Creating a model antigen system to test the mechanism of GCC-specific tolerance

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CREATING A MODEL ANTIGEN SYSTEM TO TEST THE MECHANISM OF GCC-SPECIFIC TOLERANCE

- Patrick Ihejirika
## Cancer Cases and Deaths

### Leading New Cancer Cases and Deaths – 2012 Estimates

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated New Cases*</td>
<td>Estimated New Cases*</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>241,740 (29%)</td>
<td>Breast</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>116,420 (14%)</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>73,420 (9%)</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>55,600 (7%)</td>
<td>Uterine corpus</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>44,250 (5%)</td>
<td>Thyroid</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>40,250 (5%)</td>
<td>Melanoma of the skin</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>38,160 (4%)</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>28,540 (3%)</td>
<td>Kidney &amp; renal pelvis</td>
</tr>
<tr>
<td>Leukemia</td>
<td>26,830 (3%)</td>
<td>Ovary</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22,090 (3%)</td>
<td>Pancreas</td>
</tr>
<tr>
<td>All sites</td>
<td>848,170 (100%)</td>
<td>All sites</td>
</tr>
</tbody>
</table>

|                      | Male                          | Female                        |
|                      | Estimated Deaths              | Estimated Deaths              |
| Lung & bronchus      | 87,750 (29%)                  | Breast                        | 72,590 (26%)                  |
| Prostate             | 28,170 (9%)                   | Lung & bronchus               | 39,510 (14%)                  |
| Colon & rectum       | 26,470 (9%)                   | Colon & rectum                | 25,220 (9%)                   |
| Pancreas             | 18,850 (6%)                   | Pancreas                      | 18,540 (7%)                   |
| Liver & intrahepatic bile duct | 13,980 (5%)   | Ovary                         | 15,500 (6%)                   |
| Leukemia             | 13,500 (4%)                   | Leukemia                      | 10,040 (4%)                   |
| Esophagus            | 12,040 (4%)                   | Non-Hodgkin lymphoma          | 8,620 (3%)                    |
| Urinary bladder      | 10,510 (3%)                   | Liver & intrahepatic bile duct| 6,570 (2%)                    |
| Non-Hodgkin lymphoma | 10,320 (3%)                   | Brain & other nervous system  | 5,980 (2%)                    |
| Kidney & renal pelvis| 8,650 (3%)                    | All sites                     | 275,370 (100%)                |

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

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The main treatment options include:
Surgery, chemo-, radiation, biological therapy.

All treatments present a risk of side effects

Current standard of care: FOLFOX, FOLFIRI, etc

✓ Developed in 1980
✓ Minimal response rate
✓ Prognosis: 5 year survival rate of 63%
Colorectal Cancer Immunotherapy

Meta-analysis of clinical trials reveal a very weak clinical response rate (<1%) for active specific immunotherapy procedures currently available for colorectal cancer


Failure is due to the lack of molecularly defined tumor-associated antigens that can be reliably considered:

• Tumor-specific
• Sufficiently immunogenic
• Shared among different patients


<table>
<thead>
<tr>
<th>Colorectal Cancer TAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant Self Proteins</td>
</tr>
<tr>
<td>K-ras p53</td>
</tr>
<tr>
<td>Oncofetal / Cancer Testis Antigens</td>
</tr>
<tr>
<td>βhCG Gastrin 5T4</td>
</tr>
<tr>
<td>Overexpressed Self Antigens</td>
</tr>
<tr>
<td>p53 MUC1 SART</td>
</tr>
<tr>
<td>Sialyl-Tn Her2/neu ART</td>
</tr>
<tr>
<td>Survivin CD55 Ep-CAM</td>
</tr>
<tr>
<td>Carcinoembryonic Antigen (CEA)</td>
</tr>
<tr>
<td>Tissue-Specific Differentiation Antigens</td>
</tr>
<tr>
<td>?</td>
</tr>
</tbody>
</table>
Guanylyl Cyclases

