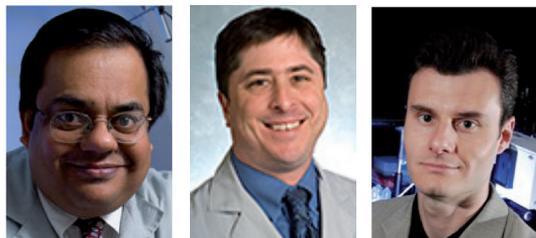# Nanocytology for field carcinogenesis detection: novel paradigm for lung cancer risk stratification

"PWS nanocytology may allow for a minimally intrusive, highly accurate modality to personalize … lung cancer screening."

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Despite being curable at early stages, lung malignancies remain the leading cause of cancer deaths among North Americans, underscoring the need for more effective early diagnostic strategies. Lung cancer should be eminently screenable since the at-risk population is well defined (~90% of lung cancer occurs in current or former smokers) [1]. While previous trials with chest x-ray and sputum cytology has been disappointing, there has been renewed interest in screening using low-dose computerized tomography (LDCT). The landmark results came from a randomized trial of 55,000 smokers (National Lung Cancer Screening Trial) that demonstrated that LDCT screening resulted in a 20% reduction of lung cancer deaths. However, the false positives rate with LDCT was substantial (cumulative rate of 33% after two LDCTs). Importantly, 7% of these false positive LDCTs mandated an invasive procedure (generally bronchoscopy) [2]. The poor positive predictive value results from the low prevalence of lung cancer in the asymptomatic smokers (~1%) and represents one of the most vexing barriers to LDCT screening [3]. Indeed, even with excellent test performance (e.g., 90% sensitivity and 90% specificity), more than nine in ten positives are actually false positives in conventional lung cancer screening groups. Thus, the concerns over cost and harms from false positives (e.g., patient anxiety, cost and complications from unnecessary invasive procedures) represent major impediments to the implementation of LDCT for population screening.

A potential solution would be to identify smokers at highest risk for lung cancer, since 90–95% of all smokers will never develop this malignancy during their lifetimes. However, the factors that portend a smoker developing lung cancer are poorly understood. One source of complexity is that tobacco smoke has more than 60 mutagens that interact with the host genome in a multitude of ways, making it difficult to predict of the occurrence of 'driver' mutations in lung carcinogenesis [4]. In addition, while numerous genetic susceptibility loci have been identified (e.g., chromosome 15), there are probably many more heretofore undiscovered genes [5]. Given the uncertainties engendered by the variability in carcinogens and host response, gauging the tissue response would be a more promising approach.

"…PWS nanocytology is exquisitely sensitive to the subtle genetic/epigenetic alterations of field carcinogenesis…"

The predilection to neoplastic transformation should be identifiable throughout the organ of interest based on field carcinogenesis. Field carcinogenesis is the notion that the genetic/environmental milieu that results in a neoplastic lesion in one area of an organ should be detectable, at least in some form, throughout the organ. This is well established in most cancer types. In the aerodigestive tract, this is manifested clinically as the increased incidence of secondary tobacco-related primaries in the field of injury (e.g., the lung, esophagus, head and neck). In the bronchial epithelium, the foci of dysplasia or genetic mutations (e.g., p53) can be found widely dispersed in lung cancer patients [6].

There has been considerable interest in exploiting field carcinogenesis for risk stratification in lung cancer by focusing on the earliest stages of

Keywords

■ biophotonics ■ field carcinogenesis ■ lung cancer ■ risk stratification ■ screening

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neoplastic transformation (microscopically normal bronchial epithelium). In a seminal report, Spira and colleagues demonstrated 80-gene signatures from brushings of the endoscopically normal bronchial epithelium differentiated patients with lung cancer from smoking-matched cancer-free patients [7]. However, using the right mainstem bronchus may be too intrusive for population screening. On the other hand, the field of injury from tobacco should be more diffuse throughout the aerodigestive tract. Therefore, the buccal (cheek) mucosa represents a clinically and biologically attractive site to interrogate (i.e., extended field carcinogenesis concept [6]). Indeed, there is an abundance of evidence that the buccal mucosa may be the 'molecular mirror' of lung carcinogenesis [8]. The key issue is identification of field carcinogenesis in a buccal mucosa with a modality that is both accurate and practical. Genomics are powerful techniques, but they can be confounded by the genetic heterogeneity in tumorigenesis. Further complicating this analysis is the impact of numerous epigenetic factors (e.g., DNA methylation and miRNA).

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An alternative approach is to assess the ultrastructural consequences of these genetic/epigenetic alterations. Many critical molecular events in carcinogenesis (e.g., Src, E-cadherin and antigen-presenting cell) would be predicted to interact with both cytoskeletal and nuclear alterations. These alterations are generally not apparent with conventional light microscopy because of the diffraction limit of light. Therefore, even though there may be profound alterations during carcinogenesis in structures less than approximately 500 μm (e.g., ribosomes, mitochondria and macromolecular complexes), these cell would be histologically normal given the limit of the resolution of light microscopy [9].

