Characterization of Spermatogonial Stem Cells and Testicular Cells in Chicken

Bo Ram Lee[1], Young Mok Lee[1], Tae Sub Park[1], Jin Geyoung Jung[2], Yeong Ho Hong[2], Jeong Mook Lim[1], and Jae Yong Han[1]

[1]School of Agricultural Biotechnology, Seoul National Uni. / [2]Avicore Biotechnology Institute, Korea

Abstract

정원세포는 수컷의 정소에 존재하며, 감수분열을 통하여 정자를 형성하고 지속적으로 자신의 복제가 가능한 세포이다. 이러한 정원세포는 마우스를 중심으로 현재 수컷불임 치료의 연구, 멸종위기 종의 보존을 위한 연구 그리고 형질전환 동물 생산 등 다양한 분야에 응용되고 있다. 그러나 조류에서는 정원세포 연구에 있어서 그 세포의 형태적·면역학적 특성이 아직 구명되지 못하여 연구진행에 어려움이 많다. 따라서 본 연구에서는 전자주사현미경을 이용하여 세포내 구조와 형태의 분석을 진행하였고, 조직염색법을 통하여 특이적 마커를 분석하였다. 이후 본 연구결과를 토대로, 포유류에서 이루어진 정원세포 이식기술의 개발 및 새로운 형질전환 기술의 개발을 조류응용이 가능할 것이다.

(Key words : 정원세포, 닭, 특이적 마커, 전자주사현미경(TEM), 조직염색법)

Introduction

Spermatogenesis that is closely related to spermatogonia is a complex process to generate sperms. The spermatogensis can be divided four phases: self-renewal, multiplication, differentiation and maturation. In these phases, the number of spermatogonia is important points because it need very large number of spermatogonia to initiate maturation and produce spermatozoa. In mammals, spermatogonia locate basement of seminiferous tubules and divided very slowly to form daughter cells. In birds, however, the ultrastructural and histochemical properties of testicular cells as well as spermatogonial stem cells have not been characterized yet and there is a room to be elucidated for identifying spermatogonia as a stem cell and developing the specific markers.

Materials and Methods

Testes were obtained from White Leghorn males between 1 week and 20 weeks. Anti-stage specific embryonic antigen-1(SSEA-1) antibody was used for the detection of chicken spermatogonia. The anti-SSEA-1 antibody was obtained from the Developmental Studies Hybridoma Bank(the University of Iowa). Whole-mount immunostining was performed. Testis tissues from adult White Leghorn males were fixed with 2 % paraformaldehyde and 2 % glutaraldehyde in 0.05 M sodium

cacodylate buffer(pH 7.2) at 4 ℃ for 2 hours and then post-fixed with 1 % OsO4 in PBS for 1 hour. The fixed samples were dehydrated through graded ethanols and embedded in Spurr's resin. The blocks were cut at 60 nm, stained with uranyl acetate, and then examined with a JEM-1010 transmission electron microscope(JEOL, Japan) at 80 kV.

Result

1~20 weeks testis of White Leghorn male were analyzed using the microscopy. Tesitis sections were observed for standardizing the patterns and detecting the initiation timin of spermatogenesis. Transmission electron microscopy was conducted to identify the cellular properties and location of spermatogonia. Immunohistochemical analysis data showed that some of antibodies and lectin could be specific markers for chicken spermatogonia.

Summary

According to topographical methods, the chicken spermatogonia was located in basal membrane of seminiferous tubules. It has large nuclei and mitochondria and proliferated with cellular bridges. Immunohistochemistry data showed that anti-SSEA-1 antibody specifically reacted with A_{pr} and A_{al} type spermatogonia. Lectin, STA and integrin-6, -1 were also specific to A_s type spermatogonia.

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