THE ESTABLISHMENT OF IN VITRO CULTURE SYSTEM OF BUFFALO SPERMATOGONIAL STEM-LIKE CELLS

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Introduction

Spermatogonial stem cells (SSCs) are precursor cells of sperm, providing continual motility for spermatogenesis, while also ensuring the transfer of genetic material between parent and offspring. The research on SSCs is of great significance for regenerative medicine, species preservation, and production of transgenic animals. In the previous studies, we used DMEM/F12 as basal medium, got the compact and round cell clusters, however, they were different from the morphology of SSCs in model organisms. The objective of this paper was to isolate, characterize and establish a new culture system of buffalo spermatogonial stem-like cells (SSC-like cells) in vitro.

Materials and Methods

We used the specific markers OCT4, DDX4, THY-1, PGP9.5 and PLZF to perform in situ staining of prepubertal buffalo testis (3 to 7 months) by paraffin section immunofluorescence staining. The single SSC-like cells were purified by two-step enzymatic digestion combined with differential planting method. The $DDX4^+$ and $PGP9.5^+$ cells were counted before and after the purification, meanwhile the cells also were detected by the quantitative real-time PCR. After the SSC-like cells were cultured for three passages, they were identified at the protein level and transcription level, respectively. Afterwards, we conducted the in vitro induction experiment with RA in this culture system.

Results and Discussion

The immunofluorescence staining showed that all of the specific markers were expressed in the SSC-like cells, which means that the materials we used really contain SSC-like cells, which could provide a source of cells for our follow-up study. Meanwhile, about 60% of SSC-like cells could be enriched through differential planting method. During our in vitro culture processes, a remarkable result was found that, serum-free culture system appeared as more suitable for the proliferation of buffalo SSC-like cells. At the initial stage of culture, SSC-like cells were mainly in the state of A_{single} or A_{paired}, which were typical proliferation forms of stem cells, with the prolongation of the culture time, showed a typical string-like proliferation mode, notably. The result of in vitro induction experiment illustrated that the differentiation genes such as Stra8 and Rec8 were highly expressed in the induced SSC-like cells in vitro, while the expression of the pluripotency gene NANOS2, PLZF and germ line gene DDX4 were decreased. In conclusion, we established a method to obtain a large number of buffalo SSC-like cells, and optimized the in vitro culture system. These results could provide the basic platform for buffalo SSC-like cells studies, such as its long-term culture, gene editing, the mechanism of self-renewal and differentiation and so on.