生物化学与生物物理进展
Progress in Biochemistry and Biophysics

肾癌相关基因克隆——肾癌cDNA消减文库的构建

Cloning of Renal Cell Carcinoma Relation Gene:Construction of a cDNA Subtractive Library of Human Renal Cell Carcinoma and Its Significance

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中文摘要：

应用抑制性消减杂交技术，构建人肾癌与正常肾差异表达的cDNA消减文库.分别从肾癌及正常肾细胞系中提取poly(A)$^+$RNA，依次合成单链及双链cDNA，经酶切成平均大小为400～600 bp的片段，将肾癌cDNA分为两组，分别与两种不同的接头衔接，再与正常肾cDNA进行两次消减杂交及两次抑制性PCR后，将产物与T/A载体连接构建成功cDNA消减文库，并转染大肠杆菌进行文库扩增.构建成功具有高消减效率的人肾癌cDNA消减文库，非特异性cDNA片段被有效地消减，特异表达的cDNA得到富集.文库扩增后得到6 500个克隆，随机挑取350个制备质粒，酶切分析均得到400～600 bp插入片段.所构建的人肾癌cDNA消减文库为进一步大批量筛选、克隆肾癌特异性表达的未知新基因奠定了基础.

英文摘要：

To construct a cDNA subtractive library of human renal cell carcinoma (RCC) with technique called suppression subtractive hybridization. The library only contains the differently expressing cDNAs between RCC and normal kidney. Poly(A)$^+$ RNA were isolated from cell lines of RCC and normal kidney respectively.Moreover, single-strand cDNAs and double-strand cDNAs were synthesized in turn. After enzyme restriction,cDNAs between 400～600 bp were obtained. RCC cDNAs then were divided into two groups and ligated to the specific adaptor 1 and adaptor 2 respectively .After RCC cDNAs hybridized with normal kidney cDNA twice and underwent two times of nested PCR,then with arms of T/A plasmid vectors to set up the subtractive library. Amplification of the library was carried out with the *E.coli* strain Top 10F′. Human RCC subtrctive library with high subtractive efficiency was set up sucessfully. The amplified library contains 6 500 positive clones.Random analysis of 350 clones with enzyme restriction shows that all plasmids in the clones contain 400～600 bp inserts. The constructed cDNA subtractive library of human RCC is a highly efficient one and lays solid foundation for screening and cloning new and specific oncogenes or tumor suppressor genes of RCC.

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