Epidermal growth factor promotes proliferation of chicken germ cells (PGCs) and differentiation potency of embryoid bodies derived from PGCs

Yanqun Wu¹, Chutian Ge¹, Caiqiao Zhang¹

¹College of Animal Sciences, Zhejiang University, Hangzhou 310029, China

The objective of this study was to investigate the effect of epidermal growth factor (EGF) on proliferation of chicken primordial germ cells (PGCs) and the differentiation potency of embryoid bodies (EBs) derived from PGCs. PGCs were isolated from the genital ridge of 3.5-4 day embryos and cultured in Medium 199 supplemented with 5%FCS and 10 ng/ml LIF. The PGCs subcultured on chicken embryonic fibroblast feeder were challenged with EGF alone or in combination with PKC inhibitor H₇ or activator phorbol 12-myristate 13-acetate (PMA). Results from immunocytochemical staining, RT-PCR and Western blotting analysis demonstrated that both PGCs and somatic cells expressed EGF receptor (EGFR). Treatment with EGF at 1-100 ng/ml significantly increased the number and area of PGC colonies in a dose-dependent manner. However, the proliferating effect of EGF on PGCs was obviously attenuated by combined treatment of 10⁻⁶-10⁻⁸ M H₇. Furthermore, treatment of 10 ng/ml EGF enhanced EGFR intensity of PGC colonies and caused a parallel increase in the abundance of EGFR mRNA and protein. The above results indicated that EGF stimulated proliferation of chicken PGCs with involvement of the PKC-mediated system. Meanwhile, purified PGCs colonies were dissociated into small cell clusters less than 20 μm in size and suspended in the 1% agar treated low-adhesive plates with M199 medium in addition with 15% FCS to form the embryoid bodies (EBs). After 4 days in suspension culture, EBs were characterized with PAS, AKP, *c-kit* and SSEA-1 staining, and plated into 96-well plates to attach and differentiate. The differentiated cells were identified by neuron specific enolase and keratin immunocytochemical staining. *In vivo* differentiation by subcutaneous implantation in embryos and RT-PCR analysis was also performed on EBs. Results showed that these 4-day-old EBs could differentiate *in vitro* into various cell types including epithelial cells, neuronal cells and skeletal myoblast cells, form teratomas *in vivo* and express mRNA of a pluripotency specific *Nanog* gene, thus indicating that they have pluripotency. Furthermore, some rhythmic beating cells were also observed in EBs, which beat spontaneously from 20-100 times per minute. In conclusion, cells derived from PGCs of chicken genital ridges are pluripotent embryonic germ cells with the ability to undergo self-renewal and to differentiate into multiple cell types.

Keywords: primordial germ cell, epidermal growth factor, embryoid bodies

Cell Research (2008) 18:s45. doi: 10.1038/cr.2008.135; published online 4 August 2008

Correspondence: Caiqiao Zhang
E-mail: cqzhang@zju.edu.cn