·基础研究·

缺血再灌注条件下内皮祖细胞促进单核/巨噬细胞向M1型极化的研究

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[摘　要]　**目的**:探讨在缺血再灌注条件下内皮祖细胞(endothelial progenitor cell,EPC)促进单核/巨噬细胞向M1型极化的作用及机制。**方法**:获取健康志愿者的外周血,原代培养EPC和单核/巨噬细胞;体外构建细胞缺血再灌注损伤模型;流式细胞术检测EPC表面细胞间黏附分子-1(intercellular adhesion molecule -1,ICAM-1)、血管细胞黏附分子-1(vascular cell adhesion molecule-1,VCAM-1)和E-选择素的表达;ELISA检测上清中ICAM-1、VCAM-1和E-选择素浓度;采用Transwell小室进行EPC和单核/巨噬细胞共培养,流式细胞术检测M1和M2型单核/巨噬细胞比例。**结果**:缺血再灌注条件下EPC表面表达及其分泌ICAM-1和VCAM-1均没有显著变化,两组之间差异均没有统计学意义;对照组EPC表面E-选择素平均荧光强度为10.89,缺血再灌注组为33.93(t=3.895,P=0.018);对照组EPC上清中E-选择素浓度为3.69 ng/mL,缺血再灌注组为18.17 ng/mL(t=4.568,P=0.010);缺血再灌注条件下EPC能够促进单核/巨噬细胞向M1型极化,对照组M1型单核/巨噬细胞比例为58.83%,缺血再灌注组为81.43%(t=5.394,P=0.006),E-选择素阻断能抑制这种作用(t=5.950,P=0.004);缺血再灌注条件下EPC抑制单核/巨噬细胞向M2型极化,对照组M2型单核/巨噬细胞比例为60.57%,缺血再灌注组为35.30%(t=6.424,P=0.003),E-选择素阻断能抑制这种作用(t=4.179,P=0.014)。**结论**:在缺血再灌注损伤条件下,EPC能够通过高表达和分泌E-选择素,促进单核/巨噬细胞向M1型极化,为缺血再灌注损伤的治疗提供了新的靶点。

[关键词]　内皮祖细胞;缺血再灌注;单核/巨噬细胞;心血管疾病

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The role and mechanism of endothelial progenitor cells to promote mononuclear/macrophage to type M1 polarization under ischemic reperfusion

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[**Abstract**]　**Objective**: This study aims to explore the role and mechanism of endothelial progenitor cells(EPC) promoting monocyte/macrophage to type M1 polarization under ischemia and reperfusion. **Methods**: the peripheral blood of healthy volunteers was obtained, the primary EPC and mononuclear/macrophage were cultured. The model of cell ischemia-reperfusion injury was established *in vitro*, and the expression of intercellular adhesion molecule -1(ICAM-1), vascular cell adhesion molecule -1(VCAM-1)and E- selectin on EPC surface were detected by flow cytometry. ELISA was used to detect the concentration of ICAM-1, VCAM-1 and E- selectin in the supernatant. Co-culture of EPC and mononuclear/macrophages was performed by Transwell chamber, and the ratio of M1 to M2 type monocytes/macrophages was detected by flow cytometry. **Results**: There was no significant change in the expression and the secretion of ICAM-1 and VCAM-1 of EPC under the ischemic reperfusion condition, and there was no significant difference between the two groups. The average fluorescence intensity of E- selectin on the surface of EPC was 10.89 in the control group and 33.93 in the ischemia reperfusion group(t=3.895, P=0.018). The concentration of E- selectin in the EPC supernatant was 3.69 ng/mL in the control group and 18.17 ng/mL in the ischemia-reperfusion group(t=4.568, P=0.010). Under ischemia reperfusion condition, EPC promoted

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monocyte/macrophage to M1 type polarization. The proportion of monocyte/macrophage type M1 was 58.83% in control group and was 81.43% in ischemia reperfusion group (t=5.394, P=0.006), E- selectin blockage could inhibit this effect (t=5.950, P=0.004). Under ischemia reperfusion condition, EPC inhibited monocyte/macrophage to M2 type polarization. The ratio of M2 monocyte/macrophage was 60.57% in control group and was 35.30% in ischemia reperfusion group (t=6.424, P=0.003), E- selectin blockage could inhibit this effect (t=4.179, P=0.014). **Conclusion**: Under the condition of ischemia - reperfusion injury, EPC can promote the monocyte/macrophage to M1 polarization through the high expression and secretion of E- selectin. Our research provides a new target for the treatment of ischemia-reperfusion injury.

