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乳腺癌特异性多肽介导的HSV-TK/GCV系统的构建及其靶向杀伤效应 点此下载全文

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摘要：

目的： 研究乳腺癌MDA-MB-231细胞特异性转导多肽PI介导的HSV-TK/GCV抗瘤系统对乳腺癌MDA-MB-231细胞的体外靶向杀伤作用。 方法: 以PCR从质粒pORF-HSV-TK中扩增目的基因PI-TK，克隆到原核表达载体pET-28a（+）中，构建pET-28a (+)-PI-TK载体，转化宿主菌，经IPTG诱导表达PI-TK融合蛋白，利用His-Tag对其进行纯化，SDS-PAGE和Western blotting鉴定PI-TK融合蛋白。将不同质量浓度的PI-TK融合蛋白与MDA-MB-231细胞共培养，联合更昔洛韦（ganciclovier，GCV）作用后，倒置显微镜下观察细胞的形态变化，CCK-8法检测MDA-MB-231细胞的增殖。 结果: 成功构建了重组原核表达载体pET-28a (+)-PI-TK，获得纯化的PI-TK融合蛋白，SDS-PAGE及Western blotting鉴定PI-TK融合蛋白表达正确。单独PI-TK融合蛋白不影响MDA-MB-231细胞的形态和增殖，但PI-TK融合蛋白联合GCV能剂量依赖性靶向抑制MDA-MB-231细胞的增殖，200 μg/ml PI-TK+10 mg/L GCV作用的抑制率达（68.9±7.57）%；PI-TK联合GCV抑制MDA-MB-231细胞的IC_{50}值为152.64 μg/ml。上述各作用对MDA-MB-435细胞均无影响（ P <0.05）。 结论: 乳腺癌特异性转导多肽PI介导的HSV-TK/GCV抗瘤系统可靶向杀伤MDA-MB-231细胞。

关键词：乳腺癌特异性多肽 PI 乳腺癌 MDA-MB-231细胞 HSV-TK/GCV抗瘤系统 靶向性

Construction and targeted effects of breast cancer specific peptide-mediated HSV-TK/GCV anti-tumor system Download Fulltext

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Abstract:

Objective : To investigate the targeted killing effect of breast cancer specific peptide PI-mediated HSV-TK/GCV system against human breast cancer MDA-MB-231 cells *in vitro*. Methods: PI-TK gene was amplified from pORF-HSV-TK plasmid by PCR, and was re-inserted into the prokaryotic expression plasmid pET-28a (+); the pET-28a (+)-PI-TK plasmid was constructed and transfected into the host bacteria. After induction with IPTG, PI-TK fusion protein was purified by His-Tag and further identified by SDS-PAGE and Western blotting analysis. MDA-MB-231 cells were cultured with different dosages of PI-TK fusion protein; after further treatment with ganciclovier (GCV), the morphology changes of MDA-MB-231 cells were observed under inverted microscope, and the cell proliferation was evaluated by CCK-8 assay. Results: Recombinant prokaryotic expression plasmid pET-28a (+)-PI-TK was successfully constructed. The purified PI-TK fusion protein was obtained and confirmed by the SDS-PAGE and Western blotting analysis. PI-TK fusion protein alone failed to affect the morphology and proliferation of MDA-MB-231 cells, but when combined with GCV, PI-TK fusion protein specifically inhibited the proliferation of MDA-MB-231 cells in a dose-dependent manner, with inhibitory rate of 200 μg/ml PI-TK + 10 mg/L GCV being (68.9±7.57)%, and IC_{50} being 152.64 μg/ml, but it had no effects on MDA-MB-435 cells (P <0.05). Conclusion: The breast cancer specific peptides PI-mediated HSV-TK anti-tumor system can specifically kill MDA-MB-231 cells.

Keywords:breast cancer specific peptide breast cancer MDA-MB-231 cell HSV-TK/GCV suicide gene system targeted

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