

反义封闭MMP-9基因表达对胶质母细胞瘤细胞系增殖的影响

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Effects of MMP-9 Gene Suppression by Antisense on Proliferation of Human Glioblastoma Cell Line *in vitro*

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- 摘要
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摘要 目的 探讨反义封闭胶质瘤细胞MMP-9基因表达对胶质瘤细胞增殖的影响。方法 利用基因重组的方法构建正义和反义MMP-9重组体;经脂质体介导,分别将pcDNA3.0空载质粒、正义重组体、反义重组体转染TJ905细胞;通过RT-PCR和Western blot检测转染细胞MMP-9的mRNA和蛋白表达水平;利用免疫组织化学法分析了转染细胞中MMP-9和Ki-67的表达。结果经限制性酶切鉴定和DNA测序分析,基因重组成功,且正义与反义重组体插入位点间的序列方向正好相反。与TJ905对照组、空载体组和正义对照组相比较,转染反义重组体后的TJ905细胞中MMP-9 mRNA和蛋白的表达水平明显下降(P<0.001)。转染空载体和正义重组体后,TJ905细胞内MMP-9 mRNA和蛋白的表达水平与未进行转染的TJ905细胞相比差异无统计学差异(P>0.05)。免疫组织化学结果显示反义封闭有效,MMP-9的表达下调,TJ905细胞增殖活性下降。结论 转染反义MMP-9重组体可以有效抑制胶质母细胞瘤细胞的MMP-9基因的表达,同时可以抑制肿瘤细胞的增殖。

关键词: 基质金属蛋白酶 胶质瘤 反义RNA 基因治疗

Abstract: Objective To evaluate the effects of MMP-9 gene down-regulation by antisense on proliferation of a human glioblastoma cell line. Methods A 528bp cDNA fragment of MMP-9 was amplified by RT-PCR with synthetic primers and subcloned into the pcDNA3.0 vector in the sense and antisense orientations. Then TJ905 cells were transfected with sense construct, antisense construct and pcDNA3.0 by using Lipofectamine 2000. MMP-9 RNA and protein were detected by RT-PCR and Western blot respectively. MMP-9 and Ki-67 expression were detected by immunohistochemistry assay. Results Restriction endonuclease analysis and sequence analysis of recombination plasmids verified that 528bp internal sequence was 100% homology with the published sequence of MMP-9 cDNA. Compared with controls, vector-transfected clones and sense MMP-9 construct-transfected clones, antisense MMP-9 construct-transfected TJ905 cells had decreased MMP-9 mRNA and protein levels (P<0.001). The proliferation of antisense MMP-9 construct-transfected TJ905 cells was inhibited. No significant difference in expression of MMP-9 was observed among vector-transfected clones, sense MMP-9 construct-transfected clones and controls (P>0.05). Conclusion Proliferation of glioblastoma cell was able to be effectively inhibited through antisense MMP-9, suggesting antisense MMP-9 may be useful for malignant glioma treatment in future.

Key words: Matrix metalloproteinases Glioma Antisense RNA Genetic therapy

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