[**Key words**] endothelial progenitor cells; ischemia-reperfusion; mononuclear/macrophage; cardiovascular disease

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随着人民生活水平的提高,我国心血管疾病的发病率和死亡率均显著升高,给社会和家庭带来了极大的负担[1]。血管内支架植入疏通了堵塞的血管,显著改善患者的临床症状,然而血管再通后的缺血再灌注(ischemia reperfusion, I/R)会激活单核/巨噬细胞,促进其向损伤部位浸润和释放炎症分子,引起心律失常和心肌细胞进一步损伤等严重后果[2]。因此,调控炎症反应对于治疗I/R损伤至关重要。炎症反应与血管生成紧密相关,低强度的炎症反应会诱导内皮祖细胞(endothelial progenitor cells, EPC)从骨髓动员[3]。EPC是内皮细胞的前体干细胞,能归巢到血管损伤部位,促进再内皮化和发挥细胞保护作用[4]。EPC表面则表达有3个促炎分子:细胞间黏附分子-1(intercellular adhesion molecule -1, ICAM-1)、血管细胞黏附分子-1(vascular cell adhesion molecule-1, VCAM-1)和E-选择素,介导了EPC与单核/巨噬细胞之间的相互作用。然而在I/R条件下,EPC与单核/巨噬细胞相互作用还未见相关报道。

1 材料和方法

1.1 材料

RPMI1640培养基、胎牛血清、EGM-2完全培养基和胰酶(Hyclone公司,美国);人淋巴细胞分离液(天津TBD公司);CCR7、CD206、CD14磁珠、ICAM-1、VCAM-1和E-选择素流式检测抗体(BD公司,美国);ICAM-1、VCAM-1、E-选择素ELISA试剂盒(深圳欣博盛生物科技公司)。

1.2 方法

1.2.1 细胞分离和培养

取健康志愿者的外周血,通过梯度离心的方法分离出单个核细胞,一部分细胞置入EGM-2完全培养基中进行培养,连续培养7 d;另一部分细胞采用CD14磁珠进行分选,PBS清洗3次后,将分选的细胞置于含有10%胎牛血清的RPMI1640培养基中培养。

1.2.2 体外缺血再灌注损伤模型构建

采用文献报道的方法构建I/R损伤模型[5]。EPC培养7 d后,弃上清,PBS清洗3次。之后加入不含葡萄糖的D-Hank's溶液,将其置于通有95% N_2、5% CO_2的37 ℃培养箱中缺氧培养3 h。之后更换为正常培养基,置于通有95% O_2、5% CO_2的培养箱中继续培养24 h,设置为I/R组。

1.2.3 EPC促炎分子检测

EPC I/R损伤处理24 h后,用胰酶消化。加入ICAM-1、VCAM-1和E-选择素流式细胞检测抗体,室温孵育15 min,PBS清洗3次后,在流式细胞仪上检测各个分子表达的平均荧光强度(mean fluorescence intensity, MFI)。同时收集细胞培养上清,ELISA检测上清中ICAM-1、VCAM-1和E-选择素的含量。

