COMMENTARY

Diverse cells at the origin of lung adenocarcinoma

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Oncogenic mutations in Kirsten rat sarcoma viral oncogene homologue (*K-RAS*) are found in a broad range of aggressive cancers in many tissues and are common in the most prevalent form of lung cancer, adenocarcinoma (1). Despite knowledge of *K-RAS* mutations for many years, patients with *K-RAS* mutant tumors remain without an effective targeted therapy option. This has generated considerable interest in the mechanisms of oncogenesis and the cell types susceptible to this form of transformation. In PNAS, Mainardi et al. (2) and Sutherland et al. (3) take complementary approaches to interrogate the ability of different epithelial cell populations to give rise to proliferative lesions and tumors in the lung. Like earlier studies by Xu et al., also published in PNAS (4), the authors conclude that several distal lung epithelial cell types are potentially capable of hyperproliferation or tumor initiation in response to oncogenic *K-Ras* activation. Taken together with the first reports of *K-Ras*–induced lung tumors in mouse models (5, 6), the important take-home message is that the precise identity of cells that are transformed and the kind of tumors that are generated depend on multiple factors. These include the cell type in which *K-Ras* is activated, the developmental timing, the potential for inflammation by use of adenoviral vectors, and the specific genetic modifiers. These results will help to inform ideas about tumor initiation in the human lung.

Adenocarcinoma is the most prevalent type of non-small cell lung cancer in the United States. In patients, adenocarcinomas often stain positively with antibodies to markers of the alveolar type II cells (AT2 cells), the surfactant-producing epithelial cells in the alveolar space, or the bronchiolar epithelial club (Clara) cells, the secretory cells lining the airways. These findings originally led to hypotheses that AT2 cells and club cells could be cells of origin in this tumor type. To explore the problem experimentally, most investigators have exploited conditional *K-Ras* alleles with a G12D/V point mutation driven by the endogenous *K-Ras* promoter. Removal of a stop codon by lox Cre recombination yields potentially oncogenic *K-Ras*$^{LSLG12D/V}$ alleles. By using different Cre drivers, *K-Ras* can be activated in different cell types and at different times. The earliest studies focused on AT2 cells and a rare population found within the bronchioalveolar duct junction (BADJ) termed bronchioalveolar stem cells (BASCs). BASCs express both AT2 marker surfactant protein C (SPC, Sftpc) and the club cell marker CC10 (also CCSP or Scgb1a1) and are sometimes referred to more simply as "double positive cells." Double positive cells were shown to expand in early *K-Ras* mutant tumors initiated with adenoviral-CMV-Cre; these and related data led to the idea that BASCs are the cells of origin of adenocarcinomas (6). Manipulation of pathways or repair processes regulating BASCs also affected the rate of tumorigenesis in *K-Ras* mutant adenocarcinoma (6–8). Subsequent studies used SPC-CreER and CC10-CreER knock-in alleles to conditionally activate *K-Ras* either in SPC-positive or CC10-positive cells, together with fluorescent reporter alleles to lineage trace cells in which recombination had occurred (4). Using the SPC-CreER allele, they found that tumors arose only in the alveoli, even though recombination also occurred in double positive cells in the BADJ. Using the CC10-CreER allele, recombination was also seen throughout the bronchioles and the BADJ, as well as in a small population of double positive cells in the alveoli. However, tumors only arose in the alveoli, and only hyperplasia was seen in the BADJ. Similar results were reported recently using the same CC10-CreER allele (9).

Mainardi et al., Sutherland et al., and Xu et al. provide evidence that AT2 cells are the predominant cell of origin of *K-Ras*–driven adenocarcinoma.

