

论文 PBCA纳米粒介导的hTERT反义寡核苷酸对A549细胞的抑制作用

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摘要:

目的以聚氰基丙烯酸丁酯纳米粒(polybutylcyanoacrylate nanoparticles, PBCA-NPs, NPs)作为端粒酶逆转录酶(human telomerase reverse transcriptase, hTERT)反义寡核苷酸(antisense oligodeoxynucleotide, ASODN)载体转染A549细胞,研究其对A549细胞的影响。方法采用MTT法检测NPs的细胞毒性和细胞增殖情况;流式细胞仪(flow cytometry, FCM)检测5'-FITC标记的ASODN(FASODN)转染细胞后胞内荧光强度和ASODN各组(ASODN1-NP, ASODN2-NP和ASODN3-NP)转染细胞后细胞周期的变化;倒置显微镜观察A549细胞生长状态;免疫细胞化学法检测hTERT蛋白表达。结果NPs质量浓度超过 $2.5 \text{ g} \cdot \text{L}^{-1}$ 时细胞毒性较大。转染24 h后, FASODN-NP组较FASODN组胞内荧光明显增强($P < 0.01$)。与空白对照组和正义寡核苷酸(sense oligodeoxynucleotide-nanoparticle, SODN-NP)组相比, ASODN-NP处理后A549细胞形态改变,细胞增殖减慢;各组细胞生长抑制率随时间延长而增高,72 h时ASODN1-NP, ASODN2-NP, ASODN3-NP各组最高抑制率分别可达62.4%, 44.6%和36.4%;细胞周期改变,细胞被阻滞于 G_1 期, S期细胞明显减少($P < 0.01$);且hTERT蛋白表达明显减少。结论NPs介导的hTERT ASODN能有效抑制A549细胞增殖、改变细胞周期及降低hTERT蛋白表达,对该细胞生长有明显抑制作用。

关键词: 聚氰基丙烯酸丁酯纳米粒 端粒酶逆转录酶 反义寡核苷酸 A549细胞

Inhibition of A549 cells by polybutylcyanoacrylate nanoparticles loaded with antisense oligodeoxynucleotide of hTERT mRNA

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Abstract:

Aim To investigate the effect of nanoparticles for antisense oligodeoxynucleotide (ASODN) of hTERT mRNA on A549 cells. Methods The cationic polybutylcyanoacrylate nanoparticles (NPs) were prepared by an emulsion polymerization process in the presence of DEAE-dextran. Antisense oligodeoxynucleotides were loaded on the particles by adsorption. The cytotoxicity of NPs and proliferation of A549 cells were detected by MTT assay. Intracellular fluorescence intensity after transfecting the 5'-FITC-labelled ASODN (FASODN) and cell cycles were determined by flow cytometry (FCM). Inverse microscope was used to observe the modality of A549 cell transfected by NPs for ASODN. The protein expression of hTERT was measured by immunocytochemistry. Results The cytotoxicity increased evidently with the increasing concentration of NPs over $2.5 \text{ g} \cdot \text{L}^{-1}$. The intracellular fluorescence in FASODN-NP group was obviously stronger than that in FASODN group (NPs free) after transfection for 24 h ($P < 0.01$). The inhibitory rate for cell modality change and proliferation after the treatment with ASODN-NP at 72 h reached peak, 62.4%, 44.6% and 36.4% for ASODN1-NP group, ASODN2-NP group and ASODN3-NP group, respectively; The cell cycle in ASODN-NP group varied observably compared with control group and sense oligodeoxynucleotide-nanoparticle (SODN-NP) group and the cell cycle was blocked in G_1 phase, the cell number in S phase decreased obviously ($P < 0.01$); The hTERT protein expression of ASODN-NP group reduced clearly. Conclusion ASODN-NP of hTERT can inhibit the proliferation of A549 cells effectively and cause the change of cell cycle, restraint of protein expression of hTERT and cell viability.

Keywords: human telomerase reverse transcriptase antisense oligodeoxynucleotide A549 cells polybutylcyanoacrylate nanoparticle

收稿日期 2005-07-21 修回日期 网络版发布日期

DOI:

基金项目:

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