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Detection of high PD-L1 expression in oral cancers by a novel monoclonal antibody L_1Mab-4

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ABSTRACT

Programmed cell death-ligand 1 (PD-L1), which is a ligand of programmed cell death-1 (PD-1), is a type I transmembrane glycoprotein that is expressed on antigen-presenting cells and several tumor cells, including melanoma and lung cancer cells. There is a strong correlation between human PD-L1 (hPD-L1) expression on tumor cells and negative prognosis in cancer patients. In this study, we produced a novel anti-hPD-L1 monoclonal antibody (mAb), L_1Mab-4 (IgG_{2b}, kappa), using cell-based immunization and screening (CBIS) method and investigated hPD-L1 expression in oral cancers. L_1Mab-4 reacted with oral cancer cell lines (Ca9-22, HO-1-u-1, SAS, HSC-2, HSC-3, and HSC-4) in flow cytometry and stained oral cancers in a membrane-staining pattern. L_1Mab-4 stained 106/150 (70.7%) of oral squamous cell carcinomas, indicating the very high sensitivity of L_1Mab-4. These results indicate that L_1Mab-4 could be useful for investigating the function of hPD-L1 in oral cancers.

1. Introduction

Oral cancer is the eleventh highest of all cancer types [1] and constitutes approximately 2% of all cancer cases worldwide [2]. In oral cancers, there are certain histological tumors, including squamous cell carcinoma (SCC), adenosquamous cell carcinoma, adenoid cystic carcinoma, osteosarcoma, and mucoepidermoid carcinoma. SCC is the most common type, accounting for > 90% of oral cancers [3]. Because of improvements and progression in therapeutic techniques such as surgery, chemotherapy, and radiotherapy, the 5-year survival rate has reached > 80% [4,5]. In contrast, it is sometimes difficult to provide sufficient therapeutic effects because of the risks of adverse events [6–8].

Recently, tumor immunotherapies, which is focused on several immune checkpoint molecules, such as programmed cell death-1 (PD-1), programmed cell death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), have emerged. Clinically, nivolumab, a complete human IgG_4 against PD-1, was firstly approved for the treatment of recurrent and/or metastatic head and neck cancer, which was previously treated with platinum-based chemotherapy [9]. Avelumab, a complete human IgG_1 against PD-L1, was recently approved for the treatment of metastatic Merkel cell carcinoma [10,11], which is detected in oral cavity [12].

PD-L1, also known as B7-H1 and CD274, is a type I transmembrane glycoprotein, which is expressed on antigen-presenting cells and some tumor cells, including melanoma, ovarian, and lung cancer cells [13–15]. PD-L1 is a ligand for PD-1 and is involved in inhibiting T-cell effector functions [16], leading to the escape of tumor cells from immune response. Recent studies have revealed a strong correlation between PD-L1/PD-L2 expression on tumor cells and negative prognosis in cancer patients [17–19].

Lin *et al.* have revealed a correlation between high PD-L1 expression and metastasis and poor prognosis in oral SCC [20]. Several reports have shown that PD-L1 could be an effective target for treatment [21–26]. However, PD-L1 expression in oral cancers has not been completely investigated. In this study, we established a novel anti-PD-L1 antibody and performed immunohistochemistry for oral cancers.

2. Materials and methods

2.1. Cell lines

Ca9-22, HO-1-u-1, SAS, HSC-2, HSC-3, HSC-4, and HEK-293T cells were obtained from the Japanese Collection of Research Bioresources

Abbreviations: PD-L1, Programmed cell death-ligand 1; PD-1, programmed cell death-1; CBIS, cell-based immunization and screening; SCC, squamous cell carcinoma; ACC, adenoid cystic carcinoma; MEC, mucoepidermoid carcinoma; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; HNC, head and neck cancer; APC, antigen-presenting cell; DMEM, Dulbecco's Modified Eagle's Medium; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; PBS, phosphate-buffered saline; FBS, fetal bovine serum; DAB, 3,3-diaminobenzidine tetrahydrochloride

