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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>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estimated New Cases</strong></td>
<td><strong>Estimated Deaths</strong></td>
<td><strong>Estimated New Cases</strong></td>
<td><strong>Estimated Deaths</strong></td>
</tr>
<tr>
<td><strong>Prostate</strong></td>
<td><strong>Breast</strong></td>
<td><strong>Lung &amp; bronchus</strong></td>
<td><strong>Breast</strong></td>
</tr>
<tr>
<td>241,740 (29%)</td>
<td>226,870 (29%)</td>
<td>87,750 (29%)</td>
<td>72,590 (26%)</td>
</tr>
<tr>
<td><strong>Lung &amp; bronchus</strong></td>
<td><strong>Lung &amp; bronchus</strong></td>
<td><strong>Prostate</strong></td>
<td><strong>Breast</strong></td>
</tr>
<tr>
<td>116,470 (14%)</td>
<td>109,690 (14%)</td>
<td>28,170 (9%)</td>
<td>39,510 (14%)</td>
</tr>
<tr>
<td><strong>Colon &amp; rectum</strong></td>
<td><strong>Colon &amp; rectum</strong></td>
<td><strong>Colon &amp; rectum</strong></td>
<td><strong>Colon &amp; rectum</strong></td>
</tr>
<tr>
<td>73,420 (9%)</td>
<td>70,040 (9%)</td>
<td>26,470 (9%)</td>
<td>25,220 (9%)</td>
</tr>
<tr>
<td><strong>Urinary bladder</strong></td>
<td><strong>Uterine corpus</strong></td>
<td><strong>Pancreas</strong></td>
<td><strong>Pancreas</strong></td>
</tr>
<tr>
<td>55,600 (7%)</td>
<td>47,130 (6%)</td>
<td>18,850 (6%)</td>
<td>18,540 (7%)</td>
</tr>
<tr>
<td><strong>Melanoma of the skin</strong></td>
<td><strong>Thyroid</strong></td>
<td><strong>Liver &amp; intrahepatic bile duct</strong></td>
<td><strong>Ovary</strong></td>
</tr>
<tr>
<td>44,250 (5%)</td>
<td>43,210 (5%)</td>
<td>13,980 (5%)</td>
<td>15,500 (6%)</td>
</tr>
<tr>
<td><strong>Kidney &amp; renal pelvis</strong></td>
<td><strong>Melanoma of the skin</strong></td>
<td><strong>Leukemia</strong></td>
<td><strong>Leukemia</strong></td>
</tr>
<tr>
<td>40,250 (5%)</td>
<td>32,000 (4%)</td>
<td>13,500 (4%)</td>
<td>10,040 (4%)</td>
</tr>
<tr>
<td><strong>Non-Hodgkin lymphoma</strong></td>
<td><strong>Non-Hodgkin lymphoma</strong></td>
<td><strong>Esophagus</strong></td>
<td><strong>Non-Hodgkin lymphoma</strong></td>
</tr>
<tr>
<td>38,160 (4%)</td>
<td>31,970 (4%)</td>
<td>12,040 (4%)</td>
<td>8,620 (3%)</td>
</tr>
<tr>
<td><strong>Oral cavity &amp; pharynx</strong></td>
<td><strong>Kidney &amp; renal pelvis</strong></td>
<td><strong>Urinary bladder</strong></td>
<td><strong>Brain &amp; other nervous system</strong></td>
</tr>
<tr>
<td>28,540 (3%)</td>
<td>24,520 (3%)</td>
<td>10,510 (3%)</td>
<td>5,980 (2%)</td>
</tr>
<tr>
<td><strong>Leukemia</strong></td>
<td><strong>Ovary</strong></td>
<td><strong>Non-Hodgkin lymphoma</strong></td>
<td><strong>All sites</strong></td>
</tr>
<tr>
<td>26,830 (3%)</td>
<td>22,280 (3%)</td>
<td>10,320 (3%)</td>
<td>6,570 (2%)</td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td><strong>Pancreas</strong></td>
<td><strong>Kidney &amp; renal pelvis</strong></td>
<td><strong>All sites</strong></td>
</tr>
<tr>
<td>22,090 (3%)</td>
<td>21,830 (3%)</td>
<td>8,650 (3%)</td>
<td>275,370 (100%)</td>
</tr>
<tr>
<td><strong>All sites</strong></td>
<td><strong>All sites</strong></td>
<td><strong>All sites</strong></td>
<td><strong>All sites</strong></td>
</tr>
<tr>
<td>848,170 (100%)</td>
<td>790,740 (100%)</td>
<td>301,820 (100%)</td>
<td>275,370 (100%)</td>
</tr>
</tbody>
</table>

