Co-mutations in tumor immune microenvironment and immunotherapy

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Tumor microenvironment mainly includes fibroblasts, macrophages, endothelial cells, and immune cells. The heterogeneity of tumor immune microenvironment (TIME) affects the response of different patients to immunotherapy. Cancer immunotherapy using immune checkpoint inhibitors (ICIs), which can be achieved by antibodies blocking the programmed cell death 1 (PD-1)/programmed death ligand-1 (PD-L1) or the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) pathway alone or in combination, prompts a new paradigm shift in oncology. However, the response rates to the ICIs in cancer patients are relatively low and the results of the reported biomarkers for predicting the treatment efficacy are conflicting. Therefore, novel and reliable biomarkers are urgently needed to monitor tumor-specific immune responses, avoid immune-related adverse events, and improve clinical efficacy.

Genomic alterations influence the tumor biology, microenvironment, and treatment susceptibility of lung cancer. Co-occurring genomic alterations/co-mutations have been reported as core determinants of the molecular and clinical heterogeneity of oncogene-driven lung cancer subgroups. Herein, we focus on the association of co-mutations with TIME and immunotherapy, especially in lung cancer [Supplementary Figure 1; http://links.lww.com/CMJ/A568].

Co-mutations Within Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)-mutations Associated with TIME and Immunotherapy

KRAS mutations, accounting for 25% to 30% of lung adenocarcinoma (LUAC), are the most common oncogenic drivers in non-small-cell lung cancer (NSCLC). Skoulidis et al[1] identified three subgroups of KRAS-mutant LUAC: the KL subgroup, co-mutations in KRAS and serine/threonine kinase 11 (STK11)/liver kinase B1 (LKB1); the KP subgroup, co-mutations in KRAS and tumor protein p53 (TP53); the KC subgroup, inactivation of cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) and suppressed neurokinin A (NK2) homeobox 1/thyroid transcription factor 1 expression, respectively. In addition, KRAS-mutation is reported to co-occur with some other genes mutations, such as kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1 (KEAP1)/nuclear factor erythroid 2-like 2 (NRF2; also known as NFE2L2), RNA binding motif protein 10, protein tyrosine phosphatase receptor type D, and SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) mutations, respectively.[1,5] The co-occurring mutations contribute to dividing KRAS-mutant NSCLC patients into clinically heterogeneous subgroups, as well as offering potential predictive biomarkers associated with survival and therapy.

KL: KRAS and STK11/LKB1 co-mutations

An analysis from 154 NSCLC patients by an immunohistochemistry assay showed that LKB1 loss was found to be more frequent in tumors with KRAS transversion mutations, and NSCLC patients with KL had a higher risk of brain metastasis.[1] STK11/LKB1 inactivation is associated with a “cold” TIME, with a reduced density of infiltrating CD3+, CD4+, and CD8+ T lymphocytes, lower PD-L1 expression in tumor cell, reduced stimulator of interferon genes (STING) level, increased neutrophil recruitment, more T cell dysfunction, and increased proinflammatory cytokine production.[1] The KL tumors also demonstrated a comparative lack of immune system engagement and exhibited suppressed expressions of immune markers, such as PD-L1 and CD274 messenger RNA (mRNA) levels. Koyama et al[2] validated that T-cell numbers and function were significantly improved by depleting the neutrophils or blocking the cytokine feedback loop using a neutralizing anti-IL6 antibody in...
compared with KL and KC LUACs combined or the KC tumors had significantly higher chromosome aberrations. KC tumors possessed a complex and strong immune response system as well as higher chromosome aberrations. KC tumors showed higher proliferation and immune cytolytic activity-high cohort of gliomas which might contribute to faster tumor growth and decreased overall survival (OS) in KC mutant LUAC patients.

**KP: KRAS and TP53 co-mutations**

The tumor suppressor gene TP53 is mutated in around half of all human tumors as the most frequently mutated gene in cancer. Cha et al. 

KP: KRAS and TP53 co-mutations

In metastatic KRAS-mutant NSCLCs, somatic genomic alterations in CDKN2A and CDKN2B account for ∼20% and ∼12%, respectively. Wang et al. analyzed the immune cytolytic activity of 1000 gliomas in Chinese Glioma Genome Atlas dataset and The Cancer Genome Atlas (TCGA) dataset. They revealed that the deletion region of 9p21.3 (CDKN2A/B) was among the most frequently identified regions in the immune cytolytic activity-high cohort of gliomas which possessed a complex and strong immune response system as well as higher chromosome aberrations. KC tumors demonstrated a mixed immune system engagement with moderate CD274 mRNA expression compared to KP or KL tumors. From these studies, we proposed that the KC tumors might have a better clinical outcome from ICIs. In addition, more clinical data of immunotherapy combined with chemotherapy in the patients with KC will be on the agenda to validate these correlations.

