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论文

正常细胞与镉转化细胞抑制消减cDNA文库构建

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摘要：

目的 构建正常人支气管上皮细胞(16HBE)与氯化镉诱导转化16HBE细胞间差异表达基因的消减cDNA文库。方法 以16HBE为驱动子,氯化镉诱导转化16HBE细胞为检测子,应用抑制性消减杂交(SSH)方法构建cDNA文库,经2次消减杂交和2次PCR后,将巢式PCR产物插入载体,随机挑选克隆进行鉴定。结果 得到纯度高及完整性好的总RNA和mRNA,并扩增出良好的双链cDNA,cDNA与接头的连接效率＞25%,最终使差异表达基因得到富集;经蓝白菌落筛选,获得1 200余个白色阳性克隆;随机挑选50个白色克隆进行PCR扩增,显示96%克隆均有100~600 bp的插入片段,这些片段可能是差异表达基因cDNA片段,提示用SSH法及T/A克隆技术有效构建了两细胞株间差异表达基因的消减cDNA文库。结论 成功创建正常16HBE细胞与镉转化16HBE细胞差异表达基因消减cDNA文库。

关键词： 氯化镉 人支气管上皮细胞(16HBE) 抑制性消减杂交(SSH) cDNA文库

Construction of suppression subtractive cDNA libraries of normal and cadmium-transformed cells

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Abstract:

Objective To construct a subtractive cDNA library of differentially expressed genes in normal and cadmium-transformed cells.Methods The human bronchial epithelial cells(16HBE cells) transformed by cadmium chloride was used as a tester,and the normal 16HBE cells as a driver,to construct a cDNA library using suppression subtractive hybridization(SSH).The products were inserted into TA vector after two substractive hybridization and nested PCR.The clones were picked up randomly and analyzed with PCR.Results The total RNA and mRNA of purity and good integrity were extracted,and the duble-stranded cDNA was well produced.The link efficiency of cDNA and connector was greater than 25%.And finally the differentially expressed genes were enriched with substractive hybridization and nested PCR.After blue-white screening,the amplified library contained more than 1 200 white positive clones,and fifty of them were selected randomly and analyzed with PCR.Totally 96% of the clones had the inserted 100-600 bp segment and the segments might be the gene cDNA segments with differential gene expressions.Conclusion The subtractive cDNA library of differential genes in transformed 16HBE cells induced by cadmium was successfully established.

Keywords: cadmium chloride human bronchial epithelial cell(16HBE cells) suppression subtractive hybridization cDNA library

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参考文献：

[1] Swaddiwudhipong W,Mahasakpan P,Funkhiew T,et al.Changes in cadmium exposure among persons

living in cadmium-contaminated areas in northwestern Thailand:a five-year follow-up[J].J Med Assoc Thai,2010, 93:1217-1222.
[2] Arita A,Costa M.Epigenetics in metal carcinogenesis:nickel,arsenic,chromium and cadmium [J].Metallomics,2009,1(3):222-228.
[3] Zhou ZH,Lei YX,Wang CX.Analysis of aberrant methylation in DNA repair genes during malignant transformation of human bronchial epithelial cells induced by cadmium[J].Toxicol Sci,2012,125(2):412-417.
[4] Lei YX,Wei L,Wang M,et al.Malignant transformation and abnormal expression of eukaryotic initiation factor during human bronchial epithelial cells induced by cadmium chloride[J].Biomed Environ Sci,2008, 21(4):332-338.
[5] 王敏,魏莲,雷毅雄.氯化镉诱发人支气管上皮细胞系恶性转化[J].中国职业医学,2005,32(6):2-4.
[6] Murat C,Zampieri E,Vallino M,et al.Genomic suppression subtractive hybridization as a tool to identify differences in mycorrhizal fungal genomes[J].Fems Microbiol Lett,2011,318(2):115-122.
[7] 白图雅,常福厚,王敏杰,等.GSTT1及CYP1A1基因多态性与肺癌易感性关系[J].中国公共卫生,2011,27(6):723-725.
[8] Lei YX,Wang M,Wei L,et al.Alterative expression and sequence analysis of human elongation factor-1δ during malignant transformation of human bronchial epithelial cells induced by cadmium chloride [J].Biomed Environ Sci,2010,23(2):151-157.
[9] Zhang L,Cilley RE,Chinoy MR.Suppression subtractive hybridization to identify gene expressions in variant and classic small cell lung cancer cell lines[J].Journal of Surgical Research,2000,93(1):108-119.
[10] Shin HJ,Park KK,Lee BH,et al.Identication of genes that are induced after cadmium exposure by suppression subtractive hybridization[J].Toxicology,2003,191(2-3):121-131.

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