1.2.4 细胞共培养实验

采用Transwell小室进行EPC和单核/巨噬细胞共培养。EPC培养7 d后,分为5组:对照组(EPC细胞与单核/巨噬细胞共培养)、I/R组(I/R损伤EPC细胞与单核/巨噬细胞共培养)、ICAM-1阻断组(I/R损伤EPC细胞与单核/巨噬细胞共培养,同时加入ICAM-1中和抗体)、VCAM-1阻断组(I/R损伤EPC细胞与单核/巨噬细胞共培养,同时加入VCAM-1中和抗体)和E-选择素阻断组(I/R损伤EPC细胞与单核/巨噬细胞共培养,同时加入E-选择素中和抗体)。共培养48 h后,收集上室的单核/巨噬细胞,加入CCR7和CD206流式细胞检测抗体,室温孵育15 min,PBS清洗3次后,在流式细胞仪上检测$CD14^+CCR7^+$M1单核/巨噬细胞和$CD14^+CD206^+$M2单核/巨噬细胞比例。

1.3 统计学方法

使用SPSS 22.0进行数据分析,结果用均数±标

准误($\bar{x} \pm s_{\bar{x}}$)表示，两组之间的比较采用t检验，$P \leqslant 0.05$为差异有统计学意义。

2 结 果

2.1 缺血再灌注条件下EPC细胞表面促炎分子E-选择素表达增加

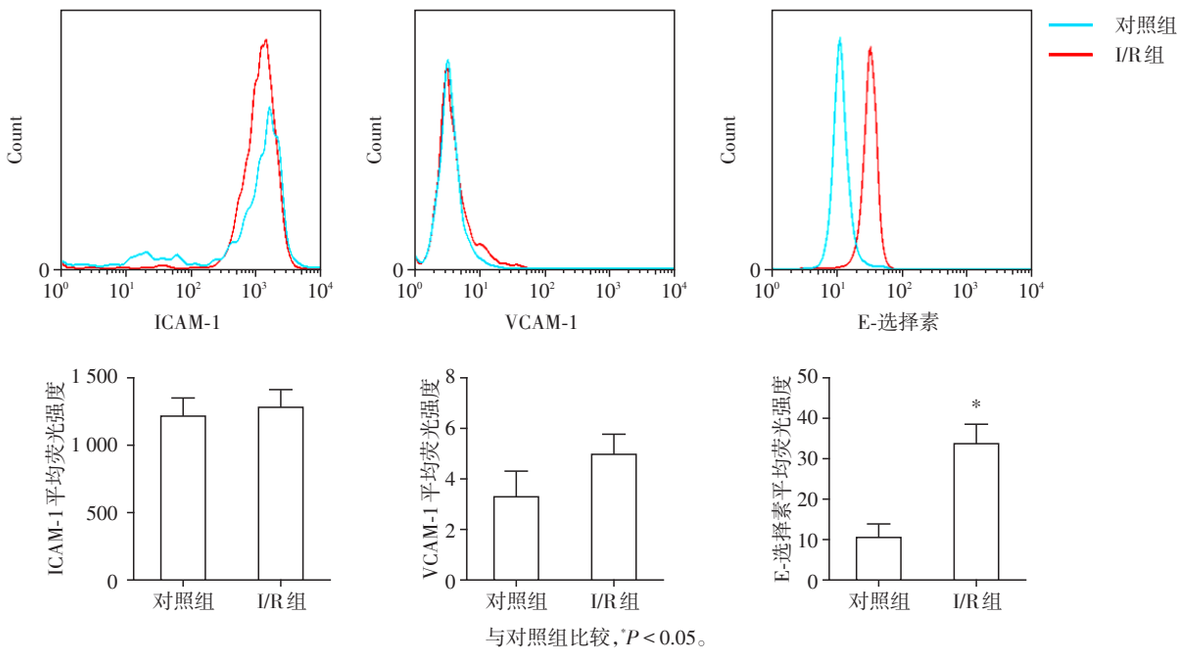
流式细胞术检测结果显示对照组EPC表面ICAM-1和VCAM-1平均荧光强度分别为1 214和3.380，I/R组为1 282和5.023，两组之间没有显著的统计学差异(t=0.354，P=0.742；t=1.322，P=0.257)；对照组EPC表面E-选择素平均荧光强度为10.89，缺血再灌注组为33.93，两组之间具有显著的统计学差异(t=3.895，P=0.018，图1)。

