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利用抑制消减杂交技术研究结核分支杆菌强毒株H37Rv和弱毒株H37Ra的基因差异

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摘要 为了解结核病的致病分子机理和筛选结核病致病菌的毒力基因，利用抑制消减杂交（SSH）技术分析了结核分支杆菌强毒株H37Rv和弱毒株H37Ra间的基因组DNA间差异。通过Southern杂交验证及序列分析得到仅在强毒株H37Rv基因组中有的DNA片段8个，其中一个编码已知的毒力因子mce蛋白，1个编码PE家族蛋白，1个编码purC合成酶，和4个潜在蛋白，另1个为非编码区片段。其中有2个基因经PCR方法已证实在强毒株H37Rv和临床分离的强毒株中存在，而在H37Ra和临床弱毒株中无；仅在弱毒株H37Ra基因组中的DNA片段3个，其中2个为新基因片段，已被GenBank收录。

关键词 结核杆菌；抑制消减杂交技术；差异基因

分类号

Identification of Differential Genomic Genes of Mycobacterium tuberculosis H37Rv and Attenuated Strain H37Ra by Suppression Subtractive Hybridization

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Abstract

To study the virulence-related genes in Mycobacterium tuberculosis, we used suppression subtractive hybridization to clone the differential genomic genes between Mycobacterium tuberculosis virulence strain H37Rv and attenuated strain H37Ra. All of 54 different genes were cloned, sequenced and analyzed by Southern-blotting. 2 different DNA fragments in H37Ra are new genes so far, and get the new Genbank number AY53450 and AY560011. 8 different DNA fragments in H37Rv were obtained. One is fragment of gene coding virulence factor mce, which is the virulence factor. one fragment belongs to gene coding for enmyze, one for PE family protein, the other 4 fragments for putative gene, and the last one for non-coding fragment. Revealed by PCR analysis, 2 of the different genes were present exclusively in the clinical virulent strain and H37Rv, but not in the clinical avirulent strain and H37Ra. The novel differential genes may provide an important clue for studying the mechanism of M. tuberculosis pathogenesis.

Key words Mycobacterium tuberculosis Suppression subtractive hybridization Different genes

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