

大鼠肝纤维化中细胞外信号调节激酶的作用

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Extracellular signal-regulated kinase in liver fibrogenesis of rat

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Abstract

AIM: To explore the role of ERK signal transduction pathway in the pathogenesis of liver fibrosis via investigating the expression and distribution of ERK1 in rats with liver fibrosis.

METHODS: Liver fibrosis model of rats were made by subcutaneously injecting with CCl₄. Thirty-two male SD rats (weight 250-300 g) were randomly scarified at 1, 4 and 8 weeks after injection of CCl₄ respectively, and their liver were used to detect ERK1 expression by immunohistochemical staining.

RESULTS: The expression of ERK1 in rats after injection with CCl₄ were found chiefly in hepatic stellate cells(HSC) and all significantly higher than those in normal rats ($P < 0.05$). Moreover, it presented with a progressive tendency for the expression of ERK1 in rats respectively at 1st, 4th and 8th week after injection with CCl₄ ($P < 0.05$).

CONCLUSION: The activation of ERK signal transduction pathway enhances HSC proliferation, and it may play an important role in liver fibrogenesis in rat.

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摘要

目的: 通过研究大鼠肝纤维化模型肝组织中细胞外信号调节激酶(extracellular signal-regulated kinase, ERK)的表达和分布规律, 初步探讨 ERK 信号传导通路在肝纤维化发病机制中的作用.

方法: ♂ SD 大鼠 32 只, 质量 250-300 g, 皮下注射 CCl₄ 制备大鼠肝纤维化模型, 分别于注射 CCl₄ 后 1, 4, 8 wk 处理动物, 采用免疫组织化学方法检测肝组织中 ERK1 的表达及分布.

结果: ERK1 主要表达于肝星状细胞中. CCl₄ 注射诱导后, 大鼠肝组织中 ERK1 的表达较正常对照明显增强 ($P < 0.05$). 且 CCl₄ 注射 1, 4, 8 wk 组肝组织中 ERK1 的表达强度呈明显的逐级递增的趋势 ($P < 0.05$).

结论: ERK 信号传导通路的激活促进肝星状细胞的活化增生, 可能与大鼠肝纤维化的发生发展有关.

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0 引言

细胞外信号调节激酶(extracellular signal-regulated kinase, ERK)可能与器官纤维化的发生有关^[1-4]. 但 ERK 通路是否参与肝纤维化过程尚无明确报道. 我们应用免疫组织化学方法对 CCl₄ 诱导的肝纤维化大鼠肝组织进行检测, 初步探讨该信号转导通路在肝纤维化发生中的作用如下.

1 材料和方法

1.1 材料 健康 ♂ SD 大鼠(由华中科技大学实验动物中心提供)32 只, 质量 200-300 g, 随机均分为 1, 4, 8 wk 及正常对照 4 组. 主要试剂: 兔抗人 ERK1 抗体和 ABC 二抗试剂盒购自北京中山生物技术有限公司, 内源性生物素封闭液(ABB 液)购自武汉博士德公司.

