WT1 Peptide Therapy for a Patient with Chemotherapy-resistant Salivary Gland Cancer

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Abstract. Wilms’ tumor (WT1) protein is one of the most promising target antigens for cancer immunotherapy. In fact, clinical responses, such as growth stabilization or shrinkage of tumor with immunological responses, have been reported in patients vaccinated with WT1 peptide. Here, we performed WT1 peptide-based immunotherapy for a patient with chemotherapy-resistant salivary gland cancer, whose histologic type was carcinoma ex pleomorphic adenoma. The patient with its pulmonary metastasis, refractory to chemotherapy, was intradermally injected with 3 mg of WT1 peptide emulsified with Montanide ISA51 adjuvant at one-week intervals for 12 weeks. The considerably rapid growth of tumor was inhibited after WT1 vaccination, and stable disease, lasting three months, was achieved. Concomitantly, immunological responses, i.e. an increase in frequencies of WT1 tetramer+ CD8+ T cells and delayed type hypersensitivity response, were detected after the vaccination. These results indicate the potential of WT1 peptide-based immunotherapy for the treatment of chemotherapy-resistant salivary gland cancer.

The Wilms’ tumor gene WT1 was first isolated and categorized as a tumor suppressor gene that was inactivated in Wilms’ tumor and mutated in the germline of children with genetic predisposition to Wilms’ tumor, a kidney neoplasm of childhood (1). The WT1 gene encodes a zinc finger transcription factor, controls the expression of many genes associated with cell growth, cell differentiation, and apoptosis, and plays a role in mRNA splicing (1).

Our group and others have demonstrated high expression of the WT1 gene and/or WT1 protein in leukemia and various kinds of solid cancers (2). Based on a series of experimental evidence, we proposed that the WT1 gene has an oncogenic rather than a tumor-suppressive function in most malignant diseases (1). These results indicated that the wild-type WT1 gene product could be the most promising target antigen for cancer immunotherapy (2). WT1 peptide or WT1 cDNA-vaccinated mice rejected the challenge by WT1-expressing tumor cells without damage to normal tissues that physiologically expressed WT1 (2). Human WT1 protein-derived peptides that were able to elicit human leukocyte antigen (HLA)-class I-restricted WT1-specific cytotoxic T lymphocyte (CTL) response were also identified by us and other groups (2-5).

Based on these pre-clinical findings, we performed a phase I clinical study of WT1 peptide vaccination for patients with acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), lung cancer, and breast cancer (2, 6). In this study, 0.3-3.0 mg of natural or modified 9-mer WT1 peptide emulsified with Montanide ISA51 adjuvant were intradermally injected at biweekly intervals. This study demonstrated that WT1 vaccination was able to induce WT1-specific CTLs and cancer regression without damage to normal tissues in the clinical setting (6). In the present study, we report a case of chemotherapy- and radiotherapy-resistant salivary gland cancer, histologically diagnosed as carcinoma ex pleomorphic adenoma, in which WT1 peptide vaccination...
considerably inhibited rapid growth of the tumor, leading to stable disease (SD) for three months.

Case Report

A 56-year-old man was diagnosed as having salivary gland cancer in February 2004. The histological diagnosis was carcinoma ex pleomorphic adenoma. The tumor was estimated as cT3N2bM1, and there was enlargement of the mediastinal lymph nodes. Left neck dissection, including submandibulectomy, was performed in February 2004, followed by postoperative radiotherapy, concurrent with S-1, targeted for the locoregional area and mediastinum. Since recurrence occurred with enlargement of mediastinal lymph nodes and elevation of tumor marker cytokeratin 19 fragment (CYFRA) in March 2005, chemotherapy with cisplatin and 5-fluorouracil was performed for three courses. However, since enlargement of mediastinal lymph nodes and lung tumor appeared after the three courses, chemotherapy with nedaplatin and docetaxel was performed for four courses. However, the disease eventually progressed with enlargement of mediastinal lymph nodes and lung tumor after the four courses. Metastasis in the right frontal lobe of brain was found in August 2006. The metastatic lesion in the brain was completely resected, followed by whole-brain radiotherapy. Histological examination of the brain tumor was compatible with metastasis of salivary gland cancer.

Since the patient was HLA-A*2402-positive, and WT1 expression of cancer tissue was proven by immunohistochemical examination (Figure 1), he was enrolled into this clinical study of WT1 peptide vaccination (7,8). The clinical course and the immunological responses, including frequencies of WT1-Tetramer (Tet)+ CD8+ T-cells in peripheral blood (PB) (Figure 2) and the WT1 peptide-specific delayed type hypersensitivity (DTH), were evaluated (8). During the month before the start of vaccination (weeks -4 to 0), the tumor was considerably rapidly growing and a small amount of pleural effusion at the right side appeared. Representative data of the computed tomographic examination from weeks -4 to 12 (the end of the clinical study period) are shown in Figure 3. The CT examinations revealed that the sum of the longest diameter (SLD) of target lesions increased from 67% at week -4 to 100% at the vaccination start (week 0) (SLD at week 0 was defined as 100%) (Figure 4, upper panel). Furthermore, necrotic lesion in the tumor was found on CT examination at week 12 (Figure 3, upper panel). Since the tumor growth was rapid before the beginning of WT1 vaccination, it is reasonable to consider that WT1 vaccination induced a clinical response to suppress tumor growth.

