Molecular Clonality Studies of Cutaneous Lymphomas

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Clonality Studies in Hematopathology

• Immunophenotypic
  – B-cell light chain restriction
    • Protein expression
    • RNA detection
  – T cell receptor Vβ repertoire analysis
  – NK-cell killer inhibitory receptor (KIR) repertoire analysis
Clonality Studies in Hematopathology

- Molecular genetic
  - Detection of a recurring genetic abnormality
    - PCR or RT-PCR
    - FISH
  - Detection of a clonal antigen receptor gene rearrangement
    - PCR
Recurring Genetic Abnormalities in Cutaneous Lymphomas

• Many genetic abnormalities are described, but most are not recurring and are not associated with a specific disease

• Detection of a clonal abnormality is helpful, but most cases are not studied by routine karyotype or array-based methods
Recurring Genetic Abnormalities in Cutaneous Lymphomas

- Primary cutaneous marginal zone lymphoma
  - Translocations associated with extranodal marginal zone lymphomas are not usually present
- Primary cutaneous follicle center lymphoma
  - Usually lacks $IGH@/BCL2$ translocations by FISH (controversial)
  - More frequently $IGH@/BCL2$ positive by PCR
Recurring Genetic Abnormalities in Cutaneous Lymphomas

- *IRF4* translocations in primary cutaneous anaplastic large cell lymphoma
  - Located at 6p25.3 (aka MUM1)
  - Detected in 20-57% of cases of cALCL by FISH
- Compared to
  - 0% of systemic ALCL
  - 0-3% of lymphomatoid papulosis
  - 0-18% of transformed mycosis fungoides

Wada DA et al. Modern Pathology 24:596-605, 2011
**IRF4 Translocations in cALCL**

**a**

**Frequency of IRF4 Translocations by FISH**

- Various lymphoma subtypes are shown, with a bar graph indicating their frequency.

**b**

**Frequency of Other IRF4 Abnormalities by FISH**

- Similar to the IRF4 translocations, other abnormalities involving IRF4 are shown with frequency bars.

**c**

**Frequency of IRF4 Protein Expression by Immunohistochemistry**

- A bar graph shows the frequency of IRF4 protein expression, categorized by percentage of positive cells.

*Wada DA et al. Modern Pathology 24:596-605, 2011*
Antigen Receptor Gene Rearrangement Studies

• There are 3 B-cell associated and 4 T-cell associated antigen receptor genes that are normally rearranged in most B or T lymphocytes
• These genes rearrange in a defined sequence in lymphocyte development
  • B-cell
    – *IGH* (14q32)
    – *IGK* (2p11)
    – *IGL* (22q11)
  • T-cell
    – *TRD* (14q11)
    – *TRG* (7p15)
    – *TRB* (7q34)
    – *TRA* (14q11)
B-cell Development

stem cell

early pre B precursor (pro-B)

pre B

mature B

plasma cell

CD34 → CD19 → TdT → TdTmu → CD20 → clg

IGH@

IGK@

IGL@
T-cell Development

stem cell → pro T → pre T → Thymocyte (cortical-T) → helper T lymphocytes

helper T lymphocytes → suppressor T lymphocytes

TRG@, TRD@

TRB@

TRA@
Antigen Receptor Gene Rearrangement Studies

D-J rearrangement

V-D rearrangement
IGH@
IGH@

\[ D_H - J_H \]
V(D)J recombination creates antibody diversity

PCR Targeting of the VDJ Rearrangement

5′- V V N D N J J Cμ - 3′
L FR I CDRI FR II CDR II FR III CDR III FR IV

≈ 350 bp

Primers True Positives
FRIII only 57%
FRIII + FRII 74%
FRIII + FRI 73%

Bagg et al. J Molec Diagn 4:81, 2002
PCR Detection: Gel vs. Capillary Electrophoresis

MW 1 1 2 2 3 3 4 4 MW – – – 10^{-2}+
PCR Targeting of the VDJ Rearrangement

With good annealing of primers and good amplification, all three primer sets may detect the same clone

≈ 350 bp
T-cell Receptor Beta Chain Gene (7q34)

V = ~65
D = 2
J = 13

D/J rearrangement
V/D rearrangement
T-cell Receptor Gamma Chain Gene (7q34)
TRG@ PCR

Monoclonal

Oligoclonal

Polyclonal
T-cell Receptor Gamma Chain Gene (7q34)

- In contrast to IGH@ assay, the primers for TCR assays target specific V regions; therefore, a single rearrangement should be specific to a primer set.
- You will not see the same rearrangement with multiple primers and such findings support an oligoclonal proliferation.
Issues Related to Antigen Receptor Assays

• Amplify DNA, so they work in paraffin
  – Up to 20% increase in false negative rate
  – Need to confirm DNA amplification by internal control, including internal amplification to the expected size
• Use consensus primers for either the V or FR regions studied
  – Consensus primers are less specific than translocation-specific primers
  – Detect down to the 0.1-1% range compared to 0.001-0.0001% range of translocation specific PCR assays
  – Will still miss some rearrangements
Confirm DNA Amplification
Issues Related to Antigen Receptor Assays

