

· 论 著 ·

新生大鼠大脑皮质神经干细胞的分离培养与胆碱能神经元的鉴定

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[摘要] **目的:** 体外分离和培养新生大鼠大脑皮质神经干细胞并进行鉴定, 为用于脑梗死动物模型梗死区移植细胞作准备。

方法: 分离新生大鼠皮质神经干细胞, 进行体外培养, 使用免疫细胞荧光染色技术对细胞的特性进行鉴定, 并对细胞的胆碱能特征进行鉴定。 **结果:** 获得了 Nestin 阳性的神经干细胞, 其分化后可获得 MAP-2、GFAP 和 CNPase 阳性的神经元、星形胶质细胞和寡突胶质细胞; 通过胆碱能鉴定发现大脑皮质干细胞可分化为胆碱能神经元。 **结论:** 体外分离和培养的大脑皮质神经干细胞具有增殖分化的能力, 并可以分化为胆碱能神经元, 有望应用于皮质损伤后认知障碍及运动障碍的细胞移植治疗。

[关键词] 神经干细胞; 皮质; 胆碱能神经元; 分离; 培养

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Isolation and proliferation of neural stem cells from brain cortex of new born SD rats and identification of cholinergic neurons

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[ABSTRACT] **Objective:** To isolate and culture neural stem cells(NSCs) from brain cortex of new born SD rats *in vitro* and to identify the cholinergic neurons, providing data for cell transplantation to the infarcted area in cerebral infarction animal model.

Methods: Neurons in cortex of new born rats were isolated and cultured *in vitro*. Immuno-fluorescence staining was used to study the character of cells and to determine cholinergic neurons. **Results:** Nestin positive stem cells were obtained and became MAP-2, GFAP-2 and CNPase positive cells(neurons, astrocytes and oligodendrocytes). Cholinergic neurons were obtained from stem cells from brain cortex through cholinergic identification. **Conclusion:** NSCs isolated and cultured *in vitro* from brain cortex have the ability of proliferation and differentiation. NSCs can differentiate into cholinergic neurons, so they may be used as graft for the cell transplantation therapy in cognitive handicap and dyskinesia after cortex infarction.

[KEY WORDS] neural stem cells; cortex; cholinergic neuron; isolation; culture

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脑梗死是威胁人类健康的一大疾病。脑梗死 3~6 h 后, 缺血损伤就会导致神经元不可逆性死亡。皮质梗死是常见且预后不良的梗死类型, 由于是神经元本身的损伤, 因此比皮质下梗死导致的传导束性损伤所致的运动障碍更为严重, 且多伴有认知和行为学的异常, 成为脑梗死致残率高的主要原因^[1]。目前, 临床上仍缺乏对脑梗死后遗症的有效治疗^[2]。神经干细胞移植是近年来兴起的一种神经替代疗法, 目前用以治疗帕金森病、多发性硬化、脑卒中、运动神经元病等中枢神经系统疾病已开始起步, 但尚未见将皮质来源的神经干细胞移植于梗死皮质的进一步报道^[3~6]。

本实验分离培养新生 SD 大鼠大脑皮质运动区的神经干细胞, 对其自我更新和多潜能的特点进行了鉴定, 并鉴定其是否具有胆碱能神经元的特性, 为进一步为脑梗死皮质缺血损伤模型的细胞移植作准备。

1 材料和方法

1.1 试剂和动物 B27、DMEM/F12(1:1)、Neurobasal Medium、DMEM 为 Gibco 公司产品。胎牛血清和小牛血清为杭州四季青公司产品, GFAP 抗体购自 DAKO 公司, 多聚赖氨酸、碱性成纤维细胞生长因子(bFGF)、Hoechst33258、其他各种一级抗体和二级抗体购于 Sigma 公司。出生 1 d 的新生 SD 大鼠购自第二军医大学实验动物中心。

1.2 神经干细胞的分离培养 切取大鼠大脑, 解剖液漂洗, 体视镜下剥离脑膜后, 切取大脑皮质, 将所取皮质组织剪碎, 用火焰刨光的玻璃吸管反复吹打,

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区的神经元。且我们通过胆碱酯酶抗体染色鉴定取自大鼠大脑皮质的神经干细胞经体外自然分化可以得到胆碱能神经元,具有皮质神经元的特性,有望用于治疗皮质梗死后出现的认知、行为障碍及运动障碍,从而为进一步的移植工作提供实验依据。

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A novel diagnostic marker, p28(GANK) distinguishes hepatocellular carcinoma from potential mimics

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[ABSTRACT] PURPOSE. To investigate the sensitivity, specificity, and spatial distribution of the product of p28 gene (p28 (GANK) protein) in human hepatocellular carcinoma (HCC) and nonhepatocellular carcinomas in relation to immunostaining with Cytokeratin 18 (CK18), alpha-fetoprotein (AFP), and Hepatocyte paraffin 1 (HepPar1). METHOD. In this retrospective study, formalin-fixed paraffin-embedded tissues from 24 HCCs, five intrahepatic cholangiocarcinomas (ICC), five combined hepatocellular cholangiocarcinomas (C-HCC-CC) and nine metastatic hepatic carcinomas (MHC) were immunostained for p28(GANK) as well as CK18, AFP and HepPar1. Only cases with more intensified staining in carcinoma contrast to the adjacent liver tissues were accepted as positive. RESULT. In HCC, p28(GANK) was expressed restrictively in hepatocytes of both para-lesion and carcinoma liver tissues, while absent in the bile duct epithelial cells, Kupffer cells, and other interstitial cells. The positive staining of p28(GANK) was noted in 16 (66.7%) specimens of HCC and three (60.0%) specimens of C-HCC-CC, and no specific lesion staining was found in all tested specimens of ICC and MHC. Sensitivity and specificity for hepatocyte-originated carcinoma were, respectively, 65.5% and 100% for p28(GANK), 79.3% and 85.2% for CK18, 20.7% and 100% for AFP, 79.3% and 92.0% for HepPar1. CONCLUSION. The hepatocytic staining for p28(GANK) is clearly useful in differentiating hepatocyte-originated carcinoma from non-HCC. p28(GANK) may be used as an ancillary marker for the diagnosis of HCC.

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