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Original Research

Indoleamine 2,3-dioxygenase 1 and programmed cell death-ligand 1 co-expression correlates with aggressive features in lung adenocarcinoma

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Abstract ***Background:*** Indoleamine 2,3-dioxygenase 1 (IDO1) is an immunosuppressive effector, and its expression is associated with prognosis in several cancer types. Here, we investigated the relationship between IDO1 expression in lung adenocarcinoma and patient prognosis and clinicopathological features, including programmed cell death-ligand 1 (PD-L1) expression.

Materials and methods: In this study, surgically resected primary lung adenocarcinoma specimens from 427 patients were evaluated for IDO1 and PD-L1 expression by immunohistochemistry, and lung adenocarcinoma cell lines were evaluated for IDO1 and PD-L1 protein expression by enzyme-linked immunosorbent assay and flow cytometry and for messenger RNA levels by real-time reverse-transcriptase polymerase chain reaction analysis.

Results: IDO1 was expressed in 260 patients (60.9%) at 1% cut-off and 63 patients (14.8%) at 50% cut-off. Tissues from 145 patients (34.0%) were positive for PD-L1 using the cut-off of 1%. Multivariate analysis showed that ≥1% IDO1 positivity was significantly associated with higher tumour grade, vascular invasion and PD-L1 expression. IDO1 and PD-L1 proteins were co-expressed in 123 patients (28.8%), and co-expressing tumours exhibited significantly

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more malignant traits than those positive for one or neither protein. In multivariate analysis, co-expression of IDO1 and PD-L1 was significantly associated with shorter disease-free survival and overall survival. Both proteins were upregulated in lung adenocarcinoma cell lines by treatment with interferon-γ and transforming growth factor-β.
Conclusion: These results suggest that IDO1 and PD-L1 co-expression may define an aggressive form of lung adenocarcinoma.

1. Introduction

Lung cancer is a major health burden worldwide and is associated with high mortality [1]. Recent preclinical and clinical studies have considerably increased our understanding of the molecular pathogenesis of lung cancer and have facilitated the development of improved treatment strategies.

Targeting of immune checkpoint factors, such as the programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) pathway, has emerged as a novel and promising therapeutic option [2]. Immune checkpoint inhibitors, such as the anti-PD-1 antibodies nivolumab and pembrolizumab and the anti-PD-L1 antibody atezolizumab, have shown survival benefits compared with conventional standard therapy in non-small-cell lung cancer (NSCLC) [3–5]. However, most patients who initially respond to these inhibitors acquire resistance, and several resistance mechanisms have been identified, including lack of tumour antigens or effective antigen presentation, impaired interferon-γ (IFN-γ) signalling, somatic Janus Kinase 1/2 mutations, impaired immune suppressive cells and/or immunoinhibitory cytokines, upregulation of other immune checkpoints and T-cell exhaustion [6–8]. Therefore, next generation immunotherapeutic drugs or combinations with cytotoxic chemotherapy and other molecularly targeted therapies should be explored to improve the response rate and to overcome resistance to immune checkpoint inhibitors.

Indoleamine 2,3-dioxygenase 1 (IDO1) catalyses the rate-limiting step in the kynurenine pathway that catabolizes tryptophan, an essential amino acid critical for cell survival, into a stable metabolite [9]. In the tumour microenvironment, IDO1 is expressed on antigen-presenting cells, such as macrophages, dendritic cells and tumour cells [9], whereas in normal settings, IDO1 is only expressed in tissues with large mucosal surface areas (lungs, gut and placenta) that experience chronic inflammation and lymphoid tissues [9,10]. IDO1 exerts its immunosuppressive effects in several ways, including induction of T cell dysfunction and apoptosis, promotion of naive T cell differentiation into regulatory T cells and impairment of natural killer cell function through the depletion of tryptophan and generation of kynurenine [11,12]. Aberrant expression of IDO1 has been shown to correlate with poor clinical outcome in breast, gastric, colorectal and ovarian cancers [13–16]. However, the clinical significance of IDO1 expression in lung adenocarcinoma has not been fully clarified. Furthermore, the association between IDO1 and immune checkpoint factors, such as PD-L1, remains unclear.

In this translational study, we investigated the association between IDO1 expression and clinicopathological factors and the prognostic value of IDO1 in patients with primary lung adenocarcinoma. We also evaluated the relationship between IDO1 and PD-L1 expression in these tumours. Finally, we examined IDO1 and PD-L1 expression and their modulation by cytokines in lung adenocarcinoma cell lines.

2. Materials and methods

2.1. Patients and samples

We performed a retrospective analysis of 427 patients who underwent surgical resection for primary lung adenocarcinoma between January 2003 and December 2012 at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. Patients with stage IV disease were excluded. Clinicopathological features, including age at surgery; sex; smoking history; tumour differentiation; pathological tumour, node and metastasis stage (seventh edition of the American Joint Committee on Cancer lung cancer staging system); pleural or lymphovascular invasion; histological subtype (World Health Organization Classification 2015); surgical procedure and epidermal growth factor receptor (*EGFR*) mutation status were recorded. The *EGFR* status of 250 specimens had previously been determined [17]. Clinical information and follow-up data were obtained from medical records. This study was approved by our Institutional Review Board (Kyushu University, IRB No. 29-318).

2.2. Immunohistochemical analysis

Formalin-fixed and paraffin-embedded (FFPE) tumour tissue sections were used for immunohistochemical