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# hCaMKⅡNα对结肠肿瘤分泌免疫抑制因子的抑制作用

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摘要：

探讨钙离子/钙调素依赖性蛋白激酶Ⅱ抑制蛋白α（human calcium/calmodulin-dependent protein kinase Ⅱ inhibitory alpha，hCaMKⅡNα）对结肠肿瘤细胞分泌免疫抑制因子的影响及其作用机制。方法：将hCaMKⅡNα基因表达载体（pKⅡNα）或siRNA（si-KⅡNα）转染至结肠肿瘤细胞（LoVo细胞、SW620细胞和HT29细胞），形成过表达或干扰表达细胞。RT-PCR检测结肠癌LoVo细胞过表达hCaMKⅡα后白细胞介素-8（interleukin-8, IL-8）、白细胞介素-10（interleukin-10, IL-10）和血管内皮生长因子（vascular endothelial cell growth factor, VEGF）mRNA表达水平，ELISA法检测转染pKⅡNα或si-KⅡNα对SW620和LoVo细胞中VEGF、PGE2、 IL-8和IL-10分泌的影响。为观测细胞外调节蛋白激酶1/2（extracellular regulated protein kinases，ERK1/2）在hCaMKⅡNα介导的细胞因子调控中的作用，利用U0126抑制HT-29细胞中ERK1/2活性后，检测hCaMKⅡNα表达下调对VEGF和IL-8分泌的影响。结果： hCaMKⅡ 可显著抑制结肠癌LoVo细胞中VEGF、IL-8和IL-10 mRNA水平的表达；可抑制SW620和LoVo细胞中IL-8和VEGF蛋白的分泌，但对PGE2和IL-10的分泌没有影响。相应地，利用RNA干扰技术下调hCaMKⅡNα表达可显著上调HT29细胞中IL-8和VEGF的分泌；并且发现MEK1/2活性的抑制可完全阻断hCaMKⅡNα对IL-8的影响，但只能部分阻断对VEGF的影响。结论：hCaMKⅡNα通过抑制ERK活性下调结肠肿瘤细胞VEGF和IL-8的分泌，在结肠肿瘤免疫逃逸中起到负相调控作用。

关键词：钙离子/钙调素依赖性蛋白激酶Ⅱ抑制蛋白 结肠肿瘤 LoVo细胞 SW620细胞 HT29细胞 VEGF IL-8 IL-10

# The inhibitory effect of hCaMKⅡNα on the production of immunosuppressive factors in colon cancer cells in vitro

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Abstract:

To investigate the effect of (human calcium/calmodulin-dependent protein kinase Ⅱ inhibitory alpha (hCaMKⅡN-α) on the production of immunosuppressive factors in colon cancer cells and the mechanisms underlying the effect in vitro. Methods: Overexpression and silencing of the hCaMKⅡN-α gene in human colon adenocarcinoma (LoVo, SW620 and HT29) cells were achieved by transfection with a hCaMKⅡN-α-expressing plasmid (pKⅡN-α) and an siRNA (si-KⅡN-α ) vector, respectively. Messenger RNA levels of interleukin-8 (IL-8), interleukin-10 (IL-10) and vascular endothelial cell growth factor (VEGF) in LoVo cells transfected with pKⅡN- were analyzed by RT-PCR. Protein levels of IL-8, IL-10 and VEGF in SW620 and LoVo cells transfected with pKⅡN- and in HT29 cells transfected with si-KⅡN- were determined by ELISA. The differences in IL-8 and VEGF protein levels in HT29 cells transfected with pKIN-α in the presence or absence of U0126 (10 M), a selective ERK1/2 inhibitor, were analyzed to elucidate the role of ERK1/2 in hCaMKⅡN-α-mediated IL-8 and VEGF production. Results: Overexpression of hCaMKⅡN- significantly decreased the mRNA abundance and protein levels of VEGF and IL-8 ($P<0.05$) but not PGE2 ($P>0.01$). Silencing of hCaMKⅡN- by siRNA significantly increased the secretion of VEGF and IL-8 in HT29 cells, but had no effect on the secretion of PGE2 and IL-10. U0126 treatment resulted in a complete reversion of increased IL-8 secretion but only a partial reversion of increased VEGF secretion in HT29 cells overexpressing hCaMKⅡ-α. Conclusion: Our observations suggest that hCaMKⅡ- may inhibit the secretion of VEGF and IL-8 and thus down-regulate the immune response in rectal tumor cells through an ERK signaling pathway-dependent mechanism.

Keywords:human calcium/calmodulin-dependent protein kinase Ⅱ inhibitory alpha (hCaMKⅡNα) colon carcinoma LoVo cell SW620 cell H29 cell VEGF IL-8 IL-10