In the two recent PNAS papers under discussion (2, 3), the investigators further explore the origin of lung adenocarcinomas using *K-Ras* conditional recombination and cell lineage tracing. Mainardi et al. (2) demonstrate that even when *K-Ras* is activated throughout many mouse tissues using a tamoxifen-controlled Cre-ER fusion driven by *polr2a*, tumors only arise in the lung. At early times after Cre activation, *K-Ras* expression read out by an X-gal reporter was found within the alveoli, bronchioles, and the BADJ, and *K-Ras* mutant cells proliferated to form small lesions. At later times however, only alveolar lesions proliferated beyond ∼20 cells to form more advanced adenomas and adenocarcinomas. Cells from these tumors were exclusively SPC$^+$, and the smaller BADJ and bronchiolar lesions contained a mix of CC10$^+$ and SPC$^+$ CC10$^+$ cells. More heterogeneous lesions containing more double positive cells were at the BADJ where these cells are ordinarily located. Interestingly, when Mainardi et al. (2) activated *K-Ras* by intratracheal injection of Cre-expressing adenovirus, a greater proportion of bronchiolar and BADJ lesions were observed, some of which progressed to more advanced papillary hyperplasias and adenomas. This was found to be dependent on inflammation induced by viral infection, demonstrating that environmental factors influence the progression of *K-Ras*–driven tumors initiated in different locations. When Mainardi et al. (2) used Cre driven by the Sca-1 promoter, expressed in SPC-expressing embryonic cells at E13.5 or CC10-expressing cells at E17.5, hyperplasia and adenomas were seen in the bronchioles and BADJ. It is not known if carcinomas would have developed due to shortened life span. Activation of Sca1-driven CreER in adults, however, permitted adenocarcinoma development only in the alveolar space. This result is interesting given that, in adult lung tissue flow cytometry studies, AT2 cells have been characterized as Sca1

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Table 1. Summary of the cell of origin experiments

Study	Oncogenic drivers	Initiation method	Location of tumors	Tumor lineage markers	Probable cell of origin
2	*K-Ras*^{LSLG12V}	*Polr2a-CreERT2* +4OHT	Alveoli: adenoma Bronchioles, BADJ: hyperplasia	Alv: SPC Bron/BADJ: SPC, CC10, CC10/SPC	Alv: AT2 cell Bron/BADJ: club cell/BASC
2	*K-Ras*^{LSLG12V}	Adeno-Cre	Alveoli: ademona, adencarcinoma Bronchioles, BADJ: hyperplasia, adenoma	Alv: SPC Bron/BADJ: SPC, CC10, CC10/SPC	Alv: AT2 cell Bron/BADJ: club cell/BASC
2	*K-Ras*^{LSLG12V}	*Sca-1-Cre*	Bronchioles: hyperplasia, adenoma	CC10, CC10/SPC	Club cell precursor
2	*K-Ras*^{LSLG12V}	*Sca-1-CreERT2* +4OHT	Alveoli: adenoma	SPC	AT2 cell
3	*K-Ras*^{LSLG12D}	Adeno SPC-Cre	Alveolar hyperplasia	SPC	AT2 cell
3	*K-Ras*^{LSLG12D}	Adeno CC10-Cre	BADJ papilloma	CC10, SPC, CC10/SPC	Club cell/BASC
3	*K-Ras*^{LSLG12D} *Trp53*$^{fl/fl}$	Adeno SPC-Cre	Alveoli adenoma, adencarcinoma	Not shown	AT2 cell
3	*K-Ras*^{LSLG12D} *Trp53*$^{fl/fl}$	Adeno CC10-Cre	BADJ papillary carcinoma	Not shown	Club cell/BASC

Summary of the different initiation methods and oncogenic drivers used by Mainardi et al. (2) and Sutherland et al. (3). The location, histological description, and lineage markers of the resulting tumors, as well as the probable cell(s) of origin that can be inferred from these data are also shown. Alv, alveolar; Bron, bronchiolar.

negative, whereas BASCs are the Sca1-expressing cells (6, 10).

