Comparison of two Synaptophysin Immunohistochemistry Antibodies using Tissue Microarray and Image Analysis: The Basis for Objective Antibody Validation

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Background

• Biobanking is about the rational and systematic use of human tissue for scientific investigation.
• Tissue science requires uniform tissue handling to allow comparative analyses.
• Immunohistochemistry allows the presence, location and amount of specific proteins to be identified\textit{ in situ} within tissue.
• Digital pathology creates machine readable data for pathologists.
• Computers the new microscopes!!
Questions

• Human Eye vs Computer?
• How does small changes in digital signal ... (i) translate into observable visual signal? (ii) represent biological change?
• Accurate assessment of IHC signal: will subjective visual assessment do?
• Comparative staining – dealing staining variation?
• Will Pathology be affected by ’big data’??
Study Design
Comparative Tissue Analysis

- How to study many samples under same conditions: design and build a tissue microarray.
- 50 childhood neuroblastoma tumours collected over 60 years
- Pathologist review to current standards
- 10 Tumours/ decade (50’s, 60’s, 70’s, 80’s, 90’s, 00’s).
- Duplicate cores.
- 40 normal control tissue – normal tissue panel
- Map of the NB TMA layout.
Image Analysis – Pixel Counting

- Create high resolution digital image - Aperio Scanscope (0.2um²)
- The pixel intensity (transmissive light) was measured.
- A high value (> 230) represents no antibody staining. A low value (<30) represents maximal antibody staining.
- Pixel partitioned into 4 grades based on amount of light detected coming through each for a particular colour hue.
- Immunohistochemistry (IHC) Index: Weighted sum of proportions pixel in each staining grade. Range 0.0 to 3.0

\[
\text{IHC Index} = \left[ (\% \text{ of } -ve) \times 0 \right] + \left[ (\% \text{ of } 1+) \times 1 \right] + \left[ (\% \text{ of } 2+) \times 2 \right] + \left[ (\% \text{ of } 3+) \times 3 \right]
\]

Chetcuti et al, Microarrays, 3(1), 72-88, 2014
Four algorithms were used to segregate the range into 10 subdivisions.
Regional Signal Distribution

Chetcuti et al, Microarrays, 3(1), 72-88, 2014
Synaptophysin

- Marker of neuroblastoma tumour.

Zymed’s Rabbit anti-Synaptophysin (Z66) vs Bond™ RTU anti-Synaptophysin (27G12)

- Stained adjacent TMA slides with Bond Automatic Immunostainer
- Specific and comparable cellular staining was seen with both antibodies.

**IHC Indices**

<table>
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<tr>
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<th>0.32</th>
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<tr>
<td>Z66</td>
<td>0.52</td>
<td>0.46</td>
<td>0.90</td>
<td>1.14</td>
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<tr>
<td>27G12</td>
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Polyclonal having greater signal at lower intensity $\Rightarrow$ non-normal distribution
Signal Differences (=0)

- Identifies those cores that have signal differences worth noting.
- Red and blue boxes indicate 1x standard deviation.
- Spots falling outside central box will best indicate the cores where staining differences between the antibodies is most apparent.

\[ y = 0.8685x + 1 \]
\[ R^2 = 0.4922 \]
Spot Comparison

- Identification and comparison of individual cores showing subtle but significant differences following digital analysis
- Compare mean IHC Index for each paired spot vs relative ratio of IHC index
- Low IHC Index – low staining or highly specific cellular staining?
Signal Distribution

- Distribution of signal across cores grouped according to decades for each antibody.
- The **monoclonal 27G 12** antibody shows a shift in peak to the right indicating more contrast in the signal over the core. ⇒ 1970’s – acetic acid fixation?
- This suggests an improved specificity (clean signal, less background) in signal from and not sensitivity of signal.
Summary

• Tissue Microarrays allow for comparative assessment of IHC.
• Comparative staining – synaptophysin signal variation shown.
• Computers will be the new microscope.
• Simple maths works – don’t be complicated
• Digital assessment of histopathology images allows quantitative assessment – see new things!
• Will Pathology be affected by ’big data’?? –Yes.
Image Analysis – Deep Learning

Sohelia Gheisari
Amanda Charlton

“Patched Completed Local Binary Pattern is an Effective method for Neuroblastoma Histological Image Classification”
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“Wait, where's Daniel?”

Any Questions  ???