

小鼠胚芽干细胞同种移植治疗慢性肝脏损伤的实验研究

Experimental Research of the Treatment of Chronic Damaged Liver by Transplantation of Mouse Embryonic Germ Cells

刘晓萍/LIU Xiao-ping¹,高英茂/GAO Ying-mao²,邴鲁军/BING Lu-jun²,郭菲菲/GUO Fei-fei¹,徐 璐/XU Luo¹

1. 青岛大学医学院组织胚胎学教研室, 山东青岛 266021
2. 山东大学医学院组织胚胎学教研室, 济南 250012

1. Department of Histology and Embryology, Medical School, Qingdao University, Qingdao 266021, Shandong Province, China
2. Department of Histology and Embryology, Medical School, Shandong University, Jinan 250012, China

[摘要] 将分离和体外扩增的小鼠胚芽干细胞,用 BrdU 标记后,经尾静脉同种移植于 CCL4 制备慢性肝损伤小鼠体内。分别于移植后 2 w、4 w 取出肝脏,进行连续冰冻切片,用免疫组织化学和免疫荧光双重染色检测移植细胞在受体小鼠肝内的存活、分布和肝细胞特异性白蛋白的表达;用 PAS 染色检测移植细胞的糖原代谢;HE 染色观察移植动物肝组织的结构变化。探索胚芽干细胞在慢性损伤肝脏中的存活、分化和治疗作用。实验结果表明:胚芽干细胞经静脉移植后可以进入损伤肝脏中,并分化为肝样细胞和肝小叶内的其他细胞,并明显改善损伤肝脏的组织结构,具有较好的治疗作用。

[关键词] 胚芽干细胞;细胞移植;慢性肝脏损伤;小鼠

[文献标识码] A

[中图分类号] R332

[文章编号] 1000-7857(2006)07-0054-05

Abstract: The EGCs were proliferated and labeled with 5-bromo-2-deoxyuridine (BrdU), then transplanted into the acute damaged liver by CCL4 through tail vein. 2 and 4 weeks later, the liver was extracted and 10μm-cryostat continuous sections were obtained. The existing and differentiation of the transplanted cells were identified by immunohistochemistry, immunofluorescence double staining and PAS histochemistry for BrdU and hepatic-specific albumin (ALB), and the glycogen. The BrdU positive cells were found in the chronic damaged liver, some cells were BrdU and ALB double positive and PAS positive staining, the structures of damaged liver were also significantly improved. We demonstrated that the transplanted EGCs could be incorporated into the chronic damaged liver and differentiated into hepatocytes in this microenvironment, and its transplantation also could compensate for chronic liver failure. These results suggest that EGCs can be used for potential cellular hepatoplasty to treat damaged liver.

Key Words: embryonic germ cells; cell transplantation; damaged liver; mouse

Document Code: A

CLC Number: R332

Article ID: 1000-7857(2006)07-0054-05

1 引言

细胞替代方法是将细胞移植入体内,替代因退变、损伤、基因缺陷或自身免疫而受损的细胞,重构原组织器官的结构和功能。用细胞替代方法治疗疾病具有广阔的应用前景。但是,细胞来源的缺乏和免疫排斥是制约细胞替代方法被广泛应用的两大障碍。干细胞具有体外大量增殖和多向分化潜能,可为细胞移植提供无限的细胞来源,使细胞移植技术的广泛应用成为可能。应用干细胞移植治疗肝损伤是当前干细胞研究的热点之一。

胚芽干细胞(embryonic germ cells, EGCs)又称原始生殖细胞(primordial germ cells,PGCs)具有多向分化潜能,增殖力强、免疫

源性低,作为细胞移植的种子细胞有不可比拟的优势。本研究将胚芽干细胞经静脉移植于 CCL4 造成慢性肝损伤的模型小鼠体内,观察移植细胞在慢性损伤肝内的存活和分化情况,为肝损伤的干细胞移植治疗提供实验依据。

