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论著

间质细胞衍生因子1α对外周血内皮干细胞衰老的影响

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摘要 目的 观察间质细胞衍生因子1α（SDF-1α）对外周血内皮干细胞（ESC）衰老的影响，探讨其可能机制。方法 密度梯度离心法获取人外周血单核细胞，培养4 d后，收集贴壁细胞。实验分为正常对照组及SDF-1α 1，10，50 和100 μg·L^{-1}组。采用SA-β-半乳糖苷酶染色试剂盒检测衰老细胞；MTT比色法和集落生成能力测定实验检测ESC的增殖和集落形成能力；端粒重复序列扩增法（TRAP）-ELISA定量检测端粒酶(端粒末端转移酶)活性；Western蛋白印迹法检测ESC Akt Ser473磷酸化水平。结果 与正常对照组相比，SDF-1α能显著减少SA-β-半乳糖苷酶染色阳性细胞，SDF-1α 100 μg·L^{-1}最为明显（40.8±7.1 *vs* 17.5±3.0；*P*<0.01）；SDF-1α 100 μg·L^{-1}也能显著促进ESC增殖能力（0.22±0.02 *vs* 0.39±0.04；*P*<0.01），集落形成能力（7.8±2.2 *vs* 22.4±3.4；*P*<0.01）；SDF-1α 100 μg·L^{-1}增加ESC端粒酶活性（0.34±0.05 *vs* 0.57±0.09；P<0.01）；SDF-1α能促进ESC Akt磷酸化。结论 SDF-1α能减缓ESC衰老，伴随ESC增殖和集落形成能力的改善，提示细胞衰老可能是SDF-1α影响ESC功能的机制之一；SDF-1α减缓ESC衰老可能与增加ESC端粒酶活性及Akt磷酸化水平有关。

关键词 间质细胞衍生因子1α 干细胞 内皮 细胞衰老 端粒酶

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Effects of stromal cell-derived factor-1α on senescence of endothelial stem cells from peripheral blood

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Abstract

OBJECTIVE To investigate whether stromal cell-derived factor-1α (SDF-1α) might be able to prevent senescence of endothelial stem cell (ESC) and also study its effects on the telomerase activity. **METHODS** Total mononuclear cells (MNCs) were isolated from peripheral blood by density gradient centrifugation, and then the cells were plated on fibronectin-coated culture dishes. After cultured for 4 d, attached cells were divided into control and SDF-1α 1, 10, 50 and 100 μg·L^{-1} groups. ESC became senescent as determined by acidic β-galactosidase staining. The proliferation of ESC was assessed by MTT assay and colony-forming capacity. Telomerase activity was measured by telomerase-PCR ELISA and the phosphorylation of Akt was determined by using Western blotting. **RESULTS** *Ex vivo* prolonged cultivation of ESC led to rapid onset of ESC senescence. Compared with control group, SDF-1α concentration-dependently inhibited the onset of ESC senescence, maximum at 100 μg·L^{-1} (40.8±7.1 *vs* 17.5±3.0; *P*<0.01). Moreover, SDF-1α 100 μg·L^{-1} increased ESC proliferation (0.22±0.02 *vs* 0.39±0.04; *P*<0.01) and ESC colony-forming activity (7.8±2.2 *vs* 22.4±3.4). Compared with control group, SDF-1α 100 μg·L^{-1} also increased telomerase activity (0.34±0.05 *vs* 0.57±0.09; *P*<0.01). In addition, SDF-1α treatment of ESC stimulated a concentration- and time-dependent Akt phosphorylation. **CONCLUSION** SDF-1α-induced prevention of ESC senescence leads to the potentiation of proliferative activity, and clonal expansion, which may be related to the activation of telomerase and Akt phosphorylation.

Key words stromal cell-derived factor-1α endothelium stem cell cellular senescence telomerase

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