肿瘤防治研究 2011, Vol. 38 Issue (8): 861-865 DOI: 10.3971/j.issn.1000-8578.2011.08.003

鞘氨醇激酶-1激活ERK通路介导人结肠癌细胞株LoVo侵袭与迁移的实验

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Sphingosine Kinase-1 Regulates LoVo Cell Invasion and Migration via Activation of ERK1/2 Pathway

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全文: PDF (1256 KB) HTML (0 KB) 输出: BibTeX | EndNote (RIS) 背景资料

摘要 目的研究Sphk1对人结肠癌LoVo细胞侵袭与迁移能力的影响并探讨其作用机制。方法将人结肠癌LoVo细胞分成Sphk1激活组，Sphk1抑制组，空白对照组。以Phorbol 12-myristate 13-acetate（PMA）为Sphk1激活剂（终浓度为100 nM），N,N-dimethyl-D-eryt-hro-sphingosine（DMS）为Sphk1抑制剂（终浓度为50 μM），NaCl（终浓度为0.9%）为空白试剂处理LoVo细胞24 h后，用Transwell Boyden小室模型测定LoVo细胞的相对侵袭率与迁移率；用Western blot方法测定细胞Sphk1、ERK1/2与p-ERK1/2蛋白水平的变化；用ELISA方法检测细胞培养上清中MMP-2、MMP-9及uPA的蛋白含量；用半定量RT-PCR检测细胞中MMP-2、MMP-9和uPA的mRNA水平。结果Sphk1激活剂可促进LoVo细胞侵袭与迁移,同时明显增强LoVo细胞中Sphk1、ERK1/2及p-ERK1/2的蛋白表达，并促进MMP-2、MMP-9及uPA的mRNA表达与蛋白分泌。Sphk1抑制剂则抑制LoVo细胞侵袭与迁移，同时抑制Sphk1、ERK1/2与p-ERK1/2的蛋白表达，并抑制MMP-2、MMP-9及uPA的mRNA表达与蛋白分泌。结论Sphk1可促进人结肠癌细胞株LoVo细胞的侵袭与迁移，其机制可能与激活ERK1/2信号通路从而促进MMP-2、MMP-9及uPA mRNA表达与蛋白分泌有关。

关键词： 鞘氨醇激酶-1 人结肠癌细胞 侵袭 迁移

Abstract： ObjectiveTo investigate the effect of Sphk1 on colon cancer cell invasion and migration.MethodsHuman colon cancer LoVo cells were divided into three group: LoVo cells were treated using 100 nM Phorbol 12-myristate 13-acetate (PMA) as the Sphk1 activation group, 50 μM N,N-dimethyl-D-erythro-sphingosine (DMS) as suppression group, and 0.9%NaCl as control group. Cell invasiveness and migration were detected by Transwell boyden chamber model. Sphk1, ERK1/2,p-ERK1/2 protein expressions were detected by Western blot, MMP-2, MMP-9 and uPA protein levels in the culture medium were detected by enzyme-linked immunosorbent assay (ELISA), and MMP-2, MMP-9 and uPA mRNA expressions in LoVo cells were detected by semi-quantitative reverse transcription-polymerase chain reaction.ResultsThe Sphk1 activator induced the expression of Sphk1 and obviously enhanced LoVo cell invasion and migration capacity, accompanied with the up-regulating of ERK1/2, p-ERK1/2 protein expressions: moreover the protein in culture medium and the mRNA in cells levels of MMP-2, MMP-9 and uPA were elevated. On the contrary, the inhibitor obviously suppressed the protein expression of Sphk1 and cell invaseness and migration, associated with the suppressing of ERK1/2, p-ERK1/2 protein expressions; furthermore, the protein and the mRNA levels of MMP-2, MMP-9 and uPA were down-regulated. ConclusionSphk1 was able to promote the invasion and migration of LoVo cells, through the activation of ERK1/2 signaling pathway, MMP-2, MMP-9 and uPA mRNA expression were up-regulated.

Key words： Sphingosine kinase 1 Human colon cancer cells Invasion Migration

收稿日期: 2010-06-24;

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ZHONG Yue-yuan,LIU Shi-quan,HUANG Jie-an et al. Sphingosine Kinase-1 Regulates LoVo Cell Invasion and Migration via Activation of ERK1/2 Pathway[J].

CHINA RESEARCH ON PREVENTION AND TREATMENT, 2011, 38(8): 861-865.

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