中国医学科学院学报
ACTA ACADEMIAE MEDICINAE SINICAE
Medline收录 核心期刊
ISSN 1000-503X CN 11-2237/R

中国医学科学院学报 » 2010, Vol. 32 » Issue (3) :283-288 DOI: 10.3881/j.issn.1000-503X.2010.03.010

锌α2糖蛋白真核表达载体的构建和体外表达鉴定

张韶君,龚凤英,邓洁英,朱惠娟,潘慧,张殿喜,李乃适*

中国医学科学院 北京协和医学院 北京协和医院内分泌科卫生部内分泌重点实验室,北京100730

Construction of Zinc-α2-glycoprotein Expression Plasmid and Its Expression in 3T3-L1 Preadipocytes

ZHANG Shao-jun,GONG Feng-ying,DENG Jie-ying,ZHU Hui-juan,PAN Hui,ZHANG Dian-xi,LI Nai-shi*

Key Laboratory of Endocrinology of Ministry of Health,Department of Endocrinology,PUMC Hospital,CAMS and PUMC,Beijing 100730,China

摘要 目的 构建小鼠锌α2糖蛋白(mZAG)真核表达载体,在体外培养细胞中鉴定其mRNA和蛋白水平表达。方法 提取小鼠肝组织总RNA,采用RT-PCR法扩增mZAG全基因表达序列,酶切法将mZAG cDNA全序列插入表达质粒载体pcDNA3.1(-)中构建pcDNA3.1(-)-mZAG真核表达质粒。采用阳离子脂质体转染法将不同浓度mZAG表达质粒(0、0.4、0.8、1.6 μg)pcDNA3.1(-)-mZAG和4对mZAG小干扰RNA (siRNA)序列转染到3T3-L1前脂肪细胞中,实时荧光定量RT-PCR测定mZAG mRNA表达水平,Western blot检测mZAG蛋白表达情况。结果 测序鉴定证实成功构建mZAG真核表达质粒pcDNA3.1(-)-mZAG。实时荧光定量RT-PCR检测结果显示,0.4、0.8、1.6 μg mZAG转染组3T3-L1细胞中的mZAG mRNA表达水平分别是0μg mZAG转染组的2.58(P=0.002)、3.67(P=0.000)、5.19倍(P=0.001);mZAG-siRNA1组和mZAG-siRNA4组小鼠3T3-L1细胞中的mZAG mRNA表达水平显著减少,分别是不转染mZAG siRNA 干扰序列对照组的49%(P=0.002)和41%(P=0.000)。Western blot检测结果显示,0.8μg mZAG质粒转染组的体外mZAG蛋白表达水平是不转染 mZAG质粒对照组的2.75倍(P=0.017);mZAG-siRNA1和mZAG-siRNA4干扰序列转染组的体外mZAG蛋白表达水平仅为对照组的55%(P=0.004)和62%(P=0.025)。结论 成功构建mZAG真核表达载体,该载体能够在体外细胞中良好表达。筛选出能够显著抑制mZAG表达的siRNA1和siRNA4,为今后进一步深入研究ZAG提供了有用工具。

关键词： 锌α2糖蛋白 3T3-L1细胞 小干扰RNA 质粒构建

Abstract： Objective To construct mouse Zinc-α2-glycoprotein (mZAG) eucaryotic expression plasmid and identify its expression in 3T3-L1 preadipocytes. Methods The total RNA from mouse liver tissue was extracted. The reverse-transcript(RT)-PCR method was used to amplify the complete domain sequence of mZAG,and the confirmed PCR products was inserted into expression plasmid by DNA ligation. The mZAG expression plasmids with various concentrations (0,0.4,0.8,and 1.6 μg) were transfectd into 3T3-L1 preadipocytes,and ZAG expression in mRNA and protein level was determined by real-time fluorescence quantitative PCR and Western blot,respectively. Results DNA sequencing confirmed the right sequence of mZAG expression plasmid pcDNA3.1(-)-mZAG. After the mZAG expression plasmid with different concentrations were transfected into 3T3-L1 preadipocytes,mZAG mRNA level significantly increased and reached 2.58 folds (P=0.002),3.67 folds (P=0.000） and 5.19 folds (P=0.001) of that in the control group (no mZAG transfection). mZAG protein level also significantly increased and reached 2.75 folds of that in the control group (P=0.017). Treating 3T3-L1 cells with small interferencing RNA (siRNA) sequence siRNA1 and siRNA4 resulted in a decrease of mZAG mRNA to 49% and 41% of those in the contro1 group(no siRNA sequence trasfection) (P=0.002,P=0.000)and a decrease of mZAG protein to 55% and 62% of that in the contro1 group (P=0.004,P=0.025). Conclusions mZAG expression plasmid pcDNA3.1(-)-mZAG was successfully established in this study. This plasimd can be well expressed in 3T3-L1 preadipocytes. siRNA1 and siRNA4 can effectively inhibit the expression of mZAG in these cells.

Keywords： Zinc-α2-glycoprotein 3T3-L1 preadipocytes small interferencing RNA plasmid construction

Received 2009-11-23; published 2010-09-10

Fund:国家自然科学基金(30540036、30771026)和北京市自然科学基金(7082079)

Corresponding Authors: 龚凤英 Email: fygong5074@yahoo.com.cn

About author: 010-65295074,传真010-65295073,电子邮件:fygong5074@yahoo.com.cn

引用本文:
张韶君,龚凤英,邓洁英,朱惠娟,潘慧,张殿喜,李乃适.锌α2糖蛋白真核表达载体的构建和体外表达鉴定[J] 中国医学科学院学报, 2010,V32(3): 283-288

ZHANG Shao-jun,GONG Feng-ying,DENG Jie-ying,ZHU Hui-juan,PAN Hui,ZHANG Dian-xi,LI Nai-shi.Construction of Zinc-α2-glycoprotein Expression Plasmid and Its Expression in 3T3-L1 Preadipocytes[J] CAMS, 2010,V32(3): 283-288