Potential Predictive Value of TP53 and KRAS Mutation Status for Response to PD-1 Blockade Immunotherapy in Lung Adenocarcinoma

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Abstract

Purpose: Although clinical studies have shown promise for targeting programmed cell death protein-1 (PD-1) and ligand (PD-L1) signaling in non–small cell lung cancer (NSCLC), the factors that predict which subtype patients will be responsive to checkpoint blockade are not fully understood.

Experimental Design: We performed an integrated analysis on the multiple-dimensional data types including genomic, transcriptomic, proteomic, and clinical data from cohorts of lung adenocarcinoma public (discovery set) and internal (validation set) database and immunotherapeutic patients. Gene set enrichment analysis (GSEA) was used to determine potentially relevant gene expression signatures between specific subgroups.

Results: We observed that TP53 mutation significantly increased expression of immune checkpoints and activated T-effector and interferon-γ signature. More importantly, the TP53/KRAS comutated subgroup manifested exclusive increased expression of PD-L1 and a highest proportion of PD-L1+/CD8A+. Meanwhile, TP53- or KRAS-mutated tumors showed prominently increased mutation burden and specifically enriched in the transversion-high (TH) cohort. Further analysis focused on the potential molecular mechanism revealed that TP53 or KRAS mutation altered a group of genes involved in cell-cycle regulating, DNA replication and damage repair. Finally, immunotherapeutic analysis from public clinical trial and prospective observation in our center were further confirmed that TP53 or KRAS mutation patients, especially those with co-occurring TP53/KRAS mutations, showed remarkable clinical benefit to PD-1 inhibitors.

Conclusions: This work provides evidence that TP53 and KRAS mutation in lung adenocarcinoma may be served as a pair of potential predictive factors in guiding anti–PD-1/PD-L1 immunotherapy. Clin Cancer Res; 23(12); 3012–24. ©2016 AACR.

Introduction

Recent clinical trials with anti-programmed cell death 1 (PD-1) and its ligand PD-L1 signaling in non–small cell lung cancer (NSCLC), the factors that predict which subtype patients will be drug sensitive or resistant are not fully understood. Therefore, it has become a primary priority to identify the biomarkers that determine the responsiveness to checkpoint blockade, and to develop strategies that could potentially increase the patient response rates. Encouragingly, recent studies had demonstrated that tumor mutational load (4–6), DNA mismatch repair (MMR) deficiency (7), the intensity of CD8+ T cell infiltrates (8, 9) and intratumoral PD-L1 expression (10, 11) have each been proposed as distinct biomarkers of response to anti–PD-1/PD-L1 therapies. Meanwhile, these factors are functionally interrelated and are often found coordinately in individual tumor specimens (12). This raises the question of whether there exist some other variables simultaneously affect two or more of these above factors so as to provide stronger predictive value for therapeutic outcomes.

The identification of subsets of lung adenocarcinoma with oncogenic drivers has transformed the treatment of NSCLC, particularly for patients whose tumors harbor activating mutations in EGFR. However, the goal of developing specific therapeutic strategies for those bearing activating mutations in KRAS has thus far proven elusive. Meanwhile, mutations in tumor suppressor genes TP53 and STK11 are also common in lung adenocarcinoma and frequently co-occur with KRAS mutations (13–15). Given that activation of specific oncogenic pathways can have broad effects on gene expression, it is reasonable to imagine that the genetic make-up of cancer cells could have major effects on the immune tumor microenvironment (TME), by driving specific immune-related pathways. This could be through induction of immune checkpoints, secretion...
**Translational Relevance**

Programmed cell death ligand 1 (PD-L1) expression, tumor mutational load, and the intensity of CD8+ T-cell infiltration have recently been proposed as predictive biomarkers for response to PD-1 blockade immunotherapy. However, there are still many treatment responses beyond the explanation of these factors. It is increased need for more effective biomarkers for PD-1 blockade. We demonstrated TP53 and KRAS mutation had remarkable effects on increasing PD-L1 expression, facilitating T-cell infiltration and augmenting tumor immunogenicity. More important, we confirmed that patients with TP53 and/or KRAS mutation showed sensitivity to PD-1 blockade. These findings represent the first demonstration of potential predictive value of TP53 and KRAS mutation for response to PD-1 blockade immunotherapy in lung adenocarcinoma.

