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论著

一种人神经母细胞瘤辅酶II-依赖性视黄醇脱氢酶cDNA的克隆及特征分析

李一凡;刘戈飞;宋旭红;杜 昆;黄东阳

汕头大学医学院分子生物学中心，广东 汕头 515041

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摘要 背景与目的: 检测神经母细胞瘤中新的辅酶II-依赖性视黄醇脱氢/还原酶[NADP(H)-dependent retinol dehydrogenase/reductase，NRDR]选择性剪接亚型。 材料与方法: 我们用NRDR特异引物，从人神经母细胞瘤细胞系SK-N-SH 和SK-SY-5Y cDNA中分别经PCR扩增出635 bp 和429 bp DNA 片段，测序证实635 bp片段是NRDR,而429 bp片段是选择性剪接新亚型。再用快速cDNA末端扩增 (Rapid amplification of cDNA ends, RACE)方法得到429 bp片段cDNA 全长序列。用绿色荧光蛋白与新亚型的融合蛋白做亚细胞定位。 结果: 得到新亚型全长并命名为human NRDR A2 (humNRDRA2)(AY616182)。已知NRDR有8个外显子，而新亚型humNRDRA2由选择性剪接造成了第4和第6外显子丢失，从第5外显子开始读码框架前移1 bp，导致随后的编码区框码漂移(frame shift)，同时蛋白翻译终止信号提前出现，产生仅有188个氨基酸的蛋白，其中第137 氨基酸以后的序列相对于NRDR完全发生改变，同时C-末端的过氧化物酶体定位信号-SRL丢失， 却在160～176 氨基酸处出现了一个细胞核定位信号。我们在ESTs库中未找到与其相同的剪接形式，提示它可能是神经母细胞瘤特有的一种NRDR剪接亚型。用GFP-NRDRA2融合蛋白做该蛋白的亚细胞定位，发现发绿色荧光的细胞均已悬浮，但悬浮状态不佳，而作为对照的NRDR另一亚型则能定位成功，提示NRDRA2蛋白可能具有一定的细胞毒性。 结论: 人神经母细胞瘤NRDRA2选择性剪接亚型羧基端框移突变并有核定位序列；该蛋白可能是一种细胞毒性蛋白。

关键词 NADP(H)-依赖的视黄醇脱氢酶 选择性剪接； 框移突变

cDNA Cloning of a Short Isoform of Human Neuroblastoma NADP(H)-Dependent Retinol Dehydrogenase/Reductase and Analysis of Its Characteristics

LI Yi-fan;LIU Ge-fei;SONG Xu-hong,DU kun;HUANG Dong-yang

Center for Molecular Biology, Shantou University Medical College, Shantou 515041, Guangdong, China

Abstract BACKGROUND & AIM: This study was aimed at the detection of novel alternative splicing variants of NRDR in human neuroblastoma. MATERIAL AND METHODS: We obtained 635 bp and 429 bp PCR fragments using NRDR specific primer from human neuroblastoma cell lines SK-N-SH and SK-SY-5Y. The two fragments were then respectively confirmed as NRDR and a novel isoform by alternative splicing. We performed 3'RACE and 5'RACE to obtain the cDNA full sequence of the spliced isoform, and tried to determine its subcellular location by GFP-humNRDRA2 fusion protein. RESULTS: We named the alternative splicing variant as humNRDRA2 with Genbank accesstion No.AY616182. Comparing with NRDR which is composed of eight exons, the novel variant NRDR A2 lost the exon4 and exon6 by alternative splicing, leading to frameshift in coding region starting from exon 5 and emergence of a premature stop codon. Thus, it produced an 188 aa protein with an altered 3'terminal sequence starting from 137 aa. The frameshift generated a bipartite nuclear targeting sequence at 160～176 aa, with the loss of the original peroxisomal targeting signal -SRL tripeptide as in NRDR. Absence of this splice variant in EST pool suggested that NRDR A2 probably was unique to human neuroblastoma. Comparing with the successful subcellular location of another splice variant of NRDR in the control experiment, we found HeLa cells that were transformed by GFP-NRDR A2 fusion protein all floaing rather than sticking to the bottom of flask, suggesting

that NRDR A2-encoded protein was probably toxic to cells. CONCLUSION: Human neuroblastoma produces a novel alternative splicing isoform NRDRA2 with frameshift and a bipartite nuclear targeting sequence. Its translated protein is probably cytotoxic.

Keywords NADP(H)-dependent retinol dehydrogenase/reductase alternative splicing frameshift

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通讯作者 黄东阳 huangdy@stu.edu.cn