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论著

RNA干扰抑制肝星状细胞CTGF表达对细胞外基质分泌的影响

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摘要 目的：利用pEGFP质粒载体构建介导结缔组织生长因子(CTGF)短发夹RNA表达的质粒，筛选有效的抑制序列后，研究其对HSC-T6中TGF-β_1、CTGF及细胞外基质表达的影响。方法:1.分别设计3对针对CTGF的有小发夹结构的两条DNA序列及1对非特异对照序列，构建重组体成功后转染HSC-T6，24 h后通过RT-PCR及Western blotting分析HSC-T6 CTGFmRNA及蛋白表达水平，筛选出有效抑制CTGF表达的序列。2.将已构建并筛选出有最高抑制效率的pEGFP-CTGFshRNA转染HSC-T6，培养24、48 h后用RT-PCR和/或Western blotting检测各组细胞中TGF-β_1、Ⅰ型胶原、Ⅲ型胶原基因的表达;放免法分析细胞上清液中Ⅲ型前胶原、Ⅳ型胶原、透明质酸和层黏连蛋白的含量。结果:将构建成功的3组pEGFP-CTGFshRNA转染肝星状细胞后，筛选出两组能高效抑制CTGF表达的序列; pEGFP-CTGFshRNA能明显抑制HSC-T6 CTGF、Ⅰ型、Ⅲ型胶原mRNA及蛋白的表达，降低上清液中Ⅲ型前胶原、Ⅳ型胶原、透明质酸和层黏连蛋白的含量(P<0.01或P<0.05)，而对TGF-β_1的基因表达则无影响。结论:pEGFP-CTGFshRNA能高效抑制肝星状细胞中CTGF及细胞外基质的表达;CTGFshRNA介导的RNA干扰有望对慢性肝病所致肝纤维化具有治疗潜力。

关键词 RNA干扰； 短发夹RNA； 结缔组织生长因子； 肝星状细胞； 转化生长因子β； 细胞外基质

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Effect of plasmid delivered shRNA knocking down CTGF on extracellular matrix secretion in rat hepatic stellate cells

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Abstract

<FONT face=Verdana>AIM: To clone the recombinant plasmids expressing CTGF short hairpin RNA (shRNA), to screen the highly efficient shRNA and to investigate the effect of CTGF shRNA on the expression of TGF-β₁, type Ⅰ procollagen, type III procollagen mRNA and extracellular matrix secretion in HSC-T6 cells. METHODS: (1) Three pairs of two DNA sequences containing small hairpin structure and one pair of unspecific control sequence were designed and synthesized, respectively. Four pairs of recombinant plasmids were transfected into HSC-T6. The expressions of CTGF mRNA and protein levels were determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting 24 h later. (2) The CTGFshRNA plasmids contained EGFP were transfected to HSC-T6 with transfection reagent metafectene. Another three HSC-T6 groups: one with plasmids and without CTGFshRNA; one only with metafectene; and one was untreated. The expression of TGF-β₁, type Ⅰ procollagen and type III procollagen mRNA level were determined by reverse transcription-polymerase chain reaction (RT-PCR) and/or Western blotting after 24 and 48 h. Precollagen type Ⅲ, Ⅳ-type collagen, hyaluronate (HA), laminin (LN) in the supernatants were determined by radioimmunoassay. RESULTS: (1) Compared to the controls, the expression of CTGF mRNA and protein levels in HSC-T6 transfected with CTGFshRNA recombinants were markedly down-regulated respectively in two groups of recombinant plasmids. (2) After 24 h and 48 h, the expressions of type Ⅰ procollagen and type Ⅲ procollagen mRNA levels in HSC-T6 transfected with pEGFP-CTGFshRNA were markedly down-regulated. The contents of precollagen type Ⅲ, Ⅳ-type collagen, HA, LN in the supernatants were decreased. No difference in the expression of TGF-β₁ mRNA and protein was observed. CONCLUSION: CTGFshRNA regulates connective tissue growth factor, extracellular

matrix molecule expression and secretion in HSC-T6. CTGF shRNA may inhibit the development of liver fibrosis.</FONT>

Key words RNA interference Short hairpin RNA Connective tissue growth factor Hepatic stellate cells Transforming growth factor beta Extracellular matrix

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