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Treatment with a programmed cell death-1-specific antibody has little effect on afatinib- and naphthalene-induced acute pneumonitis in mice

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ABSTRACT

Although several antibodies developed to target programmed cell death-1 (PD-1) and its ligand (PD-L1) have demonstrated great promise for the treatment of non-small cell lung cancer (NSCLC), and other malignancies, these therapeutic antibodies can cause pneumonitis. Furthermore, epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI)-induced pneumonitis was reported after treatment with anti PD-1 antibodies. We previously demonstrated that mice with naphthalene-induced airway epithelial injury developed severe gefitinib-induced pneumonitis through a neutrophil-dependent mechanism. The present study aimed to investigate whether treatment with afatinib, an EGFR-TKI that effectively targets EGFR mutation-positive NSCLC, and anti PD-1 antibodies induces pneumonitis in mice. C57BL/6J mice were treated intraperitoneally with naphthalene (200 mg/kg) on day 0. Afatinib (20 mg/kg) was administered orally on days −1 to 13. An anti-PD-1 antibody (0.2 mg/mice) was also administered intraperitoneally every 3 days from day 1 until day 13. The bronchoalveolar lavage fluid (BALF) and lung tissues were sampled on day 14. As observed previously with gefitinib, afatinib significantly increased the severity of histopathologic findings and the level of protein in BALF on day 14, compared to treatment with naphthalene alone. A combined anti-PD-1 antibody and afatinib treatment after naphthalene administration had yielded the same histopathological grade of lung inflammation as did afatinib treatment alone. Our results suggest that anti-PD-1 antibody treatment has little effect on afatinib-induced lung injury.

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1. Introduction

Afatinib, a second-generation epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI), is an effective therapeutic agent for EGFR-mutated non-small cell lung cancers [1,2]. However, afatinib may induce severe interstitial lung disease (ILD) in some patients [3,4]. Similarly, although immune-checkpoint inhibitors such as monoclonal antibodies specific for programmed cell death-1 (PD-1) have transformed the treatment for several tumor types [5], these agents have also been associated with occasionally fatal ILD [6–8]. Several cases of serious EGFR-TKI-associated ILD have recently been reported in patients with non-small cell lung cancer who had been treated with the anti-PD-1 antibody nivolumab [9,10]. The Japanese Ministry of Health, Labor, and Welfare (MHLW) and the Pharmaceuticals and Medical Devices Agency (PMDA) have confirmed the details of these cases, and published a reminder regarding the necessary precautions associated with EGFR-TKIs to relevant academic societies and similar groups on July 22, 2016 [11]. However, it remains unclear whether and how the extended EGFR-TKI therapy after nivolumab administration would increase the risk of ILD.

The club cell is a type of pulmonary stem cell found in the distal airway, and abnormalities in this population are thought to promote chronic lung injury and lung fibrosis [12]. As naphthalene is

Abbreviations: EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; NSCLC, non-small cell lung cancer; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; ILD, interstitial lung disease.

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selectively cytotoxic against club cells [13], we previously used naphthalene to generate an animal model of lung injury that would be at risk of developing gefitinib (first-generation EGFR-TKI)-induced pneumonitis [14,15]. In this model, gefitinib administration after naphthalene treatment prolonged lung injury and associated neutrophil infiltration on day 14. In the present study, we investigated whether afatinib treatment would induce pneumonitis in our naphthalene-induced lung injury model and whether anti-PD-1 antibody treatment and afatinib would synergistically affect the development of pneumonitis.

2. Materials and methods

2.1. Animal treatment

All animal experiments were approved by the Committee on Ethics Regarding Animal Experiments of Kyushu University (protocol no. A28-272-0). Female C57BL/6 mice (7 weeks old; SLC Inc., Japan) were used in all experiments. Mice were injected intraperitoneally with naphthalene (200 mg/kg; Wako Pure Chemical Industries, Japan) on day 0.

A solution of afatinib (SelleckChem, USA) in 0.5% methyl cellulose 400 and 0.4% Tween-80 (Wako Pure Chemical Industries, Japan) was prepared for oral administration administered orally at a dose of 20 mg/kg once daily from day −1 to day 13. In our preliminary experiments, we evaluated a dose range of afatinib (5–20 mg/kg). Afatinib at the concentration of 15 mg/kg strongly inhibits growth of HKESC-2 tumor cells in vivo [16]. Mice also received intraperitoneal injections of an anti PD-1 antibody (0.2 mg/mouse; RMP1-14, BioLegend, Japan) or control IgG2a (BioLegend, Japan) in saline every 3 days from day 1 to day 13, in accordance with a previous report [17]. RMP1-14 was proven not to deplete PD-1-expressing cells in vivo and exhibits no agonistic activity [18]. A scheme of the administration schedule is shown in Fig. 1A.

