# Effect Of Epithelial Growth Factor On Growth and Differentiation Of Embryonic Neuroepithelial Stem Cells*

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**Abstract Objective** To evaluate the effects of epithelial growth factor (EGF) on the growth and differentiation of embryonic neuroepithelial stem cells. **Methods** Neuroepithelial stem cells were dissociated from the neural tube of embryonic day 11(E11) rats and cultured with EGF and control medium. Micromeasure technique and immunocytochemical staining were employed to detect neurite growth and expression of neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP) of neuroepithelial stem cells. **Results** The neurite length in EGF groups was longer than that in control group at 24h、48h and 72h after culture. Immunohistochemical staining showed that more NSE-positive and GFAP-positive cells were found in EGF groups. **Conclusion** EGF could promote the growth and differentiation of neural tube-derived neuroepithelial stem cells.

**Key words** Neuroepithelial stem cells; Differentiation; EGF

Neural stem cells are a potential transplantation source for neurodegenerative disease. It is known that these cells have the ability to differentiate into different kinds of neurons and glial cells. Many growth factors, like EGF, can regulate the expansion and differentiation of neural stem cells. As an indispensable growth factor, EGF may promote neural stem cells proliferation in serum free culture medium. Furthermore, EGF is capable to induce the differentiation of neural stem cells derived from embryonic cortex, embryonic cerebellum, and from adult brain, adult spinal cord, et al [1-3].

During the process of development, neural plate folds into the cylindrical neural tube, which is the embryonic structure that develops into the brain and spinal cord. Neuroepithelial stem cells in neural tube are the most primitive neural stem cells which can form the whole central nervous system [4]. The expression of EGF and EGF receptor was high in neuroepithelial stem cells [5]. However, to this day, there have been very limited studies on the effects of EGF on these primitive cells. Here, we give a study in that direction.

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## MATERIALS AND METHODS

### Reagents and animals

DMEM/F12 (Gibco), EGF (sigma), new calf serum (Hangzhou Sijiqing Company), NSE and GFAP antibodies were bought from Beijing Zhongshan Biological Product Company. Wistar Pregnant rats were used in experiment.

### Cell culture

Neural tube was obtained from 11 days embryo (post-conception) under compliance with National Institute of Health Guidelines. The tissue of neural tube was dissected in sterile saline, and neuroepithelial stem cells were isolated. Then, cells were cultured in DMEM/F12 medium supplemented with 10% heat-inactivated new calf serum, streptomycin 100mg.$L^{-1}$, benzylpenicilin 100kU.$L^{-1}$, in a 5% $CO_2$ humidified incubator at 37℃, at a density of 100,000cells/ml. The cultured cells were divided into two groups: the medium of experiment group (n=6) containing EGF 60ng/ml, and control group (n=6) only containing normal medium.