<table>
<thead>
<tr>
<th>Guanylyl Cyclases</th>
<th>Isoforms</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natriuretic Peptide Receptors</td>
<td>GCA, GCB</td>
<td>ANP, BNP, CNP</td>
</tr>
<tr>
<td>Intestinal Peptide Receptor</td>
<td>GCC, ST</td>
<td>Guanylin, Uroguanylin</td>
</tr>
<tr>
<td>Orphan Receptors</td>
<td>GCD, GCE, GCF, GCG</td>
<td></td>
</tr>
<tr>
<td>Soluble</td>
<td>sGC</td>
<td>NO</td>
</tr>
</tbody>
</table>
Mucosa-Restricted GCC Expression

Swensen et al. 1996. Biochemical and Biophysical Research Communications, 225: 1009-1014

S Schulz et al, unpublished data

E Lin et al, unpublished data

Anti-Tumor Immunity
Predicted CD4 Tolerance Mechanism

Central Tolerance
- Thymus gland
- Deletion
- Treg

Peripheral Tolerance
- Deletion
- Treg
- Anergy

T cell precursor

Mature

Naive

Effector Response
Model System

- Thymus
- GCC TCR Tg
- Equivalent to GCC TCR Tg
- OT-II TCR Tg
- Inducible Antigen Tg
- Equivalent to GCC TCR Tg
<table>
<thead>
<tr>
<th>signal sequence</th>
<th>HA Tag</th>
<th>Ova_{257-264}</th>
<th>Ova_{329-337}</th>
<th>Tac</th>
<th>Transmembrane domain</th>
</tr>
</thead>
</table>

**B16**

**B16-Tac**

6μm slice
Tac FACS

Extracellular

Intracellular

Anti-HA

Anti-Tac

Anti-HA

Anti-Tac
Epitopes and surface expression
Creating GCC-based model antigen

Steps:

- cloning different epitopes and signal sequences into GCC:
  - Bip and Prp

- clone the constructs into mammalian expression plasmid (retrovirus)

- create cell lines expressing the constructs by infecting them with retrovirus containing our constructs

- test the new cell lines for 1) Chimeric antigen expression, 2) Chimeric antigen subcellular location, 3) epitope presentation

- work with Dr Eisenlohr’s lab to see how different signal sequences and model epitopes affect our new model antigen.
Overhang PCR

(a) PCR #1
Primers a + b, c + d

AB
CD

PCR #2
Primers a + d

a
AB
CD

AD
Insert into expression vector sequence to verify mutation

(b) PCR #1
Primers a + b, c + d

AB
CD

PCR #2
Primers a + d

a
AB
CD

Chimeric AD
Insert into expression vector sequence to ensure accurate junction
PCR Epitopes and GCC

BipS  BipT  PrpS  PrpT  TGCC

SGCC
Overhang PCR

b

PCR #1
Primers a + b + c + d

PCR #2
Primers a + d

Insert into expression vector sequence to ensure accurate junction

Chimeric AD

AB
CD

AD

Bip-S
S-GCC
Bip-S-GCC
Screening pENTR clones
Cutting inserts out

- Sequenced pENTR clones
- Cut inserts out with NotI and SalI
- Cut pMSCV-Puro with NotI and SalI
- Ligated inserts with pMSCV-Puro
- Transformed bacteria
- Screened colonies
Results

![Image of a gel electrophoresis result with labeled lanes and bands indicating Bip and Prp proteins. Lanes 8-11 show a positive result (+).]
Summary/Future Goal

- Create stable cell lines expressing the constructs by target cell lines with retrovirus containing our constructs.

- Test the new cell lines for 1) chimeric antigen expression, 2) define the subcellular location or our antigen, 3) quantify epitope presentation.

- Work with Dr. Eisenlohr’s lab to see how different signal sequences and model epitopes affect our new model antigen.
Thank You!

- Thomas Jefferson University:
  - Elizabeth Rappaport MD
  - Scott Waldman MD/PhD
  - Adam Snook PhD
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  - Bo Xiang
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- Audrey Fritzinger