2 材料与方法

2.1 动物

昆明种小鼠,由山东大学实验动物中心提供。晚 6:00-8:00 雌雄鼠 2:1 合笼,第 2 天早 8:00 检查阴栓,阳性者记为孕 1 d。

收稿日期: 2005-05-06

基金项目: 山东省科技厅攻关计划项目(01BB1DADA1)

作者简介: 刘晓萍,女,青岛市青岛大学医学院,教授,主要从事干细胞的定向诱导分化及其机理的研究工作;

E-mail: xpliu572001@yahoo.com.cn

高英茂(通讯作者),男,山东大学医学院组织胚胎学教研室,教授,主要从事胚胎畸形学和干细胞研究;E-mail: he@sdu.edu.cn

2.2 试剂

基础培养基为含 15% 胎牛血清的 1:1 DMEM/F12 (Gibicol 公司); 兔抗小鼠白蛋白抗体 (anti-albumin, ALB) (北京中山公司); BrdU 和 BrdU 单克隆抗体 (5-bromo-2-deoxyuridine, BrdU) (美国 Sigma 公司); Cy3 标记抗鼠 IgG 和 FITC 标记的抗兔 IgG (Sigma 公司)。

2.3 胚芽干细胞的扩增和标记

取孕 11 d 的昆明种小鼠胚胎。无菌条件下分离生殖腺嵴, 置含 D-Hanks 液的平皿中剪碎, 然后加入消化液, 37℃ 消化 10 min, 用含 15% 胎牛血清的 DMEM/F12 培养液调细胞浓度为 $1 \times 10^5/\text{ml}$, 接种培养瓶中 37℃ 5% CO_2 培养, 进行体外扩增。传至第 3 代, 加入 BrdU 培养 72 h, 以标记干细胞。

2.4 慢性肝损伤动物模型的制备, 动物分组和处理

2 月龄昆明种小鼠 38 只, 雌雄各半, 配制 1% 的 CCL_4 油剂, 以 0.15 ml 的剂量腹腔注射, 每周 2 次, 连续注射 5 w。将 38 只模型鼠随机分为 2 组: 非移植对照组 (C-C) 24 只; 干细胞移植组 (C-T) 14 只。

慢性肝损伤移植组模型小鼠于第 8 次注射后 48 h, 通过尾静脉将细胞密度为 $1 \times 10^5/\text{ml}$ 的 BrdU 标记的胚芽干细胞移植于模型小鼠体内, 每只注射 0.2 ml。慢性损伤对照组经尾静脉注入等量的培养液。

干细胞移植组动物分别于移植后 2 W、4 W 时取出肝脏, 每个时间点取材 7 只。非移植对照组动物, 分别于最后一次 CCL_4 腹腔注射后 2 d、2 W 和 4 W 取出肝脏。连续冰冻切片, 厚 10 μm 。冷丙酮固定 10 min 后, 4℃ 保存, 备用。

2.5 BrdU 免疫细胞化学染色

附有 BrdU 标记后胚芽干细胞盖片和移植小鼠的肝组织切片入 1:1 甲酰胺/2×SSC, 65℃ 孵育 2 h; 2N HCl 浸泡 60 min 使 DNA 变性; 0.1 mol/L 硼酸缓冲液 (pH=8.5) 中和 10 min, PBS 冲洗; 3% H_2O_2 甲醇溶液孵育 30 min, 以消除内源性过氧化物酶的活性; 滴加 BrdU (1:100) 单克隆抗体, 阴性对照用 0.01 mol/L PBS 代替 BrdU 单抗, 4℃ 孵育过夜; 滴加生物素化的二抗, 37℃ 孵育 30 min; 滴加辣根过氧化物酶标记的链霉卵白素, 37℃ 孵育 30 min; DAB 避光显色; 常规梯度酒精脱水, 中性树胶封片。