### Micromeasure the length of neurite

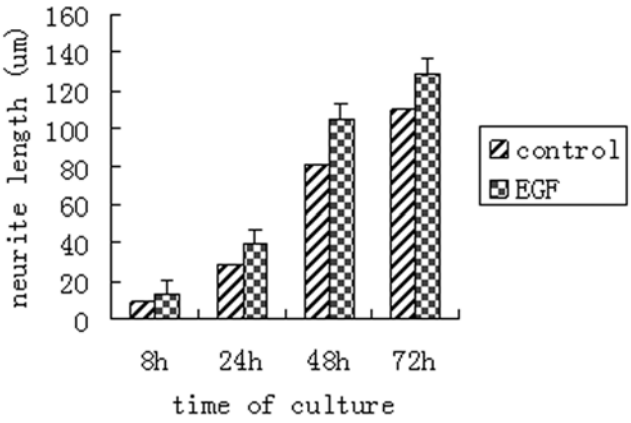


**Fig.1** EGF promotes neurite growth of neuroepithelial stem cell

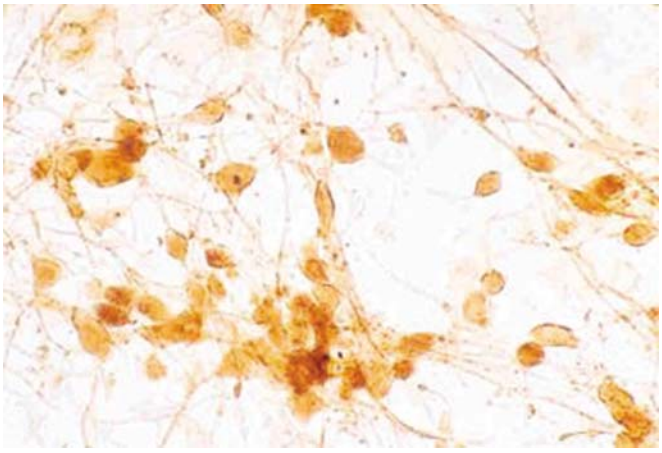

**Fig.2** Embryonic neural stem cells differentiate into NSE–positive cells at 7 days cultured with EGF. (SABC ×200)

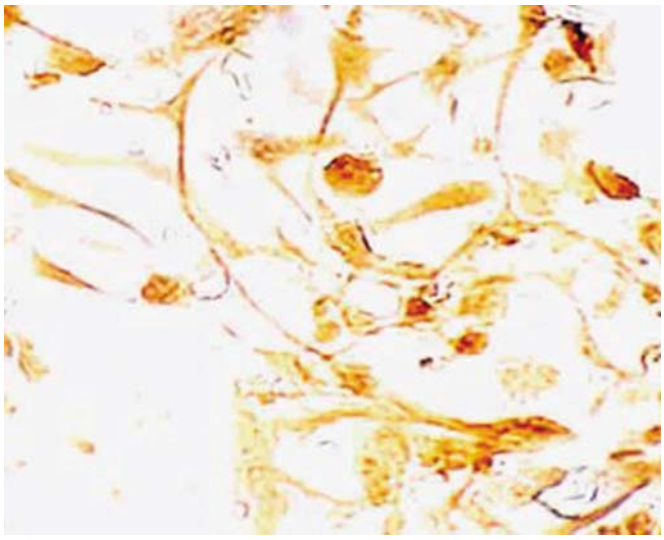

**Fig.3** Embryonic neural stem cells differentiate into GFAP–positive cells at 7 days cultured with EGF. (SABC ×200)

Under microscope, the length of neurite was examined after 8h, 24h, 48h and 72h in vitro culture. 10 visual fields (200×) were selected randomly both in experiment group and control group and the neurite lengths of every cell in the visual fields were measured. Among many neurite of cell body, the longest one was observed.

### Immunohistochemical staining

At 7 days in vitro culture, cells were fixed in 4% paraformaldehyde in PBS for 10 minutes at room temperature, then pretreated with 50% methanol and 3% hydrogen peroxide in PBS for 20 min, washed three times in PBS, and incubated in 10% normal goat serum (NGS) in PBS for 60 min prior to overnight incubation on a shaking platform with the primary antibody (NSE 1:200, or GFAP 1:200). After a 10–min rinse in PBS and two 10–min washes in 5% NGS, sections were incubated in biotinylated secondary antibody (goat–anti–rabbit) at a dilution of 1:200 in 2% NGS in PBS at room temperature for 60~90min. The cells were then rinsed three times in PBS and incubated in avidin–biotin complex (vectastain ABC kit.) in PBS for 60~90 min at room temperature. Following thorough rinsing with PBS and Tris–buffered saline, cultures were developed for 5~10 min in 0.04% hydrogen peroxide and 0.05% 3,3 –diaminobenzidine (sigma) in Tris–buffered saline. Controls with omission of the primary antibody were performed to verify the specificity of staining.

### Statistical analysis

The length of cell neurite was presented as meanSEM. A student t–test was used for statistical analysis.

## *RESULTS*

### EGF promotes neurite growth of neuroepithelial stem cell

Neuroepithelial stem cells were cultured in the presence of EGF or normal medium. In these cultures, EGF may promote more neurite growth than control. Furthermore, the neurite length of EGF groups was all higher than that in control at 24h, 48h and 72h in vitro

culture (all $P$<0.0.5), though there was no difference between two groups at 8h culture ($P$>0.05) ,see Fig.1.

**EGF induces differentiation of Neuroepithelial stem cell**

Immunochemical staining was done to assess the expression of NSE and GFAP, which were the markers of neuron and astrocyte respectively. At 7 days in vitro culture, more NSE-positive cells were detected in EGF groups than that in control groups (Fig.2), with many neurite protruded from cell body. Although GFAP-positive cells could be detected in both groups, there were more GFAP-positive cells in the presence of EGF in culture (Fig.3).

## *DISCUSSION*

Our result indicated that EGF indeed could promote the neurite growth of neuroepithelial stem cell. In the presence of EGF, cell bodies protrude longer neurite than that in control group. Further, we detected more NSE-positive cells and GFAP-positive cells in EGF group. This denotes that a high percentage neurons and glial cells have differentiated from the embryonic neuroepithelial stem cells. In fact, during early embryo development, expressions of both EGF and EGFR are high in neural tube, which suggests that EGF may play an important role in neural development [6]. Our results provide this evidence that EGF indeed promotes growth and differentiation of early embryonic neuroepithelial stem cells in neural tube. Some similar reports said that EGF might stimulate differentiation of neural stem cells derived from other regions [7].

Except for EGF, many other growth factors also have the ability to promote the growth and differentiation of neural stem cells , such as basic fibroblast growth factor (bFGF), brain derived neurotrophic factor, etc. Kelly and his colleagues reported that bFGF-may induce the proliferation of embryonic neural precursors in vitro. And, they also demonstrate that embryonic age is a crucial determinant of the number and differentiation potential of rat embryonic neural precursor cells responding to either EGF and/or bFGF [8]. Growth factor BDNF is known to promote neuronal survival and differentiation [9] and its role in neural stem cell development need to be clarified.

Neural tube is the tissue that will develop into brain and spinal cord. And, Neuroepithelial stem cells are the main component of early neural tube. Besides, these cells keep lively ability to proliferation, and, also can differentiate into all kinds of neurons and glial cells in central nervous system. Thus, these cells may be a potential transplantation source for neurodegenerative disease [10]. More studies should be focused on these cells.

In summary, we conclude that EGF could promote growth and differentiation of neuroepithelial stem cells in neural tube, Here, we only give a very primitive experiment, the mechanisms should be studied further.

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