Airway basal cell heterogeneity and lung squamous cell carcinoma

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Abstract

Basal cells are stem/progenitor cells that maintain airway homeostasis, enact repair following epithelial injury and are a candidate cell-of-origin for lung squamous cell carcinoma (LUSC). Heterogeneity of basal cells is recognized in terms of gene expression and differentiation capacity. In this Issue, Pagano and colleagues isolate a subset of immortalized basal cells that are characterized by high motility, suggesting that they might also be heterogeneous in their biophysical properties. Motility-selected cells displayed an increased ability to colonize the lung *in vivo*. The possible implications of these findings are discussed in terms of basal cell heterogeneity, epithelial cell migration and modelling of metastasis that occurs early in cancer evolution.

Background

The human upper airway epithelium is a pseudostratified barrier that protects the lungs from infection and inhaled particulate matter using the mucociliary escalator. Broadly, this function is supported by three major cell types: mucosecretory cells, ciliated cells and basal progenitor cells that function to regenerate the differentiated luminal populations [1]. Malignant transformation of this epithelium leads to lung squamous cell carcinoma (LUSC), which accounts for approximately 35% of non-small cell lung cancers, occurs predominantly in the central airways and has a poor prognosis. LUSC tumors are thought to arise through a step-wise epithelial transformation involving squamous metaplasia, dysplasia and carcinoma-in-situ (CIS) before progression to invasive cancer. Increasingly, it is recognized that significant genetic diversity, the basis of tumor evolution, exists in these pre-malignant disease stages and even in normal epithelia. It is noteworthy that in LUSC not all pre-invasive lesions inevitably progress to malignancy and the genetic, cellular and epigenetic basis of these changes are areas of active investigation [2]. Ultimately, metastases - tumors derived from the primary tumor found in regional lymph nodes and distant organs - are the predominant cause of mortality among LUSC patients but, despite forming part of the late-stage clinical presentation of the disease, it remains unclear how and at what stage metastatic seeding occurs. Importantly, recent discoveries in other cancer types suggest that metastases can

evolve in parallel with the primary tumor from an early stage of the disease course [3].

How the cellular context in which mutations arise influences LUSC progression is not currently well understood but airway basal stem cells are the leading cell-of-origin candidate. These cells represent the major proliferative airway epithelial cell type, share expression of basal cell markers such as keratin 5 (KRT5) with LUSC tumors and can give rise to LUSC-like tumors in genetically engineered mouse models (GEMMs). In the current issue, Pagano and colleagues describe a novel approach to isolate a subpopulation of immortalized human basal cells that are highly motile in culture by sequential selection of cells capable of migration through micrometer-scale transwell inserts [4]. The motility phenotype is cell intrinsic, RAC1-dependent and can be exaggerated by overexpression of Snail. Through microarray analysis the authors identify a gene expression signature associated with increased motility in which many genes are also associated with poor outcome in LUSC. Further, motility-selected cells demonstrated enhanced lung colonization after intravenous injection in mice, suggesting the possible relevance of a motile phenotype to micrometastatic seeding. The development of a platform to isolate more motile cells from heterogeneous basal cell populations in a step-wise manner has potential implications for airway basal cell heterogeneity, airway epithelial cell migration and modelling metastatic seeding during early LUSC.

Airway Basal Cell Heterogeneity

Airway basal cells have long been thought to be a heterogeneous population of cells. Early data separated distinct populations of basal cells and ‘intermediate’ cells based on morphological properties such as the proximity of nuclei to the basement membrane and basal cells also exhibit differences in marker gene expression; for example, only a subset of KRT5+ basal cells express the transcription factor P63 or the injury-induced protein KRT14. More recent studies support the functional importance of these basal cell subsets in regard to differentiation. During homeostasis, parabasal ‘intermediate’ cells express markers of both basal and luminal cells (KRT5+P63-KRT8+) and are thought to represent differentiating cells. Indeed, in the murine trachea, Notch3 activation is a hallmark of parabasal cells that

can then respond to Notch1/2 signaling to be become secretory precursors and ultimately mature ciliated or mucosecretory cells [5, 6]. Interestingly, Notch3 is expressed by human parabasal cells and is downregulated in areas of squamous metaplasia, suggesting that aberrant differentiation of parabasal cells might be relevant in pre-invasive lesion formation [6]. In injury models, further heterogeneity has been described among P63+ basal cells as these rapidly become dual-positive for P63 and either MYB or Notch2 intracellular domain (N2ICD) and these cells only give rise to ciliated or mucosecretory cells, respectively [7]. As such, different basal cell lineage trajectories might be relevant to homeostasis and injury repair. However, despite these findings, little is known about basal cell heterogeneity assessed by measures other than differentiation capacity. As such, the suggestion that a subset of premalignant basal cells might be intrinsically migratory in nature is an exciting hypothesis.