Current imaging techniques have lacked the ability to practically assess cellular nanoarchitecture. We have developed a novel optical technology, partial wave spectroscopic (PWS) nanocytology, that is able to quantify length scales that are an order of magnitude less (~20 nm) than those detectable with visible light. PWS nanocytology quantifies the optical refractive index by the parameter disorder strength (Ld). Since optical refractive index is linearly proportional to the local density of macromolecules (e.g., proteins, lipids and DNA), Ld reflects the quantity and organization of these cellular building blocks.

We have noted that PWS nanocytology is exquisitely sensitive to the subtle genetic/epigenetic alterations of field carcinogenesis in both cell culture and animal models. Importantly, our studies indicated that Ld invariably paralleled the malignant potential despite the cellular phenotype remaining normal [10]. Thus, PWS may be able to identify the neoplastic consequences of smoking in lung carcinogenesis. We postulated that buccal PWS analysis may represent a powerful approach for field carcinogenesis detection without being impacted upon by factors such as salivary RNases (which have impeded microarray studies) [11].

Therefore, we performed a proof-of-concept trial with brushings from the visually normal buccal mucosa from 135 smokers being subjected to PWS analysis [12]. We observed that the Ld was increased in the buccal mucosa of patients who harbored lung cancer versus smokers who were cancer-free. The area under the receiver operator curve was excellent at 0.85. With reference to potential screening applications, there is the observation that the performance characteristics appeared equivalent for early- and late-stage disease. Moreover, the effect size seemed to be comparable between histologies of primary lung cancers – for instance, small-cell and non-small-cell lung cancer were equivalent. Finally, the degree of smoking (pack–years) or other demographic factors did not seem to be confound the relationship between buccal Ld and lung cancer status [12].

It bears reiteration that the biological underpinning of this approach is to focus not on the heterogeneous genetic or epigenetic alterations, but rather the nanoarchitectural manifestations that we posit to be a common denominator in early neoplastic transformation. Therefore, the nanocytological detection of field carcinogenesis appears to be a platform that can be used for a variety of malignancies. For instance, we have used the technique via brushing the endoscopically normal rectum and noted that the Ld was increased in a stepwise fashion for patients harboring adenomas and advanced adenomas elsewhere in their colon. This can be useful in the primary care setting coupled with the simple digital rectal examinations to improve the yield of screening colonoscopy, since the current yield of colonoscopy in average risk patients (age ≥50 years) is only approximately 6%. With regard to field carcinogenesis detection in pancreatic cancer, the most promising surrogate site is the duodenum given the shared embryological origins. In addition, studies have demonstrated that the methylation patterns in uninvolved

duodenal mucosa was altered in pancreatic cancer patients [13]. Therefore, we performed PWS analysis on brushings of the endoscopically normal peri-ampullary duodenum and noted the ability of nanocytology to discriminate between pancreatic cancer patients and matched controls [14]. It should be underscored that in all organ systems evaluated to date (lung, colon, pancreas, esophageal and ovarian), the Ld elevation appears to be a universal marker of field carcinogenesis (both organ and extended) [9,14].

Aside from population screening, this approach could have several other potential clinical applications. Thus, in the colon, rectal PWS analysis may be useful in tailoring screening regimens in the common clinical scenario of a patient with a family history of colorectal cancer but for whom the responsible gene(s) have not been identified. Similarly, guidance for surveillance (follow-up of patients with history of neoplasia) would be useful since this is the indication for approximately 20% of all colonoscopies but still has a yield of clinically relevant neoplasia of only 5–15%. In the lung, one could envision utilization of buccal nanocytology as being an important adjunct in the management of patients with solitary lung nodules. From a therapeutic perspective, this may represent evaluation of novel targets for intervention or, equally importantly, monitoring response to therapy (chemoprevention and chemotherapy).

In summary, we believe that nanocytological assessment of field carcinogenesis (both direct and extended) represents an important biological and clinical breakthrough. This appears to be an extraordinarily powerful approach to deal with one of the major conundrums of population screening for cancer: identifying whom to screen given the low prevalence rate. Thus, PWS nanocytological risk stratification may allow:

- Enrichment of the yield of patients undergoing conventional screening (e.g., LDCT for lung cancer);

- Equally importantly, the identification of low-risk patients who are unlikely to benefit from screening and hence should forego the expense, discomfort and risk/harm (from radiation/false positives) of conventional cancer screening.

Therefore, PWS nanocytology may allow for a minimally intrusive, highly accurate modality to personalize lung (and other) cancer screening.

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