like cells*

Differentiation of SSC- derived ES like cells into mesodermal cells, such as hematopoietic or muscle cells and Neural lineages formed neurons or glial proved by Kanatsu-Shinohara *et al* (18).

Baba *et al.* showed that ES like cells have the potential to differentiate into cardiomyocytes and endothelial cells (29). Guan *et al.* differentiated ES like cells into Neuroectoderm, Endodermal and mesodermal lineages such as vascular endothelial and cardiac, skeletal smooth muscle cells using hanging drop method (19).

### *Potential applications of spermatogonial stem cells for regenerative medicine*

New strategies in regenerative medicine are focused on recovery via cell transplantation because this exogenous cells can recover the function efficiently more than mechanical devices or chemical materials (30). New cell therapeutic strategies propose a wide variety of stem cells for treatment of diseases and genetic disorders. But high propagation activity and flexible differentiation capacity of SSCs proposed them as appropriate sources for treatments based on cell transplantation (23, 31-33). These properties provide spermatogonial stem cells as an appropriate source for therapeutic use in regenerative medicine (34, 35). Researches show fetal cell transplantation has more therapeutic effect in animal models of neurodegenerative diseases (36-38). Because of limitation in access to fetal tissues, selecting an alternative sources for therapeutic intervention seems logical. It is desirable to identify an alternative stem cell population with differentiation potential similar to that of the ESCs (39, 40). SCs are the best candidate for transplantation in neurodegenerative diseases because of their differentiation ability to neural progenitors, as well as functional neurons and glial cells (41, 42).

Spermatogonial cells may represent a possible alternative to embryonic stem cells for cell therapy in replacing neurons and glia (10). ES like cells derived from testis could be used in cell line production from a specific patient and this is helpful in regenerative medicine (23, 37). Until now generation of ES like cells with differentiation capacity to all three germ layers have reported from mouse testis (19, 23). Spermatogonial stem cells have practical application in regenerative medicine in particular as a natural and novel source for treatment of neurodegenerative disease, so, it is considered as an alternative source that is able to differentiate into various cells.

### **Conclusion**

Generation of pluripotent ES like cells from spermatogonial stem cells provides models that facilitates the mechanisms involved in switching of fate map destination of committed progenitor cells into a pluripotent cell (4,5). Derivation of ES like cells from SSCs with the same ES cells or induced pluripotent stem cells (iPS) cellular and molecular profile, produces an alternative source for replacement of every cell types usable in regenerative application (10,11). Pluripotent stem cells derived from testis is an alternative source usable in the basic and clinical research such as neurodegenerative diseases, heart diseases, diabetes, etc. (7-10). They can be propagated and differentiated in vitro into the desired cell type

before being transplanted to the patient. ES like cells can differentiate to various types such as neural cells, cardiomyocytes, endothelial cells, and pancreatic cells. Researches show the derivation of functional endothelial cells from ES like cells that participate in blood vessels generation and also functional neuron and glia cells which contribute to demyelination model (27, 41). Also, surprisingly, ES like cells is actually more efficient than ES cells in generation of some mesodermal type cells such as cardiomyocytes. This confirms that they can be used as an alternative source for ES cells in regenerative medicine (29). It is believed that more experiments must be done to improve the efficiency and safety of ES like cells before their clinical application.

### **Acknowledgments**

Authors appreciate the Vice-Chancellor for Research of Mazandaran University of Medical Sciences.

### **Conflict of Interest**

The authors declare that they have no conflict of interest in this work.

### **Author Contributions**

Bojnordi developed the original idea, writing and revising the manuscript. Ghasemi provided some parts of discussion as well as revising the manuscript.

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