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Geminin基因过表达对VSMCs表型转化的影响(PDF)

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Title: Geminin over-expression promotes differentiation in rat vascular smooth muscle cells from dedifferentiation

作者: 李林峪; 张园园; 舒茂琴

第三军医大学西南医院心血管内科，重庆市介入心脏病学研究所

Author(s): Li Linyu; Zhang Yuanyuan; Shu Maoqin

Department of Cardiology, Southwest Hospital, Third Military Medical University, Chongqing, 400038, China

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摘要: 目的 探讨Geminin基因过表达对血管平滑肌细胞（vascular smooth muscle cells，VSMCs）表型转化的影响。 方法 构建真核表达载体pEGFP-N1-Geminin，脂质体2000介导转染不同表型（去分化型和分化型）的大鼠VSMCs株，设置阳性组、阴性组和空白组（*n*=3），检测转染效果、VSMCs主要表型标志物（α-actin、OPN）和肌动蛋白相关蛋白1（actin related protein，ARP1）的变化情况。 结果 Geminin基因成功过表达VSMCs株。与2个对照组（阴性组和空白组）比较，分化型VSMCs中表型标志物（α-actin和OPN）及ARP1含量变化不大；去分化型VSMCs中，OPN在阳性组中表达下调，与2个对照组（阴性组和空白组）比较差异具有统计学意义（*P*<0.05），α-actin和 ARP1在阳性组中表达上调，与2个对照组（阴性组和空白组）比较差异具有统计学意义（*P*<0.05），免疫共沉淀实验结果提示经protein G plus-Agarose沉淀ARP1相互作用蛋白复合物后，用Geminin抗体进行Western blot检测，可以检测到Geminin的表达。 结论 Geminin基因过表达有助于VSMCs由去分化型向分化型转化；免疫共沉淀结果提示Geminin和ARP1之间存在相互作用， ARP1可能参与了上述VSMCs表型转化过程。

Abstract: Objective To determine the effect of Geminin in the phenotypic transformation of rat vascular smooth muscle cells (VSMCs). Methods After an eucaryotic plasmid vector pEGFP-N1-Geminin was constructed, VSMCs strains of different phenotypes (dedifferentiated phenotype by cultured in present of serum, and differentiated phenotype by cultured without serum) were transfected with aid of lipofectamine 2000. Cells transfected with pEGFP-N1 and blank plasmids served as negative control and blank control. The expression of Geminin, actin related protein 1 (ARP1), and VSMCs phenotypic makers α-actin and osteopontin (OPN) were detected with Western blotting. The interaction between Geminin and ARP1 were analyzed with co-immunoprecipitation. Results Geminin was successfully overexpressed in VSMCs strains after transfection. Comparing with negative and blank controls, Geminin overexpression resulted in obviously down-regulation of OPN protein in the dedifferentiation cells, but significantly up-regulation in α-actin and ARP1 (*P*<0.05). However, these changes of above-mentioned markers and ARP1 were not significantly changed in differentiated cells. Co-immunoprecipitation indicated that interaction between Geminin and ARP1 was found in Geminin-overexpressed VSMCs strains with dedifferentiated phenotype. Conclusion Geminin may play a role in the phenotypic transformation of VSMCs from dedifferentiation to differentiation. The interaction between Geminin and ARP1 suggests that ARP1 may be involved in this process.

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