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论著

尖吻蝮毒腺cDNA文库的构建、金属蛋白酶基因克隆和序列分析

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摘要 目的 构建非标准化尖吻蝮（五步蛇，*Agkistrodon acutus*）毒腺cDNA文库，随机挑取克隆测序，分析金属蛋白酶基因。方法 以Trizol试剂提取新鲜尖吻蝮毒腺总RNA，以superscript Ⅱ反转录酶合成cDNA第一链并以DNA聚合酶Ⅰ连续合成第二链。双链DNA经过含EcoRⅠ酶切位点接头加接，末端磷酸化并以 XhoⅠ内切酶酶切，按照< 0.25 kb, 0.25～0.5 kb, 0.5～1 kb, 1～2 kb 和>2 kb 5个片段大小分别回收，随后与pBluescript Ⅱ SK (+) 载体相连转化E.coli DH10B，构建成尖吻蝮毒腺cDNA文库。随机挑取克隆5′端测序，共获得8696条高质量表达序列标签，经过序列拼接和聚类，这些序列在经过功能注释后最终被聚类成2855个基因聚类。其中，发现一个由74个克隆组成的基因聚类（*Agkihagin*）为新的金属蛋白酶基因。经反转录和巢式PCR扩增该基因并对其进行结构分析。结果 构建好的文库含有2.048×10^{6}个重组子，新的金属蛋白酶开框读码序列全长1827个核苷酸，编码608个氨基酸，属于 PIII型金属蛋白酶。其Zn^{2+}结合模序HEMGHNLGIDH和去整合素模序DECD在进化上高度保守。结论 该文库符合建库标准库容要求，为构建尖吻蝮毒腺基因表达谱和筛选新的目的基因提供了有效平台；克隆的金属蛋白酶基因与GenBank中其他蛇毒金属蛋白酶氨基酸序列同源性最高达87%，为研究蛇毒金属蛋白酶结构与功能的关系奠定了良好基础。

关键词 基因文库 蝮蛇类 金属内肽酶类 序列分析
分类号 R996.3

Construction of a cDNA library from *Agkistrodon acutus* venom gland and identification of *Agkihagin*, a novel transcript for metalloproteinase

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Abstract

AIM To construct a non-normalized cDNA library from *Agkistrodon acutus* venom gland as an imtial step to develop new and more effective venom by genetic engineering technique for screening and expressing target genes. **METHODS** The total RNA was extracted from fresh venom gland using Trizol. mRNA was reversely transcripted to cDNA using superscript Ⅱ reverse transcriptase. Second-strand synthesis was performed using DNA polymerase Ⅰ. After adding EcoR Ⅰ adaptor, phosphorylating the end and digesting with Xho Ⅰ, the cDNA was collected in five fractions (<0.25 kb, 0.25-0.5 kb, 0.5-1 kb, 1-2 kb and >2 kb) using the QIAquick Gel Extraction kit and ligated to pBluescript Ⅱ vectors. The five libraries obtained were plated by infecting E.coli DH10B, constructing a cDNA library of *Agkistrodon acutus* venom gland. Sequencing clones at random, 8696 high quality 5′ end expressed sequenced tags (ESTs) were obtained and analyzed. The initial sequences were assembled into 2855 clusters. Among which, one of the clusters (*Agkihagin*) consisting of 74 ESTs was identified as a novel metalloprtoteinase based on RT-PCR and sequence analysis. **RESULTS** The titers of library were 2.048×10^6. The novel metalloproteinase belonged to PIII type metalloproteinase. Its open reading frame was composed of 1827 nucleotides and coded a pre-zymogen of 608 amino acid with zinc-binding domain for metalloproteinase and Asp-Glu-Cys-Asp(DECD) domain for disintegrin. **CONCLUSION** The capacity of cDNA library of venom gland is above the general level of cDNA library. It would be a helpful platform to construct a catalog for transcripts in the venom gland of the *Agkistrodon acutus*. The sequence analysis indicates that the deduced amino acid sequence of the identified gene for metalloproteinase share the highest 87% identity with the metalloproteinase genes of other snakes in the GenBank. It lays a good foundation for the study of structure-function relationships of snake venom metalloproteinases.

Key words gene library Agkistrodon acutus metalloendopeptidase sequence analysis

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