Epithelial Cell Motility and Lung Cancer

That the motility phenotype described by Pagano et al. is cell intrinsic, i.e. retained over serial cultivation, is suggestive of a relevance to *in vivo* basal cell heterogeneity and airway cell migration but care must be taken in extrapolating these results due to the possibility of changes introduced by cell culture. Basal cells were immortalized using retroviruses carrying *Cdk4* and *hTERT* [8] and it remains to be determined whether this migratory subset is present in primary human basal cells prior to immortalization. Although the protocol avoids the use of viral oncogenes, these immortalized cells still contain structural genomic rearrangements that could theoretically influence cellular behavior. Beyond this, many important questions are raised. Firstly, what is the basis of heritability of the motility phenotype? Although highly motile cells are found in all three donor lines investigated, the authors find important differences between these, suggesting that multiple mechanisms to achieve similar phenotypes might exist. Moreover, does such a motile basal cell subpopulation exist *in vivo*? And what proportion of cells are motile? Previous lineage tracing of homeostatic airways has suggested low levels of lateral motility of epithelial cells but low frequency, highly motile cells might be missed in such assays and frequently clonal relationships cannot be proven conclusively. In one human patient, spatially distinct pre-invasive CIS lesions appeared to be clonally related

based on a common, rare P53 mutation [9], so further studies to delineate the relationship between epithelial cell migration and field carcinogenesis are warranted.

Pre-clinical studies of metastasis tend to focus on very late stage disease by using cell lines with high mutational burden and extensive genomic rearrangement but recent evidence has called into question the hypotheses that individual cells from the primary tumor seed metastases and that seeding of metastases occurs only during the late stages of tumor evolution. As such, novel strategies such as those described by Pagano et al. to investigate early micrometastatic seeding in LUSC are welcome. The authors demonstrate that dual *TP53*/*KRAS*-mutant immortalized basal cells do not survive long term in the lungs following intravenous injection in mice. However, the same cells were able to survive in the lungs following motility selection, suggesting that they are better suited for lung colonization. The basis of this is an important open question as gene expression data suggest that motility-selected cells are not undergoing epithelial-mesenchymal transition (EMT). The authors show that their motility-based selection does not favor cells with small size, small nuclei or high deformability but future studies on the nuclear integrity of motility-selected cells will be of interest. Nuclear rupture leading to DNA damage occurs during migration of cancer cells through pores of 5-20 μm [10] so aberrations sustained during migration - rather than motility itself – could potentially lead to selection of cells with enhanced colonization potential. While the current study employs *KRAS*-mutant cells, these mutations are rarely seen in the LUSC patient population so extension of this protocol into cell lines with P53 mutations in combination with other early events in the progression of lung squamous carcinogenesis might be very informative.

Modelling Pre-Invasive Lung Squamous Cell Carcinoma

Overall, the lack of available model systems to study pre-invasive LUSC is a major limitation for the field: tumors from GEMMs typically do not share comparable mutation profiles with human disease and do not recapitulate the progressive nature of human lesions. A recent study investigated the role of *SOX2* - a transcription factor located on chromosome 3q, which is amplified prior to LUSC invasion - in early LUSC using the same immortalized cell lines as those used by Pagano et al. [11]. Combined P53 knockdown and SOX2 overexpression lead to dysplastic

transformation of organotypic cultures. In combination, these studies demonstrate significant recent progress in modelling key aspects of pre-invasive disease. To our knowledge, primary cell culture methods for expansion of CIS cells have yet to be described and so studies that manipulate immortalized normal airway basal cells to provide a platform amenable to testing much needed therapeutics for early intervention LUSC studies are a welcome advance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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