2.6 BrdU 和 ALB 免疫荧光双重染色

DNA 变性处理步骤同前; 正常山羊血清封闭; 弃血清, 滴加一抗 (鼠抗 BrdU 和兔抗 ALB), 4℃ 孵育过夜; 滴加 Cy3 标记的抗鼠 IgG 和 FITC 标记的抗兔 IgG, 室温孵育 2 h; 封片; 激光共聚焦显微镜下观察, 拍照。每组染色均设有空白对照, 以 0.01 mol/L PBS 代替一抗。

2.7 PAS 染色

切片入 1% 过碘酸水溶液 5 min, 水冲洗; Schiff 试剂 15 min, 亚硫酸盐溶液洗 3 次, 水洗, 吸干; 从 95% 的酒精开始梯度脱水, 二甲苯透明, 中性树胶封片。镜下糖原颗粒呈紫红色着色。以胰淀粉酶处理片作为阴性对照。

2.8 BrdU 阳性标记率和双阳性细胞的计算

BrdU 免疫细胞化学染色, 细胞核呈圆形或椭圆形、黄褐色的细胞, 为 BrdU 阳性标记细胞; 计数细胞总数和 BrdU 阳性标记细胞数; 计算细胞标记率。

BrdU 和 ALB 双阳性细胞为移植后向肝细胞分化的细胞; 在 40× 倍显微镜下, 每张切片随机选择 7 个视野, 在目镜测微计下计数每 0.4 mm² 肝组织区域内, 双阳性细胞的数量; HE 染色后, 镜下 0.4 mm² 肝组织区域内肝细胞的数量, 取均值。折算成每平方毫米 BrdU 阳性细胞数和肝细胞数。

2.9 统计学分析

数据以均数 (X) ± 标准差 (S) 表示, 应用 SPSS 12 统计软件进行 *t* 检验及 *t'* 检验 (方差不齐时)。

3 结果

3.1 胚芽干细胞的 BrdU 标记率

BrdU 标记阳性细胞呈集落样聚集, 标记率达 90% 以上 (图 1)。

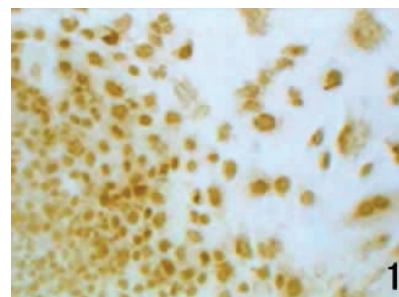


图 1 BrdU 标记胚芽干细胞图 (×400)

注: EGC 标记 72 h, 可见 BrdU 阳性, 细胞核呈黄褐色斑点状聚集呈集。

Fig. 1 The structure of EGCs labeled with BrdU (×400)

Immunohistochemistry showed the embryonic germ cells (EGCs) labeled with BrdU for 72 h. The BrdU positive cell nucleus were yellow brown motting.

3.2 非移植对照组小鼠肝组织切片 HE 染色检测

与正常小鼠肝脏组织切片相比 (图 2), 非移植肝损伤模型小鼠肝脏中肝细胞明显减少, 有些细胞呈空泡变性; 血管周围大量炎性细胞浸润; 汇管区纤维组织增生, 并向小叶内延伸, 小叶结构凌乱 (图 3)。2 w 后, 模型小鼠肝内仍可见炎性细胞浸润和局造性损伤 (图 4)。4 w 时慢性损伤鼠肝小叶结构基本正常, 有的血管周围仍有少量炎性细胞 (图 5)。

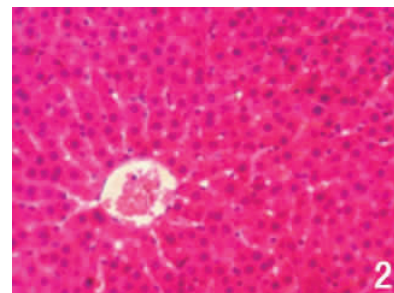


图 2 正常鼠肝脏 HE 染色 (×400)

注: 可见肝细胞索排列规则, 细胞核圆居中央, 核膜清晰, 肝细胞核染色浅, 核仁清楚。

Fig. 2 The structure of mouse liver by HE staining (×400)

Hepatocytes cord arranged regularly, the hepatocyte nucleus were round, center located and light staining in HE.