The first injection of WT1 vaccine was performed on December 20, 2006. The WT1 vaccine was composed of 3 mg of a modified WT1 peptide (amino acids 235-243: CYTWNQMNL) for HLA-A*2402 type and Montanide ISA51 adjuvant, and the vaccination was scheduled to be performed 12 times at weekly intervals (8). After WT1 vaccination was begun, rapid growth of the tumor declined (Figure 4, upper panel). SLD slightly decreased from 100% at week 0 to approximately 90% at week 5, and was then stable for a further 7 weeks until the end of this clinical study period (week 12) (Figure 4, upper panel). Furthermore, necrotic lesion in the tumor was found on CT examination at week 12 (Figure 3, upper panel). The amount of pleural effusion, which appeared before the vaccination, did not increase during the 12 weeks. The patient’s quality of life was also maintained (performance status: 0) and he was able to carry out his daily life without any limitation during the three months of this clinical study period. At the end of this clinical study (week 12), the clinical response was assessed as stable disease (SD). As for adverse effects, only local skin erythema at the injection sites of the WT1 vaccine was observed.

Delayed-type hypersensitivity (DTH) skin test for WT1 peptide was performed for the monitoring of immunological response (9,10). The DTH test was negative at the beginning of WT1 vaccination, but turned positive at weeks 5, 9 and 13 (Figure 4). Frequencies of WT1-Tet+ CD8+ T-cells among CD8+ T-cells was 0.098% before the vaccination, but increased to 0.16% at week 4, and the increased percentage was maintained at week 8 (Figure 4, lower panel).

Discussion

This report demonstrates the potential of WT1 vaccination for the treatment of salivary gland cancer, histologically diagnosed as carcinoma ex pleomorphic adenoma. WT1 peptide vaccination for a patient with recurrent, chemotherapy-resistant, considerably rapidly growing submandibular gland cancer induced a cessation of tumor growth, followed by a slight decrease in tumor size (10% decrease of SLD). The decrease in tumor size was revealed with CT examination at week 4 and remained unchanged until the end of this clinical trial (week 12) (Figure 4). Furthermore, necrotic lesion in the tumor was found on CT examination at week 12 (Figure 3, upper panel). Since the tumor growth was rapid before the beginning of WT1 vaccination, it is reasonable to consider that WT1 vaccination induced a clinical response to suppress tumor growth.

The frequency of WT1-Tet+ CD8+ T-cells in PB of the patient before the vaccination was 0.098%, while the mean value of those from healthy donors was 0.094%, indicating that the frequency of WT1-Tet+ CD8+ T-cells in the PB of the patient was at the same level as the ones of healthy donors. However, the frequency of WT1-Tet+ CD8+ T-cells increased after the vaccination from 0.098% at week 0 to 0.16% at week 4 (1.63-fold increase). We previously reported a clear correlation between clinical response and 1.5-fold increase in the frequency of WT1-Tet+ CD8+ T-cells after WT1 vaccination (6). The change of DTH from negative to positive reaction after the vaccination also supports the elicitation of a WT1-specific immunological response by the vaccination. Taken together, these data strongly suggest that
a WT1 peptide vaccination-induced immunological response, detected by tetramer assay (*ex vivo* immune monitoring) and DTH reaction (*in vivo* immune monitoring), led to the clinical response, *i.e.* stabilization of the disease.

Surgical resection, followed by local radiotherapy, when needed, is a standard therapy for patients with stage I or II salivary gland cancer, and the prognosis is not so poor (11).

However, the prognosis of patients with advanced stages of the disease, such as patients with its distant metastasis, is very poor (11). Carcinoma ex pleomorphic adenoma, presented in this report, accounts for about 12% of salivary malignancies and is a subtype of highly malignant tumor (12). Although novel therapies, including a combination therapy of trastuzumab and capecitabine, are being tested,
standard therapy for patients with advanced disease stages has not been established (12). In this context, immunotherapies, including WT1 peptide vaccine, may become alternatives for the treatment of this disease. In the course of the preparation of this manuscript, Sasabe et al. reported a case of pulmonary metastasis from adenoid cystic carcinoma of salivary gland that was successfully treated with WT1 peptide vaccination (13). Their report along with the current study strongly suggest that WT1 peptide vaccination has therapeutic potential for salivary gland cancer.

Besides salivary gland cancer, the favorable response of WT1 immunotherapy in various types of malignancies such as AML, MDS, multiple myeloma, glioblastoma multiforme, rhabdomyosarcoma, lung, breast, renal, ovarian cancers has been previously reported, which strongly suggests the superiority of WT1 protein as a target antigen for cancer immunotherapy (2-6, 8, 10, 14-16). In fact, WT1 protein was rated as the most promising target antigen in a recent review article (17). On this basis, WT1 peptide-based immunotherapy is expected to become a novel treatment for salivary gland cancer.

Conflict of Interest Statement

None declared.

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References


