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论著

MTS 1基因β启动子E 2 F 1结合位点序列突变重组质粒的构建与表达

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摘要 目的： 深入研究MTS 1基因β启动子的转录激活与E 2 F 1转录因子的相互作用关系，阐明该基因转录水平的调控机制。 方法： 用PCR定点突变方法或酶切连接法，构建β启动子0.38 kb SacⅡ-SacⅠ酶切片段中E2 F 1 A、B、C任意2个位点或3个位点均突变的pGL 3重组质粒。用脂质体介导的基因瞬时转染法，将构建的重组质粒转染MTS 1基因双等位缺失的急性T淋巴细胞白血病Jurkat细胞，检测pGL 3重组质粒中荧光素酶报告基因的表达。结果：构建的E 2 F 1A、B、C结合位点突变的重组质粒经SacⅠ或NaeⅠ酶切鉴定和DNA序列分析得到证实。与E 2 F 1位点野生型重组质粒比较，突变型重组质粒在Jurkat细胞中荧光素酶报告基因的表达量减少，以3个位点均突变的重组质粒为明显。结论：构建的E 2 F 1A、B、C2个或3个结合位点均突变重组质粒成功，可通过基因转染用于研究MTS 1基因的功能试验中；MTS 1基因β启动子的转录活性可能与E 2 F 1转录因子的反式激活有关。

关键词 MTS 1基因；E 2 F 1转录因子；基因突变

CONSTRUCTION AND EXPRESSION OF THE RECOMBINANT PLASMID OF THE MTS 1 GENE β PROMOTER ON E 2 F 1-BOUND LOCUS MUTATION

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Abstract Purpose: To further study the effect of E 2 F 1 transcription factor on the transcriptional activation of β promoter, and explain the transcriptional regulation mechanism on MTS 1 gene. Methods:The recombinant plasmid containing 2 or 3 mutant or deleted on E 2 F 1 A, B, C-bound locus sequence on the 0.38 kb fragment cut by SacⅡ and SacⅠ of the β promoter was constructed by PCR site-directed mutagenesis and enzymatic cutting and ligating. The recombinant plasmids containing 2 or 3 mutant or deleted on E 2 F 1 A, B, C-bound locus sequence were transfected into Jurkat cells, which were biallelic deletion of MTS 1 gene by transient transfection. Luciferase report gene was used to observe β promoter transcriptional activation. Results: Four new recombinant plasmids containing the mutant with two bound loci of E 2 F 1 A,B,C were obtained separately by PCR, and a recombinant plasmid containing all the three mutant on locus was constructed by enzymatic cutting and ligating, and identified by SacⅠ or NaeⅠ enzymatic cutting, and sequencing. The luciferase expression of recombinant plasmid in Jurkat cells decreased, especially the mutant 3 bound locus sequence of E 2 F 1 A, B, C, as compared to the wild-type recombinant plasmid on E 2 F 1-bound locus sequence. Conclusion: These newly-constructed recombinant plasmids can be used to study the function of the transcriptional activation of MTS 1 gene by gene transfection. There is a potential relation between the transcriptional activation of MTS 1 gene β promoter and the transactivation of E 2 F 1 transcription factor.

Keywords MTS 1 gene E 2 F 1 transcriptional factor Gene mutation

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