武强,李露斯,范文辉,杨忠.小鼠胚胎神经干细胞的体外培养及增强型绿色荧光蛋白基因转染研究[J].中国康复医学杂志,2006,(10):877~

小鼠胚胎神经干细胞的体外培养及增强型绿色荧光蛋白基因转染研究

点此下载全文

武强 李露斯 范文辉 杨忠

[1]第三军医大学附属西南医院神经内科,重庆400038 [2]第三军医大学神经生物教研室,重庆400038

基金项目：国家自然科学基金资助项目（30100058）

DOI：

摘要：

目的：探索小鼠胚胎神经干细胞（neural stem cells. NSCs）的体外培养，探讨NSCs作为基因靶细胞. 以及增强型绿色荧光蛋白（enhanced green fluorescent protein，EGFP）标记NSCs的可行性。方法：胚胎小鼠脑组织分离神经干细胞，无血清细胞培养，免疫细胞荧光染色技术鉴定。NucleofectorTM转染仪将增强型绿色荧光蛋白基因转导入NSCs，用G418筛选，在荧光显微镜下观察并挑选EGFP表达最强的克隆. 并做免疫荧光鉴定及诱导分化细胞的鉴定。结果：从胚胎小鼠脑组织分离的细胞具有连续克隆能力。表达神经上皮干细胞蛋白，诱导分化后的细胞表达神经细胞和星形胶质细胞特异性的蛋白。增强型绿色荧光蛋白报告基因转染神经干细胞后能高效表达，且不影响其增殖、分化能力。结论：小鼠胚胎神经干细胞能够在体外适宜的条件下进行长期培养；神经干细胞可直接作为基因靶细胞；转染EGFP的神经干细胞表达稳定且对NSCs的增殖和分化无明显影响。

关键词：神经干细胞 增强型绿色荧光蛋白 转染

A study on culture of mice embryonic neural stem cells in vitro and transfection of enhanced green fluorescent protein

Download Fulltext

WU Qiang LI Lusi FAN Wenhui et al.

Dept. of Neurology,Southwest Hospital,Tbe Third Military Medical University,Chongqing 400038

Fund Project:

Abstract:

Objective:To explore the culture of mice embryonic neural stem cells(NSCs) in vitro and the feasibility of NSCs being gene target cells and enhanced green fluorescent protein(EGFP) labeling NSCs.Method:The NSCs were isolated from brain tissue of embryonic mice,then cultured in serum free medium,and identified by immunocytochemistry. PEGFP were transferred into NSCs by NucleofectorTM. After selected by G418,the colonies with the best expression of EGFP were observed and selected using fluorescence microscope,and identified with immunocytochemistry technology.Result:The neural stem cells isolated newborn mice had the potential to form clones,express neuroepithelial stem cell protein(nestin) and differentiate into mature neurons and astrocytes. Moreover,enhanced green fluorescent protein vector could be efficiently transfected into this cell line. The capacity of self-renewal,proliferation and pluripotentiality of NSCs-EGFP were consistent with those of NSCs.Conclusion:The NSCs originated from brain tissue of embryonic mice can be cultured in vitro under appropriate conditions,and it can be directly used as gene target cells. Moreover,the biological features of the NSCs expressing EGFP were consistent with those of NSCs,which can be further applied for the transplantation study of labeling cells.

Keywords:neural stem cells enhanced green fluorescent protein transfection

查看全文 查看/发表评论 下载PDF阅读器