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Rescue of iCIKs transfer from PD-1/PD-L1 immune inhibition in patients with resectable tongue squamous cell carcinoma (TSCC)

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ABSTRACT

Objectives: The purpose of this study is to evaluate the therapeutic efficacy and the role of PD-1/PD-L1 pathway in tongue squamous cell carcinoma (TSCC) patients treated with radical operation combined with chemotherapy and improving cytokine induced killer cells (iCIKs) transfer.

Methods: Thirteen patients who received radical resection and chemotherapy were enrolled in this study. PD-1/PD-L1 expression was evaluated in TSCC patients. ICIKs were cultured from patient-derived peripheral blood mononuclear cells (PBMCs) in vitro. The immunological differences underlying iCIKs transfer were investigated through phenotype, cytokine secretion and PD-1/PD-L1 inhibition analysis.

Results: The serum PD-L1 levels were elevated in the TSCC patients. PD-L1 was detected on both human TSCC cells and tumour tissue sections. PD-1 expression was much higher on the PBMCs of TSCC patients than on in vitro cultured iCIKs. Interruption of PD-1/PD-L1 interaction enhanced the cytotoxicity of iCIKs in vitro. CD3 + CD8+ T cell proportion and cytokine IL-6 secretion decreased after chemotherapy. The infusion of iCIKs effectively reversed the immunosuppression through the upregulation of the CD3 + CD8+ T cell proportion and Th cell cytokine secretion (IFN-γ, TNF-α, IL-4 and IL-6). Twelve responders are currently alive (95.7+ months), another patient 83 months.

Conclusion: Our findings indicated that the PD-1/PD-L1 interaction contributes to the immunosuppression in TSCC patients. ICIKs transfer is an effective therapy to reverse the immunosuppression caused by surgical procedures and chemotherapy and improve immune system function.

1. Introduction

Oral squamous cell carcinoma (OSCC) represents the eighth most common malignancy in the world, with a poor prognosis of < 60% patient survival for > 5 years [1]. The most common subtype of OSCC is tongue squamous cell carcinoma (TSCC), which is also associated with a poor treatment outcome [2]. Over the years, surgical resection and neck dissection have been the primary therapies for TSCC patients. Chemotherapy is considered an adjuvant strategy for recurrence and/or advanced inoperable TSCC patients. However, the side effects of chemotherapy severely reduce the life quality of these patients. Thus, chemotherapy is not a sufficient strategy to improve the prognosis of TSCC patients.

Immunosuppression was observed in TSCC patients [3]. In the PBMCs population of HNSCC patients, the proportion of CD4 + CD8 + T cells, CD3-CD56 + NK cells and CD3 + CD56+ NK-T cells was down-regulated, indicating the suppression of antitumour function by the immune system [4]. The PD-1/PD-L1 interaction is an

Abbreviations: OSCC, Oral squamous cell carcinoma; TSCC, Tongue squamous cell carcinoma; HNSCC, Head and neck squamous cell carcinoma; iCIKs, Improving cytokine-induced killer cells; TILs, Tumour-infiltrating lymphocytes; PBMCs, Peripheral blood mononuclear cells; IRB, Institutional Review Board; GMP, Good manufacturing practice; OR, Overall response; PFS, Progression-free survival; OS, Overall survival

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important mechanism for immunosuppression in cancer patients [5]. Soluble PD-L1 was detected in human plasma, and the plasma level of PD-L1 was elevated in cancer patients [6]. Studies have reported a large proportion of TSCC cases with PD-L1 expressed on tumour cells and PD-1 expressed on tumours infiltrating lymphocytes (TILs), suggesting the PD-1/PD-L1 interaction as the potential mechanism underlying the immunosuppression in TSCC patients [7,8].

Improving cytokine-induced killer cells (iCIKs) transfer is a personalized therapeutic procedure, whereby autologous lymphocytes were isolated from PBMCs of tumour-bearing hosts, cultured and expanded to large quantities in vitro, and finally transferred back to the host [9]. Chemotherapy directly induces tumour cell death and enhances tumour sensitivity to the host immune system along with moderate to severe toxicities, while iCIKs transfer improves the immune function of the host with an in vitro expanded large number of T cells that counteract the side effects of chemotherapy. Clinical trials have shown that iCIKs transfer acts synergistically with chemotherapy as an effective and safe neoadjuvant therapy with less toxicity for postoperative solid tumour patients [10,11]. We previously reported that iCIKs transfer effectively reversed the immune system suppression and improved the survival time in HNSCC patients [12]. Given that the PD-1/PD-L1 interaction plays an important role in tumour immune escape, whether iCIKs transfer affects the PD-1/PD-L1 interaction in TSCC patients is yet to be further confirmed.

