

Notch 通路在大鼠肝纤维化发生发展中作用的初探

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摘要 目的:研究 Notch 通路在肝纤维化发生发展中作用及可能的分子机制。方法:Wistar 大鼠 40 只随机分为正常对照组与病理模型组,病理模型组皮下注射四氯化碳制备肝纤维化模型。8 周后将大鼠处死,取肝组织行病理 HE 染色评价肝纤维化程度并采用免疫组织化学法检测 Notch-1 蛋白、E-cadherin 蛋白与 TGF- β 1 蛋白的表达。结果:肝组织病理 HE 染色示肝纤维化大鼠肝脏肝细胞坏死、再生明显,胶原纤维沉积明显增加,肝实质结构紊乱。与正常对照组相比,病理模型组 notch-1 与 TGF- β 1 蛋白表达明显增加,而 E-cadherin 蛋白的表达明显下降($P < 0.01$)。结论:Notch 通路在大鼠肝纤维化发生发展中可能起重要作用。

关键词 肝纤维化; Notch 通路; 上皮间充质转化; 转化生长因子 β 1

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Effects of Notch Signaling in Rats with Hepatic Fibrosis

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ABSTRACT Objective: To investigate the effect of Notch signaling on the progression of hepatic fibrosis in a rat model induced by CCl₄. **Methods:** Forty healthy wistar rats were randomly divided into 2 groups: control group (n=15) and liver fibrosis group (n=25). Experimental liver fibrosis was induced by subcutaneous injection of CCl₄. At the end of 8 weeks, histopathological study of liver tissue was done with hematoxylin-eosin (HE). Notch-1, E-cadherin and Transforming growth factor- β 1(TGF- β 1) in liver were examined with the immunohistochemical technique. **Results:** A significant collagen deposition, hepatocyte necrosis and rearrangement of the parenchyma were noted in liver tissue of liver fibrosis group. The expression levels of notch-1 and TGF- β 1 increased in model group than those in control group. The expression levels of E-cadherin increased in the model animals ($P < 0.01$). **Conclusions:** Notch signaling play important role in the occurrence and progression of hepatic fibrosis in a rat model induced by CCl₄, Notch signaling and TGF- β signaling may have many consistency in the occurrence and progression of hepatic fibrosis.

Key words: Hepatic fibrosis; Notch signaling; Epithelium-mesenchymal transition; Transforming growth

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前言

肝纤维化是许多肝脏疾病的共同环节,其特点是以胶原为主的肝脏细胞外基质(extracellular matrix, ECM)的过度沉积^[1,2]。已有研究证明上皮间充质转化(epithelium-mesenchymal transition, EMT)是许多器官与组织的纤维化基础,肝脏的多种细胞可通过 EMT 转化为肌成纤维细胞,促进肝纤维化的形成和发展,而这一过程还伴随着 Notch 通路的活化^[3-6]。本研究将探讨 Notch 通路在肝纤维化发生发展中的可能作用及相关的分子机制。

1 材料与方法

1.1 材料

Notch-1 抗体(ZS-6041, 1:100), E-cadherin 抗体(ZS-7870, 1:100), TGF- β 抗体(ZS-146, 1:30)及山羊抗鼠 IgG 抗体

(PV-6002)均购自北京中杉金桥生物技术有限公司,四氯化碳分析纯购自青岛沃尔科生物技术有限公司。

1.2 方法

1.2.1 分组及处理 清洁级雄性 Wistar 大鼠 40 只,体质量 200 ± 20 g,由青岛市药检所提供(许可证号 SCXK(鲁)20090007)。实验前适应性饲养 1 周,普通饮食。随机分为正常对照组(N 组, n=15)与病理模型组(M 组, n=25)。动物模型制备:CCl₄ 与橄榄油以 4:6 配成 40%油剂,首剂量 5 mL/kg 皮下注射,以后 3 mL/kg 皮下注射,每周 2 次,共 8 周。自实验第 6 周开始,每周周末处死 2 只大鼠,取肝脏组织作肝纤维化病理学检查,按照 2000 年西安会议《病毒性肝炎防治方案》肝纤维化分期标准来判断肝纤维化程度^[7]。正常对照组给予同等剂量的橄榄油皮下注射。

1.2.2 实验结束时取材和处理 实验第 8 周末, Wistar 大鼠 3% 水合氯醛麻醉,解剖取肝脏,取每只大鼠肝左叶的相同部位,大小约 5 mm×10 mm×10 mm, 10% 中性甲醛溶液固定,常规石蜡包埋,连续切片,厚 2 μ m,用免疫组织化学法检查大鼠肝脏 Notch-1、E-cadherin 和 TGF- β 1 蛋白的表达。

