A role for club cells in smoking-associated lung adenocarcinoma

Sabine J. Behrend [1,2], Georgia A. Giotopoulou[1,2], Magda Spella[3] and Georgios T. Stathopoulos [1,2]

[1]Comprehensive Pneumology Center (CPC) and Institute for Lung Biology and Disease (iLBD); Helmholtz Center Munich-German Research Center for Environmental Health (HMGU) and Ludwig-Maximilian-University (LMU) Munich, Munich, Germany. [2]German Center for Lung Research (DZL), Giessen, Germany. [3]Laboratory for Molecular Respiratory Carcinogenesis, Dept of Physiology, Faculty of Medicine, University of Patras, Patras, Greece.

Corresponding author: Georgios T. Stathopoulos (stathopoulos@helmholtz-muenchen.de)

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Multiple lung epithelial cells are targets of carcinogenic hits. Club cells are such cells that can metabolically activate tobacco pre-carcinogens, being thus positioned as cells of origin of lung adenocarcinomas in smokers. https://bit.ly/3iOshcy

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Abstract

The cellular origin of lung adenocarcinoma remains a focus of intense research efforts. The marked cellular heterogeneity and plasticity of the lungs, as well as the vast variety of molecular subtypes of lung adenocarcinomas perplex the field and account for the extensive variability of experimental results. While most experts would agree on the cellular origins of other types of thoracic tumours, great controversy exists on the tumour-initiating cells of lung adenocarcinoma, since this histologic subtype of lung cancer arises in the distal pulmonary regions where airways and alveoli converge, occurs in smokers as well as nonsmokers, is likely caused by various environmental agents, and is marked by vast molecular and pathologic heterogeneity. Alveolar type II, club, and their variant cells have all been implicated in lung adenocarcinoma progeny and the lineage hierarchies in the distal lung remain disputed. Here we review the relevant literature in this rapidly expanding field, including results from mouse models and human studies. In addition, we present a case for club cells as cells of origin of lung adenocarcinomas that arise in smokers.

Introduction

Lung cancer is the most lethal cancer worldwide causing more than 1.7 million deaths in 2018 [1]. Lung adenocarcinoma (LUAD) is the most prevalent histologic subtype of lung cancer and accounts for almost half of all lung cancer deaths because of its indolent clinical presentation and its peripheral location in the lung parenchyma [2, 3]. As most lung cancers, and especially LUAD, are diagnosed when they have already become locally advanced or metastatic, the 5-year survival rate amounts to only 15% [4]. Despite rapid improvements in lung cancer prevention through smoking cessation and screening programmes, as well as targeted and multi-modality therapies in the last few decades, lung cancer remains a dreadful disease [5, 6]. While the incidence and mortality of many other types of lung cancer such as squamous cell lung carcinoma and small cell lung carcinoma are continuously dropping in more developed countries where smoking incidence is declining, LUAD incidence and mortality are constantly rising, a phenomenon ascribed to the changing face of manufactured cigarettes and the increasing occurrence of LUAD in nonsmokers [7–12].

Cancers are defined by both their genetic alterations and their cells and tissues of origin [13]. These precancerous cells and tissues of origin define which cells can potentially lead to cancer, and are likely distinct from stem cells in established tumours, which constitute the subset of cancer cells that possesses stem cell characteristics and can drive tumour progression, therapy resistance, relapse and metastasis [13]. It has been demonstrated that self-renewal pathways such as Wnt, Hedgehog, and Notch that are upregulated in embryonic stem cells are also commonly reactivated in tumour-initiating and cancer stem cells as well as in mature lung cancers, driving proliferation, resistance to apoptosis, epithelial-to-mesenchymal transition, metastasis, acquisition of new blood vessels and further genomic permutation [14]. Such lung

cancer initiating and stem cells possess self-renewal properties and are able to execute programmes of repair and normal tissue replacement during precarcinogenesis and established carcinogenesis [15].

