THE PRIMARY RESEARCH OF OVER EXPRESSION OF GDNF GENE IN BUFFALO TESTIS SERTOLI CELLS

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Introduction

Glialcellline-Derived Neurotrophic Factor (GDNF) is a neurotrophic factor firstly cloned from mouse brain tissue by Lin *et al.* in 1993, which can also be secreted by Sertoli cells. It has a variety of physiological functions and plays an important role in the nervous system and maintenance of spermatogonial stem cells. Up to date, the study of in vitro culture of buffalo spermatogonial stem cells (SSCs) usually supplement with mouse-derived GDNF, but the proteins for its variety of the structure and modification in different animal species may affect the culture efficiency of SSCs in vitro. In the present research, the gene of buffalo GDNF was integrated into PiggyBac expression vector to make it stably expressed in Sertoli cells, which provided the basis for the related research of buffalo SSCs.

Materials and Methods

The buffalo brain tissue was used as material to extract total RNA which was reversed by transcription. Then the GDNF fragment was cloned, and the product was taken from the agarose gel and connected with T vector to construct the Piggybac recombinant plasmid. The prepubertal buffalo testes (3 to 7 months) were used as materials. The Sertoli cells were purified by two-step enzymatic digestion combined with differential planting method, which were identified by WT1 and GDNF antibodies. The PB-GDNF plasmid was stably transfected into Sertoli cells by lipofectamine 2000. The SSCs were co-cultured with PB-GDNF-Sertoli cells and Sertoli cells as feeder layers, respectively. Afterwards, we compared the expression of PLZF, NANOS2 and DDX4 in these two kinds of SSCs, which we obtained.

Results and Discussion

We constructed the GDNF-Piggybac eukaryotic expression vector which was transfected into Sertoli cells. The quantitative real-time PCR results showed that the expression of GDNF gene in PB-GDNF-Sertoli cells was significantly higher than that of Sertoli cells. Meanwhile, the expression of the specific markers PLZF, NANOS2 and DDX4 in the PB-GDNF-Sertoli-SSC group was significantly higher than in the Sertoli-SSC group. In conclusion, we successfully constructed buffalo-derived GDNF eukaryotic over expression vectors and a buffalo Sertoli cell line which could over express the GDNF gene in vitro. This research could provide a new method for the maintenance of the pluripotency of buffalo SSCs in vitro.