Programmed Death-Ligand 1 Expression Predicts Tyrosine Kinase Inhibitor Response and Better Prognosis in a Cohort of Patients With Epidermal Growth Factor Receptor Mutation-Positive Lung Adenocarcinoma

Cheng Lin,[1] Xiong Chen,[1] Meifang Li,[2] Jingnan Liu,[1] Xingfeng Qi,[3] Wenting Yang,[3] Hairong Zhang,[4] Zhongfu Cai,[5] Yun Dai,[6] Xuenong Ouyang[1]

Abstract

The oncogenic driver epidermal growth factor receptor (EGFR) mutations upregulate immune checkpoint proteins programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) in in vitro and in vivo models of non–small-cell lung cancer (NSCLC). Immunohistochemistry revealed that PD-L1 and PD-1 were positive in 53.6% and 32.1% of tumor specimens in a cohort of 56 patients with NSCLC carrying EGFR mutations, respectively. PD-L1 expression correlated with a better response to EGFR tyrosine kinase inhibitors and longer survival.

Background: The immune checkpoint proteins programmed death-1/ligand (PD-1/PD-L1) play a critical role in immune escape of tumor cells. In models of epidermal growth factor receptor (EGFR)-driven non–small-cell lung cancer (NSCLC), EGFR signal upregulates PD-1/PD-L1. However, data on the clinical significance of PD1/PD-L1 expression in patients with the subtype of NSCLC carrying EGFR mutations remain limited. **Materials and Methods:** Immunohistochemistry was performed to evaluate the expression of PD-1, PD-L1, and CD4+ and CD8+ tumor-infiltrating T lymphocytes (TILs). **Results:** In a cohort of 56 patients, PD-L1 and PD-1 was positive in 53.6% and 32.1% of tumor specimens, respectively. PD-L1$^+$ patients had a significantly greater disease-control rate ($P = .004$), in association with longer progression-free survival ($P = .001$) after EGFR-tyrosine kinase inhibitor (TKI) therapy and overall survival ($P = .004$), and no correlation between PD-1 positivity and clinical outcomes was observed. PD-L1 expression was not significantly associated with either clinicopathologic features or TILs. **Conclusions:** These findings suggest that this subtype of EGFR mutation-positive NSCLC is highly eligible for PD-1/PD-L1 immunotherapy. PD-L1 might represent a favorable biomarker candidate for the response to EGFR-TKIs and outcomes of these patients with NSCLC.

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C.L., X.C. and M.L. contributed equally to this work.

[1]Department of Oncology, Fuzhou General Hospital of Nanjing Military Command, Fuzong Clinical College of Fujian Medical University, Fuzhou, Fujian, China
[2]Department of Oncology, Fujian Provincial Cancer Hospital, Jinan District, Fuzhou, Fujian, China
[3]Department of Pathology, Fuzhou General Hospital of Nanjing Military Command, Fuzhou, Fujian, China
[4]Department of Epidemiology and Health Statistics, Fujian Medical University School of Public Health, Fuzhou, Fujian, China
[5]Department of Oncology, 180th Hospital of People's Liberation Army, Quanzhou, Fujian, China
[6]Division of Hematology and Oncology, Department of Medicine, Virginia Commonwealth University, Massey Cancer Center, Richmond, VA

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Address for correspondence: Xuenong Ouyang, MD, PhD, Department of Oncology, Fuzhou General Hospital of Nanjing Military Command, Fuzong Clinical College of Fujian Medical University, 156 Xierhuan Northern Road, Fuzhou, Fujian 350025, China
E-mail contact: oyxn@public.fz.fj.cn

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Introduction

Non–small-cell lung cancer (NSCLC), including squamous cell carcinoma and adenocarcinoma, accounts for ~80% of lung cancers, a leading cause of cancer death worldwide. Although squamous cell carcinoma used to be the most commonly diagnosed type of NSCLC, adenocarcinoma has become the most frequent histologic type of lung cancer in the past several decades.[1] Recent advances in the discovery of oncogenic driver gene mutations/translocation (eg, epidermal growth factor receptor [EGFR], echinoderm microtubule-associated like 4-anaplastic lymphoma kinase) and the development of tyrosine kinase inhibitor (TKI) therapies targeting such mutations have led to a new era in the treatment of NSCLC, which have substantially improved the survival of patients with these fatal diseases, lung adenocarcinoma in particular.[1] Activating EGFR mutations represent one of the most common genetic alterations in patients with lung adenocarcinoma, especially women, those with no previous history of smoking, and those of Asian descent (eg, ~50% of Asians vs. ~10% non-Asians).[2-4] Currently, the use of EGFR-TKIs has been virtually restricted to patients with EGFR mutation-positive adenocarcinoma. Thus, activating EGFR mutations have been widely accepted as an exclusive biomarker for EGFR-TKI therapy in standard care.[5,6] However, approximately 30% of patients harboring EGFR mutations do not respond to EGFR-TKIs (known as intrinsic resistance), and almost all initial responders eventually acquire drug-resistance within 10 to 16 months after EGFR-TKI treatment, from varied mechanisms,[7] including de novo exon 20 T790M mutation.[8] Thus, drug-resistance remains a major challenge with EGFR-TKI therapy for NSCLC, prompting efforts to predict and eventually overcome this impeding event to improve the efficacy of EGFR-TKIs.

