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巨噬细胞通过旁分泌效应杀伤小鼠乳腺癌细胞的实验研究

摘 要

目的：目前乳腺癌的研究备受世界瞩目，巨噬细胞分布于全身参与炎症反应，为淋巴细胞传递抗原信息，在免疫应答中发挥着重要的生理功能，在癌细胞所处的环境周围，与癌细胞发生、发展密切相关的巨噬细胞在特定环境下能够产生各种不同的活性物质和细胞因子，具有抗肿瘤和免疫调节的作用。本实验采用小鼠乳腺癌细胞 4T1 为模型，自中药成分中筛选具有免疫调节的成分，调节巨噬分化，并分析其对巨噬细胞旁分泌效应杀伤小鼠乳腺癌的影响。

方法：体外培养小鼠巨噬细胞 RAW264.7，去甲基斑蝥素（NCTD）为斑蝥主要药效分子，采用不同浓度 NCTD(0,2.5、5、10、20 和 40 $\mu\text{mol/L}$)处理 RAW264.7 细胞 24 小时，收集细胞。分别采用 MTT 还原法判断甲基斑蝥素对细胞增殖和生长的影响，以流式细胞仪为工具，测定去甲基斑蝥素对细胞功能和性质的影响，细胞各项参数主要包括细胞活性、细胞周期、细胞凋亡等。通过短时间内对大量细胞的分析和测量，可以判断细胞活性氧水平，以及细胞表面 CD86 和 CD206 表达；收集不同诱导细胞的培养液上清。体外培养和小鼠乳腺癌细胞 4T1 细胞，6 组条件培养上清液诱导细胞。

结果：低浓度的 NCTD 对 RAW264.7 细胞增殖、凋亡和细胞周期无明显影响，但促进小内 ROS 含量增加；10、20、和 40 $\mu\text{mol/L}$ 对细胞增殖的抑制率分别为 28.4%、55.9%和 74.2%，G2M 期细胞百分率和凋亡细胞百分率随 NCTD 浓度增加而增加。低浓度的 NCTD 可促进细胞 CD86 表达和活性氧水平增加，减低 CD206 表达。NCTD 促进 M1 极化，增强其抗肿瘤效应。

结论：NCTD 促进巨噬细胞 M1 极化，增强其抗肿瘤效应,对细胞增殖的促进效应值得关注。

【关键词】 斑蝥；巨噬细胞；旁分泌；乳腺癌细胞；流式细胞术

The Etudy of Paracrine of Macrophage Mediated Neoplasia Rsistance Effect on Breast Cancer Cells

Abstract

Objective: At present, the research of breast cancer has attracted worldwide attention, and macrophages are an important part of the human immune system. In the tumor microenvironment, the paracrine effect of tumor related macrophages has a variety of killing effects on tumor cells. In this experiment, 4T1 cells of mouse breast cancer were used as the model, and the immune regulatory components were extracted from traditional Chinese medicine and to o investigate the endocrine function of macrophages in mice with breast cancer

Methods: RAW264.7 mouse macrophages were cultured in vitro, and norcantharidin (NCTD) was the main effective molecule of cantharidin. RAW264.7 cells were treated with different concentrations of norcantharidin(0,2.5,5,10,20 and 40 μ mol / L) for 24 hours, and the cells were collected. MTT reduction method was used to determine the effect of methyl cantharidin on cell proliferation and growth.Flow cytometry was used to determine the effect of demethylcantharidin on cell function and properties,and the cell parameters include cell activity, cell cycle, apoptosis, etc.Through the analysis and measurement of a large number of cells in a short period of time, the level of reactive oxygen species and the expression of CD86 and CD206 on the cell surface can be determined.In vitro culture and mice breast cancer cell 4T1 cells, 6 groups of conditioned culture supernatant induced cells.

Result: The effect of low dose NCTD on RAW264.7 cell growth, death and cell cycle was not significantly different,But it increased the amount of ROS in the small cell.The inhibition rates of 10, 20, and 40 μ mol / L on cell proliferation were 28.4%, 55.9% and 74.2%, respectively. With the increase of NCTD

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