COX-2 \( \square \) \( \square \) \( \square \) \( \square \) H1299 \( \square \) \( \square \) \( \square \) 

Effects of cyclooxygenase-2 antisense vector on proliferation and sensitivity to cisplatin of H1299 cells

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【Abstract】Background and objective At present, it has been known that cyclooxygenase-2 (COX-2) plays a crucial role in invasion, development and metastasis of non-small cell lung cancer (NSCLC). In order to explore whether the expression of COX-2 inhibits the occurrence and development of NSCLC, antisense vector of human COX-2 gene is transfected into COX-2 highly expressing NSCLC cell line H1299 and its effects on proliferation and sensitivity to cisplatin of H1299 are analysed. Methods H1299 cells were transfected with antisense vector of human COX-2 gene using LipovecTM transfecting technique. Transfected cells were selected with Geneticin (G418). The COX-2 mRNA level was examined by using reverse polymerase chain reaction (RT-PCR). The COX-2 protein level was examined by Western Blot. The proliferative status and sensitivity to cisplatin of cells was measured by methotethiazuron (MTT) assay. Results RT-PCR showed a lower COX-2 mRNA level in transfected cells. The level of COX-2 protein was decreased apparently. The proliferative index of the transfected cells decreased significantly (P<0.05). The IC50 value of cisplatin decreased remarkably in transfected cells (1.8 mg/l) compared with that in H1299 cells without transfection (3.8 mg/l) (P<0.05). Conclusion Transfection with antisense vector of human COX-2 gene can not only inhibit the proliferation of H1299 cells, but also increase the sensitivity to cisplatin of H1299 cells.

【Key words】Cyclooxygenase-2 H1299 Lung neoplasms Reverse polymerase chain reaction Antisense vector
1.1 COX-2 mRNA  H1299-AS, H1299-P, H1299 . RNA. RT-PCR ( ) .

1.2 COX-2 mRNA  H1299-AS, H1299-P, H1299 . GAPDH . 20 μl . 1% .

1.3 SPSS10.0  P<0.05 .

2.1 COX-2 mRNA  H1299 . COX-2 .

2.2 RNA  H1299 . 70% .

2.3 RNA  H1299 . 0.25% .

2.4 1×10^5 2×10^5 100 μl . 10% . 5 min . 1 h . PBS-Tween20 . 1:1000 . 4°C . IgG . HRP . 1 h . 5 min . (DAB) .

2.5 (Western Blot) .

2.6 COX-2 . H1299-AS, H1299-P . 96 . 2×10^5 /ml . 0.5, 1, 2, 4, 8 mg/l . 100 μl . 24 . G418(300 mg/l) .

3. MTT . 1:1000 . DDP . (IC50).
H1299 (0.82), H1299-P (0.78), H1299-AS (0.21)转移酶

2.2 H1299 (0.82), H1299-P (0.78), H1299-AS (0.21)

Fig 1 The expression of COX-2 protein in H1299 cells before and after transfection of antisense COX-2

1: H1299-AS; 2: H1299-P; 3: H1299; M: DNA marker

Fig 3 The proliferative curve of H1299 cells before and after transfection of antisense COX-2

3.0 mg/l, H1299-AS (0.01), IC50 = 1.8 mg/l, H1299-P (0.01), IC50 (P < 0.05)

Fig 4 MTT drug-sensitivity test