2.2 缺血再灌注条件下EPC促炎分子E-选择素分泌增加

ELISA检测结果(图2)显示对照组EPC上清中ICAM-1和VCAM-1浓度分别为178.0、0.740 ng/mL，I/R组为199.3、0.903 ng/mL，两组之间没有显著的统计学差异(t=0.518，P=0.632；t=0.409，P=0.704)；对照组EPC上清中E-选择素浓度为3.69 ng/mL，I/R组为18.17 ng/mL，两组之间具有显著的统计学差异(t=4.568，P=0.010)。

2.3 缺血再灌注条件下EPC促进单核/巨噬细胞向M1型极化

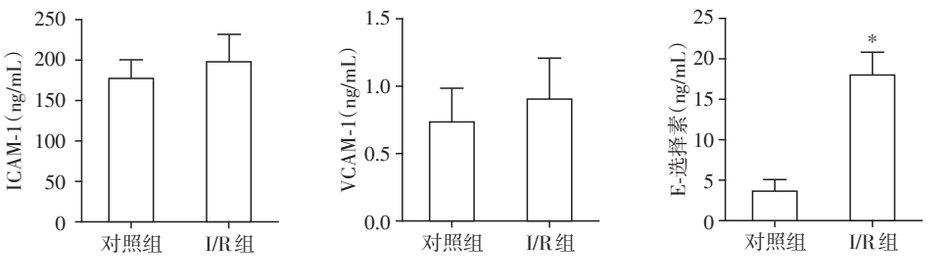
流式细胞术检测结果(图3)显示对照组$CD14^+CCR7^+$M1型单核/巨噬细胞比例为58.83%，I/R组为81.43%，两组之间具有显著的统计学差异(t=5.394，P=0.006)；ICAM-1阻断组和VCAM-1阻断组M1型单核/巨噬细胞比例分别为79.10%和76.93%，相对于I/R组，没有显著的统计学差异(t=0.531，P=0.622；t=1.061，P=0.348)；E-选择素阻断组M1型单核/巨噬细胞比例为59.70%，相对于I/R组，结果具



与对照组比较，*P < 0.05。

图1 缺血再灌注条件下EPC细胞表面E-选择素表达增加

Figure 1 Expression of E-selectin increased in surface of EPC under ischemia-reperfusion condition



