中南大学学报(医学版) 2013, 38(11) 1135-1140 DOI: 10.3969/j.issn.1672-7347.2013.11.009 ISSN: 1672-7347 CN: 43-1427/R

论著

构建p53双荧光报告载体及其功能验证

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摘要： 目的：构建p53双荧光报告载体，验证其能否模拟野生型p53的生物学活性并适用于高通量筛选。方法：用PCR在p53和萤火虫荧光素酶的开放阅读框两端引入酶切位点并移除p53的终止密码和萤火虫荧光素酶的起始密码，然后将二者插入到由内部核糖体进入位点引导的海肾荧光素酶的上游，从而构建出一个能表达P53萤火虫荧光素融合蛋白的p53FL/IRES/RL双荧光报告载体。转染该报告载体后，检测被表达的P53荧光素融合蛋白是否被MDM2降解、该融合蛋白的亚细胞定位和它对p53特异性启动子的诱导。结果：成功构建出p53双荧光报告载体。该载体在宿主细胞内表达的P53荧光素融合蛋白能被MDM2降解；主要分布于细胞核；具有野生型p53的转录活性。结论：新构建的p53双荧光报告载体p53FL/IRES/RL能有效模拟野生型p53的功能，适用于高通量筛选调节P53蛋白含量的药物、基因或化合物。

关键词： 高通量筛选 p53,野生型 双荧光载体 转染

Constructing a p53-fused dual luciferase reporter and verifying its function

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Abstract: Objective: To construct a p53-fused dual luciferase reporter and to test whether this reporter can mimic wild-type p53 activities in a high-throughput screen. Methods: A restriction endonuclease site was added to each terminus and the stop codon of the wild-type full-length p53 open reading frame (ORF) was removed by PCR. A restriction endonuclease site was added to each terminus and the start codon of the firefly luciferase ORF was removed by PCR. The two modified ORFs were inserted upstream of the IRES-induced renilla luciferase ORF in a CMV-derived vector. The p53 fusion protein was expressed in cells to test its MDM2-mediated degradation, subcellular localization, and induction of p53-responsive promoter. Results: The p53-fused dual luciferase reporter was successfully constructed. After transfection into the host cells, the reporter expressing the p53 fusion protein that was degraded by oncoprotein MDM2, was mainly located inside the nucleus, and induced the p53-responsive promoter, respectively.Conclusion: The p53-fused dual luciferase reporter (p53FL/IRES/RL) can identify modulators of P53 protein level in a high-throughput screen of genetic or chemical libraries.

Keywords: high-throughput screen p53, wild-type dual luciferase reporter transfection

收稿日期 2012-10-28 修回日期 网络版发布日期

DOI: 10.3969/j.issn.1672-7347.2013.11.009

基金项目:

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