The tumor microenvironment can vary with anatomical site to affect responses to therapy

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Presenter disclosure information:
No relationships or financial interests to disclose
Part 1 - Immunotherapy is less effective against orthotopic tumors
Tumor models and therapy

- Tumors: Renca (Ch⁺Luc⁺), CT26, RM-1

- Three agonist antibodies (TrimAb)
  - DR5 (apoptosis)
  - CD40 (antigen presentation)
  - CD137 (4-1BB, T cell activation)

- CD8⁺ T cells and IFN-γ are essential
Orthotopic renal tumors respond less than subcutaneous tumors to Trimab therapy

1 experiment representative of 3
Kidney tumors respond poorly to Trimab

**SC tumors**

- TrimAb
- Control

**IK tumors**

- TrimAb
- Control

1 experiment representative of 3
Differential responses to Trimab are also observed in other tumor models.

Renca intrahepatic

CT26 colon cancer

RM-1 prostate cancer
Part 2 - The microenvironment of tumors varies with anatomical site
No differences in frequency of immune cells in kidney tumors compared to subcutaneous tumors

<table>
<thead>
<tr>
<th>Immune Cell Type</th>
<th>In SC (%)</th>
<th>In IK (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils (TCRβ^+/Ly6G^+/CD11b^+)</td>
<td>12.3</td>
<td>11.7</td>
<td>0.75</td>
</tr>
<tr>
<td>B cells (TCRβ^+/CD19^+)</td>
<td>6.2</td>
<td>6.1</td>
<td>0.89</td>
</tr>
<tr>
<td>Macrophages (TCRβ^+/F4/80^+/CD11b^+)</td>
<td>20.5</td>
<td>20.3</td>
<td>0.93</td>
</tr>
<tr>
<td>DCs (TCRβ^+/CD11c^+)</td>
<td>22.4</td>
<td>22.5</td>
<td>0.99</td>
</tr>
<tr>
<td>CD4 T cells (TCRβ^+/CD4^+)</td>
<td>23.1</td>
<td>22.9</td>
<td>0.98</td>
</tr>
<tr>
<td>CD8 T cells (TCRβ^+/CD8^+)</td>
<td>30.6</td>
<td>30.8</td>
<td>0.97</td>
</tr>
<tr>
<td>TReg (TCRβ^+/CD8^-/CD25+/Fr4^high)</td>
<td>8.5</td>
<td>8.7</td>
<td>0.95</td>
</tr>
</tbody>
</table>

4 independent experiments pooled
More F4/80\textsuperscript{hi} macrophages are present in kidney tumors

F4/80\textsuperscript{hi} macrophages express CD206, mannose receptor

(>5 mice per group, > 5 expts)

(5 mice per group, 3 expts)
Immune gene expression differences between kidney and subcutaneous tumors (DNA microarray)

**Upregulated in orthotopic renal tumors**

- Arg1
- CD163
- CD206
- MRP-1
- MCP-1
- MCP-3
- MCP-5
- MIP-2
- CXCL5
- CX3CL1
- GM-CSF
- IL-8
- IL-33
- LIF
- SAA3
- SerpinB2
- Endothelin bR

**Upregulated in S.C. tumors**

- TGFβ3P1
- ISF10
- MMP-2
- CXCR4
- CD207
Immune gene expression differences between kidney and subcutaneous tumors (DNA microarray)

Macrophage-associated genes

Upregulated in orthotopic renal tumors

Upregulated in S.C. tumors
Immune gene expression differences between kidney and subcutaneous tumors (DNA microarray)

Macrophage-associated genes

- Upregulated in orthotopic renal tumors
- M2 macrophage-associated
Immune gene expression differences between kidney and subcutaneous tumors (DNA microarray)

Upregulated in orthotopic renal tumors

Upregulated in S.C. tumors

M2 differentiation
Immune gene expression differences between kidney and subcutaneous tumors (DNA microarray)

