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MDA-7/IL-24-HT7融合蛋白的制备及其对肿瘤细胞凋亡的诱导作用 点此下载全文

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摘要：

目的：构建MDA-7/IL-24-HT7原核及真核表达质粒，制备MDA-7/IL-24-HT7融合蛋白，研究该融合蛋白在肿瘤细胞内的定位及其对肿瘤细胞致凋亡作用。方法：PCR扩增MDA-7/IL-24基因，插入含有HaloTag (HT7)标签的载体中，构建MDA-7/IL-24-HT7原核及真核表达质粒；MDA-7/IL-24-HT7融合蛋白经IPTG诱导表达后纯化。利用带有荧光标记的HT7配基观察MDA-7/IL-24-HT7在肿瘤细胞内的定位。MTT法及AnnexinV-PI染色法检测MDA-7/IL-24-HT7对肿瘤细胞生长和凋亡的影响。结果：成功构建了表达MDA-7/IL-24-HT7融合蛋白的原核及真核表达质粒，MDA-7/IL-24-HT7融合蛋白主要存在于E.coli BL21的包涵体内。MDA-7/IL-24-HT7融合蛋白定位于肿瘤细胞的内质网上。MDA-7/IL-24-HT7融合蛋白可抑制肿瘤细胞的生长，1 mg/ml MDA-7/IL-24-HT7 融合蛋白作用大肠癌HCT116细胞、肝癌SMMC7721细胞96 h后，细胞凋亡率分别为（34.7±1.3）% 和（22.1±0.9）%，显著高于未处理的肿瘤细胞（P<0.01）。结论：带有HaloTag标签的MDA-7/IL-24-HT7融合蛋白可抑制肿瘤细胞增殖和诱导肿瘤细胞凋亡。

关键词：MDA-7/IL-24 HaloTag 融合蛋白 肝肿瘤 大肠肿瘤 凋亡

Preparation of MDA-7/IL-24-HT7 fusion protein and its apoptosis inducing activity on tumor cells Download Fulltext

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Abstract:

Objective: To construct MDA-7/IL-24-HT7 prokaryotic and eukaryotic expression vectors, and prepare purified MDA-7/IL-24-HT7 fusion protein, so as to study its cellular localization and apoptosis-inducing effect on tumor cells. Methods: MDA-7/IL-24 gene was amplified by PCR and cloned into vectors containing HaloTag (HT7) to construct MDA-7/IL-24-HT7 prokaryotic and eukaryotic expression vectors. MDA-7/IL-24-HT7 fusion protein was induced by IPTG and further puified. Cellular localization of MDA-7/IL-24-HT7 fusion protein in tumor cells was monitored by fluorescence-marked HT7 ligands. The effects of MDA-7/IL-24-HT7 fusion protein on growth and apoptosis of tumor cells were detected by MTT and Annexin V-PI staining assays. Results: MDA-7/IL-24-HT7 prokaryotic and eukaryotic expression vectors were successfully constructed. The MDA-7/IL-24-HT7 fusion protein was mainly expressed as inclusion bodies in E.coli BL21, and localized in the endoplasmic reticulum of tumor cells. MDA-7/IL-24-HT7 fusion protein inhibited growth of tumor cells. Apoptosis rates of colon cancer HCT116 cells and hepatic carcinoma SMMC7721 cells were (34.7±1.3)% and (22.1±0.9)%, respectively, after treatment with 1mg/ml MDA-7/IL-24-HT7 for 96 h, which were significantly higher than those of untreated tumor cells (P<0.01). Conclusion: MDA-7/IL-24-HT7 fusion protein containing HaloTag (HT7) can inhibit growth and induce apoptosis of tumor cells.

Keywords:MDA-7/IL-24 HaloTag fusion protein liver neoplasmas colon neoplasmas apoptosis

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