Genetically Modified Mouse Models of Lung Cancer

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Lung cancer causes more than one million cancer deaths each year and is the leading cause of cancer death worldwide (*1*). While the majority of these cases can be attributed to tobacco smoking, it is estimated that 25% of lung cancer cases worldwide involve never smokers, making cancer in never smokers the seventh leading cause of cancer death (*2*). Data from cancers that developed in atomic bomb survivors suggest that lung cancer contributes a large portion of the solid cancer risk after exposure of astronauts to space radiation (galactic cosmic rays and solar particle events) even after accounting for smoking effects (*3*). Despite the evidence that terrestrial ionizing radiation can induce lung carcinogenesis, the mechanism of radiation-induced lung cancer is unclear and the effect of radiation quality remains largely unknown. Mouse models of lung cancer have been used for decades to investigate tumor initiation, progression, and response to therapy. Recently, several new mouse models of lung cancer have been developed that may more faithfully recapitulate the human disease. These models offer a powerful system to investigate lung cancer risk following exposure to space radiation.

Lung tumors can be divided into two major forms: small-cell lung cancer and non-small cell lung cancer. The two most common histological subtypes of lung cancer, adenocarcinoma and squamous cell carcinoma, fall into the non-small cell lung cancer category. For this reason, the development of mouse models has generally focused on non-small cell lung cancer, though small cell lung cancer models also exist (*4-6*). Mouse models of lung cancer generally fall into two categories: spontaneous models in susceptible strains, and genetically engineered mouse models (GEMMs). Historically, radiation-induced lung cancer research has focused on spontaneous models of lung cancer, but genetically engineered mouse models have improved substantially with the advent of conditional oncogenes and tumor suppressors that can be manipulated in the somatic tissues of mice.

The susceptibility to lung cancer varies greatly across inbred mouse strains, and certain strains have been identified with a high propensity towards developing lung cancer. For example, strain A mice have been shown to be susceptible to spontaneous and chemically-induced lung tumors. Nearly all strain A mice develop lung tumors that mimic human tumors histologically and in their mutation profile (*7*). Chemical carcinogens such as cigarette smoke or tar can accelerate tumor development in this model. Polymorphisms in genes commonly mutated in human cancer such as the Kras oncogene are believed to influence the sensitivity of strain A mice to lung cancer (*8*). Several other mouse strains have been shown to be susceptible to lung tumor initiation with ionizing radiation, including RFM, BALB/c, B6CF1, and C3H (*9-12*). These strains have been used to investigate multiple aspects of radiogenic lung cancer including the effects of fractionation, dose-rate, and radiation quality (*13-15*). Genetically susceptible mouse strains offer a proven methodology for investigating the effects of space radiation on lung tumorigenesis.

The advent of transgenic mice has led to more sophisticated mouse models of lung cancer. Multiple mouse models of lung cancer have been developed by expressing oncogenes and

deleting tumor suppressors that are known to be mutated in human disease. Germline expression of oncogenes and deletion of tumor suppressors is often embryonic lethal. When mice are viable, germline deletion of tumor suppressors leads to a broad tumor spectrum with only a small number of lung tumors. Moreover, germline mutations of genes that drive cancer may alter development of the lung. Finally, widespread expression of oncogenes or knockout of tumor suppressors may alter the tumor microenvironment and may not recapitulate tumor initiation and progression in people (*16*). For this reason, transgenic mouse models of lung cancer have typically been developed by expressing oncogenes and deleting tumor suppressors either sporadically or conditionally in mouse lung tissues.

The two most commonly mutated genes in human non-small cell lung cancer are Kras and p53 (*17*). Activation of Kras alone via a spontaneous recombination of a latent allele of oncogenic Kras in the whole mouse predisposes mice to a range of tumor types, including lung adenomas and low grade lung carcinomas in 100% of mice and thymic lymphomas and skin papillomas at lower rates (*18*). Targeting Kras or p53 mutation specifically to the lung can be achieved by using conditional alleles in which the Kras oncogene is activated or the p53 tumor suppressor is deleted in a spatially and temporally restricted manner. The Cre-loxP system can be used to delete loxP-flanked ("floxed") tumor suppressors with Cre recombinase or activate oncogenes via removal of a stop element that prevents expression of a mutated oncogene. Cre expression can be directed to the lung epithelium by delivering an adenovirus expressing Cre recombinase (adeno-Cre) into the lungs or via tissue-specific Cre drivers.

Activation of oncogenic Kras ($Kras^{G12D}$) via intratracheal delivery of adeno-Cre into loxP-stop-loxP-$Kras^{G12D}$ mice leads to epithelial hyperplasia after four weeks and later sporadic conversion to adenocarcinoma (*19*). The number of lung tumors can be controlled by changing the titer of adeno-Cre delivered. Combining Kras activation with conditional deletion of p53 accelerates lung tumor progression, leading to the development of more advanced tumors with higher rates of metastasis (*20*). Cre expression can be directed to cellular subtypes in the lung by expressing Cre recombinase under control of cell type-specific promoters such as the Clara Cell-Specific 10 kD Protein (CC10) or the Surfactant Protein C (SPC) promoter. This approach could be useful for determining the cells of origin for radiation-induced lung cancer. Furthermore, the Cre-estrogen receptor fusion protein (Cre-ER) can be used to direct recombination only in the presence of tamoxifen to provide temporal control of gene mutation.

Oncogene expression can be switched on and off using the tetracycline-responsive expression system. In this system, oncogene expression is controlled by a tetracycline-responsive element that is regulated by a tetracycline-controlled reverse transactivator (rtTA). Oncogene expression is reversibly turned on and off by administering or withdrawing doxycycline treatment. Doxycycline-driven expression of mutant epidermal growth factor receptor (EGFR) in the lung leads to the development of bronchoalveolar carcinoma in mice (*21*). This may be a good model for lung cancer in astronauts because EGFR has been found to be mutated in up to 58% of lung cancers in never smokers (*2*).

Lung cancer development is a complex process involving cancer initiation, progression, invasion and metastasis. To accurately estimate the risk of cancer from space travel, it will be important to understand how space radiation influences all of these processes. Every mouse model has strengths and weaknesses that should be considered when determining the best system for an individual research question. While susceptible mouse strains are a proven tool for investigating radiation-induced cancer, recently developed GEMMs are powerful tools for dissecting the mechanisms and cell types involved in space-radiation induced lung cancer.

Suggested Reading

R. Meuwissen and A. Berns, Mouse models for human lung cancer. *Genes Dev* 19, 643-664 (2005). PMID: 15769940.

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