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论著

RNA-21为靶标的反义寡核苷酸对人白血病K562细胞的抑制作用

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摘要 目的： 探讨以microRNA-21为靶标的反义寡核苷酸对白血病K562细胞的生长抑制效应及可能的作用机制。方法：依据与microRNA-21序列互补的原理，设计反义寡核苷酸，人工合成并全硫代修饰，在LipofectamineTM 2000介导下，转染K562细胞。采用四甲基偶氮唑蓝(MTT)法筛选最佳作用浓度，台盼蓝拒染法检测其对细胞生长抑制的作用，姬姆萨染色观察细胞的形态学变化，流式细胞仪检测细胞凋亡及细胞周期的变化。利用荧光定量PCR技术检测反义寡核苷酸作用后细胞内microRNA-21表达水平的改变。结果：MTT结果显示反义寡核苷酸有效抑制细胞生长，分别与随机组、空白对照组进行比较有显著差异（P<0.05），反义寡核苷酸作用的最佳浓度是0.6 μmol/L。台盼蓝拒染法结果显示从转染K562细胞24 h开始，反义寡核苷酸组细胞生长明显受到抑制，抑制效应持续到72 h。姬姆萨染色显示反义寡核苷酸作用K562细胞24 h后，可在细胞中见凋亡小体。流式细胞仪检测结果显示反义寡核苷酸组出现明显的亚二倍体峰，细胞周期无发生明显变化。荧光定量PCR结果显示，反义寡核苷酸可有效抑制细胞内microRNA-21的表达水平。结论：以microRNA-21为靶标的反义寡核苷酸可有效抑制人白血病K562细胞的生长，并显著促进细胞凋亡。

关键词 microRNA 寡核苷酸类,反义 K562细胞 细胞凋亡

分类号 R342.7

Targeted inhibition of microRNA-21 with antisense oligonucleotide and their effects on human leukemic K562 cells

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Abstract

<FONT face=Verdana>AIM: To explore the inhibitory effect of anti-miRNA-21 oligonucleotide (AMO-miRNA-21) on human leukemic K562 cells. METHODS: K562 cells were transfected with AMO-miRNA-21, which was complementary to the miRNA-21 in a sequence-specific manner. Viability of K562 cells was measured by MTT assay and the optimal concentration for transfection was determined. The inhibitory effect of AMO on the K562 cell growth was examined by trypan blue dye exclusion assay at 24 h, 48 h and 72 h after transfection. Giemsa s staining was used to detect morphologic changes of the transfected cells. The cell apoptosis and cell cycle progression were assayed by flow cytometry. Expression of microRNA-21 in the cells was measured by real-time PCR. RESULTS: The growth of cells treated with AMO-miRNA-21 was obviously inhibited compared with that in control groups (P<0.05). Very low cytotoxic and high inhibitory effects of AMO-miRNA-21 were found at concentration of 0.6 μmol/L. The inhibitory effect lasted for 72 h. Apoptotic cells were increased in AMO group and typical morphologic changes were conformed by Giemsa s staining. One visible hypodiploid peak was detected in the histogram. However, the cell cycle progression was not inhibited evidently. The expression of microRNA-21 in the transfected cells was down-regulated significantly. CONCLUSION: Targeted inhibition of microRNA-21 with antisense oligonucleotide effectively suppresses leukemic K562 cells growth by inducing apoptosis. miRNA-21 might be a potential target for leukemia therapy. </FONT>

Key words microRNA Oligonucleotides antisense K562 cells Apoptosis

扩展功能

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