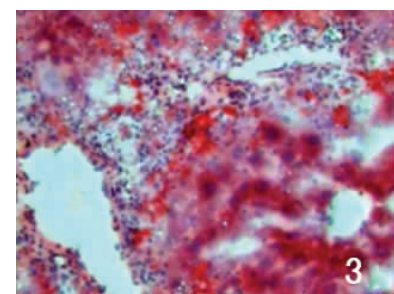


图 3 慢性损伤模型鼠肝细胞变性 (×200)

注: 可见广泛炎性细胞浸润, 纤维增生, 肝小叶结构破坏。

Fig. 3 The hepatocytes of the model mouse livers treated with CCL_4 were denature and necrosis (×200)

Inflammatory cell infiltration, fiber hyperplasia, destroyed hepatic lobules by HE staining.

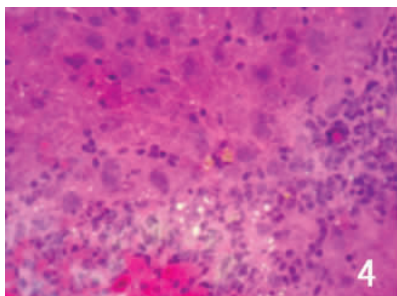


图4 慢性肝损伤 2 W 后模型鼠肝组织切片($\times 400$)
注:肝内仍见局造性损伤区及炎性浸润。
Fig. 4 2 weeks later some damaged hepatocytes of the model mouse livers($\times 400$)

Inflammatory cells in the model mice liver still could be observed.

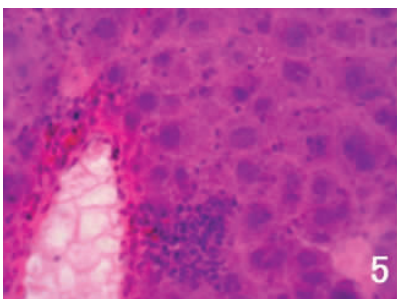


图5 慢性肝损伤 4 W 后模型鼠肝组织切片($\times 400$)
注:肝小叶结构基本修复,仅见血管周围炎性浸润。
Fig. 5 4 weeks later the liver of the damaged model mice($\times 400$)
Nearly normal showed by HE staining.

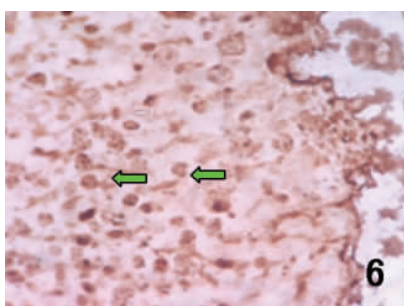


图6 慢性损伤模型鼠移植 2 W 时肝组织切片($\times 400$)
注:肝小叶内可见呈棕褐色的 BrdU 阳性细胞核大小不等
Fig. 6 The transplanted EGCs with a large BrdU positive brown nuclear ($\times 400$)
They were observed in the receptor liver in 2 weeks stained by immunohistochemistry.

3.3 移植 2 W 后肝组织切片的免疫组化染色、免疫荧光双染、PAS 和 HE 染色检测

可见 BrdU 阳性细胞散在分布,细胞核呈斑点状黄褐色,有些阳性细胞核的体积较大而圆,有的细胞核着色深、体积小(图 6);免疫荧光双重染色可见,细胞核大而圆的 BrdU 阳性细胞也呈 ALB 阳性着色;小而致密的 BrdU 阳性核多位于肝细胞边缘,ALB 免疫染色呈阴性(图 7)。与非移植组相比,纤维增生基本消失,肝小叶的结构较规则,肝细胞体积较大,细胞核大而松散、管腔周围仍有少量炎性细胞浸润(图 8)。PAS 染色显示移植组肝糖原颗粒较多,着色深(图 9)。