**Immunotherapeutic patients**

Clinical and mutation data for 34 NSCLC [29 adenocarcinoma (ADC)] patients were retrieved from chioPortal (http://www.chioportal.org/study/do/cancer_study_id=luid_rnscrc_2015). All patients treated with pembrolizumab (anti–PD-1) from 2012 to 2013 followed the protocol NCT01295827 (KEYNOTE-001). Objective response to pembrolizumab was assessed by investigator-assessed immune-related response criteria (irRC) by a study radiologist (5).

Another group consisted of 20 NSCLC (15 ADC) patients were collected prospectively in the GLCI from August 2015 to August 2016. Eleven of them were treated with pembrolizumab and nine patients were treated with nivolumab. Tumor specimens were obtained for Sanger sequencing and IHC analysis. This study was approved by the Institutional Review Board of GLCI of GGH, and all patients provided written informed consent. Clinicopathologic and molecular information are provided in Supplementary Table S2.

**Materials and Methods**

**Clinical cohorts**

The Cancer Genome Atlas (TCGA), GSE72094 and Broad cohorts were retrieved from online data repository. A total of 462 patients were included in the TCGA cohort with mRNA expression profiling and gene mutation data. The GSE72094 cohort recruited 442 patients with detailed mRNA expression data and EGFR/KRAS/TP53/STK11 sanger sequencing analysis (19). The Broad cohort contained 183 lung adenocarcinomas and matched normal tissues with detail information about mutation load and mutation spectrum (20). Most of the patients enrolled in the three cohorts were early-stage lung adenocarcinomas. A total of 85 lung adenocarcinomas from the Guangdong Lung Cancer Institute (GLCI), Guangdong General Hospital (GGH) were underwent whole genome sequencing (WGS). Key variables including demographic and clinical information are provided in Supplementary Table S1.

**mRNA expression profiling and reverse phase protein array (RPPA) analysis**

For lung adenocarcinomas included in the TCGA cohort, experimental procedures regarding RNA extraction from tumors, mRNA library preparation, sequencing (on the Illumina HiSeq platform), quality control, and subsequent data processing for quantification of gene expression have been previously reported (21). Gene expression data for the GSE72094 lung adenocarcinomas have been deposited in the GEO repository (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72094). Tumor from the GSE72094 cohort were profiled using a custom Affymetrix GeneChip. The gene expression cutoff value was chosen as median over the entire dataset (TCGA and GSE72094) to ensure all analyses of each gene were based on the same cutoff value (22, 23). Proteomic analysis was based on RPPA from the TCGA database. The RPPA methodology and data analysis pipeline have been previously described (21). For TCGA, level 3 data were downloaded directly from the TCGA portal and utilized in subsequent analyses.

**Mutation data analysis**

For the discovery set, somatic mutation data (level 2) of the 462 lung adenocarcinomas were retrieved from the TCGA data portal (https://gdc.cancer.gov/). To assess the mutational load, the number of mutated genes carrying at least one nonsynonymous mutation in the coding region was computed for each tumor. Somatic mutation data of 183 lung adenocarcinomas in Broad cohort was retrieved from chioPortal (http://www.chioportal.org/study/do/cancer_study_id=luid_broad). Somatic substitutions and covered bases within their trinucleotide sequence context were analyzed to characterize the mutation spectrum of 183 lung adenocarcinoma. Mutation spectrum for each sample was calculated as the percentage of each of six possible single nucleotide changes (AT>CG, AT>GC, AT>TA, GC>AT, GC>CG, GC>TA) among all single-nucleotide substitutions. The most frequent mutation signatures were C→T transitions and C→A transversions.