1.2.3 Notch-1、E-cadherin 和 TGF- β 1 免疫组织化学染色 切片置 60℃ 烤箱过夜;二甲苯脱蜡,酒精梯度水化;蒸馏水冲洗 2

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min , 入 3%过氧化氢溶液 10 min , 蒸馏水冲洗 2 min ;分别入 pH6.0 枸橼酸盐修复液(notch-1 ,E-cadherin)与 pH9.0EDTA 修复液 2 min(TGF-β1), 保持温度 100 ℃; PBS(pH 7.4)冲洗 4 次, 每次 1.5 min; 加一抗 37℃孵育 60 min; PBS(pH 7.4)冲洗 4 次, 每次 1.5 min;加二抗 37 ℃孵育 20 min, PBS(pH 7.4)冲洗 4 次, 每次 1.5 min; DAB 显色; 苏木素复染核 ;梯度酒精脱水 , 二甲苯透明;中性树胶封片。

1.2.4 Notch-1,E-cadherin 和 TGF-β1 蛋白表达评定标准 取 5 个较好的高倍视野, 按显色程度分弱、中、强 3 种(1、2、3 分); 按显色范围分为 4 度:显色范围占高倍视野 <25 %记为 +;显色占高倍视野 25 %-50 %记为 ++;50 %-75 %记为 +++; >75 %记为 ++++,然后按显色指数 = 显色程度× 显色范围(+ 为 1 分、++

为 2 分、+++ 为 3 分、++++ 为 4 分), 取其均数作为每个指标的最终显色指数。

1.3 统计学处理

实验数据采用 SPSS13.0 统计软件处理,计量资料采用 t 检验 ,P<0.05 确定差异有统计学意义。

2 结果

2.1 肝组织病理学 HE 染色

N 组肝脏肝小叶结构正常, 肝细胞围绕中央静脉呈放射状排列 (图 1A);M 组肝小叶结构破坏, 纤维组织增生明显 ,肝索排列紊乱 ,肝细胞广泛的脂肪变性、坏死 , 炎性细胞浸润 (图 1B)。

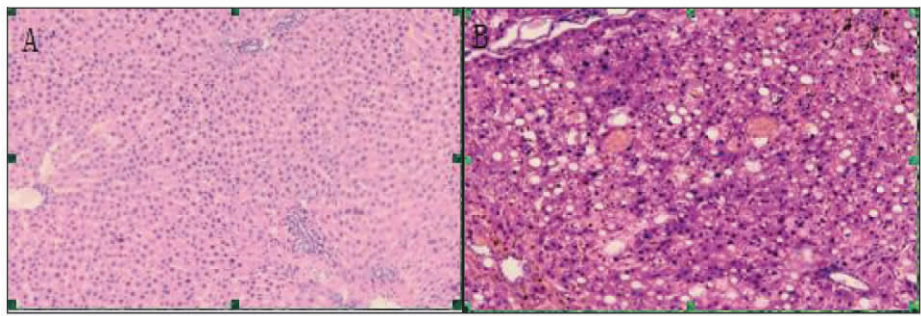


图 1 各组肝组织形态学改变 10× 10.A: N 组;B: M 组

Fig.1 Histopathological study of liver tissue10× 10 .A: control group;B: liver fibrosis group)

2.2 肝组织中 Notch-1、E-cadherin 与 TGF-β1 蛋白的表达

大鼠肝组织 Notch-1、TGF-β1 蛋白表达以细胞胞质为主, E-cadherin 蛋白表达以胞膜着色为主 ,着色均为黄色或棕黄色。

病理模型组 Notch-1、TGF-β1 蛋白的表达均明显高于正常对照组而 E-cadherin 蛋白的表达均明显低于正常对照组(P <0.01,表 1、图 2-4)。

表 1 引物序列、位置和产物大小

Table 1 Primer sequences, location and product size

	n	Notch-1	E-cadherin	TGF-β1
Control group	15	2.72± 1.11	9.69± 1.67	1.95± 1.09
Fibrosis group	25	10.37± 1.27	2.13± 1.04	9.18± 1.18
P		P <0.01	P <0.01	P <0.01

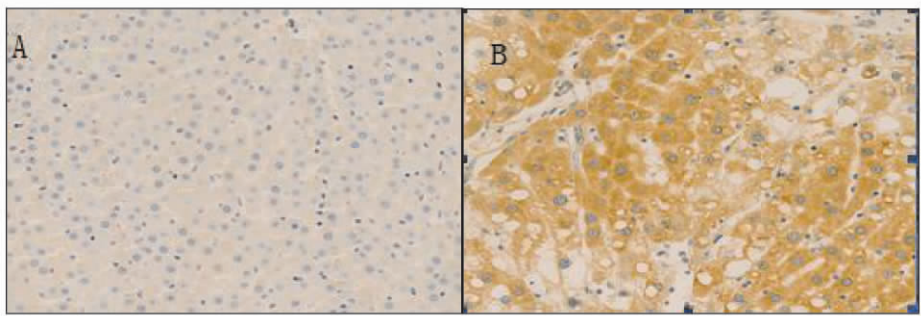


图 2 各组肝组织中 Notch-1 表达的变化(免疫组织化学× 200).A: N 组;B: M 组

Fig.2 Notch-1 express in the liver tissue 20× 10. A: control group; B: liver fibrosis group)

3 讨论

Notch 通路是一条古老的并决定细胞的命运的信号转导通路 ,Notch 通路调节的紊乱可导致包括肿瘤与纤维化在内的多

种疾病的发生^[8,9]。已有研究发现 Notch 通路参与胆管分化^[10]。本研究所检测的 Notch-1 是 Notch 通路的受体之一 ,也是在哺乳动物各个组织与器官普遍存在的 Notch 通路受体。

