Antigen-Specific T Cell Responses to Breast Cancer

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No relationships exist related to this presentation.
Why do we need to know the αβ-TCR pairs of tumor infiltrating T cells?

- The presence of TILs correlates with patient survival.
- TILs recognize tumor antigens.
- Activated tumor-specific T cells add specificity to cancer vaccines.

Our ultimate goal is to identify these epitopes and mimotopes for cancer vaccines.
T cell review

Garcia Science 1996
The first tumor-specific T cell clone we identified against the colon carcinoma CT26 was “CT”

- CT26 is an immunogenic transplantable tumor.
- The dominant MHC1-restricted antigen is gp70\textsubscript{423-431} (AH1).
- We sub-cloned AH1-specific T cells by limiting dilution. 6 of 6 clones had identical TCR sequences.
- AH1 mimotopes elicit a range of antitumor immunity.

![Graph showing tumor-free survival over days after tumor challenge with different sequences of peptides](image)

Jordan *PNAS* 2010
The 1D4 T cell clone is representative of the AH1-specific T cell response

<table>
<thead>
<tr>
<th>Source of AH1-specific T cells</th>
<th>CT-TCR TRBV13/TRBJ2-7 FCASSSGGAYEQY</th>
<th>1D4-TCR TRBV13/TRBJ2-7 FCASSDGDYEQY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mimotope vaccination n=118,994</td>
<td>6</td>
<td>10,522</td>
</tr>
<tr>
<td>AH1 vaccination n=44,138</td>
<td>0</td>
<td>1,544</td>
</tr>
<tr>
<td>TILs, no vaccine n=3,978</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL n=167,110</td>
<td>0.004%</td>
<td>7.2%</td>
</tr>
</tbody>
</table>

Buhrman JBC 2013
Prime with peptides enriched with representative TCR and tumor-antigen boost results in tumor protection

Buhrman *JBC* 2013
Emulsion RT-PCR of paired αβ TCR chains

RT-PCR reaction:
α/β V and C primers
RT-PCR reagents
T cells
50 µl

Add 300 µl Emulsion phase and vortex

Example reaction bubble (200-500 fl)

Reverse transcription
TCRα mRNA V C
TCRβ mRNA V C

Overlap extension
TCRα cDNA V C
TCRβ cDNA V C

Amplification

Vortexer

Add 300 µl Emulsion phase and vortex

Dan Munson unpublished, adapted from Turchaninova *EJI* 2013
Preparation of PCR fragment for sequencing

1. Break emulsion
2. Column purify PCR products
3. PCR with nested C-region oligos and blocking oligos
4. Final PCR to add the bridge and read sites for sequencing (450 bp product)

Dan Munson unpublished, adapted from Turchaninova *EJI* 2013
Confirmation that pairing is correct

- Confirm that all primers bind
- Clear PCR products from productive $\alpha$ chains and $\beta$ chains
- Determine number of $\alpha/\alpha$ pairs and $\beta/\beta$ pairs
- Show that pairing is not random
- Show that titration of known numbers of hybridoma cells into PBMCs emulsion
  PCR is semi-quantitative.
- Further understand the statistics, significance, and quality of the results obtained.

Dan Munson unpublished

<table>
<thead>
<tr>
<th>5KC spike</th>
<th>H/H pairs</th>
<th>% M/M pairs</th>
<th>% H/M pairs</th>
<th>% M/H pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>89</td>
<td>10.3</td>
<td>0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>94</td>
<td>4.7</td>
<td>0.2</td>
<td>1.08</td>
</tr>
<tr>
<td>1</td>
<td>98</td>
<td>1.6</td>
<td>0.2</td>
<td>0.013</td>
</tr>
<tr>
<td>5% NE</td>
<td>95</td>
<td>1.7</td>
<td>0.7</td>
<td>2.62</td>
</tr>
</tbody>
</table>

Normalized
TCR repertoire from blood and TILs of breast cancer patients are different

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Tissue</th>
<th>Cell Counts</th>
<th>Total Reads</th>
<th>Unique pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC57 CD8+ T cells</td>
<td>Tumor</td>
<td>16,500</td>
<td>283,841</td>
<td>1,194</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>30,000</td>
<td>667,982</td>
<td>3,214</td>
</tr>
</tbody>
</table>

Dan Munson unpublished
Lessons and Take Home Messages

Selected mimotopes stimulate tumor-specific T cells and augment antitumor responses.

Targeting the tumor-specific T cells that are present in the naturally responding repertoire is important in design of peptide vaccines.

Emulsion RT-PCR can be used inexpensively to identify TCR pairs (or other co-expressed messages within a cell).

Comparison of TILs and PBMCs suggests the representative TCRs of the tumor-specific T cell response.

Some “public” TCRs are shared among breast cancer patients, but the cognate antigen is still unclear.
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