Several cell types of the lungs hold tumour-initiating and stem cell properties and are thus potential cells of origin of lung cancer. To this end, p63(+)Krt5(+) distal airway stem cells likely relevant to airway basal cells have been shown to maintain and repopulate the airway and alveolar epithelium following viral injury, while club cell secretory protein (CCSP)-expressing club cells have also been shown to be capable of maintaining and repairing smaller bronchioles and alveolar structures [16–20]. Similarly, surfactant protein C (SFTPC)-producing alveolar type II (ATII) cells are responsible for maintenance of the alveolar epithelium [21, 22]. However, CCSP+SFTPC+ double positive bronchioalveolar stem cells (BASC) that reside in the terminal and respiratory bronchioles and alveolar ducts and can differentiate into club cells as well as alveolar cells were also shown to possess strong regenerative potential of both airway and alveolar epithelium [23]. An often underestimated and disputed pool of lung stem cells are of mesenchymal origin, located in all human tissues and organs and shown to migrate and differentiate into non-mesodermal cell types [24–26]. Additional cells that are lineage negative have been shown to reside in the lungs and to possess strong regenerative potential, while mesothelial cells were also shown to repopulate mesenchymal cells of the lungs and other internal organs [27, 28]. While lung stem cells and their functions are authoritatively reviewed elsewhere [29–33], the present review will summarise the current knowledge on the cells of origin of lung cancer with a special focus on club cells and their potential role as cancer stem cells of LUAD.

Methods

In addition to articles already known to the authors, PubMed (https://pubmed.ncbi.nlm.nih.gov/) was queried on 17 May 2021 using theterms: (‘lung cancer’[Title/Abstract] OR ‘lung adenocarcinoma’[Title/Abstract] OR ‘squamous cell lung carcinoma’[Title/Abstract] OR ‘squamous cell lung cancer’[Title/Abstract] OR ‘small cell lung cancer’[Title/Abstract] OR ‘small cell lung carcinoma’[Title/Abstract]) AND (‘stem cell’[Title/Abstract] OR ‘cell of origin’[Title/Abstract]), retrieving 1385 results. Titles and journal names were manually curated to yield 416 articles whose abstracts were screened to yield the articles that built the knowledgebase and reference list of the present review.

Results

The causes of cancer translated to lung adenocarcinoma

Heredity causes multiple forms of cancer that can be clinically manifest in childhood, but also in adult life, and can be spontaneous or co-precipitated by germline mutations and environmental factors such as smoking [34, 35]. Heredity can also indirectly cause cancer by influencing our interactions with the environment, as is the case with a single nucleotide polymorphism in the habenular nicotinergic acetylcholine receptor which renders individuals susceptible to nicotine addiction and thereby to COPD, lung cancer and peripheral arterial disease [36]. Environmental carcinogens are thought to be even more important than heredity in precipitating chest tumours in humans. The relationship between tobacco smoking and lung cancer is one of the best documented epidemiologic relationships, while the same goes for asbestos exposure and mesothelioma [37–44]. Radiation has also been tightly linked with lung cancer development based on a number of different data sources, including atom bomb survivors, nuclear plant workers, uranium miners, radiotherapy patients, and participants of lung cancer screening programmes [45–54]. Finally, an increasingly stronger case is in the making for the connection between urban air pollution and lung cancer [55–58]. While the list of environmental carcinogens that impact the lungs and pleura is getting longer every day, and are comprehensively reviewed elsewhere [38], a fascinating new hypothesis saw the light of day in recent years: the bad luck hypothesis by Tomasetti and Vogelstein examines the possibility of a significant proportion of human cancers resulting from stem cell divisions gone awry [59–62]. This ground-breaking work was based on measurements of cell division rates in the various organs using proliferating cell nuclear antigen (PCNA) and marker of proliferation Ki-67 staining of proliferating cells and extrapolation of the data by organ size and cell number. Indeed, PCNA+ cells in the resting lung are very sparse, and increase tremendously in lung cancers [59–62].

Hence, several different environmental and endogenous causes can precipitate lung cancer originating from the same and/or different lung lineages, and this heterogeneity is most evident with LUAD. It is highly likely that different molecular subtypes of LUAD exist, which emanate from different cells of origin that were tumour-initiated by different triggers, and such patient subgroups are evident in molecular and epidemiologic datasets. For example, we identified two subgroups of patients with LUAD in atom bomb survivors from Hiroshima and Nagasaki included in the Life Span Study that can be explained by exposures to smoking and to irradiation, and validated their existence in the TCGA cohort from the US [48]. In addition, molecular subsets of *KRAS*-mutant LUAD were identified within the TCGA cohort *via* elegant

genomic analyses [63]. This interpatient heterogeneity of LUAD needs to be addressed by future studies on the cell of origin of lung tumours, which should ascertain the tumour subtype under study.

