Study on biological characters of SGC7901 gastric cancer cell-dendritic cell fusion vaccines

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AIM: To detect the biological characters of the SGC7901 gastric cancer cell-dendritic cell fusion vaccines.

METHODS: The suspending living SGC7901 gastric cancer cells and dendritic cells were induced to be fusioned by polyethylene glycol. Pure fusion cells were obtained by selective culture with the HAT/HT culture systems. The fusion cells were counted at different time points of culture and their growth curves were drawn to reflect their proliferative activities. The fusion cells were also cultured in culture medium to investigate whether they could grow into cell clones. MTT method was used to test the stimulating abilities of the fusion cells on T lymphocytes’ proliferations. Moreover, the fusion cells were planted into nude mice to observe whether they could grow into new planted tumors in this kind of immunodeficiency animals.

RESULTS: The fusion cells had weaker proliferative activity and clone abilities than their parental cells. When they were cultured, the counts of cells did not increase remarkably, nor could they grow into cell clones in culture medium. The fusion cells could not grow into new planted tumors after planted into nude mice. The stimulating abilities of the fusion cells on T lymphocytes’ proliferations were remarkably increased than their parental dendritic cells.

CONCLUSION: The SGC7901 gastric cancer cell-dendritic cell fusion vaccines have much weaker proliferative abilities than their parental cells, but they keep strong abilities to irritate the T lymphocytes and have no abilities to grow into new planted tumors in immunodeficiency animals. These are the biological basis for their anti-tumor biotherapies.
MATERIALS AND METHODS

Reagents
The reagents used included rhGM-CSF (Promega Co. Ltd, USA), rhIL-4 (R&D System Co. Ltd), TNF-α(R&D System Co. Ltd), Polyethylene glycol (Sigma, USA), Hypoxanthine (Sigma, USA), Aminopterin (Sigma, USA), Thymidine (Sigma, USA).

Preparation for suspending SGC7901 gastric cancer cells and dendritic cells
The frozen stored SGC7901 gastric cancer cells were cultured in the DMEM culture medium, and the suspending living cancer cells were prepared for the fusion process. The peripheral blood mononuclear cells were separated from venous blood of gastric cancer patients by use of lymphocytes separating solution. rhGM-CSF (100 ng/mL), rhIL-4 (50 ng/mL) and TNF-α(20 ng/mL) were added into the cultured peripheral blood mononuclear cells to induce them to transform into dendritic cells.

Preparation for SGC7901 gastric cancer cell-dendritic cell fusion vaccines
Suspending living SGC7901 gastric cancer cells and dendritic cells were mix-cultured with the ratio of 10:1. The two kinds of parental cells were induced to be fusioned by polyethylene glycol and selectively cultured by HAT/HT culture solutions. After the culture process, we obtained pure fusion cells as anti-tumor vaccines. Simultaneously, the suspending living SGC7901 gastric cancer cells and dendritic cells were mix-cultured with the same ratios, but were not induced to the fusion process. This group was designed as control group.

Growth curves of fusion cells
The obtained pure fusion cells (cell density 1.2×10^6/mL) were inoculated into the culture media on the culture slabs. During the culture process, we counted the cell numbers daily. After fourteen days, we drew the growth curves of cells. The SGC7901 cancer cells’ and dendritic cells’ growth curves were drawn at the same time respectively, and these curves were served as control.

Cloning abilities of fusion cells
The suspending living fusion cells (cell density 1×10^3/mL) were inoculated into the agar culture medium on twenty-four holes culture slabs. The cell numbers in each hole were 1000, 2000 and 10000 respectively. After two weeks’ cultivation, the cell clones which included more than 50 cells were inoculated into the agar culture medium on twenty-four holes culture slabs with 96 holes. T lymphocytes were added. The ratios of T lymphocytes to fusion cells and mix-cultured dendritic cells were 100:1, 50:1, 10:1 and 1:1 respectively. For each ratio, six holes were repeated in order to decrease the systemic errors. After sixty hours’ cultivation, ^3H-TdR was added into each hole on the slabs (1G per hole). Twelve hours later, the CPM values were tested by Beckman liquid scintillation counting. The culture groups which included T lymphocytes only or DMEM culture medium were designed as control groups.

Abilities of fusion cells to grow into new planted tumors in nude mice
The suspending living pure fusion cells (cell density 1×10^7/mL, cell number 5×10^5) were planted subcutaneously into the back of the BALB/c nude mice. The injections were performed every three days for about two weeks. We observed whether the planted fusion cells could grow into new planted tumors in the nude mice, and the percentages of planted tumor cells growing into new planted tumors were tested. At the same time, the SGC7901 gastric cancer cells were planted into other groups of nude mice. These groups were designed as control groups.

