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基础研究

人肺腺癌GLC-82 细胞热休克蛋白70多肽复合物的提取及对细胞毒性T 淋巴细胞作用的实验研究

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Experimental Study on Purification of Heat Shock Protein 70 Antigen Peptide Complex from Human Lung Adenocarcinoma GLC-82 Cell and the Anti-tumor Effect of Cytotoxic T Lymphoc-yte acted by Heat Shock Protein

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摘要

目的 探讨GLC-82细胞中HAC-70的提纯方法及其诱导的CTL表型变化和CTL及其上清液抗瘤效应。方法 GLC-82细胞热休克诱导HSP表达，离子交换层析提纯HAC-70，SDS-PAGE和ELISA法进行定量和定性检测，活化PBMC，T淋巴细胞亚群试剂盒测定HAC-70诱导CTL细胞表型变化情况，MTT法测定CTL及上清液杀瘤活性。结果 热休克处理能使GLC-82细胞HAC-70表达增加，离子交换层析可提纯GLC-82细胞中HAC-70。经热休克处理的HAC-70诱导T淋巴细胞，其$CD3^+$、$CD4^+$、$CD8^+$细胞与对照组的CTL细胞阳性率明显提高，其诱导的CTL杀瘤活性显著提高，且其CTL培养上清液肿瘤杀伤活性最高。结论 离子交换层析可提纯GLC-82的HAC-70；HAC-70可使CTL细胞$CD4^+$／$CD8^+$倒置，活化的CTL及上清液有较高的杀瘤活性，为肺癌肿瘤疫苗的制备和临床应用提供了实验依据。

关键词： 肺腺癌 热休克蛋白70 多肽复合物 细胞毒性T 淋巴细胞

Abstract: Objective To evaluate the purification methods of heat shock protein 70 antigen peptide complex (HAC-70) f rom human lung adenocarcinoma GLC-82 cell , and to explore the phenotype varieties and anti-tumor function of CTL as well as its suspension induced by HAC-70. Methods GLC-82 cells were cultured with 43 ℃for 30 minutes to induce over-expression of HSP. The induced HAC-70 was purified with ion exchange chromatography. The purified HAC-70 was analyzed quantity and quality with SDS-PA GE and EL ISA methods. PBMC were activated by HAC-70. CTL phenotypes were determined by lymphocyte subgroup test kit . The anti-tumor effects of CTL and it s suspension on GLC-82 were determined with MTT method. Results Ion exchange chromatography was successfully applied to purify
HAC-70 from GLC-82. HAC-70 f rom heat-treated GLC-82 were over expressed with a rate of 75μg/ ml GLC-82. The positive rates of CD3 + 、CD4 + 、and CD4 + / CD8 + T cells induced by heat-treated group were much higher than cont rols. The best tumor-killer effect s of CTL were obtained in HAC-70 group while the target2effect ratio was 50∶1. Apoptosis of GLC-82 were induced by suspension of CTL in all groups. Conclusion Purification of HAC-70 from lung adenocarcinoma GLC-82 cells by ion exchange chromatography is simple and feasible. CD3 + and CD8 + T cells are increased and CD4 + / CD8 + ratio is reversed by activated HAC-70. The tumor-killer function of HAC-70-induced CTL and it s suspension is confirmed and the result s will be of valuable to the preparation of tumor vaccine as well as it s clinical application.

Key words: Lung adenocarcinoma HAC-70 CTL

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