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基础研究

ODDD调控EGFP基因的乏氧特异表达载体的构建及其意义

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摘要：

［摘 要］ 目的：构建乏氧诱导因子的氧依赖降解结构域（ODDD）调控的乏氧特异性表达荧光报告载体，探索其乏氧调控目的基因表达的可行性。方法：基因重组技术构建含有序列ODDD403-601aa、ODDD549-575aa和其2串联体调控增强型绿色荧光蛋白(EGFP)荧光报告载体。φA细胞中瞬时表达EGFP，应用RT-PCR技术、Western blotting技术和流式细胞术检测ODDD调控EGFP乏氧特异性表达。结果：克隆得到了ODDD/EGFP表达载体，进行细胞转染实验，在细胞中成功表达EGFP。Western blotting检测，ODDD可以调控EGFP乏氧特异性表达。流式细胞术检测，正常氧浓度下细胞转染pEGFP-ODDD401-603、pEGFP-ODDD549-575和pEGFP-ODDD2(549-575) EGFP的荧光强度分别为（6.06±1.97）%、（11.99±1.13）%和（23.16±2.79）%，低于缺氧条件下EGFP的表达量(P<0.05)。结论：ODDD对靶基因对氧的反应性调节受细胞内的蛋白酶影响；ODDD的调控作用发生在翻译后的蛋白水平，且与细胞内蛋白酶系统功能密切相关。

关键词： 缺氧；增强型绿色荧光蛋白；氧依赖降解结构域；基因治疗

Construction of specific expression vector of EGFP regulated by ODDD in cells under hypoxia and its significance

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Abstract:

Abstract:Objective To construct the vector of specific expression of the enhanced green fluorescent protein(EGFP) regulated by oxygen dependent degradation domain(ODDD) and detect the activity of ODDD in cells at limiting oxygen conditions. Methods The encoding sequences of ODDD 403-601aa,ODDD549-575aa,and 2ODDD549-575aa were inserted into pEGFP-C1 respectively.Transient expression of EGFP in φA cells and relative EGFP expression were measured by RT-PCR,Western blotting and flow cytometry.Results ODDD/EGFP specific expression vector was cloned and the function in cells was analyzed.The ODDD could specially regulate the expression of EGFP in φA cells under hypoxia conditions detected by Western blotting and the activity of ODDD was controlled by proteasome in cells.The fluorescence intensities of EGFP in φA cells under conditions of normoxia in pEGFP-ODDD401-603,pEGFP-ODDD549-575 and pEGFP-ODDD2(549-575) groups were （6.06±1.97）%,（11.99±1.13）%,and （23.16±2.79）%,respectively,and they were lower than that of samples at limiting oxygen conditions (P<0.05). Conclusion Luciferase reporter vectors regulated by ODDD are successfully constructed.

Keywords: hypoxia; enhanced green fluorescent protein; oxygen dependent degradation domain; gene therapy

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