For the validation set (GLCI), we conducted whole-exome sequencing of DNA from tumors and matched normal blood from 85 lung adenocarcinoma patients. Enriched exome libraries were sequenced on the HiSeq 2000 platform (Illumina) to >100× coverage. Alignment, base-quality score recalibration and duplicate-read removal were performed, germline variants were
excluded, mutations annotated and indels evaluated as previously described (4, 5, 24). Mutations between clinical groups were compared using the Mann–Whitney test.

Gene set enrichment analysis (GSEA)
For GSEA (25), the javaGSEA Desktop Application was downloaded from http://software.broadinstitute.org/gsea/index.jsp. GSEA was used to associate the gene signature with the loaded from http://software.broadinstitute.org/gsea/index.jsp. Gene set enrichment analysis (GSEA) was used to examine the gene expression signature with the loaded from http://software.broadinstitute.org/gsea/index.jsp. The gene set enrichment score (NES) is the primary statistic for examining gene set enrichment results. The normalized enrichment score (NES) is the primary statistic for examining gene set enrichment results. The normalized P value estimates the statistical significance of the enrichment score. A gene set with nominal P ≤ 0.05 was considered to be significantly enriched in genes.

Immunohistochemistry
Tumor sections were assessed immunohistochemically using PD-L1 (clone: SP142, Spring Bioscience, Inc) and CD8 (clone: C8/C21, Gen Tech (Shanghai) Co. Ltd). The IHC-stained tissue sections were scored separately by two pathologists blinded to the clinical parameters.

Results
Correlation between TP53 and KRAS mutation and PD-L1 expression in lung adenocarcinoma
To investigate the correlation between common mutations (TP53, KRAS, EGFR, and STK11) and immune checkpoint status in lung adenocarcinoma, we thus initially interrogated RNA sequencing (RNA-Seq) expression data from a repository database including 462 lung adenocarcinomas from The Cancer Genome Atlas (TCGA) and 442 lung adenocarcinomas from GEO repository (GSE72094). Both the TCGA and GEO databases showed significantly increased PD-L1 mRNA expression in the TP53 mutation subgroup than in other gene mutation. Specifically, the TP53 and KRAS comutated group manifested prominent higher PD-L1 expression than other comutation types (Fig. 1A).

We next sought to explore the impact of TP53 and KRAS mutation on PD-L1 expression in both PD-L1 mRNA expression profiling and RPPA analysis based on the TCGA database. The results demonstrated that it was TP53 mutation but not KRAS mutation that boosted PD-L1 expression (Supplementary Fig. S1A and S1B). Significantly, those with co-occurring mutations in TP53 and KRAS revealed the highest PD-L1 expression (both mRNA and protein level) than single gene mutation or wild-type tumors, indicating potential synergistic effect on activating PD-L1 expression (Fig. 1B). To confirm the association between TP53/KRAS mutation and PD-L1 expression as repository data demonstrated, we detected 93 lung adenocarcinoma surgical specimens using an IHC analysis (Fig. 1C; Supplementary Fig. S1C and Table S3) and immunostaining shows TP53/KRAS comutated specimens the strongest staining for the PD-L1 protein (Fig. 1D).

Next, we further analyzed the association between TP53 or KRAS mutation and other non–PD-L1 immune checkpoints. A heatmap depicted the expression level of key immune checkpoints to three groups (TP53, KRAS, and TP53/KRAS; Fig. 1E). The results displayed remarkable increased expression of most checkpoints in the TP53 mutation group while decreased expression in the KRAS mutation group. More interestingly, the TP53/KRAS comutated subgroup manifested exclusive increased expression of PD-L1; however, it showed decreased expression of some other non–PD-L1 immune inhibitory checkpoints, such as Lympohocyte Activating 3 (LAG3) and V-Set Domain Containing T Cell Activation Inhibitor 1 (VTCN1; ref. 27), implying a potential candidate population for anti–PD-L1/PD-L1 immunotherapy (Fig. 1F).

