Original Article

Isolation and Culture of Human Spermatogonial Stem Cells Derived from Testis Biopsy

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Abstract

Background: In cancer patients, chemo and radiotherapy can cause infertility by damaging spermatogenesis process. This process is based on self-renewal and differentiation of a rare population of the testicular cells called Spermatogonial Stem Cells (SSCs). Scientists have tried to isolate, enrich and culture Human spermatogonial stem cells, hoping to resolve infertility problems in cancer recovered patients in the future.

Methods: Spermatogonial stem cells were isolated and purified from human testicular biopsies sample consisting of at least 500,000 and at most 2,000,000 cells. Two enzymatic digestion steps were performed. Enriching methods, differential plating, and specific culture in serum-free medium with added growth factors: human GDNF, bFGF, EGF and LIF was performed on coated dishes.

Results: Human spermatogonial stem cell clusters were observed after 7 to 10 days in specific culture, then after several passages and successful expanding duration of 52 days, the cells were evaluated by three layer immunocytochemistry test (LSAB) to stain GPR125 protein as a surface marker in human spermatogonial stem cells.

Conclusion: In current study human spermatogonial stem cell were isolated and expanded with the least manipulations in comparison with the other usual isolation methods like florescent or magnetic activated cell sorting. In contrast to the other SSCs isolation and culture methods, this system is based on the testicular biopsies against large samples, thus suggested method in this study is closer to clinical usage in the future.

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Keywords: Culture, Enrichment, GPR125, Human spermatogonial stem cell, Infertility, Isolation

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Introduction

Today, treating cancer in children has been improved immensely. However, chemothera- py and radiotherapy as the methods of cancer treatment damage different types of cells es- pecially proliferating cells like spermatogo-