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研究论文

cDNA表达文库转染NIH3T3－从高转移人肺腺癌细胞系Anip973中克隆新肿瘤转移相关基因

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摘要

培养人高转移肺腺癌细胞系Anip973，构建其cDNA表达文库并转染小鼠成纤维细胞NIH3T3，将经药物筛选后的转染细胞克隆消化为单细胞，接种到软琼脂中培养2周，根据细胞明显的形态学变化挑选出有意义的细胞克隆，扩增再培养，提取DNA，PCR扩增插入片段并进行测序分析。结果表明：软琼脂中挑选出克隆100多个，PCR测序后，得到3个已知基因包括人类核糖体蛋白L23、人类假定蛋白FLJ22104和人类丝氨酸蛋白酶抑制因子6亚型以及一些氨基末端截短的核酸序列。进一步的研究表明转染人类核糖体蛋白L23的细胞与转染空载体细胞相比具有较高的侵袭能力(P<0.02)。利用 cDNA文库在NIH3T3细胞中的表达，随后筛查鉴定在软琼脂中发生形态学变化的细胞，是一种寻找恶性转化和癌转移相关基因的有效方法。人类核糖体蛋白L23基因在细胞的运动和转移中发挥重要作用。

关键词 cDNA表达文库；软琼脂；癌基因；转移；核糖体蛋白L23

分类号

Cloning of Novel Tumor Metastasis-Related Genes from the Highly Metastatic Human Lung Adenocarcinoma Cell Line Anip973

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Abstract

<P>A cDNA library was successfully constructed from Anip973, a human lung adenocarcinoma cell line with high metastatic potential. NIH3T3 cells were stably transfected using this cDNA library and screened for morphological changes in a soft agar assay. Genomic DNA was isolated from putative clones and the integrated sequence was retrieved by PCR and sequencing. Three known genes, ribosomal protein L23, hypothetical protein FLJ22104, and serine protease inhibitor, kazal type 6 and a number of 5′-terminally truncated sequences were identified. Furthermore, cells transfected with ribosomal protein L23 was highly invasive compared with the empty vector as control (P < 0.02). These results indicate that the expression cloning of cDNA libraries in NIH3T3 cells and subsequent screening for loss of contact inhibition in soft agar is a viable tool for identifying tumor-related genes and ribosomal protein L23 gene plays a role in cell movement and metastasis. </P>

Key words cDNA expression library；soft agar；oncogene；metastasis；Homo sapiens ribosomal protein L23

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