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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 inhibitiors (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.[1] Herein, we focus on the association of co-mutations with TIME and immunotherapy, especially in lung cancer [Supplementary Figure 1; http://links.lww.com/CM9/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*[2] 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.[3] 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.[4]*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.[1,5] 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.[2] Koyama *et al*[5] 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

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Kras/Lkb1-mutant mice. Among 924 LUACs, Skoulidis *et al*[2] demonstrated that *STK11/LKB1* alterations negativity correlated with PD-L1 in tumor mutational burden $(TMB)^{Intermediate/High}$ LUAC. In addition, *STK11/LKB1* is identified as the most prevalent genomic driver of primary resistance to PD-1/PD-L1 blockade in *KRAS*-mutant LUAC. These findings provide the theoretical basis for co-mutations in *KRAS* and *STK11/LKB1* in predicting clinical efficacy from PD-1 axis inhibitors in LUAC.

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.[6] Cha *et al*[7] found that PD-L1 positivity in tumor cells was significantly correlated with p53 aberrant expression, PD-L1 positivity in tumor-infiltrating immune cells, and higher stage in 323 surgically resected LUAC patients. Elevated interferon-γ (IFN-γ), PD-L1, PD-1, CTLA-4, and increased density of infiltrating $CD3^+$, $CD8^+$, and $CD45RO^+$ populations of T lymphocytes were found in the KP LUAC.[2] The expressions of PD-L1 and *CD274* are higher in the KP subtype compared with those in the KL. Skoulidis *et al*[2] also confirmed the enrichment of somatic mutations, inflammation, activated anti-tumor immunity, and immune tolerance/escape in the KP subtype. Thus, opposite to the KL tumors, the KP LUACs are associated with immunologically "hot" tumors. In addition, the KP tumors had significantly longer relapse-free survival compared with KL and KC LUACs combined or the KC subgroup alone. Dong *et al*[8] 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.

KC: KRAS mutation; CDKN2A/B inactivation

In metastatic *KRAS*-mutant NSCLCs, somatic genomic alterations in *CDKN2A* and *CDKN2B* account for ~20% and ~12%, respectively.[1] *CDKN2A/B* loss accelerated mutant *Kras*-driven lung tumorigenesis and metastasis in genetically engineered lung cancer mouse models and decreased overall survival (OS) in *KRAS* mutant LUAC patients. Wang *et al*[9] 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.[2] From these studies, we proposed that the KC tumors may help in predicting clinical efficacy from ICIs. In addition, more clinical data of immunotherapy or immunotherapy combined with chemotherapy in the patients with KC will be on the agenda to validate these correlations.

KRAS, STK11/LKB1 and KEAP1 co-mutations

Co-mutations of *KRAS* and *KEAP1* were reported to be associated with decreased OS from the start of ICIs in NSCLC patients.[3] The KL tumors had high rates of *KEAP1* mutational inactivation.[2] When co-occurring with an additional *KEAP1* mutation, the KL tumors exhibit low intra-tumoral density of infiltrating T and B lymphocytes, decreased PD-L1 expression in tumor cell, and reduced STING level. *KEAP1* gene encodes an adaptor protein which negatively regulates NRF2-mediated transcription and further reduces STING expression via post-transcriptional regulation.

Other Co-occurring Genomic Events Associated with TIME and Immunotherapy

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*[10] 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.[11] 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*[11] 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 polybromo 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.[12] Interestingly, Marinelli *et al*[12] 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 Immunoediting on Co-mutations

Antigenic oncogenic mutations can be shaped by immunosurveillance through the elimination of clones that present strong antigenic neopeptides 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.[13] Lymphocytes and IFN-γ have restrictions on tumor immunogenicity and spontaneous tumor formation.[14] 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.[15] Therefore, a "cold" TIME likely relaxes immune selection and results in a more diverse spectrum of co-mutations. Recently, Marty *et al*[16] 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 immunoediting 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 immunoediting 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, immunoediting has an impact on the patterns of co-mutations in lung cancer. Whereas, these conclusions have to be further confirmed by the muticentral 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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