Expression of Novel Genes Related to Germ Cell in Chicken

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ABSTRACT

생식 세포는 한 세대의 유전 정보를 다음세대에 전달할 수 있는 유일한 세포로서 다양한 특성을 가지고 있다. 기능 유전체 연구를 통해서 새로운 유전자의 기능을 규명함으로써 그 유전자의 생물학적 의미와 상호작용을 설명할 수 있다. 본 실험의 목적은 생식세포에서 특이적으로 발현하는 유전자를 발굴하여 그 유전자가 생식세포의 발달과 분화에 중요한 역할을 수행하는 것을 증명하는 것이다. 이에 본 실험에서는 real-time quantitative RT-PCR 기법을 이용하여 정소에서 특이적으로 발현하는 5개(AGE1, AGE2, AGE3, AGE4, AGE5)를 선발하였고, in situ hybridization 실험을 통하여 정소 조직 내에서의 발현 양상을 확인하였다. AGE1, AGE2 는 round spermatid에서 특이적으로 발현하고, AGE3, AGE4, AGE5는 spermatocyte에서 특이적으로 발현하는 것을 확인하였다. 본 실험을 통해 발굴한 유전자들은 닭의 생식선 발달에 중요한 기능을 할 것으로 예상되며, 앞으로 닭의 유전육종분야에 큰 도움을 줄 수 있을 것이다.

▶ **Key words** : chicken, germ cell, RT-PCR, in situ hybridization

INTRODUCTION

The analysis of developmental stage-specific gene expression is an essential step in understanding certain biological processes during embryogenesis as well as organogenesis. To orchestrate testis or ovary formation, a number of genes related with germline differentiation are spatiotemporally regulated and transcribed. Germ cells, the only cell-type that can transmit genetic information to the next generation have various characteristics in function. In the early embryonic developmental stages, germ cells originated from primordial germ cells (PGCs), and finally differentiate into functional gametes, sperm in male or ovum in female, after sexual maturity (Eyal-Giladi et al., 1981). Until recently, genome research in the chicken has lagged behind that in the mouse and human. However, the large expressed sequence tag (EST) database and extensive genomic sequence information available for chicken provide a foundation for studying the molecular biology of this organism using gene expression profiling and functional genomics approaches. In this study, to identify genes involved in germ-line development, we conducted five novel genes related to germ cells in chicken tissue libraries were selected from the TIGR Gallus gallus Gene Index (Kim et al., 2005). Our results show that the genes of germ cell-associated gene expression in chicken, and identifying environmental niche involved in germ cell differentiation in avian species.

MATERIALS AND METHODS

Extraction of mRNA and performance of RT-PCR

Samples were homogenized and total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. For each tissue analyzed, cDNA was synthesized from 0.5 g total RNA in 20-l reactions by reverse transcription with the Superscript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). PCR amplification of all cDNA samples was also performed using primers specific for the house-keeping gene glycerol-dehyde-3-phosphate dehydrogenase (GAPDH) as a control for RNA extraction and cDNA synthesis.

Quantitative RT-PCR

Germline-related genes from the RT-PCR analysis were chosen for confirmation studies by quantitative RT-PCR. The quantification was conducted with iCycler iQ real-time PCR detection system (Bio-Rad, USA) and SYBR Green I (Sigma, USA).

In Situ Hybridization

In situ hybridization was performed as described previously using single-stranded digoxigenin-labeled RNA probes corresponding to sense or antisense (Nieto et al., 1996).

RESULTS AND DISCUSSION

To evaluate the expression level of genes by RT-PCR, we analyzed five genes (AGE1, AGE2, AGE3, AGE4, AGE5) from chicken tissue library. The quantification of the genes expression was performed by real-time RT-PCR using SYBR Green I. All of five genes were showed exclusive highly expression in 25-week testis that not in other tissues. And five genes respectively expression were investigated in specific stage of chicken testis using in situ hybridization methods. Our results showed that these genes expressed in specifical stage of chicken testis and suggested genes be required for spermatogenesis. Germ cells possess special properties due to their unique role. In contrast to somatic tissues which cease to exist when an organism dies, germ cells link successive generations together and are, in that sense, immortal. Since germ cells ultimately give rise to all the tissues in a developing animal, they must remain pluripotency, while at the same time differentiating into highly specialized gametes. Little is known of molecular requirements for specification of chicken germ cells. However, it is likely that they are specified through the action of sequentially expressed genes just as in model organisms. Therefore, further research on these germ line-specific genes could define mechanisms involved in chicken.

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