Stromal/Tumor Interactions and Digital Analysis

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ACCME/Disclosure
Dr. Beck has nothing to disclose

The Pathologist as Data Integrator

Highly informative diagnostic profile for translational research and clinical medicine.

The central and critical role of computational analysis

Computational Pathology
Carcinomas are composed of a complex mixture of epithelial and stromal elements. Most research has focused on carcinoma cells. The role of carcinoma-associated stroma is not well understood.

Methods for Building Prognostic Model

*Signs of Prognostic Value.*

The present study was undertaken in an attempt to ascertain if there is any correlation between the histological appearance of the growth and the subsequent course of the disease, and to determine the value of such an analysis in giving a prognosis in an individual case when all the ascertainable factors have been taken into account.

largely followed, but chief importance has been attached to three factors—tubule formation, inequality in size of nuclei, and hyperchromatism.

[APRIL 21, 1928 801
THE LANCET,]
The development of C-Path for computational analysis of tumor morphology

Extract basic features from each superpixel

- 112 features extracted from each superpixel
- Features include:
  - Size, shape, texture of each superpixel
  - Relationships to neighboring superpixels
  - Characteristics of child subcellular objects (size, shape, number, variability, intensity)

Building an epithelial-stromal classifier

Basic image processing and feature construction

H&E Image
Image partitioned into superpixels
Nuclear objects identified within each superpixel
Building an epithelial-stromal classifier
Cross-validated misclassification error for epithelial/stromal classifier

Construction of higher level relational/contextual features

Construction of Higher Level/Relational/Contextual Features

Learning an image-based model to predict survival

C-Path 5YS Score Significantly Associated with Overall Survival
Multivariate Cox Regression Analysis

<table>
<thead>
<tr>
<th>Feature</th>
<th>HR</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Path SYS Model Score</td>
<td>1.78</td>
<td>1.11</td>
<td>2.86</td>
<td>0.017</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>1.8</td>
<td>1.09</td>
<td>2.96</td>
<td>0.021</td>
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<tr>
<td>Age</td>
<td>0.79</td>
<td>0.63</td>
<td>1.00</td>
<td>0.046</td>
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<tr>
<td>70-gene prognosis signature</td>
<td>2.61</td>
<td>1.94</td>
<td>3.49</td>
<td>0.010</td>
</tr>
<tr>
<td>Size</td>
<td>1.22</td>
<td>0.94</td>
<td>1.59</td>
<td>0.137</td>
</tr>
<tr>
<td>Invasiveness gene signature</td>
<td>1.84</td>
<td>0.69</td>
<td>4.99</td>
<td>0.226</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>1.3</td>
<td>0.81</td>
<td>2.07</td>
<td>0.276</td>
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<tr>
<td>Wound response signature</td>
<td>1.44</td>
<td>0.72</td>
<td>2.89</td>
<td>0.259</td>
</tr>
<tr>
<td>ERBB2 molecular subtype</td>
<td>1.88</td>
<td>0.53</td>
<td>6.67</td>
<td>0.326</td>
</tr>
<tr>
<td>Grade</td>
<td>1.19</td>
<td>0.77</td>
<td>1.83</td>
<td>0.431</td>
</tr>
<tr>
<td>Basal molecular subtype</td>
<td>0.68</td>
<td>0.45</td>
<td>1.01</td>
<td>0.612</td>
</tr>
<tr>
<td>ER</td>
<td>0.82</td>
<td>0.50</td>
<td>1.33</td>
<td>0.634</td>
</tr>
<tr>
<td>LN</td>
<td>1.15</td>
<td>0.55</td>
<td>2.43</td>
<td>0.715</td>
</tr>
<tr>
<td>Genomic grade index</td>
<td>1.09</td>
<td>0.52</td>
<td>2.32</td>
<td>0.814</td>
</tr>
<tr>
<td>Luminal A molecular subtype</td>
<td>1.14</td>
<td>0.72</td>
<td>1.77</td>
<td>0.819</td>
</tr>
<tr>
<td>Luminal B molecular subtype</td>
<td>0.83</td>
<td>0.42</td>
<td>1.68</td>
<td>0.097</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>1.04</td>
<td>0.49</td>
<td>2.21</td>
<td>0.925</td>
</tr>
</tbody>
</table>

Morphological Interpretation of Top Features

Variability in the contrast of stromal matrix superpixels to neighboring objects

Variability in epithelial nuclear texture
Minimum elliptic fit of epithelial contiguous region

How to translate new computational pathology findings and methods into the clinic?

