P41 Isolation and characterization of spermatogonial stem cells from adult mouse testis

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Objectives: The aim of this study was to investigate whether spermatogonial stem cells (SSCs) can be isolated and cultured from adult mouse testis that cryptochidism was experimentally induced (CEI testis), and to evaluate differentiation potential of SSCs into three germ layers.

Materials and Methods: Cells from CEI adult mouse testis were mechanically prepared by syringe suspension and were cultured. To establish tentative spermatogonial stem cells (TSSCs), cells were co-cultured with mitotic-arrested STO feeder cells in DMEM supplemented with recombinant murine leukemia inhibitory factor, glial cell line-derived neurotrophic factor (GDNF) and fetal bovine serum (FBS). RT-PCR analysis for markers of undifferentiated stem cells and spermatogonial stem cells was performed to characterize established TSSC lines. To examine the differentiation potential of TSSCs to three germ layers, formation of embryoid bodies (EBs) was observed in suspension culture in DMEM supplemented with FBS.

Results: TSSC colonies were established from mechanical preparation of CEI adult mouse testis and have similar characteristics with MACS-isolated SSCs, i.e., colony morphology and slow proliferation rate. TSSC colonies were established even from a single cell when subcultured. TSSC colonies expressed undifferentiated stem cell markers such as Oct-4, Nanog, Sox2, and SSC markers including GFRα-1. They also expressed differentiated germ cell markers, such as MVH, C-kit, DAZL. TSSCs formed EB-like structure by suspension culture in DMEM supplemented with FBS.

Conclusions: This study shows that spermatogonial stem cells could be established by mechanical separation method from CEI adult mouse testis. Further investigation is required to obtain pure population of SSCs. TSSCs form EB-like structures and thus they may possess capacity to differentiate into cells of all three germ layers. Hence we will observe gene expression pattern in EB-like structures to determine if they have capacity to form pluripotent cells and differentiate.

Key words: spermatogonial stem cells, cryptochidism, GDNF, OCT-4, Nanog, Sox2, GFR α-1,

P42 Effective preservation methods for human embryonic stem cell-derived neural progenitors

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Objectives: It was needed that mass production and rapid supply of transplantable neural cells for cell therapy. However, preservation of embryonic stem cell-derived neural cells or neural progenitors does not easy because of their poor ability of survival. So, we tried to development the effective preservation method using by fragmentation of the spherical neural masses into smaller masses and freezing with DMSO and without serum.

Materials and Methods: Spherical neural masses (SNMs) were made by human embryonic stem cells (SNUhES1) through selection and expansion steps and characterized by immunocytochemistry using various neural markers. SNMs were mechanically dissected 4-8 pieces into smaller masses and they were removed into freezing vials which containing neural expansion media with 10% DMSO. Vials were removed into freezing container and frozen by liquid nitrogen. Two weeks after freezing, frozen masses were thawed and analyzed for survival activity and changing of characteristics.

Results: Neural progenitors could be effectively preserved by freezing that accomplished by small mass fragmentation and DMSO without serum. More than 50% of the masses which had been thawed after 2 weeks later were survived and maintaining their proliferation and differentiation properties.

Conclusions: Our method for preservation of human embryonic stem cell-derived neural progenitors could offer the solution of the requirement for mass production and rapid supply of transplantable neural cells for cell therapy more economically and effectively.

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Key words: A, B, C, D, E, Cell preservation, Neural progenitors, Human embryonic stem cells, Cel survival, Neural differentiation