TP53 mutation facilitates CD8+ T-cell infiltration and activates T-effector and interferon-γ (IFNγ) associated gene signature
The presence of tumor-infiltrating lymphocytes (TIL) is an important biomarker for predicting responses to PD-L1 blockade therapy. We continue to analyze the correlation between these above common mutations and CD8+ TIL contents in lung adenocarcinoma based on the TCGA database. Our results revealed significantly increased expression of CD8A in TP53 mutation and TP53/KRAS comutated than other groups (Fig. 2A). It has been proposed that four different types of immune tumor microenvironments (TME) exist based on the presence or absence of TIL and PD-L1 expression. To further explore whether TP53 or KRAS mutation would influence the TME, we analyzed the correlation between TP53 or KRAS mutation and TME immune types classified based on PD-L1 and CD8A expression as previously described (28, 29). Positive PD-L1 and CD8A were defined as above-median expression. We identified that the TP53 mutation group displayed a higher proportion of dual positive PD-L1 and CD8A (PD-L1+ and CD8A+) than the TP53 wild-type group, while there was no difference between KRAS mutation and wild-type (Fig. 2B and C),
suggested an adaptive immune resistance TME existed in the TP53 mutation population. More importantly, the TP53/KRAS comutated subgroup showed the highest proportion of PD-L1+/CD8A+ than the TP53 or KRAS single mutation and wild-type group (Fig. 2D). These observations were further confirmed by our IHC analysis that TP53/KRAS comutated patients manifested a strong staining of PD-L1 and high intensity of CD8+ TILs (Fig. 2E).

Given that TP53 mutation had effects on the TME in lung adenocarcinoma, we subsequently sought to assess the relationship between TP53 mutation and T-effector and IFN-g-associated gene signature, which have previously been associated with activated T cells, immune cytolytic activity, and IFN-g release (30, 31). An integrated heatmap depicting expression levels of T-effector and IFN-g-associated genes in tumors with indicated gene mutation. Scale bar, 200 μm. E, Heatmap representation of relative mRNA expression levels of selected immune inhibitory checkpoints. F, Quantitative analysis of two typical inhibitory checkpoints (LAG3 and VTCN1) on the base of TP53 and KRAS mutation status. Mut, mutation; wt, wild-type; ***, P < 0.001; **, P < 0.01; *, P < 0.05.

TP53 and KRAS mutation shows increased mutation burden and distinct mutation spectrum

Recent studies have highlighted the relevance of tumor mutational loads and response to PD-1 blockade (5). We next speculate whether there are some common mutations in lung adenocarcinoma that affect the whole tumor mutational profile and change the tumor antigenicity. We first analyzed the TCGA and Broad databases as discovery set. The TCGA analysis showed significantly increased mutational loads in the TP53 mutation group (median, 325), followed by KRAS (median, 179) and STK11 (median, 132) mutation, EGFR (median, 60) mutation tumor had the lowest mutational loads. Meanwhile, the TP53/KRAS comutated subgroup showed significantly higher mutational loads (median, 358) than other comutated subgroup (Fig. 3A). We then tested these findings using another dataset (Broad), which consisted of 183 lung adenocarcinomas with detailed somatic mutation data, and confirmed that TP53 and KRAS mutation and the TP53/KRAS comutated group had higher mutational loads than other groups (Fig. 3A). To further verify these findings, a total of 85 lung adenocarcinomas from GLCI detected by whole genome sequencing were defined as the validation set. GLCI data manifested the
similar results with the discovery set that TP53 mutation and the TP53/KRAS comutated group had higher mutational loads than other groups (Fig. 3B). It is well known that tobacco exposure was responsible for much of the mutagenesis in NSCLC. Multivariate linear regression analysis of mutation count in patients stratified by smoking status manifested that TP53 mutation was an independent factor responsible for increased mutation burden regardless of smoking status, while KRAS mutation showed increased mutation burden only in nonsmokers (Supplementary Table S4).

