ORIGINAL ARTICLE

Genetic and Immune Profiles of Solid Predominant Lung Adenocarcinoma Reveal Potential Immunotherapeutic Strategies

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ABSTRACT

Introduction: Subtype classification of lung adenocarcinoma (LUAD) divides different survivals and therapeutic vulnerabilities; however, little is known about the disease's underlying molecular mechanism. This study sought to determine the genetic and immune profiles of histologic subtypes and identify the evidence for adjuvant immunotherapy.

Methods: We performed an integrated analysis of multidimensional data from a discovery set consisting of cohorts of The Cancer Genome Atlas and the Broad Institute data set from the LUAD public database and a validation set from the Guangdong Lung Cancer Institute. Immunohistochemical staining was carried out to determine the expression of the proteins programmed cell death 1 ligand (PD-L1) and CD8.

Results: Patients with solid predominant LUAD showed poor disease-free survival and a high frequency of relapse/metastasis compared with those with the nonsolid subtype of LUAD. The solid subtype tended to occur more frequently in those with a history of smoking. Solid predominant LUAD exclusively showed increased expression of PD-L1 and a high proportion of dual positive PD-L1– and tumor-infiltrating lymphocytes. Meanwhile, a notable increase in the tumor mutation burden and higher frequency of GC>TA transversions were specifically identified in tumors of the solid subtype. Furthermore, the solid subtype of tumor displayed an active cytotoxic immune signature and increased incidence of genetic mutations related to immunogenicity.

Conclusion: Solid predominant LUAD was identified as a subtype with adaptive immune resistance, higher cytotoxic activity, and enhanced immunogenicity. These findings suggest that patients with solid predominant LUAD may represent a potential selective group that will benefit from adjuvant programmed cell death 1 blockade immunotherapy.

Keywords: Histologic subtype; Solid predominant; PD-1/PD-L1; Adjuvant immunotherapy; Lung adenocarcinoma

Introduction

For patients with stage II to IIIA NSCLC, adjuvant chemotherapy is recommended as the standard of care. However, adjuvant chemotherapy has been reported to result in only a 4% improvement in the 5-year survival of these patients.[1] Meanwhile, this benefit was found to be partly counteracted by the toxicity of the treatment and decreased patient quality of life. Moreover, the phase III study Eastern Cooperative Oncology Group 1505 recently demonstrated no improvement in

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disease-free survival (DFS) and overall survival (OS) with the addition of bevacizumab to chemotherapy in completely resected stage IB to IIIA NSCLC.[2] However, these unsatisfactory results may be partly due to the limitation of all-comer strategies for adjuvant therapy. Therefore, it is necessary to distinguish the population that would benefit most from the therapy from the all-comer population in the adjuvant setting.

Immunotherapy has been recognized as an effective approach against minimal tumor burden and small deposits of tumor cells (TCs) that are accessible by the circulating immune cells (ICs). Furthermore, immunotherapy seeks to establish a repertoire of memory cells that can prevent recurrences.[3,4] Although early vaccine trials failed to show improvements in DFS and OS postoperatively,[5,6] immune checkpoint inhibitors have broadened the scope of adjuvant immunotherapy.[7,8] Recent studies have shown promising benefits of programmed cell death 1 (PD-1) blockade for the treatment of advanced NSCLC. These studies illustrated a group of predictive biomarkers for the efficacy of PD-1 inhibitors, including expression of the PD-1 ligand (PD-L1),[9,10] tumor mutational load,[11–13] presence of DNA mismatch repair deficiency,[14] and the intensity of tumor-infiltrating lymphocytes (TILs).[15] These factors are functionally interrelated and often found coordinately in individual tumor specimens.[16] Hence, this raises the question of whether some specific subgroups with two or more of these predictive factors may theoretically benefit the most from PD-1 blockade immunotherapy.

Furthermore, it has been recently proposed that the histologic pattern of lung adenocarcinoma (LUAD) influences the efficacy of adjuvant chemoradiotherapy.[17] Different prognoses and distinct driver mutation profiles[18,19] were identified among the histologic subtypes. In particular, solid predominant tumors associated with increased TIL counts and PD-L1 expression, which have been reported as factors favorable to PD-1 blockade.[18,20] On the basis of the aforementioned studies, we propose that the histologic subtype of LUAD may be a potential predictive factor for the efficacy of adjuvant immunotherapy. Here, we describe an integrative analysis to define the potential predictive value of the histologic subtype of LUAD for adjuvant PD-1 blockade immunotherapy.

Materials and Methods

Patient Cohort

The Cancer Genome Atlas (TCGA) and Broad Institute cohorts were defined as the discovery set. Most of the patients enrolled in the two cohorts had early-stage LUADs. A total of 194 patients with LUADs with confirmed histologic subtypes were included in the TCGA cohort, for which detailed information about the mRNA and protein expression profiles as well as gene mutation data was available. The Broad Institute cohort was obtained from the LUAD public database and contained information on 146 LUAD and matched normal tissues, with detailed information about the histologic subtypes and mutation profiles. The validation set comprised 270 patients with LUAD from the Guangdong Lung Cancer Institute (GLCI) of Guangdong General Hospital with definite histologic subtypes. Among them, 85 patients had undergone whole genome sequencing (WGS) and 194 were found by immunohistochemistry (IHC) analysis to have tumors containing PD-L1 protein, with nine patients overlapped in the whole population. In addition, 214 patients with stage I to IIIA LUADs who had completed a follow-up of at least 4 years were selected for survival analysis.

A flow diagram was provided to illustrate the study design (Supplementary Fig. 1). This study was approved by the institutional review board of GLCI of Guangdong General Hospital, and all patients provided specimens with written informed consent. Key variables, including molecular and clinicopathologic characteristics of LUAD samples in the discovery and validation cohorts, are provided in Supplementary Tables 1 and 2.

Histologic Evaluation

All available hematoxylin and eosin–stained tumor slides (a mean of five slides per patient) were reviewed by two pathologists blinded to the patient clinical outcomes (Y. F. L. and L. X. Y.). Any disagreements between the pathologists during determination of the predominant subtype were resolved through consensus by using a multiheaded microscope. Tumors were classified according to the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society classification as adenocarcinoma in situ, minimally invasive adenocarcinoma, and invasive adenocarcinoma. The percentage of each histologic pattern was recorded in 5% increments. Invasive adenocarcinomas with mixed histologic patterns were classified into one of following subtypes based on the predominant growth pattern present in the tumor: lepidic, acinar, papillary, micropapillary, solid, and invasive mucinous.[21,22] The tumors were grouped by architectural grading as low (lepidic predominant), intermediate (papillary and acinar predominant), or high (micropapillary predominant, solid predominant, and invasive mucinous adenocarcinoma).[19,23] The histologic subtype of cases in the TCGA (http://www.nature.com/nature/journal/v511/n7511/full/nature13385.html#supplementary-information)[24] and Broad Institute (http://www.cell.com/cell/fulltext/S0092-8674(12)01061-6?_returnURL=http%3A%2F%2Flinkinghub.elsevier