Development of novel markers for the characterization of chicken primordial germ cells

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Abstract

형질전환 가금의 생산은 생체반응기(Bioreactor) 가금에 의한 고부가가치의 생의약 물질을 저비용, 고효율로 생산할 수 있으며, 배 발달과정 및 유전자 조절기작 규명을 통한 학문적 이용성 등 다양한 분야에 응용될 수 있다. 형질전환 가금을 생산하기 위한 방법 중 닭의 배 발생 초기에 발생하는 성세포(정자 혹은 난자)의 전구세포인 원시생식세포를 이용한 연구가 활발하게 진행되고 있다. 그러나 이를 검증할 원시생식세포 특이적 마커의 부재로 많은 어려움을 겪고 있다.

따라서 본 연구는 원시생식세포의 특성 분석을 위해 PAS(Periodic acid-Schiff) 염색 및 특이항체 (SSEA-1,3,4 & Integrins α6, β1) 그리고 lectins (STA, DBA, ConA, WGA)를 이용하였다. 이번 연구결과를 통한 닭 원시생식세포의 특이적 마커의 개발은 원시생식세포를 이용한 가금의 형질전환 연구에 기여할 것이다.

Key words : chicken, primordial germ cell (PGC), stage-specific embryonic antigens (SSEAs), lectin, integrin

Introduction

The production of transgenic birds, and transgenic chickens in particular, through germline transmission is now considered to be the most efficient strategy for the production of animal bioreactor. Recent advances make it possible to produce germline chimeras by transfer of primordial germ cells (PGCs) into heterogeneous embryo. But Such methods are insufficient to fully characterize chicken PGCs, and additional effort to identify novel markers is urgently required for further development of avian transgenic systems. This study was designed to identify and develop novel markers for the characterization of chicken PGC.

Materials And Methods

Gonadal cells collected from 5.5-day-old chicken embryos were cultured in a DMEM-based medium, and the PGC colonies formed during the primary culture period were subcultured three times. Characterization of the PGCs with the candidate marker reagents was performed on the mixed cell population at the time of seeding, after the primary culture period(day 10), and after the third passage (day 40). Mouse embryonic stem (ES) cells were used as controls.

The cytochemical reagents investigated included periodic acid-Schiff(PAS) stain antibodies to stage specific embryonic antigens(SSEA-1, SSEA-3 and SSEA-4) antibodies to integrins 6 and 1 several

lectins(STA, DBA, ConA, and WGA), and double staining with the lectin STA and with antibodies to SSEA-1, SSEA-3, SSEA-4, integrin 6 or integrin 1. Densitometric quantification was used to identify PGC-specific markers.

Results

The results showed that chicken PGCs were stained selectively by PAS and by antibodies to SSEA-1, SSEA-3, SSEA-4, integrin 6, and integrin 1. The control mouse ES cells reacted with PAS and anti-SSEA-1 antibody, as well as with antibodies to integrins 6 and 1, but not with antibodies to SSEA-3 and SSEA-4. Chicken PGCs reacted with the lectins STA and DBA, but mouse ES cells reacted with STA and WGA.

The results of double staining of PGC colonies subcultured three times showed that the intensity of staining was not altered by concomitant use of the marker reagents. This study demonstrated that, in addition to PAS and antibodies to SSEA-1, SSEA-3, and SSEA-4, new specific markers of chicken PGCs are recognized by the lectins STA and DBA and by antibodies to integrins 6 and 1. Double staining using these newly developed markers might be the method of choice for rapid characterization of chicken PGCs.

Summary

We developed a new panel of markers for the characterization of chicken PGCs. The results of immunostaining demonstrated that anti-SSEA-3, anti-SSEA-4, anti-integrin 6, and anti-integrin 1 antibodies, and STA and DBA bound specifically to chicken PGCs. These reagents could be used to characterize chicken PGCs together with conventional marker reagents such as PAS and anti-SSEA-1 antibody. We also showed that double staining of PGCs with the newly developed markers was feasible, which might contribute to rapid detection and accurate characterization of chicken PGCs.

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References

Han J.Y., Park T.S., Hong Y.H. et al. Production of germline chimeras by transfer of chicken gonadal primordial germ cells maintained in vitro for an extended period. Theriogenology 2002;58:1531-1539.

Marani E, Van Oers J.W., Tetteroo P.A. et al. Stage specific embryonic carbohydrate surface antigens of primordial germ cells in mouse embryos : FAL(SSEA-1) and globoside (SSEA-3). Acta. Morphol. Neerl. Scand. 1986; 24:103-110.

Takagi Y., Talbot N.C., Rexroad C.E. et al. Identification of pig primordial germ cells by immunocytochemistry and lectin binding. Mol. Reprod. Dev. 1997;46:567-580.