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

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Colorectal Cancer

- 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><strong>Mutant Self Proteins</strong></td>
</tr>
<tr>
<td>K-ras</td>
</tr>
<tr>
<td><strong>Oncofetal / Cancer Testis Antigens</strong></td>
</tr>
<tr>
<td>βhCG</td>
</tr>
<tr>
<td><strong>Overexpressed Self Antigens</strong></td>
</tr>
<tr>
<td>p53</td>
</tr>
<tr>
<td>Sialyl-Tn</td>
</tr>
<tr>
<td>Survivin</td>
</tr>
<tr>
<td><strong>Carcinoembryonic Antigen (CEA)</strong></td>
</tr>
<tr>
<td><strong>Tissue-Specific Differentiation Antigens</strong></td>
</tr>
<tr>
<td>?</td>
</tr>
</tbody>
</table>
# Guanylyl Cyclases

The table below summarizes the different isoforms of guanylyl cyclases (GCA, GCB, GCC, GCD, GCE, GCF, GCG, sGC) and their respective ligands (ANP, BNP, CNP, ST, Guanylin, Uroguanylin, NO).

<table>
<thead>
<tr>
<th>Guanylyl Cyclases</th>
<th>Isoforms</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natriuretic Peptide Receptors</td>
<td>GCA</td>
<td>ANP, BNP</td>
</tr>
<tr>
<td></td>
<td>GCB</td>
<td>CNP</td>
</tr>
<tr>
<td>Intestinal Peptide Receptor</td>
<td>GCC</td>
<td>ST</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Guanylin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uroguanylin</td>
</tr>
<tr>
<td>Orphan Receptors</td>
<td>GCD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GCE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GCF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>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

Graphs showing the effects of immunization on tumor uptake, tumor number, and percent survival.
GCC-Specific Immune Responses

![Graphs showing immune response data for GCC and control groups.](image-url)
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

OT-II TCR Tg

GCC TCR Tg

Inducible Antigen Tg

Equivalent to GCC TCR Tg
<table>
<thead>
<tr>
<th>signal sequence</th>
<th>HA Tag</th>
<th>Ova\textsubscript{257-264}</th>
<th>Ova\textsubscript{329-337}</th>
<th>Tac</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Transmembrane domain**

**B16**

**B16-Tac**

6\(\mu\)m slice
Tac FACS

Extracellular

Intracellular
Epitopes and surface expression

- **Tac**
  - Intracellular
  - Surface

- **hIL-2Ra (no epitopes)**
  - Intracellular
  - Surface
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

Diagram:

**a**
- Primers: a + b, c + d
- PCR #1
- Insert into expression vector sequence to verify mutation

**b**
- Primers: a + b, c + d
- PCR #1
- Insert into expression vector sequence to ensure accurate junction
PCR Epitopes and GCC

BipS  BipT  PrpS  PrpT  TGCC

SGCC
Overhang PCR

Bip-S  S-GCC  Bip-S-GCC

Chimeric AD
Insert into expression vector sequence to ensure accurate junction

AD

PCR #1
Primers a + b + c + d

AB
CD

PCR #2
Primers a + d

AB
CD
Screening pENTR clones
Cutting inserts out

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

[Image of a gel electrophoresis result with labels Bip 1 to Bip 7 and Prp 8 to Prp 11]
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
  - Michael Magee
  - Bo Xiang
  - Laurence Eisenlohr VMD, PhD
- St. Joseph University
- Audrey Fritzinger