与对照组比较，*P < 0.05。

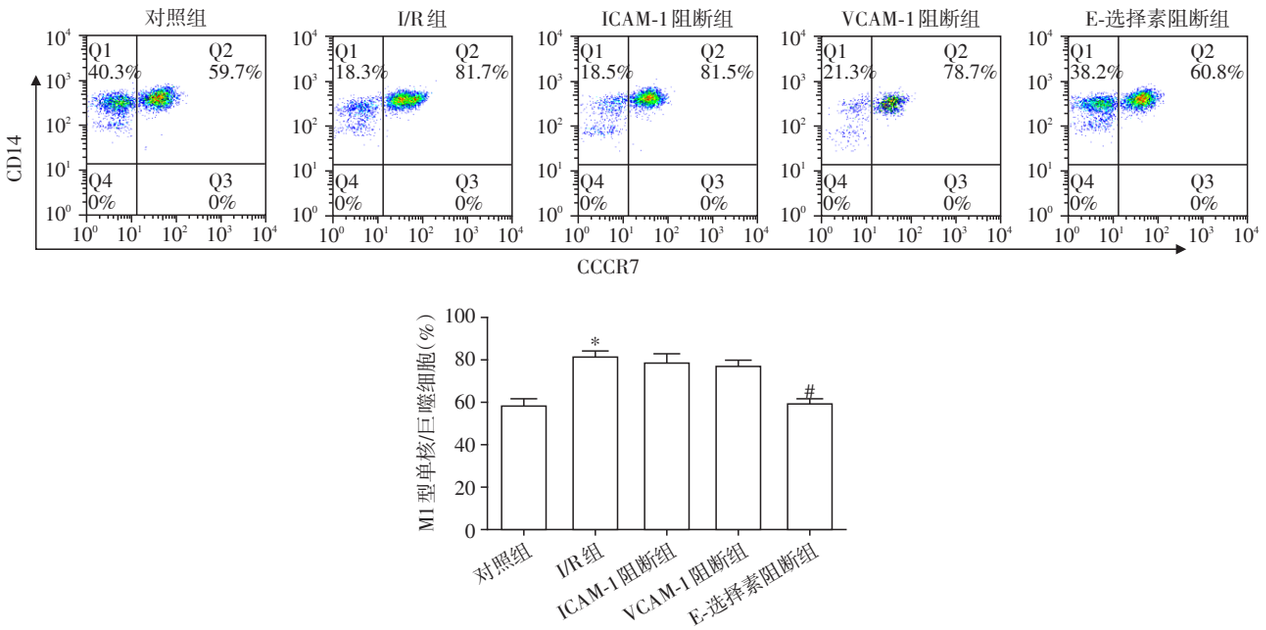
图2 缺血再灌注条件下EPC的E-选择素分泌增加

Figure 2 Secretion of E-selectin increased in EPC during ischemia-reperfusion

有显著的统计学差异(t=5.950,P=0.004)。

2.4 缺血再灌注条件下EPC抑制单核/巨噬细胞向M2型极化

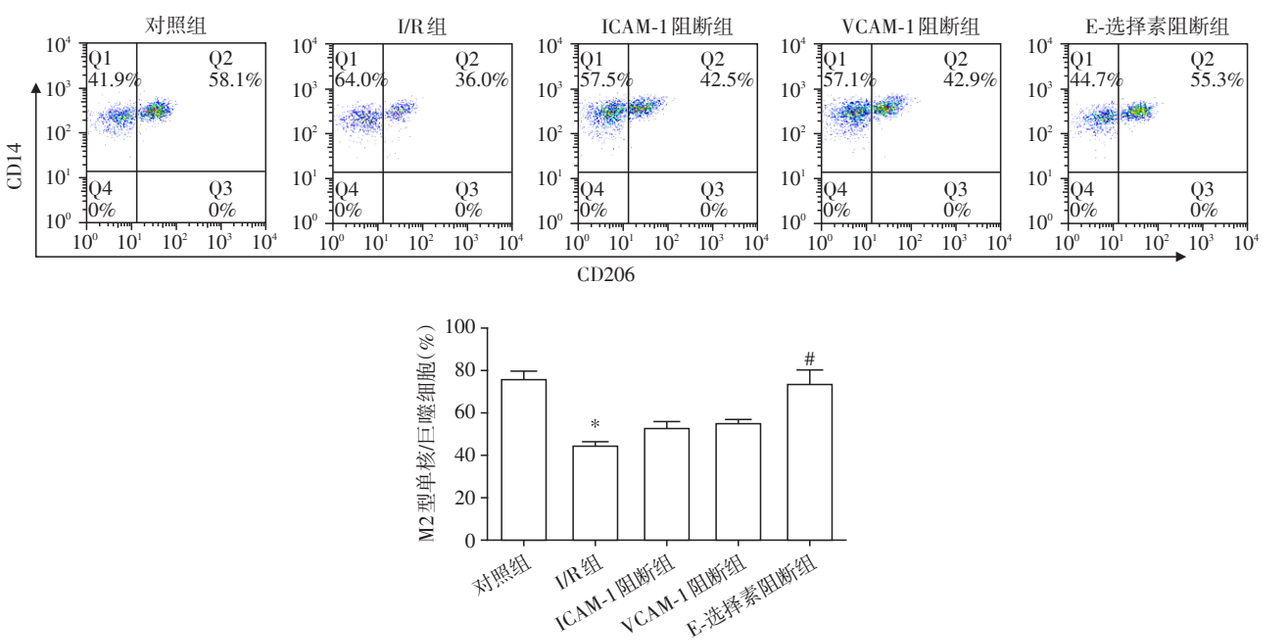
流式细胞术检测结果(图4)显示对照组CD14$^+$CD206$^+$M2型单核/巨噬细胞比例为60.57%,I/R组为35.30%,两组之间具有显著的统计学差异(t=6.424,P=0.003);ICAM-1阻断组和VCAM-1阻断组M2型单核/巨噬细胞比例分别为42.47%和42.03%,相对于I/R组,没有显著的统计学差异(t=2.676,P=0.055;t=1.838,P=0.140);E-选择素阻断组M2型单核/巨噬细胞比例为59.07%,相对于I/R组,结果具有显著的统计学差异(t=4.179,P=0.014)。