Previous studies have established the notion that somatic mutations are primarily GC > TA transversions (32). We next investigated whether these above common mutations could affect tumor mutation spectrum by using a TCGA cohort. Transversion-high (TH) and transversion-low (TL) was based on smoking history and GC > AT, GC > TA frequency as previously described (5, 21). We can identify KRAS mutations were significantly enriched in the TH cohort, while EGFR mutations were significantly enriched in the TL group (Supplementary Fig. S2). Consistent with TCGA results, the Broad dataset showed a high rate of transversion/transition (Tv/Ti) in KRAS mutation and the TP53/KRAS comutated group while the lowest rate Tv/Ti in EGFR mutation (Fig. 3C). Notably, TP53 and KRAS mutation was significantly correlated with high somatic mutations, high rate of Tv/Ti and C > A transversion and high smoking index (pack-years; Fig. 3D).
Impact of TP53 and KRAS mutation on the cell cycle, DNA replication, and damage repair–related genes

We sought to determine whether alterations in DNA replication and damage repair–related genes resulted from TP53 or KRAS mutation could account for differential mutation burden and mutation spectrum. GSEA reveals prominent enrichment of signatures relating to cell cycle, DNA replication, and DNA repair in both the TP53 and KRAS mutation groups. However, there were distinct differences between these two groups. TP53 mutation predominantly led to acceleration of cell-cycle and DNA replication, which potentially increased mutation probability, as unrepaired DNA damages that do not kill the cell by blocking replication would tend to cause replication errors and thus mutation.

KRAS mutation manifested various defects of DNA repair including MMR, nucleotide excision repair (NER), and base excision repair (BER) that greatly enhanced point mutation (Fig. 4A).

Recent studies showed that POLE mutation is associated with disruption of the exonuclease activity required for DNA proofreading and results in a high mutational burden or an “ultra-mutator” phenotype (33, 34). We identified significantly increased mutation frequencies of POLE in the TP53 mutation group (P = 0.002) while decreased mutation frequencies of POLE in the EGFR and STK11 mutation groups compare with their corresponding wild-type group, indicating TP53 mutation tends to cause DNA replication errors (Fig. 4B).

We next determined the correlation between these common mutations and DNA damage repair–related genes. DNA double-strand breaks (DSB) elicit that DNA damage response largely relies on the activity of ataxia telangiectasia mutated (ATM), which have been found to be mutated in human disorders associated with genome instability (35, 36). The results had revealed that a high frequency of ATM mutation was found predominantly in the KRAS and STK11 mutation groups, and ATM protein analysis further confirmed that waning expression of ATM protein was specifically found in the KRAS and STK11 mutation groups (Fig. 4B and C).

MMR-deficient tumors were recently shown susceptibility to checkpoint blockade immunotherapy (7). Our former GSEA identified that KRAS mutation was negatively correlated with MMR-related gene expression, and we next verified whether KRAS or other genes mutation affected the mutation status and protein expression of MMR-related genes. Four primary MMR-related genes, including MSH2, MSH6, MLH1, and PMS2, were coanalyzed. Consistent with GSEA, high mutation frequency of MMR-related genes was exclusively identified in the KRAS mutation group. Furthermore, the protein of MSH2 and MSH6 was significantly decreased in tumors with KRAS mutation; however, it was
increased in tumors with TP53 mutation, suggesting that KRAS mutation might be a potential driver agent to induce MMR deficiency and in consequence produce more neoantigens (Fig. 4B and C).

Patients with TP53 or KRAS mutation, especially co-occurring TP53/KRAS mutations, show favorable clinical benefit to anti–PD-1 treatment

TP53 and KRAS mutation showed remarkable effects on regulating PD-L1 expression, facilitating T-cell infiltration and augmenting tumor immunogenicity. We presumed that patients with these two mutations probably had increased sensitivity to PD-1 blockade immunotherapy. In support of this hypothesis, publicly available trial data (MSKCC, KEYNOTE-001) were reanalyzed. A total of 34 advanced NSCLC (29 ADC) patients were prescribed pembrolizumab from 2012 to 2013 following the NCT01295827 protocol. All tumor tissues underwent whole-exome sequencing. We observed significantly increased nonsynonymous mutation and candidate neoantigen burden in the TP53 or KRAS mutation group compared with the wild-type.
Figure 5.