Upregulated in orthotopic renal tumors

Upregulation confirmed using RT-PCR

Upregulated in S.C. tumors
Differences in protein expression determined using protein array

Higher in kidney tumors

Higher in SC tumors

N = 3
Cytokines and chemokines secreted by macrophages isolated from tumors

Higher level of CCL2 is present in the serum of mice bearing kidney tumors
Trimab works better against kidney tumors when CCL2 is neutralized

2 experiments pooled
N = 13-14 per group
Trimab works better against kidney tumors in mice deficient in IL-13

2 experiments
Summary Part 2 – markers of M2 macrophages and Th2 cells are associated with orthotopic tumors

<table>
<thead>
<tr>
<th>Kidney tumors</th>
<th>Subcutaneous tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4/80\textsuperscript{Hi} - IL-10</td>
<td>IL-1\textbeta</td>
</tr>
<tr>
<td>CD206 - IL-13</td>
<td>MMP2</td>
</tr>
<tr>
<td>CD163 - IL-23</td>
<td>CD207</td>
</tr>
<tr>
<td>MCP-1</td>
<td></td>
</tr>
<tr>
<td>MCP-3</td>
<td></td>
</tr>
<tr>
<td>MCP-5</td>
<td></td>
</tr>
<tr>
<td>MIP-1\textalpha</td>
<td></td>
</tr>
<tr>
<td>MIP-1\textbeta</td>
<td></td>
</tr>
</tbody>
</table>
Part 3 - Tumors in different sites differ intrinsically
Orthotopic tumors are similar in size to subcutaneous tumors

Renca (kidney and liver)

RM-1 prostate

CT26 cecum
Subcutaneous and orthotopic kidney tumor cells express similar levels of Trimab target molecules

CD137

CD40

DR5

2 experiments
N = 10 per group
Expression of some immune markers varies on SC and IK tumor cells

<table>
<thead>
<tr>
<th>Marker</th>
<th>SC</th>
<th>IK</th>
<th>MFI</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L1</td>
<td></td>
<td></td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Fas-L</td>
<td></td>
<td></td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>MHCI</td>
<td></td>
<td></td>
<td>6000</td>
<td>4000</td>
</tr>
</tbody>
</table>

2 experiments
N = 10 per group
Kidney tumors are more highly vascularized

(5 tumors, 10 fields/tumor)
Similar vascular permeability in SC and IK tumors

Evans blue IV injections

0.5 hour

tumours taken

Elution in formamide

24 hour

OD reading at 620 nm

Renca Ch⁺ luc⁺ cells

S.C

I.K

Similar vascular permeability in SC and IK tumors
Part 4 - The tissue can instruct the development of the tumor microenvironment
Kidney and SC tumor cells have a similar phenotype following culture ex vivo
Cell line from kidney tumors, when injected SC, responds to Trimab (cross over experiment)
Part 5 - Orthotopic tumors can affect the response of subcutaneous tumors to immunotherapy
SC tumors respond less to therapy when IK tumors are present.
Similar macrophage profile in mice bearing 1 or 2 tumours
Points to make

• Immunotherapy is less effective against orthotopic tumors
• The microenvironment of tumors varies with anatomical site
• Tumors in different sites differ intrinsically
• The tissue can instruct the development of the tumor microenvironment
• Orthotopic tumors can affect the response of subcutaneous tumors to immunotherapy
1. Inject tumor cells

2. Tissue-specific factors released

3. Tumor microenvironment sculpted

4. Immunotherapy inhibited

- LIF
- IL-33
- IL-10
- MCP-1
- MCP-5
- IL-13
- IL-23
- MIP-1α
- MIP-1β
- CXCL1
Implications

• Tumor model is important when investigating immunotherapies
• Tumor location needs to be considered when deciding best therapy
• Identification of tissue instructing factors may enable early intervention strategies
• Removing immunosuppressive tumors may permit responses of other tumors to immunotherapy
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