

## Trastuzumab F(ab')<sub>2</sub>修饰紫杉醇免疫脂质体对人大肠癌HT-29细胞的杀伤作用

吴爱国, 焦得闯, 李鹏, 邵国利, 纪术峰, 韩明阳

510282广州, 南方医科大学珠江医院普通外科

### Killing Effects of Immunoliposomal Paclitaxel Tagged with Trastuzumab F(ab')<sub>2</sub> on Colorectal Cancer Cells

WU Ai guo, JIAO De chuang, LI Peng, SHAO Guo li, JI Shu feng, HAN Ming yang

Department of General Surgery, Zhujiang Hospital of Nanfang Medical University, Guangzhou 510282, China

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#### 摘要

目的 构建Trastuzumab F(ab')<sub>2</sub>修饰的紫杉醇免疫脂质体并测定其对体外生长的人大肠癌HT-29细胞的杀伤作用。方法 采用逆相蒸发法合成紫杉醇脂质体, 使用胃蛋白酶在Trastuzumab抗体J链Fc段侧切断抗体以获得抗体F(ab')<sub>2</sub>段, 抗体交联法制备Trastuzumab F(ab')<sub>2</sub>修饰的紫杉醇免疫脂质体; 透射电子显微镜观察免疫脂质体的形态及粒径分布; 采用反相高效液相色谱(RP-HPLC)及荧光标记法测定免疫脂质体封装率和活性; MTT检测细胞的杀伤率。结果 成功构建了Trastuzumab F(ab')<sub>2</sub>修饰的紫杉醇免疫脂质体; 所得免疫脂质体平均粒径为210nm, 粒径小于200nm者占91.37%; 且其具有较高的封装率和稳定性; 相同时间内, HT-29细胞对紫杉醇免疫脂质体的摄取要明显高于对照组; Trastuzumab F(ab')<sub>2</sub>修饰的紫杉醇免疫脂质体对HT-29细胞的杀伤作用强于对照组(P<0.01), 且该作用具有时间依赖性(P<0.01)。结论 成功构建Trastuzumab F(ab')<sub>2</sub>修饰的紫杉醇免疫脂质体, 其对体外生长的大肠癌细胞具有较强的杀伤作用。

关键词: 免疫脂质体 紫杉醇 大肠癌

Abstract: Objective To construct the immunoliposomal Paclitaxel tagged with trastuzumab F(ab')<sub>2</sub> and to investigate its killing effects on human colorectal cancer cells in vitro. Methods Liposomal paclitaxel was made in a reversal phase evaporation method and trastuzumab F(ab')<sub>2</sub> was obtained by cutting the J hinge at the Fc side of the antibodies with pepsin, then the trastuzumab F(ab')<sub>2</sub> and the liposomal Paclitaxel were linked together. The figures of immunoliposomes were observed by TEM and their size distribution was measured by graphics software. The encapsulation efficiency of the immunoliposomes was estimated by RP HPLC. The immune activity of immunoliposomes was evaluated using the fluorescence microscopy after the fluorescent immunoliposomes or liposomes were incubated together with HT-29 cells. Apoptotic index of the HT-29 cells was measured using MTT assay. Results The immunoliposomal Paclitaxel tagged with trastuzumab F(ab')<sub>2</sub> that was constructed in vitro had an average diameter of 210 nm, and that the diameters under 200 nm accounted for 91.37% of the total. The encapsulation efficiency and stability of the immunoliposomal Paclitaxel tagged with trastuzumab F(ab')<sub>2</sub> were perfect and the immunoliposome was superior to liposome at the identification capability to HT-29 cells in the same condition. Immunoliposomal Paclitaxel tagged with trastuzumab F(ab')<sub>2</sub> can kill the HT-29 cells in a time dependent manner (P<0.01). Conclusion The immunoliposomal Paclitaxel tagged with trastuzumab F(ab')<sub>2</sub> was constructed successfully and it can kill the HT-29 cells effectively in vitro.

Key words: Immunoliposome Paclitaxel Colorectal cancer

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