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Enriching the molecular definition of the airway "field of cancerization": establishing new paradigms for the patient at risk for lung cancer

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Abstract

The "field of cancerization" refers to histologically normal-appearing tissue adjacent to neoplastic tissue that displays molecular abnormalities, some of which are the same as those of the tumor. Improving our understanding of these molecular events is likely to increase our understanding of carcinogenesis. Here, Kadara *et al.* attempt to characterize the molecular events associated temporally and spatially within the field of cancerization of early stage non-small cell lung cancer (NSCLC) patients following definitive surgery. They followed patients with bronchoscopies annually after tumor resection and extracted RNA from the serial brushings from different endobronchial sites. They then performed microarray analysis to identify gene expression differences over time and in different sites in the airway. Candidate genes were found that may have biological relevance to the field of cancerization. For example, expression of phosphorylated AKT and ERK1/2 was found to increase in the airway epithelium with time. Despite a number of limitations in the study design, this investigation demonstrates the utility of identifying molecular changes in histologically normal airway epithelium in lung cancer. In addition to increasing our understanding of lung cancer biology, studying the field of cancerization has the potential to identify biomarkers from samples obtained in a minimally invasive manner.

Field of cancerization

In seminal studies defining histological changes in oral malignancies, Slaughter and colleagues first used the term “field cancerization” to describe the histologically normal-appearing tissue adjacent to neoplastic lesions that display molecular abnormalities, some of which are the same as those in the tumors[1]. Since that time, investigators have attempted to define the underlying molecular events leading to field cancerization in several different epithelial malignancies, including lung cancer[2,3]. In contrast to other common epithelial malignancies, there is not yet a clinical rationale to evaluate potential premalignant events in the patient at risk for lung cancer. Thus, carefully designed clinical investigations are required to harvest airway specimens that would not otherwise be collected in these individuals. In the absence of a critical mass of such studies, knowledge regarding the molecular changes that occur in the airway epithelium in the setting of lung cancer is only fragmentary. However, it is generally accepted that there are alterations in the airway epithelium that mirror some of the changes seen in the lung cancer itself and that defining these changes could lead to the identification of biomarkers of lung cancer recurrence and provide a pathway for targeted chemoprevention.

The field of cancerization theory has been tested and shown to be present in other epithelial cell malignancies, such as prostate, head and neck, colon, esophageal, and breast cancer[4–6]. In fact, the concept of field of cancerization has led to the use of novel technologies to predict risk of cancer based on testing of cells from the field. Lee *et al.* used aberrant DNA methylation patterns of four candidate genes in the normal esophageal mucosa adjacent to squamous carcinoma to develop a risk assessment model that predicted the risk of finding squamous cell carcinoma of the upper aerodigestive tract at endoscopy[7]. Damania *et al.* used a novel imaging technique, partial wave spectroscopic microscopy, to risk-stratify patients harboring precancerous lesions of the colon[8]. In this study, an optically measured biomarker was obtained from microscopically normal but nanoscopically altered cells that may provide the potential for minimally invasive colorectal cancer risk stratification.

It is hypothesized that lung cancer has a field of cancerization similar to these other epithelial malignancies[2,9,10]. In lung cancer, mutations in *KRAS* were described in non-malignant histologically normal-appearing lung tissue adjacent to lung tumors[2,11]. Moreover, loss of heterozygosity events are frequent in cells obtained from bronchial brushings of normal and abnormal lungs from patients undergoing diagnostic bronchoscopy and were detected in cells from the ipsilateral and contralateral lungs[12]. Likewise, mutations in the *EGFR* oncogene have been reported in normal-appearing tissue adjacent to *EGFR* mutant lung adenocarcinoma and also occurred at a higher frequency at sites more proximal to the adenocarcinomas than at more distant regions[13,14]. More recently, global mRNA and microRNA expression profiles have been described in the normal-appearing bronchial epithelium of healthy smokers[15,16], and a cancer-specific gene expression biomarker has been developed in the mainstem bronchus that can distinguish smokers with and without lung cancer[17,18]. In addition, and importantly, modulation of global gene expression in the normal bronchial epithelium in healthy smokers is similar in the large and small airways, and the smoking-induced alterations are mirrored in the epithelia of the mainstem bronchus, buccal, and nasal cavities[19–21].

Recent molecular findings support the stepwise lung carcinogenesis model in which the development of the field of cancerization with genetically and epigenetically altered cells plays a central role. The hypothesis is that injury (from smoking, for example) leads to aberrant repair by stem/progenitor cells, which undergo self-renewal to form a group of indefinitely self-renewing daughter cells. Additional genetic and epigenetic alterations prevent normal differentiation of these cells and instead result in proliferation of these cells

and expansion of this field, gradually displacing the normal epithelium. Development of an expanding premalignant field appears to be a critical step in lung carcinogenesis that can persist even after smoking cessation. It is then hypothesized that further genetic and/or epigenetic changes result in the stepwise progression of premalignant lesions to full blown malignancy. If this is true for the development of lung cancer, then there is the potential for chemoprevention in at risk patients by targeting the biology underlying the field of cancerization and stepwise progression to malignancy.

Understanding the biology of field of cancerization may also be relevant for developing potentially novel therapies for lung cancer. Currently, lung cancer lags behind other fields in its studies of the field of cancerization, largely because the role of premalignant lesions in the stepwise development of lung cancer has not yet been clearly delineated. This makes it all the more important to study the field of cancerization in order to identify novel drivers for the molecular pathogenesis of lung cancer, some of which could be biomarkers in the field or novel targets for lung cancer prevention or early treatment.

