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Study on the preparation and anti-tumor biological effects of gastric cancer cell-dendritic cell fusion vaccine

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Objective: In this experiment, we used cell fusion technology to get the gastric cancer cell-dendritic cell fusion vaccine. We hoped that the fusion cells could get more tumor antigen information to increase their phenotypes and have more competence to irritate the anti-tumor immune reactions. By this experiment, we hoped to find out whether the fusion vaccine has satisfying safety and to verify its ability of inducing anti-tumor immune reactions.

Methods: Part 1- Peripheral blood mononuclear cells were separated from gastric cancer patients and co-cultured with granulocyte-macrophage colony stimulating factors; interleukin-4 and tumor necrosis factor-αto generate mature dendritic cells. The frozen stored human gastric cancer SGC7901 cell lines were resuscitated and cultured at the same time. The dendritic cells and SGC7901 cells were fusioned by using of polyethylene glycol and the pure fusion cells were screened out by cultured with HAT and HT selective culture systems. The phenotypes of dendritic cells and fusion cells were detected by flow cytometry. Part 2- The biological characteristics of fusion cells such as their morphological specialities, the expression of cell surface phenotypes (CD80\CD83\CD1a\HLA-DR), their growth curves, whether they can proliferate to be a new carcinoma in nude mice, the ability to irritate cytotoxic T lymphocytes and anti-tumor immune reactions) were tested to verify their safety when be used as anti-tumor vaccines. Part 3- The ability of fusion cells-activated T lymphocytes to kill SGC7901 gastric cancer cells was investigated by MTT method in vitro. SGC7901 cells were injected subcutaneously in nude mice to make the planted gastric cancer models. The anti-tumor effects were evaluated by detecting the growth of the new planted cancers, the apoptosis and proliferation of the planted cancer cells, the pathologic changes of the tumor tissues and the prohibitive rate for planted tumors pre and after the fusion cell vaccine's application and the cell divide cycles. Part 4- The expression of B7-1, B7-2 mRNA of fusion cells was detected by RT-PCR to detect the gene changes of these cells.

Results: 1. We obtained mature dendritic cells from gastric cancer patients' peripheral blood mononuclear cells by co-cultured with granulocyte-macrophage colony stimulating factors, interleukin-4 and tumor necrosis factor-α. These mature dendritic cells had typical morphological characteristics and their phenotypes' expressions were CD83 75.54±0.73%, CD1a 64.94±0.52%, CD80 61.56±0.27%, HLA-DR 62.50±0.66%. Dendritic cells and SGC7901 cells could be fusioned by PEG and we could get pure fusion cells by cultured with HAT\HT selective culture systems. 3. Fusion cells were much larger than dendritic cells or SGC7901 cells. They exhibited typical characteristics by light and electron microscope testing. They lived in suspension in the culture medium and could not proliferate to be new carcinomas in nude mice. The cell phenotypes were CD83 80.16±1.12%, CD1a 72.86±2.48%, CD80 81.24±2.76%, HLA-DR 79.54±1.56% respectively. 4. Fusion vaccine could induce strongly anti-tumor biological effects in vivo and in vitro: (1) Tumor growth was remarkably inhibited and the tumor size in treat group was 1.298±0.021cm3, which was extensively smaller than the size of control group 2.429±0.033cm3 ($P<0.01$). The prohibitive rate for planted tumors was 57.8%. (2) Detected by Annexin-V/PI double labeling flow-cytometry, tumor cells' apoptosis rate of treat group was 54.9±1.38%, which was promoted extensively than that of control group 20.02±0.39% ($P<0.01$) . (3) The tumor cells' proliferation was found to be greatly restrained by detecting the cell divide cycles. (4) In treat group, the tumor tissues had more infiltrated lymphocytes and apoptotic tumor cells than control group. The treat group had more hemorrhage and necrotic tissues than the control group. 5. The mRNA expressions of B7-1, B7-2 in fusion cells were highly increased and became higher than normal dendritic cells.

Conclusions: 1. Mature dendritic cells could be obtained from gastric cancer patients' peripheral blood mononuclear cells by co-cultured with GM-CSF, IL-4 and TNF-α. Gastric cancer cells and dendritic cells could be fusioned by PEG, we could obtain pure fusion cells by cultured with HAT/HT selective culture systems. 2. The fusion cells had their typical morphologies and kept the suspending living characteristic like dendritic cells. 3. By irritating the proliferation of T lymphocytes, fusion vaccine could induce more strong anti-tumor effects. 4. The fusion vaccine could inhibit the tumor growth and accelerate the apoptosis of tumor cells. 5. The mRNA expressions of B7-1, B7-2 in fusion cells were highly increased and these might be the molecular basis for fusion cells' strong anti-tumor biological effects.

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