Proteome Analysis of Chicken Embryonic Gonads: Identification of Major Proteins from Cultured Gonadal Primordial Germ Cells

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Abstract

The domestic chicken (*Gallus gallus*) is an important model for research in developmental biology because its embryonic development occurs *in ovo*. To examine the mechanism of embryonic germ cell development, we constructed proteome map of gonadal primordial germ cells (gPGC) from chicken embryonic gonads. Embryonic gonads were collected from 500 embryos at 6 day of incubation, and the gPGC were cultured *in vitro* until colony formed. After 7-10 days in culture, gPGC colonies were separated from gonadal stroma cells (GSCs). Soluble extracts of cultured gPGCs were then fractionated by two-dimensional gel electrophoresis (pH 4-7). A number of protein spots, including those that displayed significant expression levels, were then identified by use of matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry and LC-MS/MS. Of the 89 gPGC spots examined, 50 yielded mass spectra that matched avian proteins found in on-line databases. Proteome map of thistype will serve as an important reference for germ cell biology and transgenic research.

Key words : chicken, embryonic gonads, gonadal primordial germ cell (gPGC), mass spectrometry (MS), two-dimensional gel electrophoresis (2-DE)

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Introduction

Chicken (Gallus gallus) gonadal primordial germ cells(gPGCs) are an important research model for studies of transgenesis and developmental biology. Although chicken is rapidly becoming a more important model for developmental biology, research in chicken PGCs has been relatively minimal and restricted to a small scale. Several groups have attempted to identify a molecular signature for germ cells using large-scale transcriptional profiling (Reinke et al., 2004). However, a useful marker for chicken gPGCs has not been well defined. Although several groups have published proteome maps of various chickens cell types and tissues, a proteome map of chicken gPGCs has yet to be published. In our previous research, we established improved techniques for the maintenance of chicken gPGCs in long-term culture (Park and Han, 2000), and we established chicken embryonic germ cell lines for production of germline chimeras. These methods, which dramatically improve retrieval of chicken gPGCs, are critical in providing starting materials for proteomics studies, since gPGCs constitute fewer than 1% of embryonic gonadal cells. In addition, cultured chicken gPGCs provide an excellent system for study of developmental processes and germ cell biology. In the present study, we capitalized on these technical

advances in constructing proteome maps of gPGCs to investigate avian germ cell development.

Materials and Methods

Embryonic gonads were collected from 500 White Leghorn chicken embryos at stage 28. After 7-10 days in culture, gPGCs were harvested. MALDI-TOF analysis was performed in reflector mode on a Voyager-DE STR MALDI-TOF mass spectrometer. For analysis by 2-DE, pH 4-7 immobilized pH gradient (IPG) strips were rehydrated. The lysates were cup-loaded onto the rehydrated IPG strip and electrophoresed. Proteins in excised spots were identified from their MALDI-TOF spectra by manual searching using the search programs.

Result

Colloidal Coomassie-stained gels displaying typical patterns obtain from soluble protein extracts of cultured chicken gPGCs. Each horizontal series probably reflected systematic modifications, such as phosphorylations or glycosylations, of one protein. In total, 61 spots were successfully identified, which corresponded to 23.3% of the spots on the entire pH 4-7 2-DE gel. The 61 identified spots corresponded to 44 different proteins. Six of the identified proteins were represented by at least two distinct spots on the gel, and some were represented by as many as eight different spots. We classified the identified poly peptides with regard to their principal known or postulated functions. The cell structure proteins comprised the largest fraction of the identified proteins (26.9%) and included cytoskeletal proteins including a- and b-actins, vimentin, and tropomyosin. The next largest classes were comprised of those identified proteins involved in protein synthesis and processing (8.9%) and metabolism (7.9%).

적 요

배자 생식세포 발달에 관련된 메카니즘을 밝혀내기 위해서, 닭 배자 생식기에서 추출한 원시 생식세포의 단백질체 지도를 만들었다. 총 500 배자를 6일간 배양하여 배자 생식기를 획득했고, 7-10일 배양 후, 배양된 원시 생식세포는 2차원 젤 전기 영동법에 의해 분할되어 졌다. 유의적 발현 수준을 나타낸 많은 단백질 스팟 들은 MALDI-TOP 와 LC-MS/MS에 의해 확인되었으며, 89개의 단백질 스팟 중에 50개의 mass spectra 들이 데이터베이스에서 조류 단백질과 일치함을 확인하였다. 본 실험에서 행한 단백질체 지도는 형질전환 연구와 생식세포 생물학 분야에 중요한 참고 문헌으로 가치를 가질 수 있을 것이다.

참고 문헌

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