Commentary

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Recent advances in experimental animal models of lung cancer

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Lung cancer is the leading cause of cancer-related deaths worldwide [1]. In 2018, there were 2 million new cases and 1.7 million deaths attributed to lung cancer [1]. Histopathological subtypes of lung cancer include non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). NSCLC accounts for approximately 85% of lung cancer cases, which can be further divided into adenocarcinoma accounting for 38.5%, squamous cell carcinoma for nearly 20% and bronchoalveolar and large cell carcinomas accounting for the rest [2]. SCLC arises from neuroendocrine cells of bronchus and constitutes approximately 15% of cases [3].

Lung cancer is typically a lethal malignancy, primarily due to delay in definitive clinical diagnosis and consequently has a poor prognosis [1]. Thus, there is an urgent need for novel and efficacious diagnosis, as well as therapeutic options. The research in this area could be accelerated by utilizing short-term animal models that recapitulate the hallmark features of lung cancer that will be valuable in pinpointing novel therapeutic targets and appropriate interventions [4]. Importantly, cigarette smoking (both active and passive) is a major contributing factor for the development of lung cancer and chronic obstructive pulmonary disease [5]. It is likely that increasing air pollution, especially in low- and middle-income countries, is also likely to contribute to lung cancer development [6]. However, the precise underlying mechanisms that drive carcinogenesis, both molecular and cellular, remain poorly understood [4,7]. Recent advances in genetic engineering technology, including transposon-based insertional mutagenesis, RNA interference and engineered nucleases, as well as chemically-induced preclinical animal models of lung cancer have significantly contributed to furthering our understanding of carcinogenesis and associated pathophysiology. In this commentary, we will specifically focus on the recent advances in animal models of lung cancer.

Genetically modified animal models of lung cancer

Highly specific transgenic mouse models that closely resemble the genetic and pathophysiological features of human lung cancers are now available. Tumor suppressor genes (TSGs), which play a vital role in lung cancer, are often deleted or mutated to induce lung cancer development in mice. Based on the genome database and known

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mutations, Wu *et al.* selected 55 TSGs and screened them for their roles in tumorigenesis using the somatic gene editing technique, clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) in the *Kras*$^{G12D/+}$ (Kirsten rat sarcoma viral oncogene homolog) mouse model of lung cancer [8]. Interestingly, of the genes *Utx*, *Ptip*, *Acp5*, *Acacb* and *Clu*, only when the *Utx* gene was deleted, tumor progression increased [8]. Consequently, the study of specific TSGs may facilitate our understanding of lung tumorigenesis and investigation of novel pharmacotherapies. Lung cancer patients with a history of cigarette smoking can exhibit a deletion in the small arm of chromosome 3p21.3, which has several tumor suppressor genes such as *LIMD* [9]. In a study by Jamsai *et al.* the gene-trapped embryonic stem cell line was used to generate *Rbm5* deficient ($^{-/-}$) mice. When treated with the cigarette carcinogen NNK (4-[Methyl(nitroso)amino]-1-(3-pyridinyl)-1-butanone), these mice developed more aggressive cancers compared with wild-type mice with an increased number of adenocarcinoma nodules. Evidently, *Rbm5* acts as a tumor suppressor gene in cigarette smokers [10].

Elucidating the role of the protumor microenvironment and hallmark immune signatures is essential in developing novel therapies for lung cancer. To study the role of the protumor microenvironment in lung cancer, Faget *et al.* used *Kras* and p53 (KP) driven lung adenocarcinoma mice crossed with *Rag1* gene to demonstrate that Gr1^{+} neutrophil deletion leads to enhanced tumor growth and reduced the efficacy of programmed cell death-1 immunotherapy [11]. They also showed that increased angiogenesis was associated with neutrophils, which promote hypoxia and Snail production that in turn causes tumor progression [11]. The use of cell-free DNA and circulating tumor DNA is gaining attention as promising biomarkers for lung cancer. This coupled with the use of Cre-regulated genetically modified mice such as *Kras*$^{Lox\text{-}G12D}$, *Kras*$^{LSL\text{-}G12D}$ and *Kras*^{WT} and using techniques like micro-computed tomography and droplet digital PCR assay, facilitates the detection of pre-malignancy of lung adenocarcinoma, which can be a promising diagnostic tool [12]. Conditional triple deficient Rb/p53/p130$^{flox/flox}$ mice that develop SCLC with Hes1 transcriptional factor showed that the tumors had notch-active protumorigenic (non-neuroendocrine) cells that were generated from neuroendocrine cells. Inhibition of transcriptional repressor REST in notch signalling prevented cell differentiation in SCLC. Thus, notch inhibitors along with chemotherapy may be an effective therapy for SCLC [13].

The role of lung microbiota in communication between bacteria and inflammation is gaining attention. Jin *et al.* showed an interesting correlation between microbiota, inflammation and lung adenocarcinoma [14]. In KP mice it was observed that microbiota promote adenocarcinoma with increased cytokine expression via promotion of Vγ6+ Vδ1+ T(γδ T) cells. Thus, alteration in the pathway of microbiota mediated γδ T cell activation and proliferation offers it as a therapeutic target [14]. Furthermore, kinases were reported to be tumor markers, including proto-oncogene *ROS1*-receptor tyrosine kinase 1 – which has an important role in NSCLC progression [15]. Notably, Arai *et al.* developed an *EZR-ROS1* transgenic mouse model to study the role of the *EZR-ROS1* fusion gene [15]. The authors first fused the *EZR* and *ROS1* genes via the expression of a fusion kinase in NIH3T3 cells, and then injected these cells into nude mice, which resulted in the rapid development of early stage multiple adenocarcinomas. This new model can be used to study therapeutic agents for patients expressing *ROS1* rearrangements [15].

