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IL-21在肝癌细胞株H22细胞中的表达及其活性 点此下载全文

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摘要:

摘 要 目的:构建小鼠IL-21 (mIL-21) 真核表达载体mIL-21-pcDNA3.1,转染肝癌H22细胞,探讨其生物学活性。方法:RT-PCR法扩增mIL-21基因,构建mIL-21真核表达载体mIL-21-pcDNA3.1,并经DNA测序证实。脂质体法介导mIL-21-pcDNA3.1转染H22细胞,RT-PCR和Western boltting鉴定其表达,MTT法检测mIL-21-pcDNA3.1对T细胞增殖和NK细胞杀伤活性的影响。结果:DNA序列分析证实构建的mIL-21-pcDNA3.1正确无误,RT-PCR和Western boltting证实转染的H22细胞中有mIL-21的表达。MTT法显示,转染mIL-21的H22细胞培养上清刺激T细胞增殖的刺激指数(stimulation index,SI)为3.412±0.312,联合ConA的刺激指数为4.673±0.450,均显著高于转染空质粒组的1.465±0.103和未转染组的1.447±0.245,(P<0.01)。转染mIL-21的H22细胞培养上清NK细胞杀伤率为(81.66±4.26)%,显著高于转染空质粒组的(34.74±5.52)%和未转染对照组的(33.61±1.42)%。结论:mIL-21-pcDNA3.1载体在H22细胞中的表达可显著增强T细胞增殖及NK细胞的杀伤功能,为其在抗肝癌治疗中的应用奠定了基础。

关键词: 肝肿瘤 mIL-21基因 H22细胞株 真核表达

IL-21 expression in hepatoma cell line H22 and its biological activity

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Abstract:

Abstract Objective: To construct a recombinant eukaryotic expression vector mIL-21-pcDNA3.1 and transfect it into hepatoma cell line H22, so as to assess the biological activity of mIL-21. Methods: The gene fragment encoding mouse IL-21 was amplified by RT-PCR, and was then cloned into eukaryotic expression plasmid pcDNA3.1 to form recombinant plasmid mIL-21-pcDNA3.1. The recombinant plasmid is verified by DNA sequencing. mIL-21-pcDNA3.1 was transfected into H22 cells with lipofect regent, and its expression was detected by RT-PCR and Western blotting analysis. The effects of mIL-21-pcDNA3.1 on proliferation of T cells and cytotoxicity of NK cells were studied. Results: The recombinant plasmid mIL-21-pcDNA3.1 was confirmed by DNA sequencing. The expression of mIL-21 in H22 cells was confirmed by RT-PCR and Western blotting analysis. MTT results showed that stimulation index (SI) of T cells stimulated with mIL-2-H22 cell supernatant was 3.412±0.312, and the SI of ConA combination stimulating group was 4.673±0.450; both were significantly higher than those in the mock vector transfected (1.465±0.103) and untransfected groups (1.447±0.245, P<0.01). The cytotoxicity of NK cells in mIL-21-H22 cell supernatant group was (81.66±4.26)%, significantly higher than those in the mock vector transfected ([34.74±5.52]%) and untransfected groups ([33.61±1.42]%). Conclusion: The expression of mIL-21-pcDNA3.1 plasmid in H22 cells can enhance the proliferation of T cells and the cytotoxicity of NK cells, which lays a foundation for its role in the research of anti-hepatoma.

Keywords: <u>live neoplasms</u> <u>mIL-21</u> <u>H22 cell line</u> <u>eukaryotic expression</u>

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