Specific Expression of Foreign Gene in Postnatal Mouse Glial Cells using Electroporation

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Specific regulation of gene expression in glial cells may facilitate postnatal brain development and gene therapeutic studies. Now, many methods were used to express foreign gene in glial cells specifically, including transgenic techniques and virus system [1,2]. However, transgenic techniques are time-consuming and laborious. Moreover, it cannot express target gene at specific stage. While virus system has many limitations, including low capacity, integrity, potential immunogenicity, lack of tissue specificity, although it is time-saving and time specific. Compared with the above two methods, *in vivo* electroporation has many advantages, including time-saving and labor saving, higher efficiency, higher capacity, nonintegrity, no immunogenicity, better spatiotemporally specificity, more simple procedure, and so on [3,4]. However, such an efficient gene transfer method has not been applied in expressing transgene in glial cells specifically in neonatal brain until now, which may limit its further application in postnatal brain development and gene therapeutic studies. Thus, it is necessary to improve electroporation to be an efficient tool for expressing foreign gene in the postnatal glial cells.

Here, we successfully expressed GFP downstream of the GFAP promoter in the postnatal glial cells using electroporation. Briefly, the GFAP promoter sequence information was obtained from <http://epd.vital-it.ch/>, and the GFAP promoter was amplified from mouse tail genomic DNA (AseI&NheI restriction site were added) by PCR. Meanwhile, the CMV promoter was removed from the

pEGFP-N1 vector by double enzymatic digestion (AseI&NheI, as shown in Figure 1A). Next, the GFAP promoter was inserted into the digested vector, and sufficient recombinant plasmid (GFP is downstream of GFAP promoter) was prepared [5]. As a negative control, mouse nestin promoter was also cloned into pEGFP-N1 to replace the CMV promoter [6,7]. Both of the two recombinant vectors were confirmed by sequencing analysis before next step. Then, the recombinant vectors were transfected into HEK293 cells to confirm the specificity of GFAP promoter, and pEGFP-N1 (CMV promoter) and GFP (Nestin promoter) were used as control. Next, 1 μ L recombinant plasmid solution (3.5 μ g/ μ L in normal saline containing 100 μ g/mL Fast Green) was injected into the lateral ventricle of postnatal mouse brain (P0d, purchased from Experimental Center of Peking University) using mouth-controlled microinjection system as described previously [8,9]. Immediately after injection, five electric pulses at 90V (50 ms duration and 1s interval) were delivered to the operated head using BTX electroporator with tweezer type electrodes (7 mm diameter, BTX, Holliston, MA, USA). Finally, the successfully operated pups were placed on a heat pad until they recovered full mobility and subsequently returned to their mother [10]. The operated brain was examined with fluorescence microscope 24 h after electroporation, and the frozen sections (30 μ m) were prepared.

As shown in Figure 1B, mouse GFAP promoter and Nestin promoter were amplified successfully. PCR analysis (Figure 1C)

Figure 1 The CMV promoter of pEGFP-N1 was replaced by mouse GFAP promoter. (A) The map of pEGFP-N1. (B) The mouse GFAP promoter and Nestin promoter were amplified from mouse genomic DNA. (C) Positive clone was selected by PCR. The GFAP promoter and Nestin promoter were cloned into pEGFP-N1 to replace the CMV promoter.

and sequencing analysis showed that the CMV promoter was replaced by GFAP and Nestin promoter successfully. Transfection analysis on cultured HEK293 cells showed that GFP downstream of GFAP promoter cannot be expressed in the HEK293 cells, but GFP downstream of CMV and Nestin promoter can be expressed in HEK293 cells successfully (Figure 2A). This result showed that the GFAP promoter is a specific promoter. Analysis on the whole electroporated postnatal brain showed that strong GFP-positive signal was observed when using pEGFP-N1(Nestin promoter), while little GFP-positive signal can be found in the whole electroporated brain using pEGFP-N1 (GFAP promoter). Further analysis on frozen sections showed that GFP downstream of GFAP promoter was expressed successfully in the postnatal brain, although the GFP signal is less strong than that in the operated brain using Nestin promoter-controlled plasmid. The GFP-positive signal was mainly located at ventricular zone (Figure 2B). Considering the specificity of GFAP promoter, we think that the GFP was expressed in the glial cells. However, the proper enhancer should be found out, and the optimal plasmid concentration should be verified.

In summary, we successfully established the method for expressing foreign gene in postnatal glial cells using *in vivo* electroporation and may promote it to be a more useful tool to explore the gene function during the postnatal brain development and perform gene therapeutic studies.

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Figure 2 GFAP promoter can specifically control the expression of foreign gene in the glial cells. (A) GFP downstream of mouse GFAP promoter cannot be expressed in HEK293 cells, while the GFP controlled by CMV and Nestin promoter can be expressed in HEK293 cells, bar = 100 μm. (B) Less GFP-positive signal can be observed in the electroporated brain using GFAP promoter-controlled vector compared with that of Nestin-controlled vector, bar = 200 μm. (C) GFP downstream of mouse GFAP promoter can be expressed in glial cells located at VZ using *in vivo* electroporation. The nucleus was stained with DAPI. Scale bars = 80 μm.

References

1. Malatesta P, Appolloni I, Calzolari F. Radial glia and neural stem cells. *Cell Tissue Res* 2008;**331**:165–178.
2. Georg VJ, Nadine M, Claudia BK, et al. Glial promoter selectivity following AAV-delivery to the immature brain. *PLoS ONE* 2013;**8**:1–11.
3. Vry JD, Martinez PM, Losen M, et al. In vivo electroporation of the central nervous system: a non-viral approach for targeted gene delivery. *Prog Neurobiol* 2010;**92**:227–244.
4. Saito T. In vivo electroporation in the embryonic mouse central nervous system. *Nat Protoc* 2006;**1**:1552–1558.
5. Lawlor PA, Bland RJ, Mouravlev A, et al. Efficient gene delivery and selective transduction of glial cells in the mammalian brain by AAV serotypes isolated from nonhuman primates. *Mol Ther* 2009;**17**:1692–1702.
6. Kawaguchi A, Miyata T, Sawamoto K, et al. Nestin-EGFP transgenic mice: visualization of the self-renewal and multipotency of CNS stem cells. *Mol Cell Neurosci* 2001;**17**:259–273.
7. Robert DB, Muriel AC, Helen BT, et al. Nestin promoter/enhancer directs transgene expression to precursors of adult generated periglomerular neurons. *J Comp Neurol* 2004;**475**:128–141.
8. Boutin C, Diestel S, Desoeuvre A, et al. Efficient in vivo electroporation of the postnatal rodent forebrain. *PLoS ONE* 2008;**4**:1–6.
9. Ding XF, Zhao YQ, Hu ZY, et al. Efficient gene transfer into neonatal mouse brain using electroporation. *Neurochem Res* 2012;**37**:1392–1398.
10. Ding XF, Zhao YQ, Fan WH, et al. Gene transfer into the both sides of postnatal cerebral cortex using tweezer electrode. *CNS Neurosci Ther* 2013;**19**:197–198.