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-β1 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-β1 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β1

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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-β1(TGF-β1) in liver were examined with the immunohisochemistrical 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-1and TGF-β1 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 μm，用免疫组织化学法检查大鼠肝脏 Notch-1、E-cadherin 和 TGF-β1 蛋白的表达。

1.2.3 Notch-1,E-cadherin 和 TGF-β1 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)。

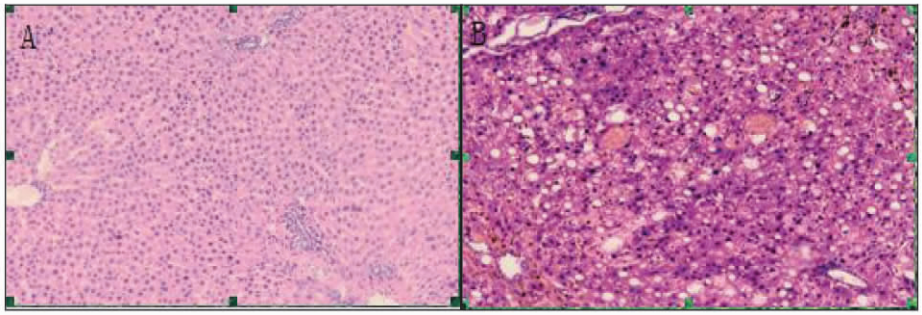

图1 各组肝组织形态学改变10× 10.A: N组;B: M组

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

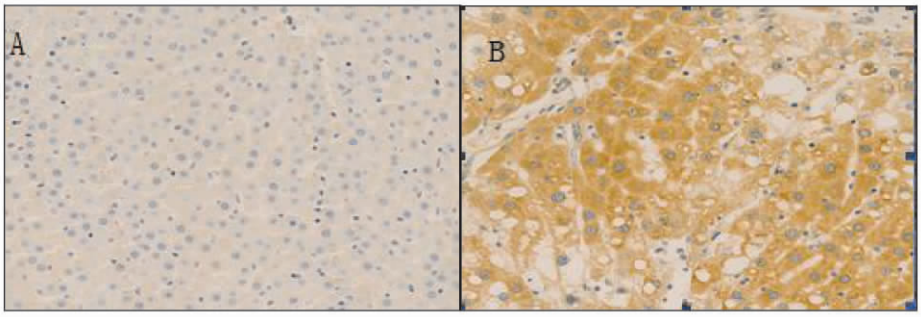

图2 各组肝组织中Notch-1表达的变化(免疫组织化学× 200).A: N组;B: M组

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

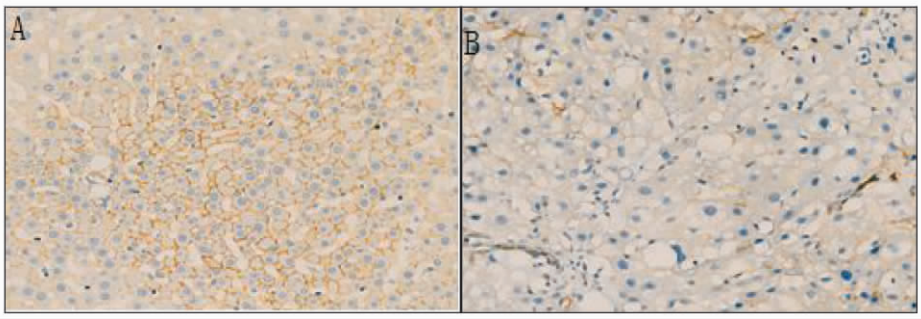

图 3 各组肝组织中 E-cadherin 表达的变化(免疫组织化学× 200).A: N 组;B: M 组

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

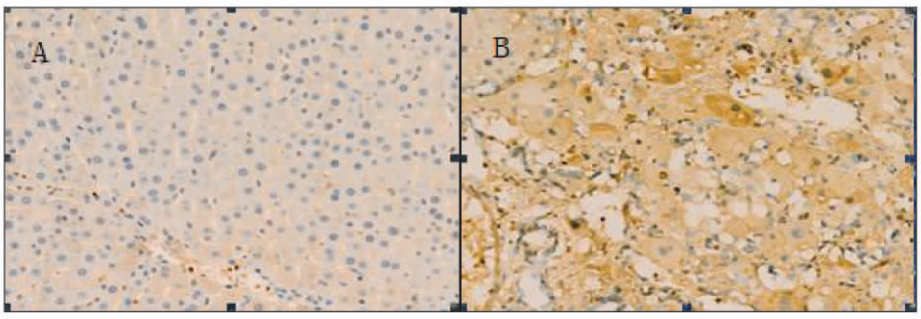

图 4 各组肝组织中 TGF-β1 表达的变化(免疫组织化学× 200).A: N 组;B: M 组

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，获得良好母儿结局。

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