



ENRICHMENT OF SPERMATOGONIAL STEM CELL BY USING SIDE POPULATION IN RAINBOW TROUT

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Background:

Spermatogonial stem cells (SSCs) provide the foundation for spermatogenesis. Using the rainbow trout (*Oncorhynchus mykiss*), we recently established a novel germ cell transplantation technique to identify SSCs, based on their biological function [1]. A portion of type A spermatogonia (A-SG) transplanted into the peritoneal cavity of the recipient embryo migrated toward and colonized within the recipient embryonic gonad, then produced a large number of functional sperm for several years. These results demonstrated the colonized A-SG were SSCs. These colonizable SSCs can be retrospectively identified after transplantation analysis; however, there is no available technique to prospectively enrich these SSCs before transplantation. In this study, we established a novel technique for SSCs enrichment by using side population (SP).

Methods:

As the method for enriching SSCs, we focused on the SP. SP cells are identified as those cells that are less stained by Hoechst 33342 dye (H33342) as detected by fluorescence activated cell sorting (FACS) analysis [2]. SP cells are firstly identified from bone marrow, and enrich the hematopoietic stem cells. However, it remains controversial whether SSCs are enriched in SP cells; some groups successfully enriched SSCs in SP cells [3-6] but not others [7, 8] in mouse. In this study, we optimized the staining conditions: concentrations of H33342, temperatures, and incubation times, to determine whether SP cells are observed among A-SG in rainbow trout. Then, to determine whether the SSCs were enriched in SP cells, we analyzed the colonizing activity of the SP cells within the recipient gonad.

Results:

SP cells were detected among A-SG by staining with 5µg/ml H33342 for 10 hours at 16°C. To analyze whether these SP cells have the higher colonizing activity, about 150 isolated SP cells or non-SP cells were transplanted into the peritoneal cavity of the recipient embryos. SP cells were colonized within the gonad in 46.6% of transplanted recipient embryos (N=84), whereas non-SP cells were colonized only in 7% of recipient embryos (N=59). These results showed the colonization efficiency was significantly increased by

the transplantation of SP cells compared with non-SP cells.

Conclusion:

In this study, we showed the SSCs were enriched in the SP cells in fish. This is very useful technique to isolate the population highly enriched SSCs without SSC-Specific surface markers. SSCs enriched with SP will provide us powerful tools to biochemically and molecularly characterize the SSCs. From an applicational point of view, this technique will increase the efficiency of surrogate broodstock technology.

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