Statistical analysis
Data were imported into Systat software (SPSS) for statistical computations and graphing. One-way analysis of variance (ANOVA), independent-samples T test and Chi-Square tests were used to evaluate the differences between groups. P < 0.05 was considered as significant.

RESULTS

Growth curves of fusion cells
We obtained the fusion cells after the selective culture process. The fusion cells’ phenotypes and morphological characters were examined by flow cytometer and histological methods. The parental SGC7901 gastric cancer cells proliferated fast during their cultivation. After the subculture, the cancer cells stayed in proliferative delitescence for a short period, and then they entered into the logarithmic growth period with cell number increasing quickly. The cancer cells’ proliferation slowed down and their number decreased gradually (Figure 1). Dendritic cells had much weaker proliferative abilities than SGC7901 cancer cells. During their cultivation, the cells’ number did not increase remarkably. Like the parental dendritic cells, fusion cells also had weak proliferative abilities with a flat growth curve. This indicated that fusion cells’ proliferative characters were much similar to parental dendritic cells (Figure 2).

Cloning abilities of fusion cells
Clone abilities effectively reflect the proliferation of single cells. If a kind of cell could grow into numerous cell clones in the agar culture medium, it indicates these cells have strong abilities to proliferate. In our study, we found that SGC7901 gastric cancer cells had strong proliferative abilities. They grew into numerous cell clones fast. Like the parental dendritic cells, fusion cells also had much weaker proliferations. During their cultivation, they could not
grow into visible cell clones.

**Abilities of fusion cells to irritate T lymphocytes’ proliferation**

The abilities of fusion cells to irritate T lymphocytes were much stronger than the mix-cultured DC group (P < 0.01) (Table 1).

**Abilities of fusion cells to grow into new planted tumors in nude mice**

After injected subcutaneously into the back of the BALB/c nude mice, the SGC7901 gastric cancer cells grew into new planted tumors quickly, whereas the fusion cells could not grow into new tumors in nude mice, even with a higher cell density and injection frequency, suggesting that fusion cells had no abilities to proliferate and grow into new planted tumors in immunodeficiency animals.

**DISCUSSION**

**Characters of fusion cells’ growth curves, cloning abilities in vitro and their biological significance**

Cell number and growth curves could directly reflect cell proliferative characters. Clone ability is an important criterion to judge cells’ growing capabilities. The stronger the growth powers, the more cell clones could be formed. Researchers reported that when the tumor antigen related genes were transmitted into dendritic cells, T lymphocytes were irritated and showed anti-tumor biological effects. T lymphocytes in vitro can be divided into several sub-groups which have different phenotypes and biological effects. The most important sub-groups are CD4+ T lymphocytes (Th) and CD8+ T lymphocytes (Te/CTL). The in-activated pre-Tc cells were irritated and became active Te cells in vitro by the tumor antigens and CD4+ Th cells. These activated Te cells exerted anti-tumor biological effects by the double signal approaches.

The cell number and activities of T lymphocytes directly affected anti-tumor immune responses of cancer patients. Our purposes of modifying dendritic cells as anti-tumor vaccines were to increase their abilities of stimulating T lymphocytes’ proliferation and their activities. Some researchers reported that when the tumor antigen related genes were transmitted into dendritic cells T lymphocytes in vitro could be strongly irritated to become CD4+ Th cells and CD8+ Th cells. In our research, we found that the SGC7901-DC fusion cells could strongly irritate T lymphocytes’ proliferation, while the mix-cultured dendritic cells could not. This indicated that fusion cells had much stronger abilities to activate anti-tumor immune responses. So, fusion cells are possible to be used as anti-tumor vaccines.

**Abilities of fusion cells to grow into new planted tumors in immunodeficiency animals and their biological significance**

New planted tumors in immunodeficiency animals are...
perfect models for oncology researches\textsuperscript{10}. We injected the SGC7901 cancer cells and SGC7901-DC fusion cells into different groups of nude mice and found that SGC7901 cancer cells could grow into new planted tumors quickly but the fusion cells could not, even when much higher cell densities and more injection frequencies were applied. This implied that SGC7901-DC fusion cells had no abilities to grow into new planted tumor in nude mice. This is the most important difference between the parental SGC7901 cells and the SGC7901-DC fusion cells. This character together with the growth curves and clone abilities indicated that fusion cells were safe when used as anti-tumor vaccines \textit{in vivo} and \textit{in vitro}.

The strong abilities of SGC7901-DC fusion cells to irritate T lymphocytes’ proliferation and the characters that fusion cells were safe when used as anti-tumor vaccines \textit{in vivo} and \textit{in vitro} are the precondition of anti-tumor biological therapy.

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