1. Transform the entire workflow of pathology
2. Try to adapt discoveries to existing workflow

Using computational pathology to re-train pathologists

Dan Xia, MD MS

Xia, ..., Beck. Under Review and USCAP 2016 Poster
Manual review of images with extreme feature scores

**Xia, ..., Beck. Under Review**

Computational analysis trains pathologists to identify human interpretable prognostic feature

Hazard ratio = 0.32, 95% CI = 0.19-0.54
p-value = 2.5 x 10^{-5}

Xia, ..., Beck. Under Review
USCAP 2016, Poster #1938

Carcinomas are composed of a complex mixture of epithelial and stromal elements.

Most research has focused on carcinoma cells

The role of carcinoma-associated stroma is not well understood.

How to computationally disentangle cellular complexity of bulk tumor expression profiling data?
Fibromatosis

Breast Cancer

Microenvironment


Functional gene set analysis of core DTF genes

<table>
<thead>
<tr>
<th>Functional Gene Set</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular matrix</td>
<td>4.13E-28</td>
</tr>
<tr>
<td>Extracellular region</td>
<td>1.05E-22</td>
</tr>
<tr>
<td>Extracellular matrix structural constituent</td>
<td>6.71E-21</td>
</tr>
<tr>
<td>Collagen</td>
<td>2.59E-16</td>
</tr>
<tr>
<td>ECM-receptor interaction</td>
<td>5.15E-14</td>
</tr>
</tbody>
</table>
The Fibromatosis signature is associated with improved survival

A. Log Rank p = 0.0004
B. Log Rank p = 0.049
C. Log Rank p = 0.014

Disease Free Survival (Years) Disease Specific Survival (Years) Overall Survival (Years)

DTF-like Cases
Non-DTF-like Cases

Most Random Gene Expression Signatures Are Significantly Associated with Breast Cancer Outcome

David Venet 1, Jacques E. Dumont 1, Vincent Debures 1,2,3

1 INRS-UCG, Université Libre de Bruxelles (ULB), Brussels, Belgium, 2 INSERM, Université Libre de Bruxelles (ULB), Campus Erasme, Brussels, Belgium, 3 UFR sciences, Université Libre de Bruxelles (ULB), Campus Erasme, Brussels, Belgium

Received: April 23, 2010. Accepted: September 7, 2011. Published: October 30, 2011

ARE PROGNOSTIC STROMAL SIGNATURES NO BETTER THAN RANDOM?

'Provocative' Paper Sparks Debate on Relevance of Breast Cancer Gene Expression Signatures

September 04, 2012

HR = 1.8 (CI, 1.2–2.9)
p=0.0072
Significance Analysis of Prognostic Signatures (SAPS) to Identify Robust Prognostic Signatures


~16% of gene sets significant by $P_{\text{pure}}$, Are significant by $P_{\text{SAPS}}$
Significance Analysis of Prognostic Signatures (SAPS)

- Our analysis identified a core set of prognostic signatures in breast and ovarian cancer subtypes
- Stromal Signatures (immune, EMT, hypoxia, angiogenesis) among most robust in genome-wide analysis
- SAPS method enables rigorous assessment of stromal signatures vs. random signatures

Summary

- Stromal morphologic phenotypes are strong prognostic factors
- Stromal expression signatures are robustly prognostic in breast cancer
- What epithelial-stromal interactions drive stromal signature expression?

Extensive rewiring of epithelial-stromal co-expression networks in breast cancer

How do the epithelium and stroma interact to promote carcinogenesis?

How to disentangle cellular complexity for unbiased transcriptomic analysis?

Laser Capture Microdissection to purify epithelial and stromal tissue compartments

Prior data-driven approaches have focused on differential expression analysis rather than directly modeling epithelial-stromal interactions.
Epithelial-Stromal coexpression network approach directly models epi-stroma interactions

Epithelial-Stromal coexpression network approach directly models epi-stroma interactions

Computing the epi-stroma co-expression network

~412,000,000 epithelial-stromal interactions tested overall

Ultra Fast computation of Epi-Stroma co-expression relationships with large matrix operations

Epi-stroma interactions show a moderate increase in number during carcinogenesis
Major increase in “self-loop” epi-stroma coexpression relationships in cancer