The majority of mutations occurring in lung cancer models are *Kras* dependent, and corresponding mutated mice are widely used models in preclinical studies [16]. One profiling study on exomes and transcriptomes of *Kras* mutations in genetically modified mouse models suggested that a greater degree of heterogenicity evolved during tumorigenesis [17]. This indicates the need for further refinement of genetically modified animal models, which would aid more effective development of therapeutics.

Chemically induced animal models of lung cancer

Chemically induced animal models of lung cancer are increasing in importance compared with xenograft and genetically modified models due to their ease of application, rapid timeframes and efficiency in reproducibility. Recently, these models have gained attention, primarily due to major increases in the number of lung cancer cases related to cigarette smoking and other environmental carcinogens, such as polycyclic aromatic hydrocarbons and aromatic amines [18]. The major chemical carcinogens in cigarette smoke include NNK and polycyclic aromatic hydrocarbons [19]. These carcinogens have been extensively used to develop different models of lung cancer. For instance, NNK is used to induce adenocarcinoma [20] whereas N-nitroso-tris-chloroethylurea (NTCU) promotes the development of squamous cell carcinoma [21]. Merval *et al.* developed a novel animal model of NSCLC by inducing adenocarcinoma using diethylnitrosamine in the FVB/N (Friend leukemia virus B) mouse strain [22]. Interestingly, no mutations in the hot spot areas of *EGFR* and *Kras* genes were observed, which highlights the importance of this particular model in investigating the pathogenesis of *EGFR* and *Kras* negative tumors [22].

Lung epithelial cell lines such as BEAS-2B and human bronchial epithelial cells that lack p53 expression when exposed to NNK exhibited activation of IGF-1R phosphorylation via β-adrenergic receptor (β-AR) [23]. Blocking β-AR with NNK antagonists in A/J mice with lung cancer suggests that blockade of β-AR may be an effective therapy for lung cancer [23]. In addition to NNK, inorganic phosphate (Pi) also plays a crucial role in signal transduction and expression of several genes like transcription factor *Nrf2* during cell division [24]. However, the role of Pi in lung cancer pathogenesis is poorly understood. A study investigating the role of high phosphate diet for 1, 2 and 4 months in *Kras* mice suggested that the diet enhanced autophagy and epithelial to mesenchymal transition in early and later stages due to cellular metabolic adaptation, resulting in the decline of cancer. Overall, it is necessary to avoid high phosphate diet with early stages of lung cancer [24]. Another hallmark feature of lung cancer is chronic inflammation, Kim *et al.* proposed that cigarette smoke exposure to C57BL/6J and IL-17a$^{-/-}$ in mice lungs causes increased Th17 differentiation with enhanced pro-Th17 and neutrophils, by analyzing blood and spleen, it was found that there was a systemic inflammation, eventually leading to intestinal inflammation [25].

The development of preclinical nonrodent lung cancer models may be crucial for the study of lung carcinogenesis that may more precisely mimic the human disease. Administration of NNK to ferrets (50 mg/kg body weight) for 4 months, followed by a rest periods of 24, 26 and 32 weeks resulted in preneoplastic and neoplastic lesions [26]. This led to the development of tumors with higher numbers of macrophages and increased expression of α7 nicotinic acetylcholine receptors, which replicates observations in humans. Thus, non-rodent models could be used to study the progression of lung cancer in conjunction with laboratory rodents [26]. In addition to carcinogens, genetically modified A549 cells with *hNIS gene* expression and an imaging reporter ^{99m}Tc-pertechnetate ($^{99m}TcO^{4-}$) cells have also been used to create a new orthotopic xenograft lung cancer model. Engrafting these cells to athymic nude mice results in the development of tumors, and the level of tumor progression was measured using single proton emission computed tomography/CT (SPECT/CT) with $^{99m}TcO^{4-}$. This enabled the identification of tumor mass at different time points and highlights the importance of this orthotopic xenograft model for use in monitoring the effects of potential anticancer therapies longitudinally [27]. In order to study the cellular and molecular changes which occur during chemically induced lung adenocarcinoma, Spella *et al.* developed a C57BL/6 mice model of lung adenocarcinoma by exposing tobacco carcinogen urethane via intraperitoneal injection for 10 weeks. This study showed that airway epithelial cell were more sensitive to Kras^{Q61R} mutations compared to alveolar type II cells, suggesting that tobacco-induced lung adenocarcinoma starts in airway epithelial cells [28].

Conclusion

Collectively, the analysis of genetically modified and chemically induced animal models of lung cancer has progressed the understanding of lung cancer pathogenesis. Lung cancer in animal models progresses in a relatively short time frame and under extremely controlled conditions and aids the identification of most relevant oncogenic pathways, which could be novel drug targets. Understanding the genetic changes that occur during cancer progression is complex and depends upon the type of cancer and the stimulus. There remains a lack of robust short-term, easily reproducible, cigarette smoking-induced mouse model which replicate similar clinical features of human lung cancer.

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