Lung Cancer Risk Determination with Low Coherence Enhanced Backscattering Spectroscopy (LEBS) Analysis of Buccal Mucosa


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Drs. Roy and Backman are co-founders/shareholders of American BioOptics LLC which has licensed to the LEBS technology
Lung Cancer Screening Issues

- Leading cause of cancer deaths in Western countries.
- Smoking responsible for ~90% of lung cancer enabling identification of appropriate screening population.
- Screening is effective as the National Lung Screening Trial demonstrated a ~20% reduction in lung cancer mortality among smokers with low dose CT (NEJM 2011)
- However lung cancer prevalence even in highly selected population was low (<2%) resulting in the vast majority of positive tests being false positives.
- False positive rates are high
  - After two rounds if 33% of subjects had significant false positive results.
  - 7% undergoing unnecessary invasive procedures.
  - Croswell et al, Ann Int Med 2010
Biophotonics for Lung Cancer Screening

- Optics widely applied to cancer screening mainly for detection of dysplasia or pathological determination (“optical biopsy”)
- Our multidisciplinary group of clinicians, biomedical engineers and cancer biologists are focused on developing light scattering technologies for risk stratification in colorectal, lung and pancreatic cancer
- The approach has been to identify field carcinogenesis as a surrogate for cancer risk.
Field Carcinogenesis (Field of Injury) in Lung

MARKERS IN THE NORMAL MUCOSA

- **Bronchial Epithelium**
  - p53
  - LOH
  - Genomic (right mainstem) with sensitivity of 80% and specificity of 84%

- **Buccal Epithelium**
  - “Molecular mirror” for lung cancer.
  - LOH studies
    - Sanz-Ortega et al., Histol Histopathol. 2007 May;22(5):541-5
  - Genomic
    - Sridhar et al., BMC Genomics 2008

Steiling et al., Cancer Prev Res 2008
Length Scales of Structural Alterations in Field Carcinogenesis

Microarchitectural consequences of Genetic/epigenetic alterations

C

Lung Carcinogenesis

EIBS
Collagen matrix alterations
Cytoskeleton
Higher order chromatin structure
Organelles

Light microscopy
>250-500 nm

Endoscopy
Field carcinogenesis (not seen)

Nanocytology
(10-200 nm)

Microvascular
Tissue ultrastructure
Cell nanarchitectue

LEBS
(150-800 nm)
Low-Coherence Enhanced Backscattering Spectroscopy (LEBS)

- Based on a self-interference phenomena in tissue optics discovered by the Backman lab in which time-reversed photons traveling in same pathway constructively interfere
  - Kim, Backman et al., Optic Lett 2004

- Can sense and quantify objects 10-20 times smaller than detectable with conventional microscopy.
  - Kim, Backman et al Appl Optics 2005
  - Kim, Backman et al Optics Lett 2005

- We have previously demonstrated that LEBS was sensitive to field alterations in the microscopically normal mucosa for colon carcinogenesis
  - Roy, Backman et al., Clinical Cancer Res 2006
  - Roy Backman et al, Cancer Res 2009
Hypothesis

- LEBS analysis of visually normal buccal mucosa would be able to discriminate between smokers with and without lung cancer.
Methods

- Case-control study with a blinded independent validation set
- Cases: Pathologically confirmed lung cancer prior to therapy
- Controls: Smokers without diagnosis (current or past) of lung cancer.
- Eight readings were taken (each requiring ~250 milliseconds) by gently applying probe to buccal mucosa.
- LEBS marker was developed attempting to minimize the number of micro-architectural and microvascular parameters
- Statistical analysis was performed by an independent biostatistician using SAS 9.2 and R 2.1.
Instrumentation
LEBS Markers

LEBS markers
(E, W, S)

Microarchitectural markers
(fractal dimension, variance and correlation length of density spatial variations) + microvascular markers

Logistics Regression

LEBS marker

Roy, Backman et al., Clin Cancer Res 2006
# Cohort (n=68)

<table>
<thead>
<tr>
<th></th>
<th>Number Of Patients (Training)</th>
<th>Number Of Patients (Testing)</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td><strong>Non Smokers</strong></td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>19</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td><strong>Lung Cancer</strong></td>
<td>21</td>
<td>13</td>
<td>34</td>
</tr>
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</table>
Two Parameter Buccal LEBS Marker : Training Set

P < 0.001
Buccal LEBS Performance

Threshold for testing set selected to maximize sensitivity at cost of specificity
Buccal LEBS Marker: Testing Set

LEBS Marker

P < 0.0001

Smokers

Lung Cancer
## Buccal LEBS: Blinded Validation

### Performance

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<table>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>93%</td>
</tr>
<tr>
<td>Area under ROC curve (AUC)</td>
<td>0.974</td>
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LEBS Marker and Pathological Subtype (Combined Training and Testing Set)

- Smokers: n=5
- Small cell carcinoma: n=29
- Non-small cell carcinoma: n=7
- Squamous cell carcinoma: n=18
- Adeno-carcinoma: n=4
- Unknown: * p<0.00001

LEBS Marker

- Smokers: p=0.07
- Small cell carcinoma: *
- Non-small cell carcinoma: *
- Squamous cell carcinoma: *
- Adeno-carcinoma: *
- Unknown: *
Buccal LEBS Marker and Tumor Stage (Combined Datasets)

- Smokers
- Stage 1 & 2 Cancer
- Stage 3 & 4 Cancer

* p<0.00001 Versus smokers

P=0.5
### Buccal LEBS and Lung Cancer Risk Factors: ANCOVA Analysis for Confounding

<table>
<thead>
<tr>
<th>Confounding Factor</th>
<th>ANCOVA P-Value</th>
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<tr>
<td>Presence of Neoplasia</td>
<td>&lt;0.0001</td>
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<tr>
<td>Smoking History</td>
<td>0.5036</td>
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<tr>
<td>Race</td>
<td>0.8068</td>
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<tr>
<td>Gender</td>
<td>0.8756</td>
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<tr>
<td>AGE</td>
<td>0.9253</td>
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</table>
Buccal LEBS marker appeared to be an accurate modality for discriminating between smokers with and without lung cancer.

The marker was comprised of two parameters mitigating risk of overfitting.

This was validated in an independent dataset.

The relationship did not seem to be confounded by lung cancer risk factors.

Importantly, buccal LEBS was sensitive to early stage (potentially curable) lung cancer.

Major limitation is modest sized data set with larger scale trials currently ongoing.
Buccal LEBS in the Clinical Armamentarium

Other Potential Applications

- Companion biomarker (response to chemotherapy or chemoprevention)
- Risk stratification of lung nodules