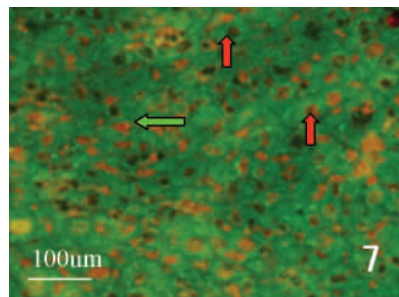


图7 慢损模型鼠移植 2 W 时肝组织切片
注:免疫荧光双染可见 BrdU(红色)和 ALB(绿色)双阳性细胞(↑)和 BrdU 单阳性细胞(↓)。
Fig. 7 The livers transplanted with EGCs for 2 weeks
The double positive cells (↑)with BrdU (red colour) and ALB (green colour) were observed with immunofluorescence double staining, some cell were BrdU single positive (↓).

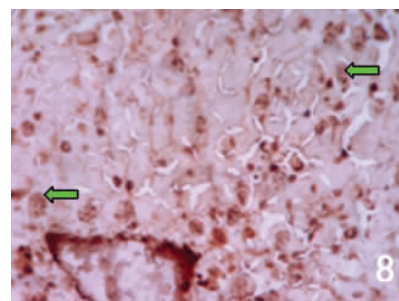


图8 慢损模型鼠移植 4 W 时肝组织切片($\times 400$)
注:有大而圆 BrdU 阳性核(↓)的肝样分化细胞较 2 W 减少。
Fig. 8 The livers transplanted with EGCs for 4 weeks ($\times 400$)
The hepatic differentiated cells with large round BrdU positive nucleus (↓) in the liver with EGCs transplantation for 4weeks were less than that for 2 weeks.

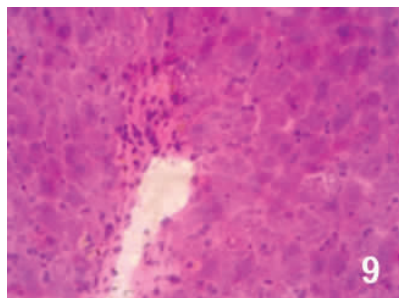


图9 移植 2 W 时慢损鼠的肝细胞切片($\times 100$)
注:肝小叶清晰,纤维增生消失,炎性浸润细胞明显减少,肝细胞核大而松散。
Fig. 9 The hepatic lobule with EGCs transplantation for 2 weeks ($\times 100$)
They were clear. Fiber hyperplasia disappeared. Inflammatory cells were a few, but some hepatic nucleus were large and the chromatin were scatter.

3.4 移植 4 W 后肝组织切片的免疫组化、免疫荧光双染、PAS 和 HE 染色检测

移植 4 W 时,肝组织内 BrdU+细胞数量与 2 W 时相比无显著减少(图 10)。此时,肝小叶结构清晰,肝细胞和细胞核的体积均较大,仅在血管周围可见少量炎性细胞(图 11);PAS 染色可见肝糖原着色变浅、颗粒细小、分布均匀(图 12)。

气候变化对血吸虫病空间分布的潜在影响

Potential Impact of Climate Changes on Spatial Distribution of Schistosomiasis in China

彭文祥/PENG Wen-xiang, 张志杰/ZHANG Zhi-jie, 庄建林/ZHUANG Jian-lin, 周艺彪/ZHOU Yi-biao, 姜庆五/JIANG Qing-wu

复旦大学公共卫生学院流行病学教研室, 上海 200032

Department of Epidemiology, School of Public Health, Fudan University, Shanghai 200032, China

[摘要] 应用地理信息系统技术,探讨了划分血吸虫病流行区与非流行区的较好气温指标,并用其研究预测气候变化对血吸虫病空间分布的潜在影响。研究表明,随着全球气候变暖以及中国南水北调工程的实施,若钉螺扩散,且适宜钉螺孳生的其他条件满足,则存在钉螺北移的风险。若干年后,全球气候变暖对钉螺的分布和血吸虫病的防治将带来挑战,对此应给予足够的重视。