The correlation between TP53/KRAS mutation and clinical response to PD-1 blockade. Comparison of nonsynonymous mutation (A) and candidate neoantigen (B) burden in TP53 or KRAS mutation and wild-type group. C, Proportion representation of transversion dominant mutation in the indicated group based on TP53 or KRAS mutation. D and E, Kaplan-Meier survival curves estimates of PFS compared TP53 or KRAS mutation with the wild-type group in patients treated with pembrolizumab. F, Proportional representation of clinical benefit of pembrolizumab in the indicated group based on TP53 or KRAS mutation. G, Individual PFS of 34 NSCLC patients coupled with their mutational status of TP53 and KRAS, pathology, PD-L1 expression, mutation burden, mutation spectrum and clinical benefit.

TP53 and KRAS Mutation Predicts Response to PD-1 Blockade
Discussion

Although the expression of PD-L1 on the surface of tumor cells, as measured by IHC, is recommended as a predictive factor to identify patients who would benefit from PD-1 blockade, not all PD-L1–positive patients respond well (10, 37). The underlying biology of such limitations has not been clearly understood until recent studies, which showed that the presence of TILs and mutational burden correlate with T-effector signature and immunogenic features that supported the response to anti–PD-1/PD-L1 therapy (5, 8, 12, 38, 39). Here, we first identified a group of oncogenic driver (EGFR and KRAS) and tumor suppressor (TP53 and STK11) mutations of lung adenocarcinoma that distinctively affected immune checkpoints expression, T-cell infiltration, and tumor immunogenicity. Specifically, our findings revealed that TP53 mutation remarkably increased PD-L1 gene expression.
Figure 6.
Antitumor activity and biomarkers analysis of PD-1 blockade in patients with NSCLC. A, Best tumor burden change from baseline in target lesions in 20 NSCLC (15 ADC) patients who received nivolumab or pembrolizumab. The presence of mutation genes in each patient was indicated. B, Time to progression and duration of response in individual patients, as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. C, PET/CT scan shows typical imaging alternation in a patient after 4 cycles of pembrolizumab treatment. D, Biomarker analysis of TP53 and KRAS mutation status and protein expression of PD-L1, CD8, MSH2, MSH6, MLH1, and PMS2 detected by DNA sequencing and IHC.
expression and facilitated CD8+ T-cell infiltration, and accompanied with a higher proportion of dual positive PD-L1 and CD8A than other mutation groups. Furthermore, TP53-mutated tumors showed prominently increased somatic mutation burden and specifically enriched in the TH subset. Previous studies have classified the TME into four groups on the basis of PD-L1 expression and TIL recruitment. These include type I (PD-L1 positive with TILs driving adaptive immune resistance), type II (PD-L1 negative with no TIL indicating immune ignorance), type III (PD-L1 positive with no TIL indicating intrinsic induction), and type IV (PD-L1 negative with TIL indicating the role of other suppressors in promoting immune tolerance; refs. 28, 29). Significantly, type I is largely thought to be associated with a high mutational burden, PD-L1 amplification, and oncogenic viral infection, which defines a subtype sensitivity to PD-1 blockade (29). These notions, to some extent, support our findings that TP53 mutation represents a state of adaptive immune resistance and a high immunogenicity, which contributes to a probable sensitivity to PD-1 blockade (40). Nevertheless, we could also discover a fact that TP53 mutation equally enhanced some other non-PD-L1 immune-inhibitory checkpoints expression, such as LAG3 and VTCN1, which might serve as potential primary resistance to TP53 mutation patients treated with anti-PD-1/PD-L1 (41).

