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Original article

The complete preventive effect of homologous tumor vaccines – Based on a 5-year experimental study in mice

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ABSTRACT

By using a frozen-thaw method, we developed homologous and heterologous cell-based tumor vaccines, which were derived from mouse H22 hepatoma, S180 sarcoma and human A549 lung carcinoma, SK-OV-3 ovarian, and SMMC-7721 hepatoma cell lines. The prophylactic and therapeutic effects of those vaccines were evaluated in mice challenged with live H22 or S180 cells. The result demonstrated that homologous vaccines and heterologous vaccines had no therapeutic effect on tumor growth. However, homologous vaccines showed a complete prevention against live H22 and S180 cell challenge and they could stimulate cross-immune response of anti-tumor in mice. Furthermore, these tumor-free mice immunized with homologous vaccines showed full protection against the repeat challenge every 3 months for 5 years. The study also revealed that tumor-free female, not male, mice transferred anti-tumor ability to some of their offsprings. Heterologous vaccines exhibited no protective effect on tumor development. Immunological analysis discovered that activities of CTLs and NK were enhanced and the levels of IL-2, IL-12 and IFN-γ were significantly increased. Our results demonstrated that homologous tumor vaccines could elicit complete cross-protection against the lethal challenge of tumor cells through enhancing cell-mediated immune response, which lasted for 5 years in mice. These observations may provide a new vaccine strategy for tumor prevention.

1. Introduction

Application of tumor vaccines for tumor treatment or to prevent tumor in high-risk population is one of the effective ways for tumor biotherapy and has been evaluated extensively in animal experiments and some clinical trials [1–5]. Tumor cell-based vaccine, such as whole tumor cells, tumor cell lysates and genetically modified tumor cell, can potentially express a set of tumor-specific antigens and elicit anti-tumor immunity. Thus, it is a promising approach in tumor vaccine research [6]. However, the full protective and therapeutic potential of tumor vaccines has not been achieved [7,8] although many vaccination approaches including autologous, allogeneic and heterologous tumor cell vaccines have been evaluated extensively in animal models and some clinical trails. In this long-term experimental study, we developed some cell-based vaccines through a simple procedure, which were from mouse and human tumor cell lines. The prophylactic and therapeutic effects of the homologous and heterologous vaccines without immune adjuvant were evaluated in mice challenged with live mouse hepatoma H22 or S180 sarcoma cells.

2. Materials and methods

2.1. Mice and cell lines

Kunming mice (24–26 g) were provided by the Experimental Animal Center of Hebei province. H22 mouse hepatoma and S180 mouse sarcoma cell lines were provided by Hebei Medical University. A549 human lung carcinoma epithelial cell, SK-OV-3 human ovarian tumor cell, and SMMC-7721 human hepatoma cell lines were purchased from the tumor institute of Chinese Academy of Science. All tumor cell lines were cultured in RPMI-1640 culture medium (Sigma) with 10% heat-inactivated fetal bovine serum (FBS).

2.2. Preparation of tumor vaccines and vaccination

All tumor cell lines were cultured at starting concentration of 0.5×10^5/ml. and then without changing media. In order to make tumor vaccines, tumor cells were deprived of nutrition without

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changing media and spontaneously died during 7 to 15 days. Dead cells were harvested when positive cells with Tappan blue staining reached to 90 percent. They were frozen at −20 °C for 2 days and kept at room temperature (25 °C) for other 3 days, and then adjusted into 1×10^8/ml. Those tumor vaccines were ready for use. The combination cell vaccine of H22 and S180 was mixer half for each. Mice were immunized by intraperitoneal injection of 1 ml vaccines every other day for total of 6 times.

To compare the histological characters of the vaccines, live-tumor cells and corresponding tumor vaccine were centrifuged, fixed, HE stained, and taken images under microscope (Olympus, Japan).

2.3. Study protocols

To test the efficacy of treatment from tumor vaccines, mice were randomly divided into 12 groups, with 20 mice each group (10 male and 10 female). Six groups were intraperitoneally inoculated with 2×10^5 live H22 cells and the other six groups with 2×10^5 live S180 cells. On the 3rd day after challenge with live H22 cells, five groups were respectively vaccinated with H22, S180, A529, SK-OV-3, or SMMC-7721 tumor vaccines. The control group was given saline at same schedule as treatment groups. The same protocol was performed in the other six of groups challenged with live S180 cells. Tumor growth was monitored and survival data was recorded.

To test the preventive effect of tumor vaccines, mice were randomly divided into 10 groups. Five groups were first vaccinated with H22, S180, A529, SK-OV-3, or SMMC-7721 tumor vaccines. Ten days after the final immunization mice were challenged with 2×10^5 live H22 cells. Some tumor-free mice were then repeatedly challenged with live H22 cells every 3 months and continued for 5 years, with a total of 20 times. The same protocol was performed in the other five groups challenged with live S180 cells. The tumor growth was monitored in every group.