[关键词] 气候变化;钉螺;气温;血吸虫病;地理信息系统

[中图分类号] R532.21,P464

[文献标识码] A

[文章编号] 1000-7857(2006)07-0058-03

Abstract: The better index of air temperature that can be used to distinguish the epidemic area from non-epidemic area of schistosomiasis using Geographic Information System (GIS), and the potential impacts of climate changes on the spatial distribution of schistosomiasis, were studied. The result shows that *Oncomelania Hupensis* has the possibility to move north if the other conditions that affect the survival of *Oncomelania Hupensis* meet, with the global climate warming and the carry-out of south-north water transfer project in China. Global climate warming will cause great challenges for the schistosomiasis control and the distribution of the *Oncomelania Hupensis* in the future.

Key Words: climate changes; *Oncomelania Hupensis*; air temperature; schistosomiasis; Geographic Information System

CLC Numbers: R532.21, P464

Document Code: A

Article ID: 1000-7857(2006)07-0058-03

收稿日期: 2006-05-10

基金项目: 国家自然科学基金重大项目(30590374), “十五”国家科技攻关项目(2004BA718B04)

作者简介: 彭文祥,男,上海市医学院路138号复旦大学公共卫生学院流行病学教研室,高级工程师,研究方向为流行病学与卫生信息学;E-mail: gisrsgps@163.com

姜庆五(通讯作者),男,上海市医学院路138号复旦大学公共卫生学院流行病学教研室,教授,研究方向为流行病学;E-mail: jiangqw@shmu.edu.cn

- [3] YIN L, et al. Derivation, characterization, and phenotypic variation of hepatic progenitor cell lines isolated from adult rats [J]. *Hepatology*, 2002, 35(2): 315-324.
- [4] WANG X, MONTINI E, Al-Dhalimy M, et al. Kinetics of liver repopulation after bone marrow transplantation [J]. *Am J Pathol*, 2002, 16(2): 565-574.
- [5] BUCHER N L R, FARMER S. Liver growth and repair [M]. Strain AJ, Dichtl A. eds, London: Chapman and Hall, 1993.
- [6] FELDMANN G. Liver transplantation of hepatic stem cells: Potential use for treating liver disease [J]. *Cell Biol Toxicology*, 2001, 17(2): 77-85.
- [7] ZIMMERMANN A. Liver regeneration: The emergence of new pathways [J]. *Med Sci Monit*, 2002, 8(3): RA53-63.
- [8] CHINZEI R, TANAKA Y, SHIMIZU -SAITO K, et al. Embryoid - body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes [J]. *Hepatology*, 2002, 36: 22-29.
- [9] THEISE N D, BADVE S, SAXENA R, et al. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced

myeloablation [J]. *Hepatology*, 2000, 31: 235-340.

- [10] MONGA S P, et al. Expansion of hepatic and hematopoietic stem cells utilizing mouse embryonic liver explants [J]. *Cell Transplant*, 2001, 10(1): 81-89.
- [11] KOLLET O, SHIVTIEL S, CHEN YQ, et al. HGF, SDF -1, and MMP (are involved in stress-induced human CD34+ stem cell recruitment to the liver [J]. *J Clin Invest*, 2003, 112(1): 160-169.
- [12] YIJUN Y, YEW K L, MANUEL S T, et al. AFP +, ESC -Derived cells engraft and differentiate into hepatocytes *in vivo* [J]. *Stem Cells*, 2002, 20: 338-346.
- [13] SHUJI T, ISAO S, NAOKI Y, et al. An *in vivo* model for monitoring trans-differentiation of bone marrow cells into functional hepatocytes [J]. *J Biochem*, 2003, 134(4): 551-558.
- [14] GOLDEN M L et al. Having it all? Stem cells, haematopoiesis and lymphopoiesis in adult human liver [J]. *Immunol Cell Biol*, 2002, 80(1): 45-52.
- [15] DOUAGI I. Identification of the earliest prethymic bipotent T/NK progenitor in murine fetal liver [J]. *Blood*, 2002, 99(2): 463-471.

(责任编辑 王 芷)