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Rock信号通路参与TNF-α刺激兔基底动脉平滑肌增

《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 35 期数: 2013年第18期 页码: 1952-1956 栏目: 论著 出版日期: 2013-09-30

Title: Rock signaling pathway mediates rabbit basilar artery smooth muscle proliferation stimulated by TNF-α

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关键词: 基底动脉; 血管平滑肌细胞; rho相关激酶类; 细胞增殖; 肿瘤坏死因子α; 兔

Keywords: basilar artery; vascular smooth muscle cells; rho-associated kinases; cell proliferation; tumor necrosis factor-α; rabbits

分类号: R322.121;R329.26;R329.28

文献标志码: A

摘要: 目的 探索Rock信号通路在肿瘤坏死因子α(tumor necrosis factor-α，TNF-α)引起兔基底动脉血管平滑肌细胞（vascular smooth muscle cells，VSMCs）增殖中的作用。 方法 组织块法原代培养兔基底动脉VSMCs，TNF-α刺激VSMCs，Rho激酶(Rho kinase，Rock)抑制剂Y-27632预处理，CCK-8法检测细胞增殖变化，流式细胞仪检测细胞周期，免疫细胞化学观察增殖细胞核抗原(proliferating cell nuclear antigen，PCNA)在VSMCs的定位及表达变化，Western blot法检测细胞PCNA表达量的变化。 结果 细胞培养24 h后，与阴性对照组比较，20 ng/mL TNF-α处理可以显著增加VSMCs的增殖率（P<0.01），PCNA蛋白表达显著增高（P<0.01），S期+G_2/M期细胞比例明显增加（P<0.05）；加入Y-27632预处理后,与20 ng/mL TNF-α处理组相比，VSMCs的增殖率受到显著抑制（P<0.01），PCNA蛋白表达明显减少（P<0.05），S期+G_2/M期细胞比例明显减少（P<0.05）。 结论 TNF-α可促进VSMCs增殖和细胞周期进程，Rock抑制剂可阻滞TNF-α对VSMCs的这一作用，Rock信号通路参与TNF-α诱导的VSMCs增殖和细胞周期进程加速。

Abstract: Objective To investigate the role of Rho kinase (Rock) signaling pathway in the proliferation of rabbit basilar artery vascular smooth muscle cells (VSMCs) stimulated by tumor necrosis factor-α (TNF-α). Methods VSMCs were isolated and primarily cultured from rabbit basilar artery. After Y-27632, Rock inhibitor at different concentrations was added into the culture medium,

followed by the stimulation of TNF-α. Cell viability was determined with CCK-8 assay. Cell cycle of VSMCs was determined by flow cytometry. The expression of proliferating cell nuclear antigen (PCNA) in the VSMCs was measured with immunocytochemical staining and Western blot analysis. Results Compared with normal control cells, TNF-α treatment at 20 ng/mL for 24 h markedly induced VSMCs proliferation (P<0.01), increased the percentage of cells in S and G_2/M phase (P<0.05), and enhanced PCNA expression (P<0.01). Pretreatment with Y-27632, however, significantly reversed the above effects induced by 20 ng/mL TNF-α, that is, significantly inhibited cell proliferation (P<0.01), decreased expression of PCNA protein (P<0.05) and the amounts of cells at S and G_2/M phase (P<0.05). Conclusion TNF-α promotes the proliferation of VSMCs and cell cycle, but Rock inhibitor, Y-27632, effectively reverses the above effects induced by TNF-α. Rock signaling pathway mediates TNF-α-induced acceleration of VSMCs proliferation and cell cycle progression.

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