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PTEN抑制人脐静脉内皮细胞ECV304的增殖和VEGF的表达

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摘要：

目的：探讨与张力蛋白同源的10号染色体缺失的磷酸酶基因（phosphatase and tensin hemology deleted on chromosome ten gene， PTEN ）对人脐静脉内皮细胞ECV304细胞增殖、凋亡，及血管内皮生长因子（vascular endothelial growth factor，VEGF）及其受体1（VEGF receptor 1，VEGFR1）的影响。方法：将携带有野生型 PTEN 及绿色荧光蛋白（green fluorescent protein， GFP ）基因的腺病毒Ad PTEN GFP及空载体腺病毒Ad GFP感染ECV304细胞，MTT实验、Hoechst3342染色法及流式细胞术检测ECV304细胞的增殖和凋亡。实时荧光定量PCR法检测Ad PTEN GFP感染后ECV304细胞中PTEN、VEGF和VEGFR1 mRNA表达水平，ELISA检测ECV304细胞培养上清中VEGF的水平。鸡胚尿囊膜（chick chorioallantoic membrane，CAM）血管生长实验检测 PTEN 对鸡胚血管生长的影响。结果：与Ad GFP相比，Ad PTEN GFP感染能明显抑制ECV304细胞的增殖，诱导细胞凋亡，5 d时增殖抑制率可达(50.38±5.42)%、细胞凋亡率达（73.3±5.3）%。当感染复数为100时，Ad PTEN GFP组ECV304细胞的 VEGF及VEGFR1 mRNA表达水平分别为未感染组的(13.40±1.32)%及(46.12±5.20)% 。同时，Ad PTEN GFP感染能够明显抑制CAM体内血管生长\[血管指数(57.6±3.37)% vs (92.2±4.37)%，P <0.05\]。结论： PTEN 能显著抑制人脐静脉内皮细胞ECV304的增殖、促进其凋亡，其机制可能与抑制VEGF和VEGFR1表达，抑制血管新生有关。

关键词：PTEN基因 血管内皮细胞生长因子（VEGF） 血管内皮细胞生长因子受体1(VEGFR1) 血管新生 ECV304细胞

PTEN inhibits proliferation and VEGF expression in human umbilical vein endothelial ECV304 cells

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Abstract:

Objective: To investigate the effect of phosphatase and tensin hemology deleted on chromosome ten gene (PTEN) on proliferation, apoptosis, VEGF (vascular endothelial growth factor) and its receptor VEGFR1 expression in human umbilical vein endothelial cell (HUVEC) line ECV304. Methods: Recombinant adenovirus containing green fluorescent protein (GFP) and PTEN (Ad PTEN GFP) or empty vector (Ad GFP) were transfected into ECV304 cells; proliferation and apoptosis of ECV304 cells were measured by MTT assay, Hoechst3342 staining and flow cytometry, respectively; PTEN, VEGF, VEGFR1 mRNA expression levels in Ad PTEN GFP transfected ECV304 cells were examined by quantitative PCR; and VEGF protein level in ECV304 cell supernatant was detected by ELISA. Chick chorioallantoic membrane (CAM) assay was used to study the effect of PTEN on angiogenesis. Results: Ad PTEN GFP transfection significantly inhibited the proliferation and induced apoptosis of ECV304 cells and the inhibitory rate and apoptotic rate were (50.38±5.42)% and (73.3±5.3)% at 5 d. VEGF and VEGFR1 mRNA expression levels were (13.40±1.32)% and (46.12±5.20)% of untransfected group after transfected with Ad PTEN GFP at MOI=100 in ECV304 cells. Furthermore, CAM assay results showed that Ad PTEN GFP transfection inhibited CAM angiogenesis in vivo . Conclusion: PTEN can inhibit the growth of and promote apoptosis of human umbilical vein endothelial ECV304 cells, which might be related to the down regulation of VEGF/VEGFR1 expression and the resulting angiogenesis inhibition.

Keywords:PTEN vascular endothelial growth factor(VEGF) VEGF receptor(VEGFR1) angiogenesis ECV304 cell

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