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The Effects of Interfering COX-2 Gene Expression on Malignant Proliferation of Human Lung Adenocarcinoma A2 Cell in vitro

Weiying Li, Hui WANG, Baitang LAI, Xuehui YANG, Chunyan ZHANG

摘要

Background and objective COX-2 was highly expressed in many tumor tissues and was involved in the initiation and development of tumors. The RNAi technique is a method to inhibit gene expression economically, quickly, efficiently and specifically. This study used RNAi technique to explore the interfering effect of COX-2 gene expression and the influence on the malignant proliferation of A2 cells after quenching COX-2 in vitro . Methods Three COX-2 siRNA expression vectors with human U6 promoter were constructed. The COX-2 siRNA vectors and the vacant
vector (pEGFP) were transfected into A2 cells with lipofectamine respectively. The cell strains transfected were selected. The change of COX-2 expression levels was examined by Western blot and RT-PCR. The effects on the proliferation of A2 cells after silencing COX-2 were detected by cell growth curve and clonogenic assay in vitro . Results The three siRNA and U6 promoter cloned into pEGFP plasmid were validated by PCR, restriction endonucleases identification, DNA sequencing and BLAST alignment. The cell strains transfected were coded as A2-3, A2-7, A2-10 and A2-p
respectively. Green fluorescence was seen in A2-p cells and not in A2-3, A2-7 and A2-10 cells in 24 h, 48 h and 72 h
after transfected. The results of RT-PCR and Western blot showed the three siRNA expression vectors acted effectively
and the expression of COX-2 was inhibited in different extent. In contrast to A2 cells, COX-2 mRNA levels of A2-3,
A2-7 and A2-10 cells were reduced 15.6%, 20.4% and 64.2% respectively, and COX-2 protein expressions of them were
reduced 23.7%, 36.7% and 60.2% respectively. The results of cell growth curve and clonogenic assay showed the growth of A2-10 cell slowed and the clonal formation rate was reduced. However the growth of A2-3 and A2-7 cells had no obvious changes vs controls (A2 and A2-p). Conclusion Silencing COX-2 gene in vitro by RNAi technique can significantly inhibit the malignant proliferation of A2 cells.

关键词

Cyclooxygenase 2; RNA interference; in vitro ; Lung neoplasms

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