To test whether the preventive effect of vaccines was inheritable, twenty mice (10 female and 10 male) were vaccinated with combination tumor vaccine of H22 and S180. Ten days after the final vaccination injection, mice were challenged with 2×10^5 live H22 and 2×10^5 S180 cells. After 40 days, two tumor-free female and male mice were bred with healthy mice. Until they grew to around 15 grams, offsprings were divided into two groups. One group was challenged with 2×10^5 live H22 cells and the other with S180 cells. Tumor-free offsprings were challenged with live-tumor cells one more time on the 45th day. Tumor growth was monitored.

2.4. Immunological analysis

At the end of the 5-year observation, blood was taken from four tumor-free mice (two female and two male). The levels of IL-2, IL-4, and IL-12 were measured by using MTT kits. Spleens were dissected under sterile conditions and placed on a 200-μm still filter. Splenocytes were prepared by mashing the spleen with the rubber end of a syringe plunger. Lymphocytes were separated with using mouse lymphocyte separation medium. Suspend splenocytes were carefully transferred into centrifugation tube, layered over the separation media in 1:2 proportion, centrifuged at 2000 rpm for 15 minutes. Lymphocytes were recovered by aspirating the layer, and washed twice with RPMI-1640 media (at 1500 rpm for 10 minutes), resuspended in RPMI-1640 media containing 10% FBS. The activity of CTL and cell proliferation of T cells were measured by using MTT kits, the measurement of NK activity was done with the chromium ^{51}Cr release assay, TNF was assayed with ELISA kit, and the level of IFN-γ was measured with cell inhibiting assay.

2.5. Statistical analysis

The data are presented as the mean values ± SD. Statistical comparisons between groups were carried out with Student's *t*-tests. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Histological character of tumor vaccines

After subjected to frozen and thaw, some of the dead tumor cells broke down into cellular fragments with shrinking nuclear and

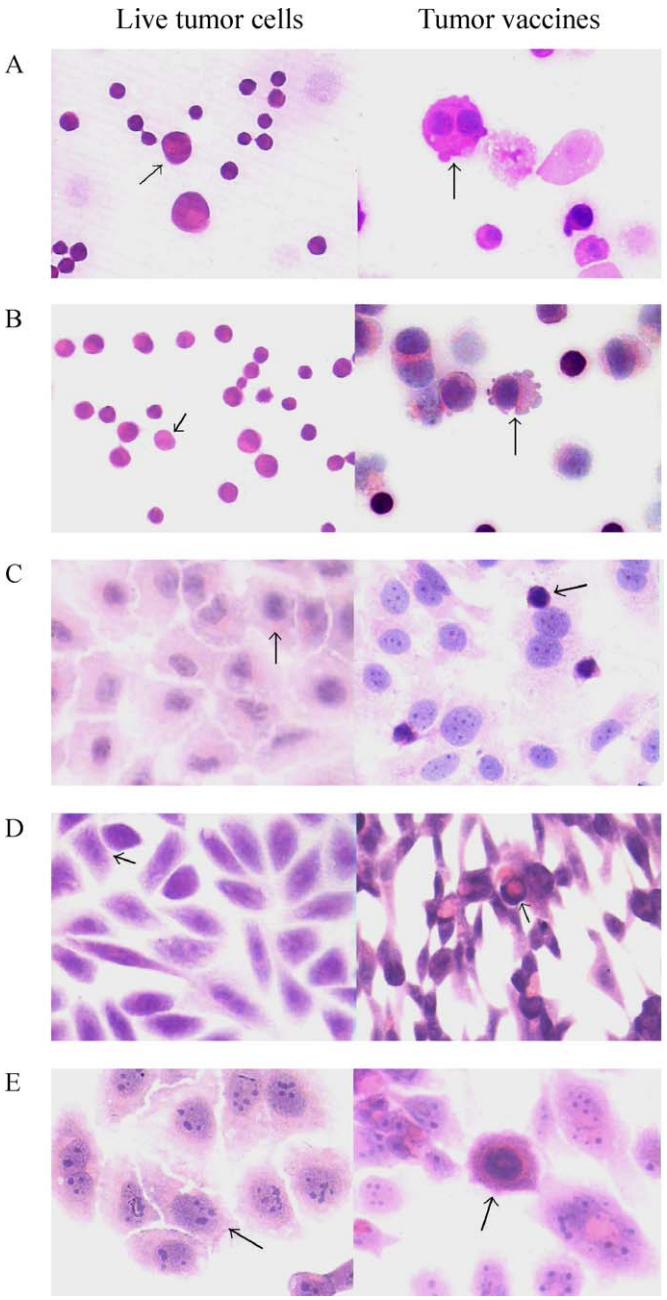

Fig. 1. Histological comparison of live-tumor cells and corresponding tumor cell-based vaccine. A: mouse H22 hepatoma cell; B: mouse S180 sarcoma cells; C: human A549 lung carcinoma epithelial cell: D: human SK-OV-3 ovarian tumor cells; E: human SMMC-7721 hepatoma cells. Tumor vaccines contained floating dead cell and cellular fragments including with shrinking nuclear and vacuolating cytoplasm. Arrows indicated typical histological characters.