中南大学学报(医学版) 2010, 35(8) 792- DOI： 10.3969/j.issn.1672-7347.2010. ISSN: 1672-7347 CN: 43-1427/R

论著

人MUC5AC基因启动子荧光素酶报告基因载体的构建及其转录活性分析

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摘要：

目的：克隆人黏蛋白/黏液素5AC(MUC5AC)基因启动子并构建该启动子的荧光素酶报告系统，探讨该启动子的活性及转录靶向性。方法：运用Vector NT1软件包对MUC5AC基因5′端1 348 bp进行启动子特征分析；以人A549细胞基因组DNA为模板分离出人MUC5AC基因5′端非翻译区大小为1 348 bp的片段并进行测序鉴定，再以扩增产物为模板对启动子进行5′端删除分析，分别扩增737 bp(-689/+48)，372 bp(-324/+48)，112 bp(-64/+48)的片段，与荧光素酶报告基因载体重组构建，通过双荧光素酶活性进行转录活性分析。应用定点突变技术，在重组质粒的基础上建立MUC5AC启动子区SP-I结合位点和核因子（NF）-κB结合位点单独突变体，并测定中性粒细胞弹性蛋白酶(NE)诱导的转染细胞荧光素酶的相对活性。结果：序列分析发现人MUC5AC基因5′端1 348 bp的区域内存在多个顺式作用元件，成功构建了4个不同长度的MUC5AC启动子报告基因载体。双荧光素酶活性分析372 bp片段为有活性的最小片段。NE可诱导含有MUC5AC启动子区NF-κB结合位点单独突变体（pGL3E-MUC5AC-NF-κB-MU）荧光素酶相对光强度增加，而NE不能诱导SP-I结合位点单独突变体（pGL3E-MUC5AC-SP-1-MU）荧光素酶表达增加。结论：上述载体的成功构建及序列分析为进一步研究MUC5AC基因的启动子活性及基因表达调控奠定了基础。MUC5AC 5′上游序列中-324/-64位点存在参与NE诱导MUC5AC基因表达的重要调控元件，位于此区域的顺式作用元件SP-1位点在NE诱导MUC5AC基因表达机制中起重要作用。

关键词： 人MUC5AC基因 启动子 基因序列分析 转录活性分析

Construction of luciferase reporter gene vector for human MUC5AC gene promoter and analysis of its transcriptional activity

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Abstract:

ObjectiveTo clone the human mucin (MUC)5AC gene promoter and construct its luciferase reporter vector for human MUC5AC gene and analyze its transcriptional activity. MethodsThe 1 348 bp DNA sequence at the human MUC5AC gene 5 end was analyzed by the Vector NTI software. After the target sequence from human A549 cells genomic DNA was amplified by PCR method， and the product of PCR was sequenced. By promoter deletion analysis， 3 promoter segments with diferent lengths were amplified by PCR， then the products were identified by DNA sequencing, and 4 promotor segments were inserted into pGL3- enhancer vectors. Site-specific mutagenesis technique was used to establish mutants of specificity protein (SP)-l and nuclear factor-kappa B (NF-κB) site in MUC5AC gene promoter. The relative luciferase activities were detected in the transfected A549 cells. ResultsSequence analysis indicated that there were many cis-acting elements in the regions of 1 348 bp DNA sequence at the human MUC5AC gene 5 end. The 4 reporter gene vectors with promoter segments with different lengths were constructed successfully. Dual-luciferase assay revealed the 372 bp fragment including activity with the minimal fragment. Neutrophil elastase (NE) could increase the expression of luciferase reporter gene plasmid containing mutated NF-κB version (P＜0.05 vs. contro1) of MUC5AC promoter in the transfected A549 cells. The induction by NE decreased markedly when the SP-l element in MUC5AC promoter were mutated. ConclusionThis research may provide an important basis for the further study of human MUC5AC gene promoter activity and regulation of gene expression. There is an up-regulative element of gene transcription in the region of -324 to -64 bp in MUC5AC gene upstream. SP-l site of the promotor mediates NE-induced MUC5AC expression in human A549 cells.

Keywords: human MUC5AC gene；promoter；gene sequence analysis；transcriptional activity analysis

收稿日期 2009-12-06 修回日期 网络版发布日期

DOI: 10.3969/j.issn.1672-7347.2010.

基金项目:

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