Immune checkpoints refer to multiple inhibitory pathways that counteract certain crucial steps of T cell-mediated immunity to maintain self-tolerance and modulate the duration and amplitude of immune responses.[9] Recently, the understanding of several checkpoints that shut down the immune system as an immunosuppressive mechanism in tumors has evoked a paradigm shift in cancer treatment.[10] Immune checkpoints are initiated primarily through T cell coinhibitory receptors and their ligands, including cytotoxic T lymphocyte-associated protein (CTLA)4:B7.1 or B7.2, programmed death-1 (PD-1):programmed death-ligand 1 (PD-L1) (also known as B7-H1 and CD274) or PD-L2 (also known as B7-DC and CD273), among many others.[9] PD-1 (CD279), a receptor of the CD28 family, is expressed on activated $CD4^+$ and $CD8^+$ T cells.[11] PD-L1 is the ligand of PD-1, a pair of which plays a critical role in the immune checkpoints responsible for immune escape of tumor cells.[12] Recent studies have revealed that PD-L1 is widely expressed in various types of cancer, providing a foundation for the development of PD1/PD-L1 antibodies as cancer immunotherapy.[9] Current clinical trials have demonstrated very promising activity for anti-PD-1 (eg, BMS-936558/nivolumab) and anti-PD-L1 (eg, BMS-936559) monoclonal antibodies in the treatment of advanced cancer, including NSCLC.[13,14] The objective response rate has been significantly greater in patients with NSCLC expressing high levels of PD-L1, supporting the notion that PD-L1 serves as a biomarker for the selection of patients who might benefit from PD1/PD-L1 immunotherapy.[15]

PD-L1 has also been demonstrated to correlate with the poor prognosis of patients with several types of cancer, including esophageal cancer,[16] renal cell carcinoma,[17] hepatocellular carcinoma,[18] pancreatic cancer,[19] colorectal cancer,[20] gastric cancer,[21] and NSCLC.[22] Recently, it was shown that oncogenic EGFR mutations directly upregulate PD-L1 expression in NSCLC cell lines, and exposure to EGFR-TKIs (eg, gefitinib) leads to PD-L1 downregulation.[23] Moreover, the anti-PD-1 antibody significantly reduces tumor growth and prolongs the survival of animals with EGFR-driven adenocarcinoma, events associated with marked increases in binding of the anti-PD-1 antibody to PD-1-expressing $CD4^+$ and $CD8^+$ T cells and decreased levels of tumor-promoting cytokines.[23] These findings raise the possibility that patients with EGFR-driven NSCLC might be particularly susceptible to PD-1 blockade immunotherapy.[24] They also suggest a potential link between PD-L1/PD-1 expression and EGFR mutations in patients with NSCLC. Therefore, we sought to explore the effects of PD-L1 expression on the clinical outcomes, including the TKI response and overall survival (OS), in a specified cohort of patients with the EGFR mutation-positive subtype of lung adenocarcinoma and who received EGFR-TKI therapy. We found that PD-L1 expression will be positive in tumors of > 50% patients with EGFR mutation-positive cancer in this cohort, which significantly correlated with a greater disease control rate (DCR) and longer survival (progression-free survival [PFS] and OS) after treatment with EGFR-TKIs.

Materials and Methods

Patients

The patients were required to have been diagnosed with EGFR mutation-positive advanced lung adenocarcinoma and must have received EGFR-TKI therapy (ie, gefitinib or erlotinib). Of the 272 patients initially screened, 56 were eligible for the present study. The treatment after recurrence included radiotherapy for metastatic lesions in the bone and brain, chemotherapy, and regional therapy, and the best supportive care was given, as appropriate. Three patients refused to receive any additional treatment after disease progression for personal reasons. The ethical committee of Fuzhou General Hospital approved the present study.

Analysis of EGFR Mutations

EGFR gene mutations in exons 18, 19, 20, and/or 21 were examined in tumor samples obtained by biopsy or surgical resection, using the amplification refractory mutation system and the fluorescence polymerase chain reaction diagnostic kit, as described previously.[25,26] Of the 56 patients, 32 had EGFR gene mutations due to exon 19 deletion, 23 had an L858R substitution in exon 21, and 1 patient had a G719A/G719S/G719C mutation in exon 18; no mutations were found in exon 20.

Immunohistochemistry

The biopsy and surgical resection specimens were fixed in formalin and embedded in paraffin using standardized procedures. Deparaffinized sections (4 μm) were stained for PD-L1 or PD-1 using standardized immunohistochemistry (IHC) procedures. In brief, endogenous peroxidase activity was blocked by hydrogen