Editorial

Studying the immune landscape in lung cancer models: choosing the right experimental tools

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Genetically engineered mouse models (GEMMs) of cancer are powerful tools to define the role of specific genetic lesions in tumor formation and progression. In the study of lung cancer, activation of oncogenes or repression of tumor suppressor genes is directly induced in lung epithelial cells—the tumor initiating cells of lung carcinoma (1)—to prevent tumor formation in extra-pulmonary organs. These genetic alterations are mediated through Cre-loxP recombination, whereby expression of Cre recombinase results in the activation of oncogenes or deletion of tumor suppressor genes flanked with LoxP sites. Lung-specific expression of Cre is achieved either by lung delivery (intra-nasal or intra-tracheal instillation, direct intra-pulmonary injection) of recombinant adenoviruses or lentiviruses expressing Cre-recombinase (2,3), or by crossing mice expressing Cre under the control of lung epithelial cell-specific promoters (4). In a recent article published in *OncoImmunology*, Tippimanchai *et al.* (5) evaluate the impact of Cre-induced recombination in immune cells after pulmonary administration of adenovirus carrying a ubiquitously expressed Cre-recombinase construct (Ad5-CMV-Cre). Using *K-Ras*$^{LSL\text{-}G12D/+}$; *mT/mG* mice, they show that Cre-mediated activation of oncogenic K-Ras^{G12D} occurs not only in lung epithelial cells, but also in alveolar macrophages, resulting in their proliferation and increased tumor growth in co-xenotransplantation models. This study highlights the importance of the method chosen for induction of Cre-mediated recombination, particularly when investigating novel immunotherapeutic approaches and tumour-immune cell interactions in mouse models of lung cancer.

The clinical utility of immunotherapies to treat cancer has renewed interest into the role of the immune microenvironment in tumor formation and progression. Investigation of the innate and adaptive immune landscape of human cancers has revealed diverse immune microenvironments that can vary according to histological subtype and tumour genomic profile (6). However, these studies in human tumors do not allow for in-depth investigations into causative roles played by the immune infiltrate in cancer initiation, progression and metastasis. Therefore, the use of GEMMs is necessary to untangle the interaction between the immune milieu and tumor cell behavior. GEMMs present a distinct advantage over xenotransplant models that require immunodeficient environments. GEMM tumors grow orthotopically in immunocompetent animals, more accurately mimicking the human disease. However, genetic manipulations *in vivo* may impact the tumor immune microenvironment. Numerous hematopoietic cells can regulate tumor growth, including T lymphocytes, macrophages and neutrophils. Increased neutrophil-to-lymphocyte ratio is correlated with poor prognosis in solid tumors (7), while presence of activated T-cells is predictor of response to immunotherapy (8). Tippimanchai *et al.* found that intranasal or intratracheal administration of Ad5-CMV-Cre to activate the K-Ras^{G12D} oncogene led not only to the activation of the oncogene in

lung epithelial cells, but also in tissue-resident macrophages. While lymphocytes and neutrophils were not significantly affected, K-Ras^{G12D} expression was induced in alveolar macrophages resulting in an increase in their proliferation. This study not only reveals the need to acknowledge potential limitations when using GEMMs of lung cancer, but also highlights the role alveolar macrophages may play in promoting lung cancer formation.