Evidence from genetic mouse models of LUAD

Studies on the origins of LUAD have been boosted tremendously by the use of genetically engineered mouse models, which are valuable tools for tumour induction and lineage tracing. Model organisms have been genetically manipulated to conditionally express oncogenic or tumour suppressive alleles, in conjunction with CRE recombinase expressed under the control of a promoter active specifically in one of the different respiratory cell lineages, and are therefore ideal for the tracing of a specific cell population carrying specific mutations in time and space. Prominent focus has been given to the development of mouse models harbouring KRAS proto-oncogene GTPase (encoded by the human *KRAS* and the murine *Kras* genes) and tumour protein 53 (encoded by the human *TP53* and the murine *Trp53* genes) mutations, as oncogenic mutations of the *KRAS* and *TP53* genes are found in 34% and 54% of human LUAD [64, 65]. As a result, several mutant *Kras/KRAS* knock-in and *Trp53* knockout mouse models have been generated, with the most widely used among them being the *Lox-Stop-Lox-KRAS*^{G12D} model, which develops LUAD within 4 months post intranasal administration of adenoviral CRE, and the *Lox-Trp53-Lox* model, in which *Trp53* can be deleted in specific lineages and can cause more aggressive LUAD when combined with the *Lox-Stop-Lox-KRAS*^{G12D} model, as well as other transgenic mouse models that cannot be all mentioned here [64–68]. Pulmonary lineage tracing studies in the respiratory epithelium of these genetically modified mice following forced expression of the *Kras*^{G12D} mutation in club airway epithelial cells expressing CCSP, or in ATII alveolar epithelial cells expressing SFTPC, or in bronchoalveolar epithelial cells expressing both markers, resulted interchangeably in LUAD formation, leading to inconclusive data as to the progenitors of LUAD in adult mice [22, 23, 69–71]. This is partly attributable to the fact that some of the above-referenced lineage tracing mouse models feature incomplete and/or promiscuous lung cell labelling, to the heterogeneity among ATII cells regarding their proliferative and to tumour-initiating cell properties [22], but also to the viral-induced injury itself, since it was also shown that adenoviral infection alone contributed to the transformation of lung cells towards LUAD [71]. Similar genetically engineered mouse models reproducing other LUAD driver mutations such as *EGFR* mutations and *EML4-ALK* fusions have also been established and have proven the oncogenicity of the respective molecular changes when forcefully expressed in alveolar cells, implying that ATII cells are the cellular origins of multiple oncogene-driven LUAD tumours [72–74].

Evidence from environmental LUAD induction in mice

Although the above-referenced genetic mouse models have enhanced our mechanistic understanding of LUAD development, oncogene function and cell of origin, they do not fully capture the mutation diversity and burden of human LUAD, which is caused by environmental carcinogens rather than single oncogenes [74, 75]. To better recapitulate the mutational acquisition pattern and dissect the complex pathobiology of human LUAD, alternative strategies can be employed that combine conditional respiratory lineage tracing with carcinogenic insults. This approach is advantageous in recapitulating pathophysiologic endogenous carcinogenic events and in unravelling key events taking place during early tumour initiation, knowledge which can prove valuable for the development of LUAD early detection of chemoprevention strategies. Along these lines, we recently showed that as early as two weeks following treatment of lineage-marked mice with urethane (ethyl carbamate, a chemical carcinogen contained in tobacco smoke) [76], *Kras*^{Q61R} driver mutations accumulate specifically in club and not in ATII cells [20], in line with evidence from human airway epithelial cells found to be sensitised by tobacco smoke to a single-hit *KRAS* mutation [77] and from a massive parallel sequencing approach [78]. These results are also in accord with earlier studies that dictate that only club cells possess the cytochrome CYP2E1 [79, 80] that is required to convert the tobacco pre-carcinogen urethane (ethyl carbamate) to carcinogenic derivatives vinyl carbamate and its epoxide [81, 82], which in turn have a half-life of a few femtoseconds and can thus only injure the DNA of the same cell that metabolically activates them [83, 84]. Thus, club cells are likely to be the cellular source of LUAD triggered by the tobacco carcinogen urethane as opposed to ATII cells as cells of origin of transgenic lung tumours in mice [20, 85].

