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小鼠canstatin及其N端片段在大肠杆菌BL21 中的表达 Expression of Mouse canstatin and its N-domain in E.coli BL21

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摘要 以小鼠肝脏组织总RNA为模板，通过RT-PCR扩增小鼠canstatin及其N端片段基因，克隆到pMD18-T载体中并进行序列分析。将小鼠canstatin及其N端片段基因定向克隆于原核表达载体pET30a(+)中,分别构建表达质粒pET/Can和pET/Can-N, 转化大肠杆菌BL21(DE3), IPTG诱导表达。结果表明: 小鼠canstatin的cDNA长度为684bp，编码227个氨基酸，与已知的人canstatin cDNA同源性为89%，氨基酸的同源性为96%。小鼠canstatin N端片段(1-95aa)与人的同源性为100%。 IPTG诱导原核表达载体pET/Can和pET/Can-N在大肠杆菌 BL21(DE3)中的表达量约占菌体总蛋白量的35% 和 18%, 重组蛋白主要以包涵体形式存在。文中报道的小鼠canstatin 及其N端片段核苷酸序列已收入GenBank, 接受号分别为: AY375463和AY502946。Abstract:The mouse canstatin and its N-domain cDNA were amplified from total RNA of mouse liver by RT- PCR and cloned into vector pMD18-T for sequencing. Prokaryotic expression vectors pET/Can and pET/Can-N were constructed and expressed in E.coli BL21(DE3) with induction of IPTG.. Mouse canstatin cDNA is 684bp in length encoding 227 amino acids. The sequences of both cDNA and amino acids share high homology with human canstatin, with cDNA identity at 89% and amino acids identity at 96% to human canstatin. N-domain of mouse canstatin is the same amino acid sequence as that of human canstatin. In the present study, prokaryotic expression vector pET/Can and pET/Can-N were expressed in E.coli BL21 with amount of 35% and 18% of the total bacterial proteins after being induced by IPTG for 4h. The expressed products existed mainly as inclusion bodies. This work has laid down the basis for further study of its angiogenic activity and potential application for tumor dormancy therapy.

关键词 canstatin 血管生成抑制素 RT-PCR cDNA克隆 Key words canstatin angiogenesis inhibitor RT-PCR cDNA cloning prokaryotic expression.
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Abstract

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