EMT(epithelium-mesenchymal transition)在正常情况下可

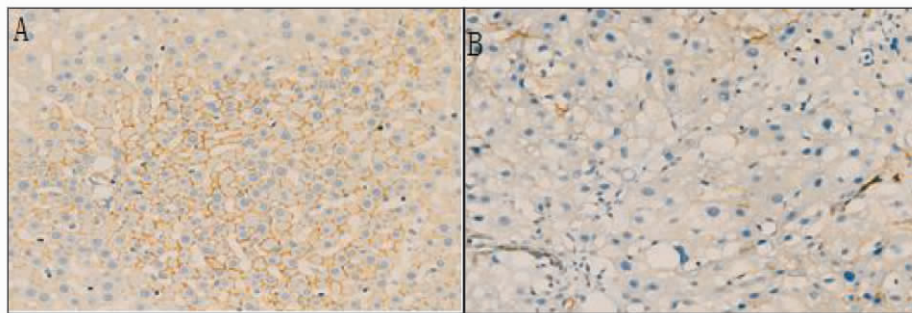


图3 各组肝组织中 E-cadherin 表达的变化(免疫组织化学× 200).A: N 组;B: M 组

Fig.3 E-cadherin express in the liver tissue 20× 10. A: control group;B: liver fibrosis group)

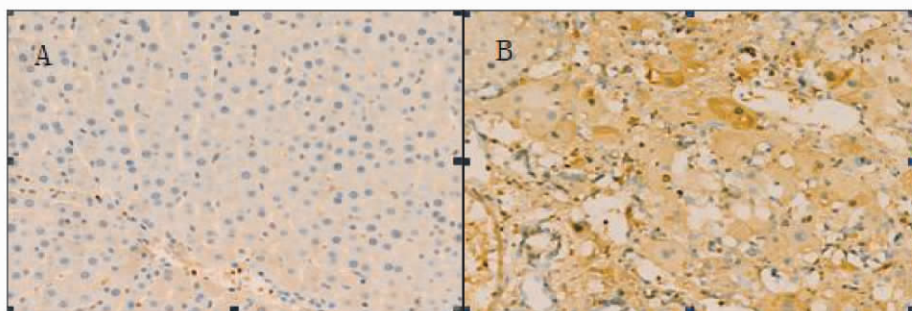


图4 各组肝组织中 TGF-β1 表达的变化(免疫组织化学× 200).A: N 组;B: M 组

Fig.4 TGF-β1 express in the liver tissue 20× 10. A: control group;B: liver fibrosis group)

使损伤的器官与组织达到修复,但 EMT 持续存在可导致纤维化^[11,12]。E-cadherin 存在人与动物的上皮细胞内,介导细胞的连接与相关的信号通路,E-cadherin 的缺失是 EMT 的特异性标志。TGF-β1 是 EMT 最主要的促发因子之一,TGF-β1 受体阻滞剂可以逆转 EMT 改善肝纤维化,而 E-cadherin 的缺失又可以反过来上调 TGF-β1 及其下游基因的水平,在各种原因所导致的纤维化中发挥重要作用^[13-15]。本研究通过免疫组化的方法检测 Notch 通路的受体 Notch-1,EMT 的特异性标志 E-cadherin 及促肝纤维化因子 TGF-β1 蛋白的表达情况发现模型组 Notch-1 与 TGF-β1 的表达均明显高于正常对照组,而病理模型组 E-cadherin 的表达明显低于正常对照组。

Notch 通路可通过 TGF-β/Smad3 途径诱导 EMT 显型,从而促进肺泡上皮细胞中肌成纤维细胞的分化,促肺间质纤维化的发展^[16]。研究发现在肾小管的 EMT 过程中,Notch 通路是 TGF-β1 诱发 EMT 的重要因素,且 Notch 通路阻滞剂可以阻止 TGF-β1 诱发 EMT^[17]。在腹膜透析所致的腹膜纤维化中 TGF-β1 可诱导大鼠腹膜间皮细胞的 Notch 通路活化,且 Notch 通路 γ-分泌酶阻断剂 DAPT 可阻断 TGF-β1 所诱导的 Notch 通路活化,进而改善腹膜纤维化,成为目前治疗腹膜纤维化的新方法^[18]。而本研究的结果表明 notch 通路具有促肝纤维化的作用,而 notch 通路的促肝纤维化作用可能与 TGF-β1 具有协调一致性,两者可能均通过诱导肝脏细胞的 EMT 过程,使肝脏的多种细胞转化为肌成纤维细胞导致纤维化。

肝纤维化是一个复杂的病理过程,其中涉及多条信号转导通路的参与,而本研究发现 Notch 通路参与了肝纤维的发生发展,但过程复杂且国内外研究较少,其具体的作用机制需待进一步研究。

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胎宫内死亡,在积极处理产科并发症的同时,B超监测活胎宫内发育情况,并配合母体的凝血功能监测,获得良好母子结局。

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