Editorial Commentary

Profiling changes in metabolism and the immune microenvironment in lung tumorigenesis

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Lung cancer

Lung cancer is the leading cause of cancer-related mortality worldwide, with 5-year survival rates still below 20% (1). Several multidisciplinary approaches are used clinically for the treatment of this disease. Depending on the type, classification, and staging of the lung tumor, patients are treated with surgery, radiotherapy, chemotherapy, or immunotherapy, alone or in combination. More than 80% of lung cancers are classified as non-small cell lung cancer (NSCLC). Although targeted therapies have transformed treatment for NSCLC patients with defined genetic alterations (driver mutations), these targeted drugs are ineffective in tumors that lack specific molecular alterations (non-driver mutated NSCLC). However, immune checkpoint inhibitors targeting either programmed death receptor 1 (PD-1), programmed death receptor-ligand 1 (PD-L1) or cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) have revolutionized the clinical approach for the management of advanced non-driver NSCLC.

Based on the results of the phase III KEYNOTE-024 trial (2), pembrolizumab (anti-PD-1 antibody) is currently approved by the FDA as a first line monotherapy for the treatment of advanced (stage IV) NSCLC with ≥50% PD-L1 expression. It has also been approved in combination with carboplatin and pemetrexed for treatment of non-squamous NSCLC (2) or in combination with a carboplatin/taxane doublet for squamous NSCLC, even with low or absent PD-L1 expression (3). A newer clinical trial (KEYNOTE 042) has shown that regardless of the percentage of PD-L1 expression, using pembrolizumab as a monotherapy improves overall survival (OS). However, these benefits appear to be driven by a subgroup of patients with high PD-L1 expression (4). High PD-L1 expression is observed in approximately 30% of advanced NSCLC patients, and clinical trials have demonstrated that high PD-L1 expression predicts the response to pembrolizumab (5).

Other checkpoint inhibitors are being investigated for use as monotherapy and in combination with chemotherapy but have not yet received FDA approval. The CheckMate 227 trial showed that patients with ≥1% PD-L1 expression and high tumor mutational burden had improved progression free survival (PFS) with a combination of nivolumab (anti-PD-1 antibody) and ipilimumab (anti-CTLA-4 antibody) compared to chemotherapy (6). However, the CheckMate 026 trial found that in patients with ≥5% PD-L1 expression, nivolumab monotherapy did not improve OS or PFS compared to chemotherapy (2). For second line therapy following the failure of platinum-based chemotherapy, pembrolizumab, nivolumab and atezolizumab (anti-PD-L1 antibodies) have been FDA-approved as monotherapies for NSCLC with ≥1% PD-L1 expression (5). Results of ongoing clinical trials will continue to change the standard of care treatments for patients with NSCLC, but combinations of various checkpoint inhibitors with chemotherapy or targeted agents will undoubtedly improve outcomes for patients that historically have been

unresponsive to therapy.

Nrf2 pathway in lung cancer

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), encoded by the gene *NFE2L2*, has emerged as an important target for cancer therapy. The Nrf2-Keap1-ARE pathway is a master cellular defense mechanism that protects against oxidative and electrophilic stresses. Activation of this pathway regulates a wide variety of cellular processes as diverse as redox-balancing, metabolism, detoxification, and inflammation, so Nrf2 is tightly regulated. Under basal conditions, Nrf2 is constantly degraded through binding with its endogenous repressor, Keap1. When stress signaling occurs, a conformational change causes the release of Nrf2 from Keap1; Nrf2 then translocates to the nucleus where it acts as a transcription factor (7). Historically, because of its regulation of detoxifying and cytoprotective enzymes, activation of the Nrf2 pathway was considered beneficial for cancer prevention. Nrf2 deficiency enhances susceptibility to carcinogens, and Nrf2 activators prevent or delay tumor development in many preclinical models, regardless of cancer type or the carcinogen involved (7). Cells in the lung, which are continuously exposed to reactive oxygen and nitrogen species as well as carcinogens, rely heavily on this important defense pathway to maintain cellular homeostasis. Clinical trials testing the ability of Nrf2 activators to prevent the development of lung cancer in smokers are underway.

However, the discovery of mutations in the *NFE2L2* and *KEAP1* genes in human cancers and the accumulation of data supporting the tumor-promoting effects of Nrf2 suggest that inhibition of Nrf2 activity might be beneficial for treating cancer. Gain of function mutations in the *NFE2L2* gene and loss of function mutations in the *KEAP1* gene have been identified in many lung cancer tumors. Constitutive activation of the Nrf2 pathway induced by these mutations is associated with chemoresistance and poor survival. Activation of the Nrf2 pathway in tumor cells not only assists their adaptation to an oxidative environment but also promotes proliferation via metabolic reprogramming (8,9). Several Nrf2 inhibitors have been developed, and proof of concept studies suggest that tumor cells can be re-sensitized to chemotherapy by inhibiting the Nrf2 pathway (7). Further research is needed to better understand the complex role of Nrf2 in cancer and how to target this pathway for cancer therapy.

