Presenter disclosure information

• David H. Munn, MD

The following relationships exist related to this presentation:

NewLink Genetics, Inc.  (Consultant, stock, SAB)
Immune Suppression by Stromal Cells

David H. Munn
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Potentially suppressive stromal cells

• tolerogenic DCs
• tumor-associated macrophages
• MDSCs
• endothelial cells
Potential molecular mechanisms

• indoleamine 2,3-dioxygenase
• arginase I
• Treg mechanisms (CTLA-4 & others)
• PD-ligands
• ROS, NO
Indoleamine 2,3-dioxygenase (IDO)

- IDO is a natural endogenous molecular mechanism of immune suppression
- IDO can create acquired peripheral tolerance *de novo*
- IDO is counter-regulatory (i.e., induced by inflammation but suppressive for immune responses)
- IDO regulates both innate and adaptive responses
  - control of local inflammation, IL-6, etc
  - suppresses effector T cells, activates Tregs
Ovarian carcinoma (sample courtesy of Dr. Kunle Odunse)
[From Qian, Odunsi and colleagues, Cancer Res 2009;69:5498–504]
IDO in melanoma sentinel LNs is expressed by plasmacytoid DCs

**IDO staining**
(From Munn et al, Science 2002)

**BDCA2 vs IDO**
Predictive value of abnormal IDO expression in human tumor-draining lymph nodes

- 40 patients with cutaneous malignant melanoma, no metastases
- sentinel lymph node obtained at time of initial diagnosis

- in collaboration with Scott Antonia at Moffitt Cancer Center

INDO expression by microarray and by qPCR correlated to clinical outcome in patients with adult AML

Brain tumors:
(IDO staining in mouse glioblastoma)

• Also may be important to stain for TDO in CNS tumors
IDO and TDO in glioblastoma

How does IDO get turned on?
DCs in prostate tumors express FOXO3, which induces IDO expression and a suppressive DC phenotype


**Table 2**
Human TADCs have elevated expression of genes associated with tolerance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold change tumor/non-tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>FASLG</td>
<td>5.2</td>
</tr>
<tr>
<td>IDO1</td>
<td>7.3</td>
</tr>
<tr>
<td>CD274</td>
<td>3.1</td>
</tr>
<tr>
<td>STAT3</td>
<td>5.1</td>
</tr>
<tr>
<td>FOXO3</td>
<td>6.9</td>
</tr>
</tbody>
</table>

RNA was isolated and hybridized to Affymetrix Human Gene 1.0 ST arrays. Fold change values have corresponding *P* values of less than 0.00001 (ANOVA). Data are representative of 5 independent microarrays for tumor and non-tumor biopsies.
Technical caveats in IDO staining

• different IDO antibodies may give different staining patterns (validated antibodies are crucial)

• tumor IDO may stain differently than host IDO (mutation, alternant splicing, etc)

• antibodies may cross-react with IDO1 and IDO2

• some epitopes are labile in room air (need to stain fresh-cut sections)

• most IDO antibodies need antigen retrieval for paraffin
Links between IDO, CTLA-4 and Tregs

IDO → CTLA4 loop

Dendritic cell

FOXO3

B7

CTLA-4

Activated Treg

AhR

KYN

TRP

GCN2
High expression of IDO and Foxp3 at baseline may be a predictor of clinical response to ipilimumab.

Why does the presence of tumor automatically create a tolerogenic microenvironment?

• Virchow said that a tumor was a “wound that never heals” …
  • constant tissue remodeling
  • new blood-vessel formation
  • chronic, macrophage-driven inflammation

• all healing wounds (if sterile) may be an inherently tolerogenic milieu for T cells
  • tissue remodeling releases many self antigens – may need active tolerance induction
  • the local milieu is rich in TGFβ, VEGF (tolerogenic cytokines)
  • macrophage-mediated inflammation may suppress rather than activate T cells
Other potentially suppressive cell types:
• Tumor-associated MΦ’s

CD169+ macrophages in TDLN (mouse)
IDO helps maintain tolerance to self antigens derived from apoptotic cells

IDO-KO mice develop lupus when challenged with apoptotic cells

From Tracy McGaha lab
Ravishankar B et al. PNAS 2012
IDO can regulate adaptive T cell immune responses

from Mellor & Munn, *Nature Reviews | Immunology*, 2008
Arginase I Expression in 3LL Tumor

Isotype

Arginase I

Courtesy of Dr. Augusto Ochoa’s lab
L-Arginine

CAT-2B

Arginase
L-Ornithine + Urea
Polyamines
Cell Proliferation
Collagen
L-Proline

NOS
NO + L-Citrulline
Vasodilatation, inhibition of inflammation
ONOO-
Cytotoxicity

Courtesy of Dr. Augusto Ochoa’s lab
T lymphocytes

L-Arg

Arg-tRNA

GCN2

P-eIF2α

HuR

Cyclin D3

Protein

Cyclin D3 mRNA stability

T lymphocytes

Courtesy of Dr. Augusto Ochoa’s lab

phospho-eIF2a (mouse)
Conclusions and future directions

- IDO and Arginase are two potential stroma-derived suppressor mechanisms in tumors and TDLNs.

- These mechanisms activate a shared pathway of amino-acid depletion (eIF2a > GCN2 > CHOP).

- They may link mechanistically to other pathways (e.g., CTLA-4, Tregs, mTOR).