Studies of the field of cancerization enrich our understanding of the molecular pathogenesis of lung cancer and have potential transformative clinical value. Biomarker signatures within the field could be used for risk assessment, diagnosis, monitoring progression of disease during active surveillance, and predicting the efficacy of adjuvant therapies following surgery. As the field of cancerization for lung cancer may extend to the nose and mouth, these are attractive areas for study of the “field”, because they represent potential minimally invasive sites for risk assessment and diagnostic testing[18,19]. Studies are now underway assessing these minimally invasive and potentially cost-effective pre-screening strategies using biomarkers obtained from the field of cancerization to identify high risk individuals from the at risk population. In the future, following validation, these biomarkers may be utilized to stratify the high-risk patients to undergo CT scans.

While the studies above have begun to characterize the molecular “field of injury” among smokers with lung cancer, a number of key questions remain to be addressed. The temporal and spatial pattern of gene expression changes in the airway of smokers with lung cancer has yet to be characterized, and it is unclear what, if any, spatial gradient in the airway transcriptome alterations exist relative to the site of tumor development. Before utilization as a screening tool for disease, we must define how far in advance of tumor development this field of cancerization arises within the airway. Importantly, the impact of histologic and molecular subtypes of lung cancer, as well as lung cancer treatment, on airway gene expression profiles remains to be defined, having implications both in terms of the underlying mechanism driving these alterations and their potential to serve as biomarkers of lung cancer recurrence.

Improving our understanding of changes in the field of cancerization over time in early stage NSCLC patients after definitive surgery

In the current issue of the journal, Kadara *et al.* attempt to address some of the gaps in our understanding of the field of cancerization by profiling whole-genome gene expression temporally and spatially within the airway of early stage NSCLC patients following definitive surgery. This study employs a unique study design by following patients’ airway epithelial biopsies over time, starting with collection of the first samples within the first year following definitive surgery, then every 12 months thereafter for up to 36 months. A total of 19 patients were followed with serial bronchoscopies with brushings during this time. Importantly, the study was limited by lack of access to airway samples from these patients prior to surgical resection. Thus, it is unclear what impact, if any, surgical treatment had on the temporal (or spatial) changes observed in the field of injury, as there is no “baseline” for

comparison. Despite these limitations, candidate genes were found that may shed light on the dynamic nature of the field of cancerization over time. For example, expression of phosphorylated AKT and extracellular signal-regulated kinase (ERK1/2) was found to increase in the airway epithelium with time following surgery. This was confirmed at the protein level by immunostaining for AKT and ERK. The potential clinical implications of this finding are unclear, however. Additional studies with larger patient populations are needed to evaluate the potential association of these temporal changes in airway gene expression with disease recurrence.

Improving our understanding of spatial changes in the field of cancerization in early stage NSCLC patients after definitive surgery

One of the strengths of this study was the ability of these investigators to collect airway brushings from at least four areas obtained from each of the 19 patients at annual bronchoscopy. This technical *tour de force* allowed unprecedented characterization of the field of cancerization by comparing gene expression levels in the airways adjacent to the tumor to brushings from the main carina and contralateral airways. Functional pathway analysis using Ingenuity Pathways Analysis revealed significantly differentially expressed signaling pathways in the “adjacent” samples as compared to the main carina and “contralateral” samples. The potential significance of this spatial “gradient” in the molecular field of cancerization, however, is not entirely clear. The large number of genes that change spatially throughout the airway may relate to the location of the tumor or may simply reflect changes related to the different cell types collected based on the location brushed. Spatial mapping of gene expression profiles in the airway of smokers without lung cancer (as a control group) is needed to shed light on the significance of the changes observed here. Additionally, profiling gene expression in the airway of smokers with lung cancer prior to tumor resection would ensure that the spatial (and temporal) changes in gene expression identified in this study do not reflect recovery from the adjacent surgical procedure.

Importantly, gene networks mediated by the phosphoinositide 3-kinase (PI3K) and ERK gene networks were upregulated in the airways adjacent to the resected tumor. Gustafson *et al.* previously reported the identification of a gene expression signature of upregulated genes involved in the PI3K pathway in the cytologically normal airways of high-risk smokers with premalignant lesions and lung cancer[22]. Dysregulation of the PI3K pathway in the field of cancerization may therefore represent an early event in lung carcinogenesis that may persist even after resection of the primary tumor. Further evaluation of the PI3K pathway in the airways of smokers pre- and post-resection of their lung cancer is needed.

Conclusions

This study provides proof of principle that it is possible to serially evaluate changes in gene expression in the airway over time in patients with lung cancer. Further studies are needed to temporally and spatially profile the airway transcriptome in smokers with and without lung cancer prior to and after surgical resection. Investigators in the field are also challenged to continue to relate newly discovered molecular events to risk, clinical outcome, and prediction of response to prevention and therapy. This will pave the way for future utilization of similar approaches to identify molecular abnormalities in the airways of high risk individuals, leading to: 1) biomarkers for lung cancer risk assessment and selection for image-based screening, 2) specific targeted chemoprevention, and 3) selection of post-operative interventions. Molecular characterization of lung cancers has led to transformative changes in the treatment of patients with advanced stage NSCLC. As a result of investigations of molecular characterization of the field of cancerization, we are now at the

dawn of a new era in which clinical practice-changing advances may be achieved for individuals at risk for lung cancer.

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