Notably, Nrf2 also plays an important role in anabolic cancer metabolism, as has been recently reviewed by Lee *et al.* (10) and confirmed by Best and colleagues (11). Nrf2 senses the nutrient status of a cell by receiving input from various metabolic signaling pathways, including the PI3K-AKT-GSK3β pathway, the AMPK pathway, and the UPR-PERK pathway. These pathways modulate Nrf2 post-translationally and thus alter the interaction between Keap1/Nrf2, Keap1-independent degradation of Nrf2, or localization of Nrf2 thus regulating the activation of the Nrf2 pathway (10). Once Nrf2 is activated, it can directly regulate the expression of metabolic enzymes and transporters, such as *G6PD* and *PGD* in the pentose phosphate pathway (8), *MTHFD2* and *PPAT* in the nucleotide biosynthesis pathway (12), *PHGDH* and *PSAT1* in the serine/glycine biosynthesis pathway (9), and *GCLC* and *CLCM* in glutathione metabolism, to favor cellular proliferation. Reprogramming cells into these anabolic pathways provides the materials needed for biosynthesis of proteins, lipids, and nucleotides, and meets the high metabolic demands of rapidly proliferating tumors.

Nrf2 is also known for its robust anti-inflammatory actions. Activation of Nrf2 can directly suppress the expression of pro-inflammatory genes such as *IL-6* and *IL-1*β. In addition, Nrf2 can modulate inflammation by regulating redox metabolism and crosstalk with NF-ΚB signaling (7). Nrf2 is widely expressed in many hematopoietic cells, especially monocytes and granulocytes, suggesting its importance in immune responses. Activation of Nrf2 has been shown to repress the expression of STING and the production of type I IFN (13). Nrf2 also suppresses the responses of pro-inflammatory T helper 1 and 17 cells and activates immunosuppressive populations like Treg and T helper 2 cells (7). As a result, many Nrf2 activators are being developed as treatments for various autoimmune diseases.

In cancer, the effects of Nrf2 on immune cells within the tumor microenvironment (TME) are not fully understood but are most likely context dependent. The present study by Best *et al.* (11) discovered an immunosuppressive microenvironment with decreased numbers of natural killer (NK) cells and T cells in the lungs of mice with tumors following the deletion of both KEAP1 and PTEN. Nrf2 is also critical for maintaining the survival and suppressive function of myeloid derived suppressor cells (MDSCs) (14). However, during the process of lung carcinogenesis, we identified an immunosuppressive signature in Nrf2 knockout (KO) mice injected with the carcinogen vinyl carbamate to induce lung cancer. In these studies, increased

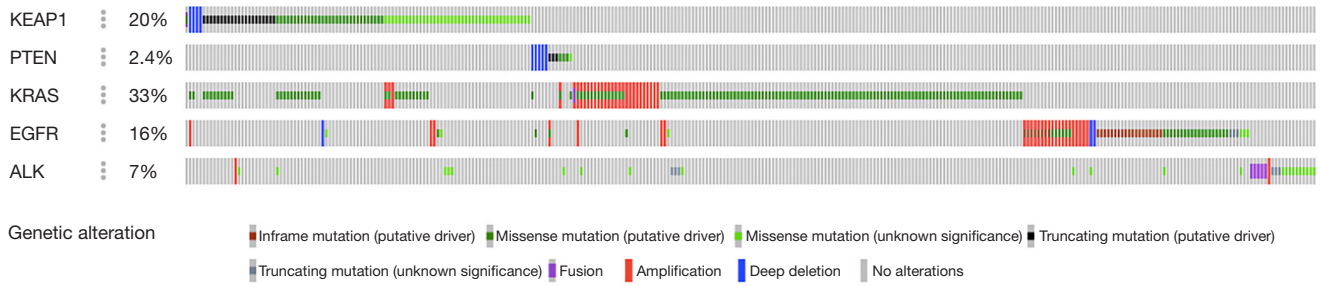

Figure 1 Oncoprint of key genetic alterations in patients with lung adenocarcinoma (507 samples), including *KEAP1*, *PTEN*, *KRAS*, *EGFR*, *AKT*. Alterations of *KEAP1* and *PTEN* are mutually exclusive. Figure was created using cbioportal.org (16,17). Unaltered patients are not shown.

expression of cytokines, a decreased proportion of T cells in the lung, and increased proportions of macrophages or MDSCs in tumor-bearing lungs or spleen, respectively, were observed in the Nrf2 KO mice compared to wild-type (WT) mice (15). These seemingly contradictory data raise the question of whether the immune signature identified in the microenvironment is a direct consequence of tumor burden or the result of Nrf2 activity. Different levels of Nrf2 activity (Nrf2 KO *vs.* Nrf2/Keap1 WT *vs.* Keap1 KO) may also lead to various outcomes on immune responses. Furthermore, the effects of Nrf2 can be cell type specific and the overall net effect (Nrf2 activation in cancer cells *vs.* immune cells) during the initiation and progression of cancer requires additional studies.

