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论著

人COX-2基因反义真核表达载体的构建及对SGC-7901细胞增殖的影响

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摘要 背景与目的： 构建人COX-2基因反义真核表达载体,研究COX-2基因对人胃癌细胞株SGC-7901增殖的影响。 材料与方法： 采用分子克隆技术,用EcoR Ⅰ从含人全长COX-2基因的质粒pBOSNeoCOX-2中切下含COX-2 cDNA的目的片段,然后分别在其两端添加EcoR Ⅰ和Bgl II酶切位点,酶切后将该片段按正、反两个方向插入真核表达载体pIRES2-EGFP的多克隆位点中,琼脂糖凝胶电泳法对重组子进行鉴定，脂质体法将重组质粒转染到人胃癌细胞株SGC-7901中,MTT法检测转染反义COX-2基因的表达载体后SGC-7901细胞增殖的变化。 结果：经酶切鉴定,正、反义目的片段成功地连接到pIRES2-EGFP中，COX-2基因正、反义真核表达载体成功构建,转染后在SGC-7901细胞中稳定表达，反义COX-2基因真核表达载体转染SGC-7901细胞后能抑制其增殖。 结论: 成功构建人COX-2基因反义真核表达载体,反义COX-2基因能抑制SGC-7901细胞增殖。

关键词 环氧合酶 反义技术 重组载体 增殖

Construction of COX-2 Gene Antisense Eukaryotic Expression Vector and Its Effect on SGC-7901 Proliferation

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Abstract BACKGROUND AND AIM: To construct the antisense expression vector of human COX-2 gene and explore the relationship between COX-2 gene and tumor proliferation. MATERIALS AND METHODS: COX-2 gene fragment cleaved from plasmid pBOSNeo COX-2 with EcoRⅠ, restriction enzyme cutting sites EcoRⅠand Bgl II was added to its two ends in two reverse directions artificially. The target fragment was inserted into the polyclone site of plasmid eukaryotic expression vector pIRES2-EGFP. The constructed recombinant was identified by agarose gel electrophoresis. The transfection of antisense COX-2 recombinant was made to gastric cancer cell line named SGC-7901 which with high expression of COX-2 gene mediated by liposome. The transfectants were screened by G418 and idendified by detection of exogenous NEO resistant gene with PCR technique. PCR and Western Blot were used to determine if the expression of COX-2 was inhibited by antisense gene in the transfectant. MTT essay and colony formation test were used to observe the effect to the proliferation of the transfectant. RESULTS: Agarose gel electrophoresis confirmed that the sense and antisense target fragment were successfully bound to pIRES2-EGFP. The test of exogenous gene showed we obtained stable transfectants. The expression of COX-2 was downregulated in transfectant with antisence gene. MTT essay and clone formation test showed the proliferation of transfectant was inhibited. CONCLUSION: The antisense eukaryotic expression vector of COX-2 was constructed successfully by means of reversely inserting the target fragment into pIRES2-EGFP. COX-2 gene could control proliferation of SGC-7901.

Keywords cyclooxygenase antisense technology recombinant vector proliferation

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