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HCMV UL97 mRNA序列特异性M1GS的构建及其体外切割活性研究

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摘要 HCMV UL97基因编码一种蛋白激酶，该酶参与调控病毒DNA的复制和衣壳的形成，且序列异常保守，可作为抗HCMV治疗的重要靶位。基于HCMV UL97 mRNA T3位点附近的序列，设计一段与该位点互补的引导序列（Guide Sequence，GS），并将其与大肠杆菌核酶P催化亚基（M1 RNA）的3’末端共价连接，构建了一种序列特异性的M1GS（M1-T3）。体外试验证实，所构建的M1-T3可与UL97 mRNA的T3位点特异性结合并产生有效的切割作用。进一步研究M1-T3的结构与其对底物片段靶向切割活性的关系，结果发现在M1 RNA与GS之间增加一段88核苷酸桥连序列的M1-T3（即M1-T3*），其靶向切割活性大大增强。此外，去除M1-T3 3’末端的CCA序列，其靶向切割活性将基本丧失。上述结果表明，这段桥连序列和3’末端的CCA序列是M1-T3 重要的结构元件。本研究一方面有助于阐明M1GS与其底物的相互作用机制，同时也为下一步评价M1-T3在体内对UL97基因表达及病毒复制的抑制活性奠定了基础。

关键词 核酶P（RNase P）； M1GS；人巨细胞病毒；UL97

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Construction of An Effective M1GS Ribozyme Targeting HCMV UL97 mRNA Segment in Vitro

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Abstract

A sequence-specific M1GS ribozyme (M1-T3) was constructed by covalently linking an oligonucleotide (guide sequence, GS) to the 3’ terminus of M1 RNA , the catalytic subunit of RNase P from Escherichia coli. The engineered ribozyme is targeted to the mRNA sequence encoding a protein kinase（UL97）of HCMV and could effectively cleave the mRNA segment in vitro. Further studies about the significance of some structural elements in the M1GS (e.g. the 3’ CCA tail sequence and a bridge sequence between the 3’ terminus of M1 RNA and the 5’ terminus of the GS) were carried out. The results showed that the bridge sequence of 88 nucleotides in a mutated M1GS (i.e. M1-T3*) dramatically increased the cleavage activity to the substrate in vitro. Moreover, the 3’CCA tail sequence was confirmed to be a necessary element for the cleavage activity of M1GS ribozyme. These data we got in the study will help in understanding the interaction between the M1GS RNA and its substrate, and will markedly facilitate the research of a general gene targeting agent for anti-HCMV applications.

Key words Ribonuclease P (RNase P)； M1GS； HCMV； UL97

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