- False positive peaks with eosin staining of tissue prior to embedding
- Degradation of DNA with heavy metal fixation
- Peaks that normally occur with the assay
Issues Specific to Skin

- Specimens are usually paraffin embedded
- Specimens are small
- Specimens may have low numbers of B-cells or T-cells
Pseudoclonality

• Samples that have too few target lymphocytes
  – $TRG@$ or $TRB@$ studies with too few T-cells
  – $IGH@$ or $IGK@$ studies with too few B-cells
• Samples that have too little target DNA
  – Cellular samples that are over-diluted
Pseudoclonality

Study of reactive lymph node with starting template of 50ng/µl

Elenitoba-Johnson KSJ, et al.
J Mol Diag 2:92-6, 2000
# Pseudoclonality

<table>
<thead>
<tr>
<th></th>
<th>Normal skins</th>
<th>Daudi</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A1 A2 A3 A mix</td>
<td>B1 B2 B3 B mix</td>
</tr>
</tbody>
</table>

Nihal, Mikkola & Wood  
J Mol Diag 2:5-10, 2000
Pseudoclonality

TRG assay
## BIOMED-2 Assays

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cases (N)</th>
<th>Cases Positive for Clone (Combined IGH and IGK), N (%)</th>
<th>Cases Positive for IGH, N (%)</th>
<th>Cases Positive for IGK, N (%)</th>
<th>Cases Positive for t(14;18), N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBCL</td>
<td>26</td>
<td>22 (85)</td>
<td>18 (69)</td>
<td>17 (65)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>MZL</td>
<td>15</td>
<td>12 (80)</td>
<td>11 (73)</td>
<td>9 (60)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>FCL</td>
<td>11</td>
<td>10 (91)</td>
<td>7 (64)</td>
<td>8 (73)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Benign lymphoid infiltrates</td>
<td>23</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>None</td>
<td>Not performed</td>
</tr>
</tbody>
</table>

BIOMED-2 Assays

Value of Duplicate Specimens
## Value of Duplicate Specimens

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cases (N)</th>
<th>Cases Positive for IGH@ and/or IGK@, N (%)</th>
<th>Identical Clones Identified, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary cutaneous B-cell lymphoma</td>
<td>20</td>
<td>19 (95)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>More than one site</td>
<td>16</td>
<td>15 (94)</td>
<td>7 (44)</td>
</tr>
<tr>
<td>Same site at different time points</td>
<td>4</td>
<td>4 (100)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Cutaneous marginal zone lymphoma</td>
<td>12</td>
<td>11(92)</td>
<td>8 (67)</td>
</tr>
<tr>
<td>Cutaneous follicle center cell lymphoma</td>
<td>8</td>
<td>8 (100)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Benign cutaneous lymphoid infiltrates</td>
<td>12</td>
<td>2 (17)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

What About T-cell Lymphomas?

**TRG@ clonal heterogeneity and persistence in MF**

<table>
<thead>
<tr>
<th></th>
<th>Common GR</th>
<th>Different GR</th>
<th>No GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concurrent skin samples (n=17)</td>
<td>11 (65%)</td>
<td>4 (24%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Sequential skin samples (n=22)</td>
<td>18 (82%)</td>
<td>2 (9%)</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>Skin/lymph node samples (n=11)</td>
<td>8 (73%)</td>
<td>2 (18%)</td>
<td>1 (9%)</td>
</tr>
</tbody>
</table>

Utility of Testing Multiple Samples

TRG@ testing in Sézary syndrome

Skin
Blood
Sorted SS cells
Sorted non-SS cells

**TRG@ vs TRB@**

<table>
<thead>
<tr>
<th></th>
<th>TRG@</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Monoclonal</td>
<td>Polyclonal</td>
<td>Oligoclonal</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>TRB@</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monoclonal</td>
<td>43</td>
<td>22</td>
<td>1</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Polyclonal</td>
<td>21</td>
<td>107</td>
<td>0</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Oligoclonal</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>129</td>
<td>7</td>
<td>202</td>
<td></td>
</tr>
</tbody>
</table>

- Studied 69 MF and 133 inflammatory dermatosis samples
- Concordance rate of 77.2%

Proposed workflow for MF specimens – 2 order types based on clinical suspicion of disease (low or high). TCR-LR (low risk), TCR-HR (high risk)

1. Low pre-test probability (0.15~0.50)
   - TCRG test
     - TCRB test
       - Clonal support for MF
       - Non-diagnostic, suggest follow-up
     - No clonal support for MF

2. High pre-test probability (0.50~0.75)
   - TCRG test
     - TCRB test
       - Clonal support for MF
       - No clonal support for MF

Final Comments

• Make sure there are enough B or T cells in the specimen before ordering molecular studies

• Avoid doing both B and T cell studies
  – Helps to avoid getting results you cannot interpret

• Run assays in duplicate and only call reproducible clones positive

• Compare clone size to those found in other samples
  – If the same size, strong support for lymphoma
  – Different size, could be a second primary (does not exclude lymphoma)
Acknowledgements

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