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研究报告

X-连锁迟发性脊椎骨骺发育不良基因剪接受体突变对mRNA加工的影响

罗　强, 高　超, 王怀立, 周建华, 高铁

郑州大学第一附属医院儿科, 郑州450052

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摘要　X-连锁迟发性脊椎骨骺发育不良（spondyloepiphyseal dysplasia tarda, SEDL）是一种少见的由SEDL基因突变引起的骨软骨发育障碍性疾病，病变主要累及腰椎和近端承重大关节。为研究SEDL基因剪接受体突变(IVS2 -2A→C)对mRNA加工的影响，从该突变所致SEDL患者，以及健康对照者外周血中提取总RNA，逆转录合成cDNA，以此为模板进行聚合酶链式反应（polymerase chain reaction, PCR），对PCR扩增产物采用双向直接测序和非变性聚丙烯酰胺凝胶电泳(polyacrylamide gel electrophoresis, PAGE)方法进行分析。测序结果发现IVS2-2A→C突变患者的一种cDNA外显子2与外显子4直接拼接，显示外显子3全部丢失；另一种cDNA外显子1与外显子4拼接，显示外显子2和外显子3均缺失；在健康对照者也发现了外显子2缺失的cDNA。PAGE发现患者和对照者都存在两种RT-PCR产物，长度分别为567bp、425bp以及679bp、537bp，证实了测序结果。这说明SEDL基因第二内含子剪接受体突变（IVS2-2A→C）导致其外显子3在mRNA加工过程中全部丢失，由于SEDL基因的翻译起始位点位于外显子3，它的缺失可能使生成的mRNA不能被翻译,从而引起SEDL发生；外显子2位于5′ UTR，它的缺失提示SEDL基因存在选择性剪接，正常人也存在缺失外显子2的cDNA，说明这种选择性剪接对临床表型的影响似乎并不大，它对基因表达水平和表达调控是否有影响还需要进一步研究。

关键词　迟发性脊椎骨骺发育不良；　SEDL基因；选择性剪接；逆转录；剪接受体

分类号　R394

Effect of A Novel Splicing Mutation（IVS2-2A→C）of SEDL Gene on RNA Processing

LUO Qiang, GAO Chao, WANG Huai-Li, ZHOU Jian-Hua, GAO Tie-Zheng

Department of Pediatrics, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052,China

Abstract

X-linked spondyloepiphyseal dysplasia tarda (SEDL) is a rare osteochondrodysplasia caused by the mutation of SEDL gene, which mainly involves vertebral bodies and hips. To explore the effect of the novel splicing mutation (IVS2 -2A→C) of SEDL gene on mRNA processing in a large Chinese family with X-linked spondyloepiphyseal dysplasia tarda and to elucidate the molecular base of SEDL, total RNA was isolated from EDTA blood samples of patients and controls. RT-PCR was performed on total RNA. cDNA was analyzed by bi-directionally direct sequencing of PCR products and Polyacrylamide gel electrophoresis (PAGE). Sequencing analysis revealed that there were two kinds of cDNA in patients. One is that exon 2 directly spliced exon 4, that is, exon 3 absence from the mature mRNA; and the other is that exon 1 directly spliced exon 4, meaning both exon 2 and 3 being spliced out completely. Meanwhile one kind of cDNA that exon 1 directly spliced exon 3 was found in normal controls. By PAGE, RT-PCR amplified products, 679bp and 537bp, were detected in normal controls, while 567bp and 425bp fragments were found in affected individuals. Our data show that the mutation of the splice-acceptor site in intron 2 causes exon 3 entirely exclusion from the mature RNA transcripts in affected individuals. As the translation start site of the SEDL gene locates on exon 3, the splicing defect causes affected individuals failure to produce sedlin, which elucidates the causative role of SEDL gene in the pathogenesis of SEDL. The absence of exon 2 indicates that there is alternative splicing in SEDL gene. The alternative splicing was also found in normal controls, which demonstrated that the alternative splicing might not be related to the phenotype of SEDL. Because the alternative splicing of exon 2 occurred in

the 5′UTR, it is not clear whether it affects the gene expression.

Key words spondyloepiphyseal dysplasia tarda； SEDL gene； reverse transcript；alternative splicing；splice acceptor

DOI:

通讯作者 高超 gaochao39@371.net