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14株肿瘤细胞P53基因突变的检测　点此下载全文

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摘要：

目的: 检测14株肿瘤细胞P53基因的突变情况。 方法：根据 P53　基因5～8外显子之间的内含子序列设计引物，分别对人肺癌、脑胶质瘤、肝癌、胃癌、前列腺癌、乳腺癌、结肠癌、脉络膜恶性黑素瘤、视网膜成细胞瘤等9种肿瘤14株细胞的P53基因5、6、7、8的外显子进行PCR扩增反应。琼脂糖凝胶电泳鉴定PCR产物；DNA测序，并与正常的DNA序列比较；抽提肿瘤细胞蛋白进行Western blotting检测P53蛋白的表达。 结果：14株肿瘤细胞P53基因热变区5、6、7、8外显子的扩增产物经电泳鉴定与预期相同。DNA测序结果表明，14株肿瘤细胞中8株存在P53　基因5～8外显子的突变，其中肺癌细胞H1299、肝癌细胞Hep3B、肝癌细胞SMMC7721、脉络膜恶性黑素瘤细胞OCM 1检测到以前未报道过的突变；突变主要发生在外显子的编码序列，多数为单个碱基替换导致的错义突变，也有部分表现为同义突变；2株正常细胞未检测到突变。Western blotting检测显示，有基因突变的8株肿瘤细胞中仅6株有P53蛋白表达；所有 P53基因未突变的肿瘤细胞株和正常细胞株均未检测到 P53蛋白。 结论： 检测的14株肿瘤细胞中有8株细胞P53　基因热变区存在突变，其中4株检测到以前未报道过的突变；2株正常细胞未发现P53基因突变。

关键词：肿瘤细胞 P53基因 基因突变

Detection of P53　gene mutations in 14 tumor cell lines　Download Fulltext

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Abstract:

Abstract Objective To investigate　P53　gene mutations in 14 tumor cell lines. Methods: Primers were designed　according　to the intron sequences locating between exons 5 8 of　P53　gene for amplification of　P53　gene exon 5 8 of the 14 tumor cell lines from 9 kinds of human carcinoma, namely, the lung cancer, gloma, hepatocarcinoma, gastric carcinoma, prostate carcinoma, mammary carcinoma, colonic carcinoma, choroidal melanoma, and retinoblastoma. The PCR products were subjected to electrophoresis; DNA sequencing was performed for the amplified product and the results were compared with wild type　P53 gene. Meanwhile, the P53 protein of 14 tumor cell lines was extracted for Western blotting analysis. Results: The products of exons 5 8 amplified by PCR were identical to that expected. The results of DNA sequencing showed that 8 of 14 tumor cell lines had　P53　gene mutation in exons 5 8. Novel mutations were found in 4 tumor cell lines, including human lung cancer cell H1299, hepatocacinoma cell Hep3B and 7721, and choroidal melanoma cell OCM 1. The mutations mainly existed in the coding areas of the exons; most of them were missense mutations due to single base replacement; some others were silent mutations; and no mutations were found in the 2 normal cell lines. The　results　of Western blotting showed that only 6 of 8 mutant lines had P53 protein expression, which was not found in the non mutant lines and the normal cells. Conclusion: We have identified 8 lines with mutant　P53　genes among the 14 tumor cell lines, novel mutations are found in 4 tumor cell lines and none in the two normal cell lines.

Keywords:tumor cell P53 gene mutation

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