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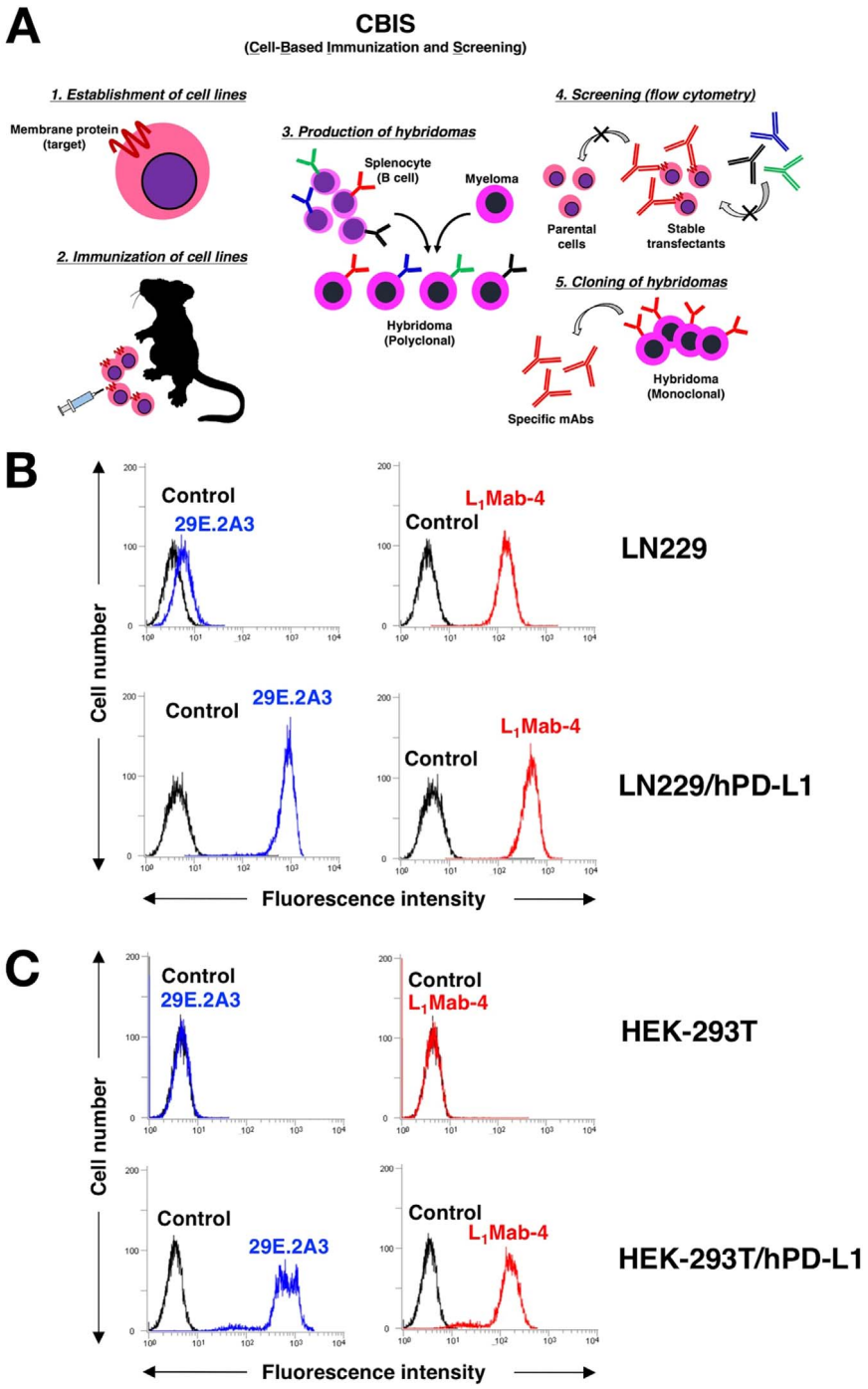

Fig. 1. Flow cytometry of L_1Mab-4 against LN229/hPD-L1. (A) Procedure of cell-based immunization and screening (CBIS) methods. (B) LN229 and LN229/hPD-L1 were treated with 1 μg/mL of 29E.2A3 (positive control; blue line) and L_1Mab-4 (red line), followed by treatment with Alexa Fluor 488-conjugated anti-mouse IgG; black line, negative control. (C) HEK-293T and HEK-293T/hPD-L1 were treated with 1 μg/mL of 29E.2A3 (positive control; blue line) and L_1Mab-4 (red line), followed by treatment with Alexa Fluor 488-conjugated anti-mouse IgG; black line, negative control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Cell Bank (Osaka, Japan). LN229 and P3U1 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). LN229/human PD-L1 (hPD-L1) was produced by transfecting pCAG/PA-hPD-L1-RAP-MAP into LN229 cells using a Gene Pulser Xcell electroporation system (Bio-Rad Laboratories, Inc., Berkeley, CA). The stable transfectant of LN229/hPD-L1 was established by limiting dilution. HEK-293T/hPD-L1 was produced by transfecting pCAG/PA-hPD-L1-RAP-MAP into HEK-293T cells using Neon transfection system (Thermo Fisher Scientific, Inc., Waltham, MA). A few days after transfection, PA tag-positive cells were sorted using a cell sorter (SH800; Sony Corp., Tokyo, Japan). PA tag system (GVAMPGAEDDVV (12 a.a.) vs. clone: NZ-1), RAP tag system (DMVNPGLEDRIE (12 a.a.) vs. clone: PMab-2), and MAP tag system GDGMVPPGIEDK (12 a.a.) vs. clone: PMab-1) have been previously established in Tohoku University Graduate School of Medicine and described in detail [27–29].

Ca9-22, HO-1-u-1, SAS, HSC-2, HSC-3, HSC-4, LN229, LN229/hPD-L1, HEK-293T, and HEK-293T/hPD-L1 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Nacalai Tesque, Kyoto, Japan), and P3U1 cell line was cultured in RPMI 1640 medium (Nacalai Tesque) at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air, both of which were supplemented with 10% heat-inactivated fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.). One hundred units/mL