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基础医学

ACE2基因转染对ApoE-/-小鼠动脉硬化黏附分子的影响

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摘要：

目的 构建血管紧张素转换酶2(ACE2) 的复制缺陷重组腺病毒Ad-ACE2，并观察其对载脂蛋白E基因敲除(ApoE-/-)小鼠动脉硬化黏附分子的影响及意义。方法 采用RT-PCR反应，从小鼠肾脏组织中扩增出小鼠ACE2基因全长的cDNA 序列，克隆到pMD18-T载体，再亚克隆到pDC316载体，构建穿梭质粒(pDC316-ACE2)。穿梭质粒与腺病毒骨架质粒进行同源重组，形成重组腺病毒质粒，重组腺病毒质粒在293细胞内包装成为复制缺陷重组腺病毒Ad-ACE2。采用高脂饲养建立动脉粥样硬化模型后，将16只ApoE-/-小鼠随机分为ACE2基因治疗组和ACE2基因对照组，每组8只。通过尾静脉注射Ad-ACE2和Ad-EGFP分别干预，采用油红O染色，免疫组化及Western blotting观察ACE2治疗后斑块的脂质含量、血管细胞黏附分子(VCAM-1)及E-选择素的变化。结果 RT-PCR反应、酶切及测序结果 证实，Ad-ACE2构建成功；ACE2基因治疗组动脉硬化斑块内脂质含量和黏附分子的表达低于ACE2基因对照组。结论 Ad-ACE2构建成功；ACE2基因过表达可降低动脉粥样硬化斑块内的脂质含量及VCAM-1和E-选择素的表达，减轻斑块严重程度。

关键词： 血管紧张素转化酶2；基因；克隆；动脉粥样硬化

Effects of ACE2 gene transfection on adhesion molecules in atherosclerotic plaque of ApoE-/- mice

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Abstract:

Objective To construct replication-deficient recombinant adenovirus Ad-ACE2 and to investigate the effects of angiotensin-converting enzyme 2 (ACE2) on the severity of atherosclerosis in apolipoprotein-E knockout (ApoE-/-) mice. Methods The full-length ACE2 encoding sequence was amplified from the RNA of mice kidney tissue by RT-PCR technique, cloned into plasmid pMD18-T vector, and then subcloned into plasmid pDC316 to form pDC316-ACE2. Homologous recombination was conducted between the shuttle plasmid and adenovirus skeleton plasmid to form recombinant adenovirus plasmid, then recombinant adenovirus plasmid was packed into replication-deficient recombinant adenovirus (Ad-ACE2) in the 293 cell. High-fat feeding was applied to establish 16 mice models of atherosclerosis, which were then divided into two groups randomly, receiving Ad-ACE2 and Ad-EGFP tail vein injection respectively. The lipid contents, protein expressions of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin were evaluated by Oil Red O staining, immunohistochemical method and Western blotting. Results The recombinant plasmid Ad-ACE2 was confirmed by polymerase chain reaction, enzyme digesting and DNA sequencing. The lipid contents, protein expressions of VCAM-1 and E-selectin were significantly lower in ACE2 gene treatment group than in ACE2 gene control group. Conclusion Ad-ACE2 is constructed successfully. Overexpression of

ACE2 gene can reduce the lipid contents and protein expressions of VCAM-1 and E-selectin in the atherosclerotic plaque, and alleviate the severity of atherosclerotic plaque in ApoE-/- mice.

Keywords: Angiotensin-converting enzyme 2; Gene; Clone; Atherosclerosis

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