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Postoperative anti-PD-1 antibody treatment to reduce recurrence in a cancer ablation surgical wound

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ARTICLE INFO

Article history:
Received 22 March 2017
Received in revised form
18 May 2017
Accepted 11 August 2017
Available online xxx

Keywords:
Head and neck cancer
Surgery
Tumor recurrence
Immune checkpoint inhibitor
Antitumor immunity

ABSTRACT

Background: Postoperative radiation and chemotherapy are routinely applied for microscopic residual diseases; however, treatment outcomes are not optimal, and patients frequently suffer from treatment-related toxicities. To search for an effective and less-toxic adjuvant treatment for patients with high risk of recurrence, the preventive effect of anti-programmed cell death protein 1 (PD-1) treatment was evaluated in an in vivo animal model of post-surgical tumor recurrence.

Materials and methods: An animal model of postsurgical tumor recurrence (SCCVII tumors in C3H mice) was established by reinoculating tumor cells (10^5 cells) into surgical wound of primary tumor resection. Initial and recurrent tumors were compared by an immunohistochemistry and complementary DNA microarray. Using this *in vivo* model, tumor recurrence rates were evaluated in the animals receiving anti-PD-1 treatments. Animals were rechallenged with tumor cells, and interferon gamma secretion from spleen cells was analyzed to determine tumor-specific antitumor immunity.

Results: FoxP3^{high} cell population was significantly elevated in recurrent tumors compared with that in primary tumors. Some immune response–related factors (granzyme F, neuronal leucine-rich repeat protein 1, myosin heavy chain 3, and transmembrane protein 8C) showed significant differences between primary and recurrent tumors. In this animal model, anti-PD-1 treatments significantly suppressed tumor recurrence. Importantly, tumor induction was significantly reduced when anti-PD-1–treated mice were rechallenged with tumor cells. Tumor cell–specific interferon gamma production was increased in these animals.

Conclusions: Postoperative anti-PD-1 treatment significantly reduced recurrence in a cancer ablation surgical wound in an *in vivo* model of tumor recurrence. Our data lay the preclinical groundwork for the future clinical validation of adjuvant anti-PD-1 treatments in patients.

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http://dx.doi.org/10.1016/j.jss.2017.08.022

Introduction

Surgical excision of tumors has been an essential therapeutic modality in cancer control. However, major vessels and nerves do not allow enough safety margins in curative surgery, particularly in head and neck cancers. This sometimes results in remnant minimal residual disease at the primary site, causing local recurrence.[1-3] Although postoperative radiation and chemotherapy are routinely applied for these cases, treatment outcomes are not optimal. Patients may even suffer from treatment-related toxicities.

In addition, it has been suggested that surgery itself or surgery-related factors may facilitate local recurrence and metastasis progression.[4-7] Surgical procedures themselves could evoke damage to the surrounding tissue and induce dissemination of tumor cells into blood and lymphatic circulations.[8,9] Moreover, elevated proangiogenic factors and decreased antiangiogenic factors can play a role in the recurrence of a tumor during perioperative period.[10-12] Suppression of cell-mediated immunity has also been considered as an additive mechanism of cancer progression after surgery.[13,14]

A malignant tumor itself could have multiple tumor-resistant mechanisms, including local immune escape and dysfunction of T-cell signal pathways.[15] One potential mechanism is that tumors can produce programmed death (PD)-L1 and PD-L2 protein surface molecules, which engage with PD-1 receptors on T-cell surfaces (immune checkpoint).[16] Their interaction might result in T-cell dysfunction and tumor progression.[16] Various types of solid tumors have been shown to have PD-L1 expression with significant prognostic value.[17] An early phase I trial of anti-PD-1 antibody for a refractory solid tumor has shown a beneficial result.[18]

Adjuvant treatment with an immune checkpoint inhibitor has also been tried in several types of solid tumor.[19-21] A clinical trial of postoperative adjuvant treatment with an anti-PD-1 antibody in melanoma patients has shown that recurrence is significantly decreased in anti-PD-1–treated arm.[19] A neoadjuvant trial with anti-PD-1 antibody in head and neck cancer patients has also demonstrated a favorable effect in 83% of patients, without causing significant complications.[21]

Even with these promising clinical evidences, clear indication for the use of immune checkpoint inhibitors to inhibit cancer recurrence remains to be determined. For example, conventional surgery alone has successful treatment outcomes in over 80%-90% of patients with early-stage head and neck cancer. Thus, more specific indications for the use of immune checkpoint inhibitors should be investigated in terms of cost-effectiveness. The objective of this study was to determine the preventive effect of an immune checkpoint inhibitor, anti-PD-1 antibody, in an *in vivo* animal model of postsurgical tumor recurrence. Our specific goal was to provide preclinical evidence for future clinical trials of postoperative anti-PD-1 treatments in patients with a high risk of recurrence.

Materials and methods

An animal model of tumor recurrence at the surgical wound of primary tumor resection

To establish an immune-competent animal model, syngeneic SCCVII tumor cells and C3H/He mice were used in this study. SCCVII cells are cutaneous, moderately differentiated, murine squamous cell carcinomas, syngeneic to C3H mice. They were originally isolated and cultured by Dr Herman D. Suit (Harvard Medical School, Radiation Oncology). These tumor cells were maintained in a humid incubator at 37°C with 5% CO_2 in Roswell Park Memorial Institute 1640 medium (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, 10 mmol/L N-2-HEPES buffer (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid), 100 IU/mL penicillin, and 100 μg/mL streptomycin. Female C3H/He mice were purchased from Orient Bio (Seongnam, Gyeonggi, South Korea) at the age of 5 wk. The mice were acclimated for at least 1 wk before experiments. They were housed five per cage in our vivarium with free access to food and water on a 12:12 light/dark cycle.

SCCVII cells (5×10^5 cells in 100-μL phosphate buffered saline) were injected subcutaneously at the right flank of C3H/He mice using a 1-mL tuberculin syringe with a 29-gauge needle (Fig. 1A). Body weight and tumor size were measured twice a week. When tumor volume reached approximately 500 mm^3, mice were anesthetized via an intraperitoneal injection of ketamine (100 mg/kg). After anesthesia, an elliptical skin incision around the tumor was made, and the entire tumor was excised with a grossly negative resection margin. The surgical wound was sutured after the excision, and then SCCVII cells (1×10^3, 1×10^4, 5×10^4, or 1×10^5 cells in 100-μL phosphate buffered saline) were reinoculated at the surgical wound to mimic minimal residual tumors after excision of the tumor. Regrowing tumors were harvested, and mice were sacrificed using CO_2 when the tumor volume reached 800 mm^3 or their body weights were diminished to 25% below their initial body weights. The experimental protocol was approved by our Institutional Animal Care and Use Committee (approval no. 20150504001).

Comparison of primary and recurrent tumors

Paraffin-embedded tissue blocks of primary and recurrent tumors were obtained. CD4 + CD25 + $FoxP3^{high}$ regulatory T cells (T_{reg}) are known to be one of the key cells in tumor-induced immune escape.[22,23] Therefore, we immunestained tumor samples of mice with FoxP3 proteins. Detection of FoxP3 was performed with an anti-FoxP3 monoclonal antibody (1:50; Abcam, Cambridge Science Park, Cambridge, UK). Immunohistochemistry result was evaluated with an image analysis algorithm; Positive Pixel Count v9 program on the Aperio ImageScope v11.1.2.760 (Aperio Technologies, Vista, CA). Percentage of FoxP3 antibody–stained area in comparison with total area of the tumor was calculated using this software. Matched paired *t*-test was used to analyze