

论著

黏着斑激酶相关非激酶质粒转染对肝星状细胞凋亡的影响

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摘要 目的: 应用黏着斑激酶相关非激酶 (FRNK) 表达质粒瞬时转染肝星状细胞 (HSCs), 探讨FRNK选择性抑制黏着斑激酶(FAK)磷酸化对FN刺激的HSCs凋亡的影响。方法: 在体外培养HSCs, 以FN刺激HSCs增殖, 采用脂质体介导的方法进行FRNK表达质粒转染。应用膜联蛋白 (Annexin-V) /碘化丙啶 (PI) 双标记流式细胞术、DNA凝胶电泳技术和透射电镜技术检测细胞的凋亡, Western blotting及RT-PCR方法检测FRNK、FAK、p-FAK (Tyr397)、caspase-3蛋白及其mRNA表达。结果: FRNK表达质粒成功转染HSCs, 在翻译后水平抑制FAK磷酸化; FRNK表达质粒转染HSCs 48 h后, 细胞凋亡率增加, 与空质粒组之间有显著差异 [(25.37±1.92) % vs (9.28±1.05) %], P<0.01, 伴随caspase-3在翻译和转录水平的增高 [(264.17±12.60 vs 185.82±9.69), P<0.01; (4.19±0.48 vs 1.07±0.27), P<0.01]。结论: 在脂质体介导下瞬时转染FRNK表达质粒, 可使外源性的FRNK在HSCs内大量表达, 在翻译后水平抑制FAK磷酸化; 可以诱导FN刺激的HSCs发生凋亡。

关键词 [肝星状细胞](#); [细胞凋亡](#); [黏着斑激酶相关非激酶](#); [半胱氨酸天冬氨酸蛋白酶3](#)

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Effect of FAK-related non-kinase on apoptosis in hepatic stellate cells

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Abstract

AIM: To evaluate the inhibitory effect of FRNK on the phosphorylation of FAK and apoptosis in hepatic stellate cells (HSCs). METHODS: After stimulated with fibronectin, HSCs was transfected with FRNK plasmid by cationic liposome method. The apoptosis of FRNK-induced HSCs was examined by Annexin-V/propidium iodide double-labeled flow cytometry (FCM), gel electrophoresis and transmission electron microscope. The protein levels of FRNK, FAK and p-FAK (Tyr397) in HSCs were assayed by Western blotting, and RT-PCR was used to detect the expression of mRNA. RESULTS: The expression of FRNK was enhanced and the phosphorylation of FAK was inhibited after FRNK was transiently transfected into HSCs in vitro. The apoptotic rate in HSCs exposed to FRNK plasmid for 48 h was higher than that in the non-FRNK plasmid group [(25.37±1.92) % vs (9.28±1.05) %, P<0.01], and accompanied by a significant increase in caspase-3 activity both in the protein and in the mRNA level [(264.17±12.60 vs 185.82±9.69), P<0.01; (4.19±0.48 vs 1.07±0.27), P<0.01] . CONCLUSION: In HSCs, the expression of FRNK is enhanced and the phosphorylation of FAK is inhibited after FRNK transfection. FRNK induces the HSCs apoptosis.

Key words [Hepatic stellate cells](#) [Apoptosis](#) [Focal adhesion kinase related non-kinase](#) [Caspase-3](#)

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