Immune Changes in Tumor-Draining Lymph Nodes as Novel Biomarkers

Peter P. Lee, M.D.
Dept. of Medicine (Hem)
Stanford University
TDLNs in Breast Cancer

- Tumor invasion of TDLNs is a key determinant of clinical outcome in breast and other cancers
- Lymph nodes are immune organs!
- Are immune cell populations (T cells, B cells, and dendritic cells) altered in sentinel and axillary lymph nodes?
- If so, do immune changes in a LN reflect tumor invasion?
- Do immune changes predict clinical outcome?
Integrated image analysis approach

1. Multicolor staining of tissue sections

2. High-res spectral imaging & automated scanning of the whole tissue section

3. Machine-learning-based cell identification by GemIdent → number of cells and Cartesian coordinates

4. Quantitative and spatial statistical analyses
Multicolor staining

Tumors

T cells
DCs
Other cells
Imaging & scanning by Vectra™

Build a classifier:
enable the program to recognize tissue vs. non-tissue (empty spaces, bubbles, fats, etc).

= tissue
= non-tissue

Build a spectral library:
enable the program to unmix signals from different chromogens

Set up the scan:
- Organize slides in cassettes
- Set up autofocus
- Take brightfield reference images
- Determine imaging area
- Determine threshold for tissue finding (for both LPF and HPF imaging)
Chromogen unmixing and image reconstruction by Vectra™

Original image

Unmixed images

Re-constructed composite image
GemIdent analysis

Whole image overview
Maps of each cell type

- Tumors
- DCs
- T cells
- Other cells

Image showing a semi-transparent tissue section with labeled cell types: Tumors, DCs, T cells, and Other cells. The cells are represented in different colors and patterns for clarity.
Analysis of Results

- Number and proportion of each cell type
- Spatial statistics: architectural pattern analysis of immune cells and tumor cells

Relationship with >5-year clinical outcome & parameters
Proof of Concept: Breast Cancer

– Total 77 stage IIA, IIB, IIIA breast cancer patients analyzed
– All patients had positive SLN biopsy and thus had ALN dissection
– Significant numbers of recurrences within 5 years allowing correlation with clinical outcome
– 10 non-cancer LNs analyzed as controls

IHC analysis of TDLN in breast CA

Breast cancer cells: AE1/AE3

Immune cells:
- CD4 ‘helper’ T cells
- CD8 ‘cytotoxic’ T cells
- CD1a dendritic cells - ‘antigen presentation’
Immune Alterations Correlates with Tumor involvement of TDLN

<table>
<thead>
<tr>
<th>Immune Profile</th>
<th>Tumor-free lymph node % (mean ± SE)</th>
<th>Tumor-involved lymph node % (mean ± SE)</th>
<th>Wilcoxon rank sum test P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph Node Predictors of Lymph Node Metastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4%</td>
<td>17.85±2.19</td>
<td>2.11±0.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8%</td>
<td>7.93±0.99</td>
<td>7.52±0.71</td>
<td>0.682</td>
</tr>
<tr>
<td>CD1a%</td>
<td>3.59±0.56</td>
<td>0.26±0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4/CD8 Ratio</td>
<td>2.47±0.28</td>
<td>0.34±0.08</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*P-values adjusted for multiple comparisons.
TDLN Immune Status Correlates with DFS
DFS Stratified by Tumor Stage (T1) and TDLN Immune Profile
DFS Stratified by Tumor Stage (T2) and TDLN Immune Profile

![DFS Survival Curves](image)
Beyond numbers: spatial patterns

• Different cell phenotypes constitute a “marked point process”
• Goal: to quantify spatial characteristics in order to understand cellular interaction
• Preliminary Findings: T and B cells from TDLN and healthy LN have different spatial distribution patterns [PLoS ONE 5(8):e12420, 2010]
Dendritic Cell Clustering and Relapse

Relapsed

Disease Free
Summary

• Numerical and spatial changes arise in immune cells in TDLNs
• Some of these changes appear to predict clinical outcome
• Quantitative, spatial analysis tools for histology have been developed for high throughput analysis
• Immune cells in TDLNs provide novel biomarkers for cancer
• Proof-of-concept in breast cancer – extend to melanoma, GI cancer
Acknowledgments

Holbrook Kohrt, MD
Francesca Setiadi, PhD
Valeria Carcamo-Cavazos
  Adam Kapelner
  Andrew Chang

Fred Dirbas, MD (Surg Onc)
Erich Schwartz (Pathology)
Susan Holmes, PhD (Statistics)

Notre Dame
Danny Chen, PhD (Computer Science)
Mark Alber, PhD (Math)
The following relationships exist related to this presentation:

None