**KC: KRAS mutation; CDKN2A/B inactivation**

In metastatic KRAS-mutant NSCLCs, somatic genomic alterations in CDKN2A and CDKN2B account for ∼20% and ∼12%, respectively. Dong et al. reported that patients with KP co-mutations undergoing pembrolizumab treatment obtained prolonged progression-free survival (PFS) and a durable clinical benefit. Therefore, ICIs may be effective therapeutic strategies for KP tumors. However, the data about the efficacy of ICIs or ICIs combined with chemotherapy in patients with KP co-mutations compared with those with KRAS single mutation are rarely reported. Thus, more clinical studies especially the multi-center prospective randomized controlled trials are taken into consideration.

**ALK receptor tyrosine kinase (ALK) and TP53 co-mutations**

ALK and TP53 co-mutations predict an unfavorable outcome of systemic therapy in NSCLC. Kron et al. reported that PD-L1 positivity is significantly associated with TP53 mutation status in 34 ALK-positive patients. Thus, ALK and TP53 co-mutations likely have a positive influence on the clinical efficacy of ICIs. The above results are from a limited clinical sample. Therefore, a large number of NSCLC patients with ALK and TP53 co-mutations are needed to analyze the association of co-mutations with TIME and immunotherapy.

**Epidermal growth factor receptor (EGFR) and mitogen activated protein kinase (MAPK) co-mutations**

The EGFR and MAPK co-mutations had higher TMB and PD-L1 levels compared to other EGFR co-mutant patterns and EGFR single-mutant patients. The EGFR and MAPK co-mutant group had longer PFS and favorable TIME, such as upregulated T cells, B cells, and Fcγ receptor-mediated phagocytosis. In addition, L858R mutations were more frequently found with a higher TMB compared with those with exon 19 deletions in the EGFR co-mutations. Most ICI studies exclude the patients with EGFR mutations, whereas the study reported by Yang et al. revealed that LUAC with EGFR and MAPK co-mutations might benefit from ICI treatment. Due to the limited data, the conclusions and underlying mechanism will be further confirmed in clinical studies.

**KEAP1-driven co-mutations**

LUAC patients with co-mutations in KEAP1 and poly-bromo 1 (PBRM1), SMARCA4 or STK11 had higher TMB and different immunogenomic landscape of T-cell receptor repertoire. T helper cell signatures, core immune signatures, and immunomodulatory genes compared with the wild-type groups. Interestingly, Marinelli et al. found that KEAP1-driven co-mutations (KEAP1, PBRM1, SMARCA4, and STK11) are more likely to be unresponsive to immunotherapy. In addition, compared with both single-mutant and wild-type tumors, the tumors harboring co-mutations had inferior survival outcomes. These KEAP1-driven co-mutations seemed to be associated with immunologically “hot” tumors, but are resistant to...
immunotherapy, which may be partly explained by the complex TIME and tumor heterogeneity.

**Impact of Immunooediting on Co-mutations**

Antigenic oncogetic mutations can be shaped by immunosurveillance through the elimination of clones that present strong antigenic neoepitopes at the early stages of tumor development. The opinions have been confirmed in mouse models. Compared with immunocompetent mice, immunodeficient mice are more susceptible to cancers with more immunogenic tumor cells. Lymphocytes and IFN-γ have restrictions on tumor immunogenicity and spontaneous tumor formation. Whereas, reduced tumor antigen expression or presentation on major histocompatibility complex class I (MHC-I) makes primary sarcomas to be less immunogenic and escape T lymphocyte attack in a genetically engineered mouse model. Therefore, a “cold” TIME likely relaxes immune selection and results in a more diverse spectrum of co-mutations. Recently, Marty et al. analyzed the interactions between patient MHC-I allele combinations and recurrent cancer mutations for thousands of tumors from TCGA. The results showed that MHC-I genotype-restricted immunooediting shapes the mutational landscape during tumor formation and an individual’s MHC-I genotype-based score can be used for the prediction of oncogenic mutations. Such immunooediting may have an impact on the patterns of co-mutations, which have to be further validated.

**Conclusions**

In this study, we summarized the co-mutations in TIME and immunotherapy. The subgroups of KRAS mutations, including ALK and TP53 co-mutations, EGFR and MAPK co-mutations, and KEAP1-driven co-mutations displayed molecular and biology diversity, which explains the different TIME and therapeutic efficacies of immunotherapy. In addition, immunooediting has an impact on the patterns of co-mutations in lung cancer. Whereas, these conclusions have to be further confirmed by the mutational and prospective clinical studies. In the new era of precision medicine, co-mutations may contribute to identifying the subset of patients who are most likely to benefit from immunotherapy and pave the way for offering personalized immune-based therapy.

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**Conflicts of interests**

None.

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