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杜氏利什曼原虫表达位点相关基因样蛋白的分子克隆及表达定位

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Molecular Cloning and Localization of Leishmania donovani Expression Site Associated Genes-like Protein

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摘要

目的 克隆杜氏利什曼原虫 (Leishmania donovani) 无鞭毛体特异表达新基因, 观察其编码蛋白的亚细胞定位。方法 制备杜氏利什曼原虫前鞭毛体和无鞭毛体mRNA, 以消减抑制杂交技术筛选无鞭毛体新的表达序列标签, 扩增含有新表达序列标签的基因全长cDNA, Northern杂交和RT-PCR检测新基因在前鞭毛体和无鞭毛体中的表达, 共表达方法观察杜氏利什曼原虫内新基因编码蛋白的亚细胞定位。结果 构建了杜氏利什曼原虫无鞭毛体表达序列标签消减文库, 克隆到一个新基因, 命名为表达位点相关基因样蛋白 (ESAGLP) 基因, 其cDNA全长为2 258 bp, 编码620 aa。ESAGLP基因仅在无鞭毛体内表达, 其编码蛋白定位于线粒体。结论 ESAGLP鉴定为杜氏利什曼原虫无鞭毛体新基因, 其编码蛋白定位于无鞭毛体的线粒体。

关键词: 杜氏利什曼原虫, ESAG样蛋白, 表达, 定位, 消减抑制杂交

Abstract:

Objective To clone the novel gene that specifically expressed in the amastigotes of Leishmania donovani, and observe subcellular localization of the gene encoding protein. Methods mRNA from promastigotes and amastigotes of L. donovani were prepared. The novel expressed sequence tag of amastigotes was selected by suppression subtractive hybridization. The expression of the novel gene in different stages of L. donovani was detected by Northern hybridization and semi-quantitative RT-PCR. The subcellular localization of the novel gene encoding protein was observed. Results The subtractive library of the specifically expressed sequence tag of amastigotes was constructed, and a novel gene designated as expression site associated genes-like protein (ESAGLP) gene was cloned. The full length of ESAGLP cDNA was 2 258 bp. The open-reading frame encoded a polypeptide of 620 amino acid residues. ESAGLP gene expressed only in amastigotes, the encoding protein was localized in the mitochondria. Conclusion The ESAGLP gene is identified as a novel gene which specifically expressed in Leishmania donovani amastigotes, and its encoding protein is localized in the mitochondria.

Key words: Leishmania donovani ESAG-like protein Expression Localization Suppression subtractive hybridization

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