Nrf2 and PI3K pathways in lung cancer

As reported by Best *et al.* (11), deletion of *KEAP1* is not sufficient to induce tumor development. Lung adenocarcinomas only develop when both the PI3K and Nrf2 pathway are activated. Significantly, an immunosuppressive signature and up-regulation of PD-L1 in tumor cells were identified in these $KEAP1^{f/f}/PTEN^{f/f}$ mice, and the combination of anti-PD-1 and anti-CTLA-4 antibodies dramatically reduced tumor burden (11). Despite these intriguing results, genetic alterations of *KEAP1* are mutually exclusive with *PTEN* alterations in human lung adenocarcinoma patients (TCGA, PanCancer Atlas, 507 patients). There is also very limited co-occurrence of *Keap1* alterations and other key drivers in lung cancer including *KRAS*, *EGFR* and *ALK* (*Figure 1*), suggesting functional redundancy among these pathways. Indeed, Nrf2 is known to be a downstream target of PI3K signaling (18) and signaling pathways activated by oncogenes including *KRAS*, *BRAF* and *MYC* (19). Metabolic changes detectable in plasma and different immune signatures, identified by the authors (11), need to be characterized for the most clinically relevant genetic alterations and pathways activated in lung cancer.

TME

The relevance of the TME in cancer development is widely known, both at the primary site and at metastatic lesions (20). Tumor cells closely interact with blood vessels, immune cells, fibroblasts, signaling molecules, and the extracellular matrix (ECM) that constitutes the TME. Physical pressure, oxidative stress, nutrient deprivation or competition, hypoxia, and immune surveillance are challenges that develop over time as tumors progress. Hypoxia leads to enhanced glucose consumption, and consequently, generation of lactate, which in turn acidifies the TME. Immune surveillance exercised by T cells, in the context of the TME, is highly dependent on the presence of glucose; the high consumption of glucose by tumor cells contributes to antitumor immunity (21,22).

Metabolic and immune profiling may predict responses to immunotherapy

Best *et al.* (11) clearly demonstrate how changes in metabolites can be detected in the plasma of mice with lung cancer. The study elegantly shows that a combination of genetic alterations that activate Nrf2 and cause the loss of PTEN leads to the development of lung cancers that arise from the bronchial tree in mice. As shown in *Figure 1*, *NFE2L2/KEAP1* mutations are clinically relevant as they are present in approximately 20% of human lung cancers.

However, inactivating mutations in *PTEN* are quite rare and are detected in less than 3% of lung tumors. Notably, Kras-driven lung tumors do not generate the same immune signature (23) as reported for the KEAP1/PTEN lung tumors (11), and it is likely that the metabolic signature will also differ. The finding that some metabolic changes can be detected in the plasma of mice during tumorigenesis is hugely relevant for the potential use of metabolites, such as Taldo1, involved in the pentose phosphate pathway, as clinically relevant biomarkers. Increased Taldo1 expression was accompanied by increased expression of NQO1, a downstream target of Nrf2, and decreased immune cell populations, both in the KEAP1$^{f/f}$/PTEN$^{f/f}$ mice and in human tumors found in the TCGA data.

After further exploring the consequences of metabolic rewiring and cytotoxic CD8 T cell activation, the authors showed that lung tumors highly expressed PD-1 in CD8 cells and PD-L1 in tumor cells in the mice. Elevated expression of PD-L1 and PD-1 increases the likelihood of a response to immune checkpoint inhibitors (24) that have forever changed clinical strategies for treating lung cancer. When KEAP1$^{f/f}$/PTEN$^{f/f}$ mice were treated with anti-PD-1 and anti-CTLA-4 antibodies, starting seven months after tumor initiation by infection with Adeno-Cre, tumor burden was markedly lower than in mice treated with vehicle controls. As the treatment was initiated before tumors had fully developed, it will be critical to test if the same treatment strategy still reduces tumor size and burden when used against established tumors. Late time points are frequently associated with exhausted T cells and the depletion of T cells that have infiltrated into the tumor; these tumors often do not respond to checkpoint inhibitors (25). Additionally, treatment with anti-PD-1 and anti-CTLA-4 antibodies increase activated splenic T cells (CD8, CD44^{hi}) and expression of INFγ. Future studies should test whether the number of activated T cells in the lungs increases or that the cytotoxic T cells are degranulating (with the use of CD107 marker), and actively killing tumor cells.

Overall the studies by Best *et al.* (11) lay an important rationale for examining metabolic alterations in tumors that can be detected in plasma. Changes in either metabolism or immune cell populations could be used as biomarkers for predicting or monitoring efficacy of checkpoint inhibitors in lung cancer (24). Further studies in relevant tumor models and patients will help accelerate the clinical translation of these important observations.

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Footnote

Conflicts of Interest: KT Liby has patent interest in synthetic triterpenoids. The other authors have no conflicts of interest to declare.

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