Induction and Gene Manipulation of Chicken Oviduct Epithelial Cells

Hee Won Seo, Sun Young Kim, Sang Su Shin, Tae Min Kim, Young Mok Lee, Bo Ram Lee, Tae Wan Kim, Jeong Mook Lim, Jae Yong Han
School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

ABSTRACT

닭의 유전자 지도가 밝혀지고 그와 관련한 생물학적 연구들이 활발히 이루어지면서 닭을 생체 반응기나 질병 모델 동물로 이용하기 위한 연구가 많이 진행되고 있다. 이 중 닭을 생체 반응기로 이용하기 위해서는 많은 양의 단백질을 생산하는 난관에 대한 연구가 필수적이다. In vivo와 in vitro에서 난관 특이적 프로모터에 의한 외래 유전자의 발현에 대한 연구를 하였고 유전자를 전이하는 방법으로는 렌티 바이러스 시스템을 이용하였으며, 프로모터는 난관 특이적 프로모터인 오브알부민 프로모터 (5' 조절 부분의 1.4kb)와 RSV 프로모터를 이용하였다. 리포터 유전자로는 형광 발현 단백질 (enhanced green fluorescence protein, EGFP)을 이용해서 마우스 배아 섬유아세포, 닭 배아 섬유아세포, 난관 상피 세포에서 발현을 유도해서 조직 특이적 발현 여부를 확인하였다. 그 결과 RSV 프로모터는 모든 세포에서 발현하였으나, 오브알부민 프로모터에 의한 리포터 유전자의 발현은 난관 상피 세포에서는 특이적으로 발현하였다. 이와 같은 연구는 산란계를 이용해서 난관으로부터 효율적인 생리 활성 물질을 생산하기 위한 가능성을 보여주었다.

INTRODUCTION

The number of proteins required for medical and industrial application is large and increasing rapidly. These proteins are typically produced in mammalian cells in industrial fermentation facilities. The low yields of recombinant proteins from these cells combined with high facility costs have led to the development of alternative production systems. The current white Leghorn hen which have been improved by breeding lays up to 330 eggs per year and the protein content in an egg is ~6.5 g, of which 3.5 g is egg white protein. Tubular gland cells belong to the magnum of the oviduct and continually express and store egg white proteins in intercellular granules (Harvey et al., 2002). The key to improving the expression of foreign in eggs is the construction of tissue-specific expression vectors for chicken oviduct bioreactors. Therefore, this study was conducted to figure out the possibility of tissue-specific transgene expression in the oviduct of laying hens using a transfection technology that involves in vitro and in vivo infection with lentiviral vector systems and the ovalbumin promoter.

MATERIALS AND METHODS

The vector construct contains a reporter

gene encoding enhanced green fluorescent protein (EGFP) driven by the human RSV -IE (Rous sarcoma virus immediate early) promoter (pRSV) and the 1.4kb ovalbumin promoter (pOV1.4). Selected female WL chicks (4day old) were stimulated with diethylstilbestrol (DES) (Sigma, USA) pellets. The magnum portion of the oviduct was excised from chicks primed with DES. The oviduct epithelial cells were cultured in OVE medium containing DMEM-Ham's F-12 supplemented with beta-estradiol, corticosterone, insulin, FBS and antibiotic - antimycotic solution (Sanders et al., 1985; Kim, 2006). Chicken embryonic fibroblasts (CEFs) and mouse embryonic fibroblasts (MEFs) were cultured in basic medium (DMEM that contained 10% FBS, penicillin, and streptomycin). The chick oviduct epithelial cells were characterized with immunohistochemistry for chicken ovalbumin. To test the specific in vivo expression of the pOV1.4 - EGFP construct, the lentiviral solution was injected into the blood vessel of 53 - hour - incubated chicken embryo.

RESULTS AND DISCUSSION

The DES-treated chicks showed increases in the weight and length of the oviduct compared to control chicks, whereas the body weight decreased slightly in comparison to the control. The primary culture of chicken oviduct tubular gland cells were successful in OVE medium containing steroid hormones. Ovalbumin gene expression of in vitro-cultured oviduct epithelial cells (OVEs) was confirmed by immunofluorescence and immunoblotting of newly synthesized ovalbumin proteins. The pRSV-EGFP lentiviral vector, which lacks tissue-specific regulatory elements, showed expression of reporter genes in chicken OVEs, as well as in MEFs and CEFs. In contrast, CEFs and MEFs did not express the reporter gene when transfected with the pOV1.4-EGFP lentiviral vector. When the pOV1.4-EGFP lentiviral vector was transfected into OVEs, the expression of the reporter proteins was successful in chicken OVEs. And the EGFP expression driven by OV1.4kb promoter was examined in each tissue (oviduct, liver, heart and intestine). The reporter gene expression was detected in the oviduct magnum of the experimental group, but not in the other tissues. These results demonstrated that the 1.4kb ovalbumin promoter regulates tissue-specific expression of transgene.

REFERENCES

1. Harvey AJ, Speksnijder G, Baugh LR, Morris JA, Ivarie R. Expression of exogenous protein in the egg white of transgenic chickens. Nature Biotechnology 2002; 20:396 - 399.
2. Sanders MM, McKnight GS. Chicken egg white genes : Multihormonal regulation in a primary cell culture system. Endocrinology 1985; 116:398-405.
3. Kim SY, Gene manipulation for chicken oviduct epithelial cells by lentiviral vector. Seoul National University : Thesis for Master 2006.