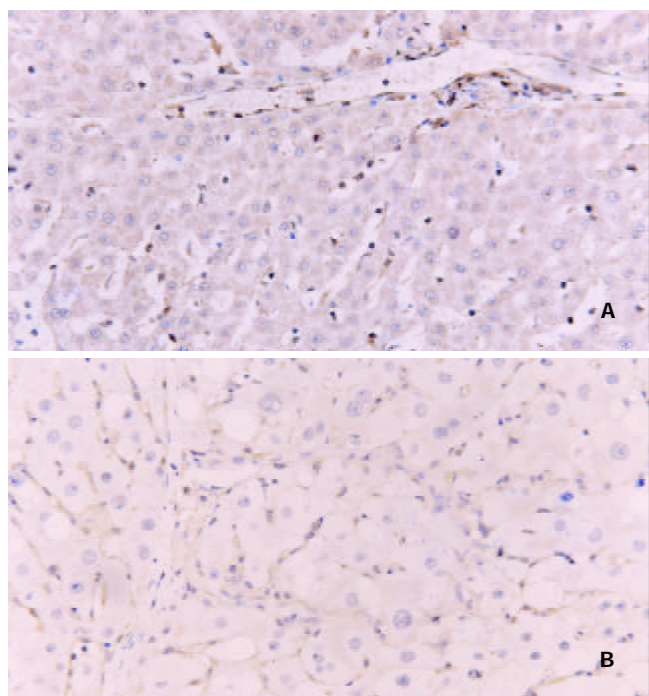
1.2 方法 肝纤维化模型制备及标本处理: 按 0.3 ml/100 g 体重的剂量, 皮下注射 40 ml/L CCl₄ 油剂, 2 次/wk. 分别在注射 CCl₄ 第 1, 4, 8 周分批处死动物, 留取肝脏组织, PBS(PH7.4)清洗后 100 ml/L 甲醛固定, 常规石蜡包埋, 连续 5 μm 切片. 免疫组织化学染色: 采用 SABC 法, 石蜡切片常规脱蜡, 30 ml/L H₂O₂ 甲醇溶液室温孵育 15 min, PBS 洗涤 5 min × 2 遍后, 用

0.01 mol/L 的柠檬酸缓冲液(pH6.0)加热至 92-96 °C 修复抗原 15 min, 冷却后依次滴加 ABB 液和正常兔血清封闭各 10 min, 而后用 ERK1 一抗 4 °C 孵育过夜; 次日取出切片以 PBS 冲洗后, 再依次滴加生物素化羊抗兔二抗、链酶卵白素; 最后 DAB 显色, 苏木素复染. 常规乙醇脱水、二甲苯透明、中性树胶封片保存. 光镜下观察并分析 ERK1 表达情况. ERK1 抗体稀释度为 1:200. 用 PBS 代替一抗作阴性对照. 图像分析和数据统计: 用 HPIAS-1000 型全自动医学图像彩色分析系统(由华中科技大学同济医学院病理教研室提纲)进行图像半定量分析, 每张切片随机选取 5 个视野, 测定肝组织中 ERK1 的棕黄色阳性表达颗粒的平均吸光度 A 值.

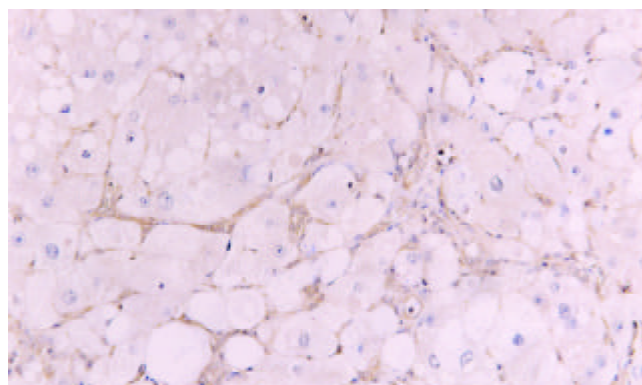
统计学处理 应用 SPSS 统计软件对四组数据进行 ANOVA 检验, 结果以均数 \pm 标准差($\bar{x} \pm s$)表示, $P < 0.05$ 为有显著性差异.