2.2. Histopathological evaluation and immunohistochemistry

Histopathology was performed as previously described [14]. The right lung was fixed in 10% buffered formalin, embedded in paraffin, and sectioned; the resulting lung sections were stained with hematoxylin and eosin. The whole midsagittal area was evaluated under ×200 magnification to determine the pathological grade of inflammation using the following criteria: 0 = no lung abnormality; 1 = presence of inflammation involving <25% of the lung parenchyma; 2 = lesions involving 25–50% of the lung; and 3 = lesions involving >50% of the lung.

Immunohistochemistry was performed as follows. First, sections were deparafinized with xylene and dehydrated in a graded ethanol series. Next, the sections were treated with 0.3% hydrogen peroxide in methanol for 30 min at room temperature to block endogenous peroxidase activity, and subsequently autoclaved (121 °C, 5 min) to activate heat-mediated antigen retrieval. Following an incubation in normal serum to block nonspecific proteins, the sections were incubated with antibodies specific for neutrophil elastase (1:50; Santa Cruz, USA), anti-PD ligand 1 (PD-L1) (final 15 μg/ml; R&D Systems, USA), and club cell-specific protein (CCSP) (1:500; Santa Cruz) at 4 °C overnight. Next, the sections were incubated with an immunoperoxidase polymer reagent, Histofine Simple Stain Mouse MAX PO (Nichirei Bioscience, Japan), for 30 min, after which positive reactions were visualized with 3,3′-diaminobenzidine tetrahydrochloride (Nichirei Bioscience). We counted the number of CCSP-positive cells and total bronchiolar epithelial cells in 10 terminal bronchi per mouse.

For immunofluorescence, the sections were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Dojindo Laboratories) and

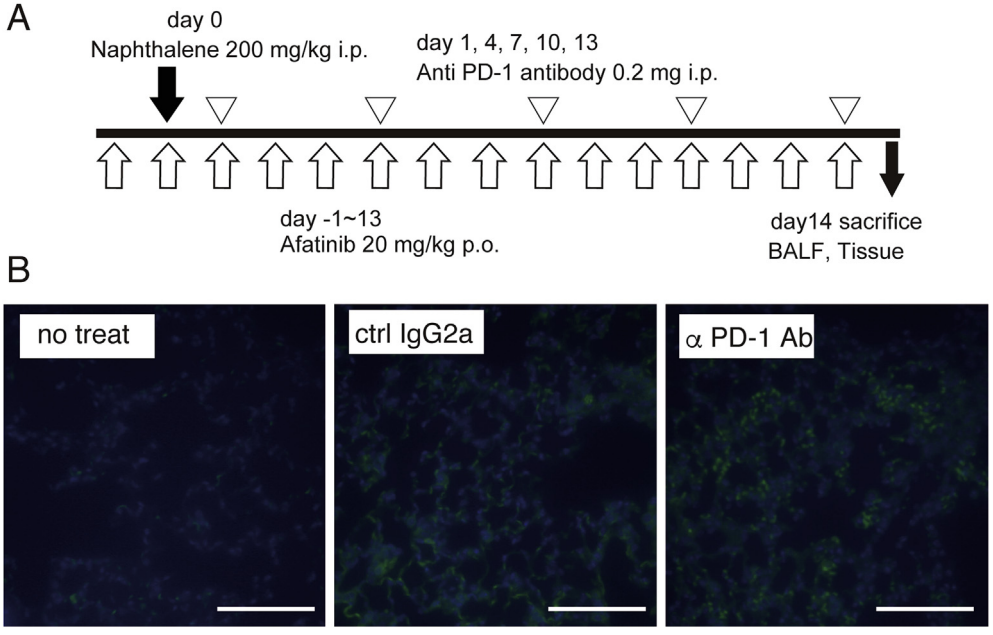

Fig. 1. Experimental scheme and detection of injected immunoglobulin in the lungs.
(A) Experimental scheme detailing the administration of naphthalene, afatinib, and PD-1-specific antibody. (B) Detection of intraperitoneally injected control rat IgG2a or rat anti-mouse PD-1 antibody in the lungs via immunohistochemistry with Alexa Fluor 488-conjugated anti-rat IgG and DAPI. Scale bars: 100 μm. p.o.: oral administration, i. p.: intraperitoneal administration, PD-1: programmed cell death-1, BALF: bronchoalveolar lavage fluid, DAPI: 4′,6-diamidino-2-phenylindole, Ab: antibody.

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