Recent studies based on a phase III clinical trial have identified that patients who harbored an EGFR mutation displayed unfavorable response to PD-1 blockade than those with a wild-type EGFR (42–44). This may be the first finding in which driver mutation of NSCLC was involved in altering sensitivity to immunotherapy. The most likely explanation is that patients with EGFR mutation were prone to produce a weak immunogenic tumor and an immunosuppressive TME. These perspectives were also confirmed in our study that EGFR mutation showed the lowest mutation burden and lowest rate of TV/Ti than other mutations. Besides, EGFR mutation did not increase the expression of PD-L1, like others reported, but with a relatively lower expression than TP53 and KRAS mutation (45), which further supported their hypersensitivity to PD-1 blockade. KRAS mutation was the second important oncogenic driver mutation in lung adenocarcinoma. The development of more effective treatment strategies for patients with KRAS mutation is hampered by the biologic and phenotypic heterogeneity of KRAS-mutant tumors. More recently, some studies suggested that patients with activating mutations in KRAS may probably benefit from PD-1 blockade, but the underlying mechanisms remained elusive and most of the researchers attributed this predilection to the association between smoking and the presence of KRAS mutations (5, 42). In this study, we uncovered potential mechanisms that account for this correlation. We discovered a significant increase of mutation load in KRAS-mutant tumors. Particularly, a predominant higher proportion of TV/Ti was also found in this subgroup. Furthermore, we observed that KRAS mutations defected DNA repair, especially in MMR, which supported the notion that MMR deficiency acted as a favorable agent for PD-1 blockade (7).

It is well known that smoking-related lung cancers were characterized by greater mutation burden, higher rate of transversion, and more frequent KRAS mutation than that occurred in never smokers (21, 32, 46, 47). More recently, studies have demonstrated the association of PD-L1 expression with significant smoking history (48). In our study, we discovered that TP53 mutation, especially TP53/KRAS comutation, showed increased PD-L1 expression and augmented tumor immunogenicity. To confirm whether these correlations are more related to tobacco exposure, a multivariate linear regression analysis of mutation count and PD-L1 expression stratified by smoking status was performed. We demonstrated that TP53 mutation was responsible for increased mutation burden and PD-L1 expression independent of smoking status (Supplementary Tables S4 and S5). Recent studies based on subgroup analysis demonstrated those with a history of current or ever smoking showed much better benefits of PD-1 blockade than non-smokers. So we can imagine current or ever-smoker patients with TP53 and/or KRAS mutation may be the optimal population for PD-1 blockade immunotherapy.

Co-occurring mutations in TP53 and KRAS have recently been defined as a specific cluster associated with activation of antitumor immunity and immune tolerance/escape (49). Interestingly, our study identified TP53 and KRAS comutant tumors manifested exclusive increased expression of PD-L1 and a highest proportion of PD-L1+/CD8A+ than TP53 or KRAS single mutation. Meanwhile, TP53/KRAS dual mutation showed predominant increased mutation burden and enriched in the TH subset. Consistent with these preclinical predictions, the clinical analysis on the base of MSKCC and our center database had further confirmed that those with co-occurring mutations in TP53 and KRAS showed remarkable clinical benefit from pembrolizumab. These results implicated a possibility that TP53 and KRAS mutation played a role with synergistic and complementary in regulating immune biomarkers, which gave rise to a responsive TME with adaptive immune resistance and high immunogenicity. However, these findings were established in a relatively small cohort and even fewer patients with TP53 and KRAS comutation. Based on the preliminary evidence, a prospective study with a larger sample size of TP53/KRAS mutation and PD-L1 expression for response to PD-1 blockade is warranted in the future.

Taken together, the results of this study provided an insight into immune regulation driving by some common mutations of lung adenocarcinoma. We discovered a prominent significance of TP53 and KRAS mutation in boosting PD-L1 expression, facilitating T-cell infiltration, and augmenting tumor immunogenicity. This work provided evidence that TP53 and KRAS mutation in lung adenocarcinoma might be served as a pair of potential predictive factors in guiding PD-1 blockade immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: Z.-Y. Dong, Y.-L. Wu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Z.-Y. Dong, W.-Z. Zhong, X.-C. Zhang, Y.-L. Wu

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