Indeed, environmental-induced LUAD in susceptible inbred mouse strains is a versatile research tool. To this end, single-hit LUAD emerge in sensitive FVB and A/J mice 6–9 months post-treatment with intraperitoneal urethane (ethyl carbamate), N-nitrosodiethylamine (DEN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosonornicotine (NNN), and N-methyl-N-nitrosourea (MNU), and uniquely recapitulate the mutational landscape of human LUAD [20, 75, 86–93]. Such models have been successfully used to study oncogene function in the genomic context and to reproduce human-relevant LUAD mutanomes in mice [75, 93]. Although chemical models of LUAD do not necessarily rely on human-relevant *Kras* mutations, they rather generate a human-relevant mutation spectrum in terms of

single nucleotide variants in the mono- and tri-nucleotide, as well as the gene contexts [75, 87]. For example, urethane-induced LUAD in mice feature the *Kras*^{Q61R} mutation, which is very rare in human LUAD, but at the same time they harbour multiple mutations in critical LUAD genes such as *Alk* and *Crebbp* that are highly relevant to human LUAD [75]. Combining such tools with lineage tracing allows spatiotemporal exploration of whole adverse outcome pathways leading from a specific carcinogen to lineage-restricted molecular initiating events, key progression events and clinicopathologic and molecular signatures that indicate the initiating agent and cell type. Using such an approach, club cells were shown to contribute to lung maintenance, repair and carcinogenesis, to possess stem cell features and to sustain chemical-induced *KRAS* mutations as LUAD cells of origin would [20, 85].

Evidence from human LUAD

Human LUAD patient cohorts have also been interrogated for cell of origin signatures, since abundant evidence supports that cell of origin is imprinted and deductible from molecular data [94, 95]. These studies have been sparse but imperative, since there are marked differences between mouse and human lung epithelial cell biology, rendering translation of mouse lineage tracing data to the human setting uncertain. Such studies are marked by inherent uncertainty, since lineage plasticity in the lungs is tremendous and even malignant lung tumours can switch histology and molecular profiles upon acquisition of new genomic alterations [96, 97]. To this end, one study exploited the finding of co-mutations of *KRAS* and *KEAP1* in 5% of LUAD [63] to ascribe different cells of origin (airway *versus* alveolar), as well as immunometabolic profiles to *KRAS*-mutant LUAD with or without *KEAP1* alterations [98]. In another effort to determine genomic imprints of cell fate, squamous and adenomatous lung tumours appeared highly similar, suggesting a common ancestor [99]. In our view, tumours of smokers and nonsmokers may very well have different cellular origins, and hence studies should focus on molecular hallmarks of smoking when examining lineage of origin, since such markers have been described, including KRAS and TP53 mutations, as well as the C>A transversions described elsewhere [100–102]. These data show the need for genetic lineage tracing models in the search for cells of origin of lung tumours, and for biomarkers of environmental and endogenous lung cancer causative agents. Furthermore, they illustrate the marked heterogeneity of LUAD, which needs to be taken into account in such lineage tracing studies.

Conclusions

The contribution of cells with stemness properties to tissue homeostasis, regeneration and tumour progression is undeniable, and this trait renders them attractive therapeutic targets. In heterogeneous tumours, stem cells will sustain tumour growth and possibly tumour recurrence. Chemical carcinogenesis mouse models faithfully recapitulate human LUAD, and have highlighted club cells as a central respiratory cell population with a key role in early initiation events leading to LUAD. Future perspectives should therefore be targeted to better characterise this cell type and to increase our comprehension of the mechanisms regulating cell biology, biomarker expression, mutational acquisition spatiotemporal patterns, molecular dynamics of tumour evolution and tumour architecture. Novel technologies, such as organoids, 3D whole organ imaging with single cell resolution, and single cell sequencing, have been developed to compliment the knowledge gained from transgenic mouse models and better understand the underlying tumour pathobiology. The new knowledge should be tested on human-relevant experimental pre-clinical models, so that effective therapies can be developed.

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