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论著

小干扰RNA 沉默MST1 基因减轻TNF-α 诱导的人脐静脉内皮细胞凋亡

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摘要： 目的： 探讨丝/ 苏氨酸蛋白激酶(mammalian sterile 20-like kinase 1,MST1) 对肿瘤坏死因子-α (tumornecrosis factor-α,TNF-α)诱导的人脐静脉内皮细胞(human umbilical vein endothelial cell,HUVEC)凋亡的影响及作用机制。方法：用不同浓度的TNF-α(0~100 ng/mL) 诱导内皮细胞凋亡, 24 h 后通过TUNEL 法观察内皮细胞凋亡率,Western印迹分析TNF-α 对 MST1 活性的影响；随后将构建的MST1 小干扰RNA(small interference RNA,siRNA) 在脂质体Lipofectamine 2000 的介导下转染原代HUVEC,转染后24 h 加入TNF-α(10 ng/mL) 诱导HUVEC 凋亡,24 h 后通过Western 印迹确定MST1 siRNA 的基因沉默效率；通过TUNEL 法观察MST1 基因的敲除对TNF-α 介导的内皮细胞凋亡的影响；并进一步用Western 印迹观察MST1 基因敲除对caspase-3 活性的影响。结果：TNF-α(10,40,100 ng/mL)能导致内皮细胞凋亡显著增多(P<0.001),并且呈剂量依赖性；随着TNF-α 浓度增加,MST1 的活化增多,MST1 激酶活性相应增加；MST1 siRNA 对内皮细胞中MST1 基因表达的抑制呈剂量依赖性,100 nmol/L MST1 siRNA 能特异性沉默内皮细胞中MST1 基因的表达(P<0.05)；MST1 siRNA 能明显减少TNF-α 诱导的内皮细胞凋亡(P<0.05),抑制TNF-α诱导的MST1 裂解活化,同时caspase-3 的活性也相应减少。结论：MST1 siRNA 通过抑制caspase-3 的级联放大效应减少TNF-α 诱导的HUVEC 凋亡。

关键词： 肿瘤坏死因子-α 内皮细胞 凋亡 MST1 caspase-3

Silencing of MST1 expression by siRNA diminishes TNF-α- mediated human umbilical vein endothelial cell apoptosis

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Abstract: Objective: To elucidate the effects of mammalian sterile 20-like kinase 1 (MST1) gene on tumor necrosis factor (TNF)-α-mediated human umbilical vein endothelial cell (HUVEC) apoptosis. Methods: Cultured HUVECs were treated with either vehicle or TNF-α (1-100 ng/mL) for 24 hours. Cell apoptosis was measured by TUNEL staining, and MST1 activity was analyzed by Western blot. In order to knock down MST1 expression in HUVECs, cells were transfected with 100 nmol/L MST1 small interference RNA (siRNA) using Lipofectamine 2000 for 24 hours, and the transfection efficiency was analyzed by Western blot. MST1 siRNA-transfected cells were treated with 10 ng/mL TNF-α for an additional 24 hours. Cell apoptosis was measured by TUNEL staining and caspase-3 activity was detected by Western blot. Results: MST1 activity was stimulated in a dose-dependent manner after TNF-α treatment (10, 40, 100 ng/mL) and reached the maximal effect at 100 ng/mL. MST1 activity also paralleled the onset of apoptosis as determined by TUNEL staining (P<0.001). Transfection with MST1 siRNA markedly diminished MST1 gene expression in a dose-dependent manner. MST1 siRNA (100 nmol/L) significantly silenced MST1 gene (P<0.05) and reduced TNF-α-induced endothelial cells apoptosis (P<0.05) by way of inhibiting MST1 gene activation and, accordingly, suppressing caspase-3 activity. Conclusion: Silencing of MST1 expression by siRNA diminishes TNF-α-mediated human umbilical vein endothelial cell apoptosis by inhibiting the cascade effect of caspase-3.

Keywords: tumor necrosis factor-α endothelial cell apoptosis MST1 caspase-3

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