The aim of this study was to evaluate the clinical outcome and the potential role of the PD-1/PD-L1 interaction in combination neoadjuvant therapy in TSCC patients. In this study, 13 patients diagnosed with TSCC were enrolled, and all of the patients received chemotherapy in combination with iCIKs transfer.

2. Patients and methods

2.1. Patient selection

A total of thirteen patients with primary or secondary TSCC, who had received radical resection, were enrolled in this study. All the selected patients provided written informed consent prior to receiving chemotherapy and iCIKs transfer. Information on the gender, age, location of the lesions, lymph node metastasis, clinical stage, number of iCIKs transfer, prognosis and survival time are shown in Table 1. The TSCC patients were subjected to the following treatment course (Fig. 1): All of the TSCC patients received surgery resection and/or neck dissection prior to chemotherapy and iCIKs transfer treatment. After iCIKs transfer, the TSCC patients were evaluated every 3 months for the first 3 years and every 6 months thereafter. In this study, peripheral blood was extracted before and after chemotherapy or iCIKs transfer to culture iCIKs to evaluate the effect of iCIKs transfer on the immune system function of the treated TSCC patients. Our treatment protocol was approved by the institutional ethics committee. This study was approved by the Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University IRB (Institutional Review Board). The date of the last follow-up was November 22, 2017.

2.2. Cell lines

A primary human TSCC cell line was obtained from a TSCC patient from the Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University. Cal27 and Jurkat cell lines were obtained from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. The CDS of PDCD1 was cloned into the Jurkat cell line, named Jurkat$^{PD\text{-}1}$. The Cal 27 cell line and human TSCC cells were maintained in DMEM/F12 media supplemented with 10% FBS. Jurkat$^{PD\text{-}1}$ cells were cultured in RPMI1640 media supplemented with 10% FBS.

2.3. Immunofluorescence

For Cal27 cell staining, the cells were fixed in 4% PFA for 20 min at room temperature. After washing once, the cells were blocked with 5% BSA for 1 h at room temperature and incubated with PD-L1 monoclonal antibody (clone 28–8, Abcam, Cambridge, UK) at 4 °C overnight. The cells were washed twice with PBS and incubated with donkey anti-mouse IgG as a secondary antibody (A10037, Thermo Fisher, Santa Clara, California, USA) for 1 h at room temperature. After washing twice, the cells were mounted in VECTASHIELD mounting medium with DAPI (Vector laboratories, Burlingame, CA, USA) and covered with a cover slip. For staining frozen human TSCC tissue sections, the samples were fixed in cold acetone for 10 min at 4 °C, anti-human PD-L1 monoclonal antibody (clone 28–8, Abcam) was used, and the other steps were performed as described above. The slides were observed on a laser scanning confocal microscope (TCS-SP5, LEICA, Buffalo Grove, IL, USA).

2.4. Cell phenotype and cytokine secretion analysis

For the phenotype analysis, the lymphocyte subpopulation of the PBMCs was analysed by using fluorescein-labelled monoclonal antibodies against the following proteins: CD3 (clone UCHT1, Dako, Glostrup, Denmark), CD4 (clone RM4–5, BD Bioscience, San Jose, CA, USA), CD8 (clone SK, BD Bioscience), CD25 (clone ACT-1, Dako) and CD56 (clone MOC-1, Dako) after 13 days of iCIKs culture. The cells were suspended and incubated with monoclonal antibodies against CD3, CD4, CD8, CD25 and CD56 in buffer (2% FBS and 1% NaN3 in PBS) for 30 min at 4 °C. After washing twice with PBS, the cells were suspended in 1 mL of staining buffer (BD Pharmingen, San Diego, CA, USA). For PD-1/PD-L1 detection, hTSCC cells, Cal27 cells and iCIKs

Table 1
Clinical characteristics of the TSCC patients.

Patient	Gender	Age	Location (Tongue)	Clinical stage	Lymph node metastasis	Number of lymph node dissection	Number of iCIKs transfer	Prognosis	Survival time (month)
1	Male	63	Right	III	Yes, both side	3	24	CR	89+
2	Female	78	Right	III	Yes, right	1	19	CR	90+
3	Female	70	Right	II	No	0	18	CR	87+
4	Female	60	Right	II	No	0	16	CR	104+
5	Male	46	Right	III	Yes, right	1	16	CR	87+
6	Female	74	Left	II	No	0	16	CR	115+
7	Female	76	Left	III	Yes, left	1	15	CR	96+
8	Male	49	Left	III	Yes, left	1	15	CR	94+
9	Female	64	Left	III	Yes, left	1	14	CR	96+
10	Male	75	Right	II	No	0	12	CR	94+
11	Female	72	Left	II	No	0	5	CR	83
12	Female	44	Left	III	Yes, left	2	2	CR	97+
13	Male	51	Right	III	Yes, left	1	2	CR	99+