Alveolar macrophages are a distinct population of resident macrophages present in the lumen of the airways and alveoli of the lung. They play a critical role in the healthy lung to clear apoptotic cells and cellular debris and facilitate immunological responses in the lung. Alveolar macrophages are distinguished from interstitial macrophages by their high expression of CD11c and SiglecF and the absence of CD11b expression (9). The role of alveolar macrophages in lung cancer has not been studied as extensively as monocyte-derived, tumor-associated macrophages that are recruited to the tumor site to promote cancer growth. Nevertheless, alveolar macrophages appear to facilitate breast cancer metastasis to the lung by suppressing anti-tumor T cell reactivity (10). Depletion of alveolar macrophages reduced tumor burden in *EGFR*-driven models of lung adenocarcinoma (LUAD) (11) and in urethane-induced models of lung cancer (12), suggesting that alveolar macrophages also promote tumor initiation and progression. A recent analysis using single cell profiling showed accumulation of suppressive PPAR gamma^{hi} (Peroxisome proliferator-activated receptor gamma) macrophages in early stage LUAD lesions, where a higher ratio of tumor-associated macrophages to resident macrophages also correlated with a survival disadvantage (13). These findings prompt further investigations into the role of distinct macrophage populations during lung carcinogenesis and highlight the necessary careful choice of the experimental model for such studies. According to Tippimanchai *et al.*, adenoviral-mediated transduction of lung cells using ubiquitous promoters to activate oncogenes or knock-out tumor suppressor genes has the potential to affect the function of alveolar macrophages (5). It remains to be determined if activation of different oncogenes in alveolar macrophages changes their immunophenotype, their ability to present antigens, or their capacity to secrete specific cytokines; all of which could alter tumor cell proliferation and metastatic potential.

The critical role of the interaction between the immune system and cancer cells is also highlighted by the diversity of the immune landscape in solid tumors. A recent RNA-sequencing analysis of more than 10,000 tumors across 33 sites defined 6 cancer immune profiles (C1–C6). LUAD were found to be associated mostly with the C3 (inflammatory), C2 (IFNγ dominant) and C1 (wound healing) subtypes, whilst lung squamous cell carcinomas (LUSC) were associated predominately with C1 and C2 classes, where the C2 subset had a higher macrophage signature than the C1 subgroup. These findings show that each histological subtype has a different immune landscape. Furthermore, each immune subclass was also found to be associated with distinct oncogenic alterations, where C1 and C2 subtypes were most commonly observed in *KRAS*, *TP53*, *PIKC3A* and *PTEN*-mutant cancers (6). The variation of the immune milieu with tumor genotype is also observed at the transcriptomic subtype level, where molecular subtypes of LUAD and LUSC showed distinct patterns of immune cell infiltrate (14). This observation was further explored in mouse models, where a higher CD8$^+$ T-cell infiltration was observed in *K-Ras* and *K-Ras/Tp53* mutant lung tumors driven by Ad5-CMV-Cre administration, compared with *EGFR*-mutant mice, which was further confirmed in a human LUAD tissue microarray (15). The percentage of alveolar macrophages was not evaluated in this study, but tumor-associated macrophages were dramatically increased in all three LUAD models (*EGFR*, *K-Ras*, *K-Ras/Tp53*), compared with small cell lung cancer mouse models driven by loss of *Rb1/Tp53* (15). Given the ability of the molecular genotype of tumor cells to dictate the immune landscape, oncogene activation or loss of tumor suppressor genes in alveolar macrophages in GEMMs may exert different effects according to the tumor molecular subtype.

The work by Tippimanchai *et al.* emphasizes how the use of a ubiquitous promoter driving Cre-recombinase expression, even through localized administration to the lung, may affect multiple cell types and impact tumor growth. Similarly, analysis of tumor progression by loss-of-function of a gene of interest may not only be the result of its inactivation in the epithelial cell compartment, but may also be due to its loss in microenvironmental cells that affect tumor growth. The use of lung specific promoters to drive Cre-recombinase expression circumvents these difficulties. Intranasal or intratracheal administration of lung epithelial cell-specific Ad5-cre viruses (Ad5-SPC-cre, Ad5-CC10-cre, Ad5-CGRP-cre; Ad5-Keratin5-cre) has been successfully used to investigate tumor-initiating cells of lung carcinomas and provides precise models of lung carcinogenesis, where the oncogenic lesion is specifically activated in epithelial cells (16-18). Other genetic tools, such as tamoxifen or doxycycline-inducible knock-in of Cre in lung epithelial

cell-specific promoters, have also successfully been used to generate mouse models of LUAD, enabling spatial and temporal activation of oncogenes such as *K-Ras*^{G12D} (4) or *EGFR* (19) only in epithelial cells. Overall, the new era of cancer immunotherapy has generated immense interest into the interaction between immune and tumor cells, and will require careful consideration of the advantages and limitations of experimental models to explore this cross-talk.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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