Automated Multispectral Image Analysis of Tissue Microarrays in a University-based Core Facility

Peter Gann, MD, ScD
Department of Pathology
University of Illinois at Chicago
Chicago, IL
First, a pause to acknowledge our historic location
What the talk will cover:

Three examples of image analysis projects presenting to our core lab:
the Research Histology and Tissue Imaging Core at the University of Illinois.

Each project involves distinct challenges addressed by multispectral imaging.

• Glucocorticoid receptor (GR) and serum/glucocorticoid regulated kinase 1 (SGK1) in breast cancer (brightfield).

• Selenium binding protein 1 (SBP1) in prostate cancer (duplex IF).

• Caveolin (CAV1), superoxide dismutase (SOD2), adenosine monophosphate kinase (AMPK) in breast cancer (triplex IF).
Does psychological stress promote the progression of various subtypes of breast cancer?

Investigators: Garth Rauscher, Abeer Mahmoud and Umaima Al-alem

- TMA from the Breast Cancer Care in Chicago study with 287 breast cancers and 36 benign tissue samples.

- 103 African-American, 84 White, and 80 Hispanic women. All molecular subtypes of breast cancer represented.


Potential pathways for GR-mediated effects of stress on breast cancer development

Hypothesis: Expression of glucocorticoid receptor α (GR) and its transcriptionally regulated target, SGK1, varies according to subcellular localization, breast cancer subtype and race.

SGK1 transmits a strong survival signal to epithelial cells.

Both GR and SGK1 are expressed in the nucleus and cytoplasm.
## Workflow for automated scoring

<table>
<thead>
<tr>
<th>Raw</th>
<th>Segment tumor</th>
<th>Segment cells</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="GCR Raw" /></td>
<td><img src="image2" alt="GCR Segment tumor" /></td>
<td><img src="image3" alt="GCR Segment cells" /></td>
<td><img src="image4" alt="GCR Score" /></td>
</tr>
<tr>
<td><img src="image5" alt="SGK1 Raw" /></td>
<td><img src="image6" alt="SGK1 Segment tumor" /></td>
<td><img src="image7" alt="SGK1 Segment cells" /></td>
<td><img src="image8" alt="SGK1 Score" /></td>
</tr>
</tbody>
</table>

1. Set up spectral library for hematoxylin, DAB
2. Use machine learning and manual editing to map epithelium vs. stroma
3. Segment nuclei, then set parameters to detect cytoplasm around nuclear border
4. Set thresholds for intensity per nucleus or cytoplasm (for display – data can be continuous)
Selected results …

GCR expression was decreased relative to benign breast. Nuclear and cytoplasmic expression were correlated ($r = 0.80$).

Strong association between nuclear GCR and expression of CK 5/6, a controversial marker of “basal-like” breast cancer.

75% of CK 5/6 (+) cases had high nuclear GCR, vs. 42% of CK 5/6 (-); $P < 10^{-4}$

Nuclear GCR and cytoplasmic SGK1 weakly but significantly correlated: $r = 0.12$, $P = 0.048$

High cytoplasmic SGK1 associated with: ER-negative, PR-negative status.

High cytoplasmic SGK1 also associated with CK 5/6 or EGFR-positive status ($P = 0.026$), both markers for “basal-like” breast cancer.

Thus, preliminary data suggest that increased GCR and SGK1 expression is associated with markers of basal-like breast cancer, which is more prevalent in African-American women.
Is selenium-binding protein 1 a tumor suppressor in human prostate cancer?

Investigators: Alan Diamond, Emmanuel Ansong

Higher expression of SBP1 is associated with better survival in colon cancer (Ansong E, Yang W, Diamond AM, 2014)

Is it also associated with better survival in prostate cancer?

CPCTR “Recurrence TMA” with 202 cases of recurrent PCa and 202 non-recurrent controls, matched on age, Gleason, pStage and race. Quadruplicate cores.

SBP1 immunostained with mouse mAb (MBLI Corp.), labeled with AF647.

Staining seen in stroma – used cytokeratin 8/18 rabbit mAb (Abcam) labeled with AF488 to create epithelial mask.
SBP1 analysis

1. Set up spectral libraries for SBP1, CK, DAPI and AF

2. Train machine learning on CK channel

3. Segment nuclei and associated cytoplasm

4. Score on a per-cell basis
### Odds ratios for risk of prostate cancer recurrence by quartile of SBP1 (mean nuclear intensity and percent nuclear-positive cells)*

<table>
<thead>
<tr>
<th></th>
<th>mean nuclear intensity</th>
<th></th>
<th>percent positive nuclei</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% CI</td>
<td>Odds ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Q1</td>
<td>1.00</td>
<td>referent</td>
<td>1.00</td>
<td>referent</td>
</tr>
<tr>
<td>Q2</td>
<td>0.36</td>
<td>(0.16 - 0.81)</td>
<td>0.62</td>
<td>(0.30 – 1.29)</td>
</tr>
<tr>
<td>Q3</td>
<td>0.44</td>
<td>(0.20 – 0.99)</td>
<td>0.43</td>
<td>(0.19 – 0.95)</td>
</tr>
<tr>
<td>Q4</td>
<td>0.34</td>
<td>(0.13 – 0.91)</td>
<td>0.41</td>
<td>(0.17 – 1.01)</td>
</tr>
<tr>
<td>PSA</td>
<td>1.06</td>
<td>(1.02 – 1.10)</td>
<td>1.05</td>
<td>(1.02 – 1.09)</td>
</tr>
</tbody>
</table>

* Adjusted for age, race, Gleason and stage by matching and for PSA in the model.
What sustains the shift to glycolysis in aggressive breast cancer? (and are there drug targets?)

Investigators: Marcelo Bonini and Peter Hart

Hypothesis:

Test: Does the data show the associations in the model between CAV1, SOD2 and pAMPK in triple-negative breast cancers, which are more dependent on glycolysis?

- Breast cancer TMA: 67 breast ca. (n = 34 triple-negative), 5 DCIS, 5 benign
- Triplex immunofluorescence stain: CAV-1 (AF647), SOD2 (AF568), pAMPK (AF488) plus DAPI
- Key challenge: score all three targets in both nucleus and cytoplasm on a per-cell basis
Separating signals in triplex immunofluorescence in a breast cancer TMA

Unmixed pseudocolor, no DAPI

- SOD2
- pAMPK
- CAV-1

CAV-1 channel  SOD2 channel  pAMPK channel
Selected results (surprising)

CAV1, SOD2 and pAMPK all trend down with increasing tumor grade.

SOD2 and CAV1 were positively correlated in triple negative cancers, but not others.

**Cytoplasm SOD2 vs. Cytoplasm CAV1**

- Not TNBC: $r = 0.13, P = 0.46$
- TNBC: $r = 0.54, P = 0.0009$

SOD2 and pAMPk expression were positively correlated in both types of breast cancer.

**Cytoplasm SOD2 vs. Nuclear pAMPK**

- Not TNBC: $r = 0.60, P = 0.0002$
- TNBC: $r = 0.62, P = 0.0009$
Conclusions

• Multispectral imaging can be used to overcome several challenges encountered by a university-based core lab.

• These challenges include heterogeneous tissue compartments, subcellular localization, and, especially - target multiplexing.

• Exploiting the full potential of multispectral image analysis requires improvements in tissue labeling (staining).

• Promising new techniques include autostainer-enabled tyramide signal amplification (TSA) and quantifiable FISH using amplified probes.
Acknowledging …

Ryan Deaton

Peter Nguyen

Milita Petrauskaite

Raj Emmadi, MD

Andrew Hall

Fall in Chicago

Thank you.