Sutherland et al. (3) investigate the cell of origin of *K-Ras*–driven lung cancer by controlling Cre from SPC and CC10 promoters. They achieved this by introducing cell type-specific adenoviruses—SPC-Cre or CC10-Cre virus—rather than using CreEr knock-in alleles as in Xu et al. (4). Sutherland et al. (3) additionally examine the contribution of *Trp53* loss, also driven by Cre recombination, another frequently observed alteration in lung adenocarcinoma (11). Notably, the mice in Xu et al. were all heterozygous for the same *Trp53* allele (4). Using Adeno-SPC-Cre, Sutherland et al. (3) observe SPC$^+$ tumors within the alveoli, whereas the Adeno-CC10-Cre gives rise to tumors with CC10$^+$ and double positive cells at the BADJ but rarely in the bronchioles, which contained mostly CC10$^+$ cells. Interestingly, the tumor spectrum was different depending on which cell type virus was delivered; bronchiolar and BADJ tumors were papillary in nature, more common in Adeno-CC10-Cre recipients, and never progressed in mice that received Adeno-SPC-Cre. In contrast, alveolar hyperplasia and alveolar carcinomas developed more often in Adeno-SPC-Cre recipients. Using fluorescent lineage tracing tools, Sutherland et al. (3) show that many CC10$^+$ cells, especially those in the proximal bronchioles, activate Cre but are unable to proliferate further. In contrast, some CC10$^+$ cells at the BADJ form tumors that include CC10$^+$, CC10$^+$/SPC$^+$, and SPC$^+$ cells. Consistent with other studies (5, 12), *p53* loss accelerated development of *K-Ras* tumorigenesis driven by SPC-Cre and CC10-Cre, inducing characteristics of invasiveness and metastasis. SPC-driven and/or alveolar tumors were more developed in *K-Ras* mice and CC10-driven and/or BADJ tumors were more invasive and metastatic in *K-Ras p53* mice (3). Thus, oncogenic genotypes differentially influence cells of origin in lung cancer, an effect seen in other cancer types (13).

Mainardi et al. (2) and Sutherland et al. (3) both confirm earlier findings that under different circumstances SPC-expressing and CC10-expressing cells and their precursors can all initiate tumors in response to *K-Ras* activation (4). Mainardi et al., Sutherland et al., and Xu et al. (2–4) provide evidence that AT2 cells are the predominant cell of origin of *K-Ras*–driven adenocarcinoma. However, under the right circumstances, club cells, BASCs, and progenitors of these cells are almost certainly able to act as cells of origin. Methods to drive Cre specifically in double positive cells will be required to definitively prove whether BASCs initiate *K-Ras* tumors.

These studies also raise the interesting possibility that *K-Ras*–activated cells pass through distinct differentiation states before giving rise to tumors composed of cells that appear more differentiated. Indeed, this has been observed in other cancer types such as basal cell carcinoma, where the nonstem tumor cells of origin are reprogrammed to resemble embryonic hair follicle stem cells (14, 15). In the steady state, CC10$^+$ cells do not appear to give rise to SPC$^+$ cells (16), but in response to bleomycin injury, CC10$^+$ cells can contribute to the SPC$^+$ AT2 population (17, 18). Sutherland et al. (3) observe single SPC$^+$ cells arising from *K-Ras*–activated CC10$^+$ cells, and they hypothesize that *K-Ras* activation may represent another specific condition that enables this differentiation to occur. Mainardi et al. (2) also speculate that viral Cre may alter the differentiation program of CC10$^+$ cells to a less mature state. Although not observed by Mainardi et al. (2) or Sutherland et al. (3), double positive cells have been observed in SPC$^+$ alveolar tumors (4).

Taken together, the emerging consensus is that the propensity of aggressive lung adenocarcinomas to develop from different initiating cells is influenced by multiple factors: developmental, environmental, and genetic. As tumorigenesis more likely occurs when cells are challenged by genetic mutation and environmental factors, it will be important to further delineate how these impact the cellular origins, pathology, progression, and therapeutic response of lung adenocarcinoma.

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