2 结果

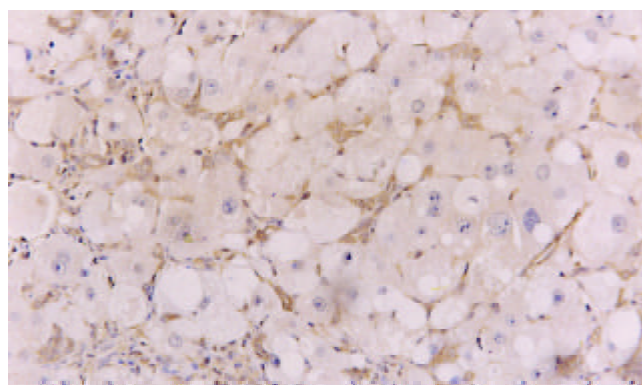
在正常肝组织中, ERK1 呈针尖状棕黄色弱阳性表达, 主要见于汇管区、小叶中央静脉周围及肝索 Disse 腔间隙中. 高倍镜下, ERK1 分布于 Disse 间隙的 HSC 胞质中, 肝细胞未见表达(图 1A). 在 CCl₄ 注射 1, 4, 8 wk 组肝组织中, ERK1 的表达明显增多、增强, 低倍镜下可见小叶中央静脉周围、汇管区以及肝小叶内均有大量条索状、星芒状的 ERK1 阳性表达. 高倍镜下, ERK1 阳性表达主要分布于 HSC 胞质、胞核中. 在 8 wk 组肝纤维化组织中, 除 HSC 表达外, 亦有零星肝细胞胞质、胞核呈 ERK1 阳性(图 1B, C, D). 随 CCl₄ 诱导时间的延长, ERK1 在正常对照组及 CCl₄ 注射 1, 4, 8 wk 组肝组织中的表达强度呈明显的逐级递增的趋势(0.3597 ± 0.0140 , 0.3849 ± 0.0199 , 0.7876 ± 0.0316 , 0.9125 ± 0.0158 , $P < 0.01$ 或 $P < 0.05$).



A: 正常.
B: CCl₄ 注射 1 wk.



C: CCl₄ 注射 4 wk.



D: CCl₄ 注射 8 wk.

图 1 大鼠肝组织中 ERK1 的表达 SABC $\times 200$.

3 讨论

肝纤维化是大多数慢性肝病向肝硬化发展的共同病理过程^[5-7]. 研究证实, 肝星状细胞(haptic stellate cell, HSC)增生、活化及分泌细胞外基质(extracellular matrix, ECM)是肝纤维化形成的重要机制^[8-12]. 近年资料表明, 肝慢性损伤及炎症反应时, HSC 最强的促分裂剂血小板衍生生长因子(platelet-derived growth factor, PDGF)及其受体的表达明显增强^[13-16]. 而 PDGF 活化信号可能是通过 Ras/raf/ERK 级联通路进行转导的^[17-20]. 为此, 我们对 ERK1 在大鼠肝纤维化发生、发展中的表达及分布进行了研究, 结果显示: CCl₄ 注射诱导后, 大鼠肝组织中 ERK1 的表达较正常对照明显增多、增强; 在正常大鼠肝组织中, ERK1 呈肝间质细胞胞质分布, 正常肝细胞内则未见表达; 在 CCl₄ 诱导各阶段, ERK1 在 HSC 胞质、胞核均有表达, 呈核浆型分布; 随着 CCl₄ 注射诱导时间的延长, 肝组织 ERK1 表达呈逐渐增强趋势.

Ras/raf/ERK 信号通路是 MAPK 众多途径中不可缺少的组成之一, 能将多种细胞外信号通过磷酸化的活化方式逐级传递至细胞核, 激活多种转录因子, 参与细胞增生、分化以及细胞恶性转化等多种生理、病理过程^[21-23]. ERK(包括 ERK1、ERK2 两种亚型)即是此通路中极为关键的一员, 负责将胞质内的活化信号传递入胞核内^[24-26]. ERK 为丝氨酸/苏氨酸激酶, 激活后可催化 c-jun、c-fos、c-myc 以及核糖体 S6 蛋白激酶

(RSK)的磷酸化,后者诱导靶基因的转录,促使细胞由G0期进入到G1期,继而调节细胞增生^[27-30].我们的结果显示,在各肝纤维化组中,ERK1的表达强度随着肝纤维化程度的加重而明显增强,而且由单纯性胞质分布转变为核质型分布,提示ERK携带信号的核转入明显增多,与HSC的增生密切相关.而HSC作为肝纤维化形成过程中起关键作用的细胞,他的激活是整个事件发生的开端,由此推测,ERK介导的信号通路促进肝星状细胞的增生、活化,参与了肝纤维化发生、发展过程.

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