Most significant relationships in ER-Positive breast cancer are self-loops

Most significant relationships in ER-Negative breast cancer are self-loops

Type I Interferon Response

Mitotic Cell Cycle
Most connected genes in the Epi-Stroma Coexpression Networks

<table>
<thead>
<tr>
<th>Normal breast</th>
<th>Epi-negative IBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene</strong></td>
<td><strong>Stromal Degree</strong></td>
</tr>
<tr>
<td>1</td>
<td>GABRA6</td>
</tr>
<tr>
<td>2</td>
<td>FGF22</td>
</tr>
<tr>
<td>3</td>
<td>POU3F1</td>
</tr>
<tr>
<td>4</td>
<td>FPR3</td>
</tr>
<tr>
<td>5</td>
<td>RPE65</td>
</tr>
<tr>
<td>6</td>
<td>ASPM</td>
</tr>
<tr>
<td>7</td>
<td>HTR2A</td>
</tr>
<tr>
<td>8</td>
<td>ABI3BP</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ER-positive IBC</th>
<th>Epi-negative IBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene</strong></td>
<td><strong>Stromal Degree</strong></td>
</tr>
<tr>
<td>1</td>
<td>BDNF</td>
</tr>
<tr>
<td>2</td>
<td>IFIH1</td>
</tr>
<tr>
<td>3</td>
<td>FUT5</td>
</tr>
<tr>
<td>4</td>
<td>KIF20A</td>
</tr>
<tr>
<td>5</td>
<td>UBE2C</td>
</tr>
<tr>
<td>6</td>
<td>FOXM1</td>
</tr>
<tr>
<td>7</td>
<td>CCNB2</td>
</tr>
<tr>
<td>8</td>
<td>KIF4A</td>
</tr>
<tr>
<td>9</td>
<td>DSC3</td>
</tr>
<tr>
<td>10</td>
<td>MGAM</td>
</tr>
</tbody>
</table>

Nervous System-Associated Factors:
- Neurotensin (NTS)
- Myelin regulatory factor (MYRF)
- Glutamate receptor, metabotropic (GRM1)
- Brain derived neurotrophic factor (BDNF)

*USCAP 2016: Francisco Bec, Oral Presentation on EZH2 Expression in Normal Breast and Breast Cancer Risk

Validation with Protein Immunohistochemistry Images from the Human Protein Atlas

Evaluation of predicted self-loops by image analysis

- **Epithelium**
- **Stroma**
- **Brown pixels**
Evaluation of predicted self-loops by image analysis

Conclusions:
Extensive rewiring of epithelial-stromal co-expression networks in breast cancer

- Epithelial-stromal coexpression network analysis represents a new approach for analyzing spatially-localized transcriptomic data
- Analysis uncovers epi-stroma network hubs and functional network rewiring in breast cancer
- Epi/Stroma self-loops appear to be a hallmark of cancer


Emerging technologies for highly multiplexed single cell analysis of biomarkers in situ

2013, PNAS
Highly multiplexed single-cell analysis of formalin-fixed, paraffin-embedded cancer tissue

2014, Na
Multiple Intact-Tissue Transcriptional Analysis at Cellular Resolution

2014, Science
Expansion Pathology

Joint work with Ed Boyden, Yongxin Zhao, Octavian Bucur

Visualization of kidney foot processes with conventional epifluorescence microscopy

Expansion microscopy to probe atypical cells with single cell resolution in situ

H&E Stain  Post-expansion, Multi-IF
Expansion pathology to improve pathological assessment of non-invasive breast disease

<table>
<thead>
<tr>
<th>Consensus Reference Diagnosis</th>
<th>No. of Interpretations</th>
<th>Overall Concordance Rate</th>
<th>Overinterpretation Rate</th>
<th>Underinterpretation Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign without atypia</td>
<td>2070</td>
<td>87 (85-89)</td>
<td>13 (11-15)</td>
<td></td>
</tr>
<tr>
<td>Atypia</td>
<td>2070</td>
<td>48 (44-52)</td>
<td>17 (15-21)</td>
<td>35 (31-39)</td>
</tr>
<tr>
<td>DCIS</td>
<td>2097</td>
<td>84 (82-86)</td>
<td>3 (2-4)</td>
<td>13 (12-15)</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>663</td>
<td>96 (94-97)</td>
<td>4 (3-6)</td>
<td></td>
</tr>
</tbody>
</table>

Elmore et al. JAMA 2015

Expansion improves performance of automated classification models

Future Directions: Epithelial-Stromal Interactions and Computational Pathology

- Highly multiplexed in situ molecular approaches should enable monitoring of epi-stromal interactions with single cell resolution on intact tissue samples
- Computational pathology will be critical for extracting knowledge from these increasingly complex data sets
- Integration of approaches for measuring and analyzing epi-stroma interactions may lead to improved cancer prevention and treatment

Nuclei Segmentation on Pre-Expansion and Expansion Images

H&E Stained
Pre-Expansion Segmentation
Expansion Segmentation

Zhao, Bucur, Beck, Bodyen. In preparation
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