与对照组比较,*P<0.05;与I/R组比较,#P<0.05。

图3 缺血再灌注条件下EPC促进单核/巨噬细胞向M1型极化

Figure 3 EPC promotes monocyte/macrophage polarization to M1 under ischemia-reperfusion conditions



与对照组比较,*P<0.05;与I/R组比较,#P<0.05。

图4 缺血再灌注条件下EPC抑制单核/巨噬细胞向M2型极化

Figure 4 EPC inhibits monocyte/macrophage polarization to M2 during ischemia-reperfusion

3 讨 论

心血管疾病是威胁我国人民生命和健康的最大杀手，据统计目前我国心血管疾病患病人数近3亿，死亡率居首位，高于肿瘤和其他疾病，占居民疾病死亡构成的40%以上。近十年，药物洗脱支架在临床广泛应用，使得很多患者能够得到及时救治，显著缓解其临床症状[6]。然而血管再通后的I/R损伤会释放大量的氧自由基，损伤的细胞则会释放大量的炎症分子，诱导单核/巨噬细胞向I/R损伤部分浸润[7]。单核/巨噬细胞分为M1型和M2型2种，M1型为促炎型单核/巨噬细胞，能够分泌大量的炎症分子，包括肿瘤坏死因子-α（tumor necrosis factor-α，TNF-α）和IL-6等，这些升高的炎症分子能够损伤血管内皮细胞，加剧炎症反应；M2型为组织修复型单核/巨噬细胞，能够分泌血管内皮生长因子（vascular endothelial growth factor，VEGF）和转化生长因子-β（transforming growth factor，TGF-β）等，促进组织损伤的修复和血管生成[8]。本研究发现在I/R条件下，EPC能够促进单核/巨噬细胞向M1型极化，引起缺血组织的进一步损伤，这为I/R损伤提供了一个新的机制。

炎症反应与血管生成紧密相关，EPC和单核/巨噬细胞之间相互作用。低强度的炎症反应能够诱导血管生成，过度或者持续的炎症反应则会损伤血管内皮细胞[9]。M2型单核/巨噬细胞能够分泌大量促血管生成分子，比如VEGF和TGF-β等。通过诱导单核/巨噬细胞向M2型极化能够促进损伤血管内皮细胞层的修复和组织的再生修复[10]。EPC表面则表达有3个促炎分子：ICAM-1、VCAM-1和E-选择素，介导了EPC与单核/巨噬细胞之间的相互作用[11]。本研究发现在I/R损伤条件下，E-选择素表达和分泌会显著升高，而ICAM-1和VCAM-1则没有显著的差异。在损伤的情况下，内皮细胞会高表达和大量分泌E-选择素进入外周血，诱导动脉粥样硬化的发生[11]。本研究发现EPC能够通过高表达和分泌E-选择素，促进单核/巨噬细胞向M1型极化，加剧局部的炎症反应，这说明I/R条件下，EPC和单核/巨噬细胞之间的相互作用是通过E-选择素来介导的。因此，在I/R损伤治疗中，应该注意调控EPC表面E-选择素的表达。

综上，本研究发现在I/R损伤条件下，EPC能够通过高表达和分泌E-选择素，促进单核/巨噬细胞向M1型极化，为I/R损伤的治疗提供了新靶点。

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