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TAT-ASPP2融合蛋白的制备及其对胶质瘤细胞增殖的抑制作用 点此下载全文

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摘要:

目的:制备TAT-ASPP2融合蛋白,探讨其对人胶质瘤U-87MG细胞和U251细胞增殖的抑制作用。方法:设计TAT-ASPP2引物,应用IN-Fusion技术构建原核表达质粒pET-TAT-ASPP2,双酶切、DNA测序鉴定后转化大肠杆菌 E.coli BL21,IPTG诱导TAT-ASPP2融合蛋白的表达,SDS-PAGE和Western blotting鉴定TAT-ASPP2融合蛋白。MTT法检测TAT-ASPP2融合蛋白对U-87MG和U251细胞增殖的作用。结果:成功构建了原核表达质粒pET-TAT-ASPP2,转化 E.coli BL21后成功表达TAT-ASPP2融合蛋白,其相对分子质量约为128 000,并可被ASPP2特异性抗体所识别。TAT-ASPP2融合蛋白对 U-87MG 和U251细胞增殖的抑制率分别为(65.0±3.0)%和(64.7±2.5)%,而ASPP2蛋白则不能抑制U-87MG和U251细胞的增殖。结论:成功地克隆、表达及纯化TAT-ASPP2融合蛋白,该融合蛋白可抑制胶质瘤细胞的增殖。

关键词: 胶质瘤 TAT-ASPP2 融合蛋白 增殖

Preparation of TAT-ASPP2 fusion protein and its inhibitory effect against proliferation of glioma cells Download Fulltext

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Abstract:

Objective: To prepare the TAT-ASPP2 fusion protein and investigate its inhibitory effect against the proliferation of glioma U-87MG and U251 cells. Methods: TAT-ASPP2 specific primer was designed and recombinant prokaryotic expression vector pET-TAT-ASPP2 was constructed using the In-Fusion cloning technique. After identified by double endonuclease digestion and DNA sequencing, pET-TAT-ASPP2 vector was transformed into E. coli BL21 and TAT-ASPP2 fusion protein was induced by IPTG. TAT-ASPP2 fusion protein was further identified by SDS-PACE and Western blotting analysis. The effects of TAT-ASPP2 fusion protein on proliferation of U-87MG and U251 cells were detected by MTT assay. Results: The prokaryotic expression plasmid pET-TAT-ASPP2 was successfully constructed, and TAT-ASPP2 fusion protein was induced by IPTG in transformed E.coli BL21; the molecular weight of the fusion protein was about 128 000 and it could be specifically recognized by ASPP2 antibody. TAT-ASPP2 fusion protein significantly inhibited the proliferation of U-87MG and U-251 cells, with the inhibitory rates being about $(65.0\pm3.0)\%$ and $(64.7\pm2.5)\%$, respectively; while ASPP2 protein did not inhibit the proliferation of U-87MG and U-251 cells. Conclusion: TAT-ASPP2 fusion protein has been successfully expressed and purified, and the fusion protein can significantly inhibit the proliferation of glioma cells.

Keywords:glioma TAT-ASPP2 fusion protein proliferation

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