IMMUNOSUPPRESSIVE MYELOID CELLS
IN PANCREATIC CANCER

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Cancer Hallmarks

Hanahan and Weinberg, Cell 2000

Next generation

Emerging Hallmarks

Avoiding immune destruction

Deregulating cellular energetics

Genome instability and mutation

Tumor-promoting inflammation

Enabling Characteristics

Hanahan and Weinberg, Cell 2011
Immune escape within tumor microenvironment: who are the actors?

TUMOR REJECTION

- CTL
- B Cell
- Dendritic cells

TUMOR PROGRESSION

- Treg
- Tumor Associated Macrophages (TAM)
- Myeloid Derived Suppressor Cells (MDSC)
Developing of Pancreatic Ductal Adenocarcinoma (PDA): model of immune reaction

C.E. Clark et al., Cancer Letters 2009
Tumor-stroma interactions: role of soluble factors and membranal co-inhibitory molecules

The bi-directional tumor-stroma interactions are both **cell contact independent** and mediated by several soluble factors, (including the inflammatory molecules S100A8/A9) and **cell contact dependent**. In this case the role of the co-co-inhibitory molecules CTLA4 and PDL-1 has been suggested.

**Focus on:**

- Soluble factors **S100A8/A9**, highly expressed by tumor infiltrating inflammatory cells and by tumor cells themselves
- **Co-inhibitory molecules CTLA-4 and PDL-1** whose altered expression in tumor infiltrating lymphocytes and myeloid cells was claimed to favour tumor progression
Soluble mediators: S100 A8/A9 heterocomplex

NORMAL CELLS

- CELL CYCLE REGULATION
- CELL GROWTH
- MIGRATION
- SIGNAL TRANSDUCTION

PANCREATIC CANCER (PC) CELL

- PROGRESSION
- INVASION
- METASTATIC PROCESS
- STRONG EXPRESSION IN STROMAL MYELOID CELLS INFILTRATING PC
S100A8/A9 in PDAC progression

**Bone marrow**

Hematopoietic stem cells → Myeloid progenitor → **Immature myeloid cells** → Dendritic Cells, Macrophage

**Tumor derived factor**

**Premetastatic niche**

**PDAC**

Tumor progression and migration

Pro-tumorigenic genes

Tumor derived factor

VEGF, TGF-β, TNFα

**S100A8/A9 heterocomplex**

**RAGE**
**Co-Inhibitory molecules**

1. **T cell activation**
   - T cell
   - Antigen
   - MHC
   - TCR
   - CoL
   - CoINH

2. **T cell activation**
   - T cell
   - Antigen
   - MHC
   - TCR
   - CoL
   - CoINH

3. **T cell proliferation**
   - T effector cell
   - Antigen
   - MHC
   - TCR
   - CoL
   - CoINH
   - ipilimumab
CTLA-4 Blockade

Anti-tumor immunity

... and autoimmunity

Lung

Skin

Colon

Colon CD3

Liver

Brain

Pardoll DM. Nature Review Cancer, April 2012
Inhibitory molecules of interest in PaCa

CTLA4

PDL1

Pardoll DM. Nature Review Cancer, April 2012
AIMS OF THE STUDY

● To assess “in vitro”, whether pancreatic cancer cells (BxPC3, Capan 1, MiaPaCa2) conditioned media induce normal mononuclear circulating cells to acquire an immunesuppressive phenotype.

● To verify whether the S100A8/A9 hetero-complex, could be responsible for any immunesuppressive effect.

● To evaluate the involvement of the inhibitory co-stimulatory molecules PDL-1 and CTLA4.
Methods: Pancreatic cancer cell lines

BxPC3
- well-differentiated
- poorly metastatic
- Express high levels of S100A8/A9

Capan 1
- Moderately differentiated
- Moderately metastatic
- Express high levels of S100A8/A9

MiaPaCa2
- poorly differentiated
- highly metastatic
- not express S100A8/A9

2x10^5 BxPC3 and MiaPaCa2 cells and 4x10^5 Capan 1 cells were cultured for four days in 1% FCS medium.
PBMC (Peripheral Blood Mononucleare Cells) were isolated from healthy blood donors’ Buffy coats.
Methods

Human PBMC were splitted in four fractions, cultured for **four** days in complete control medium or PC cells conditioned media (adjusted to 10% FCS). After collection, the cells were analyzed by flow cytometry.

PBMC cells

Control

PC Conditioned media

**Flow cytometry**

**Four antibody panels**

1. CD3/CD45/CD4 or CD8
2. CD3/CD45/CD4/CD25
3. CD33/CD45/HLA-DR/CD14
4. CD33/CD45/HLA-DR/CD14/CTLA4 or PDL1
Human PBMC were split into two fractions, cultured for two days in complete control medium or medium supplemented with 10 nM S100A8/A9 complex. After collection, the cells were analyzed by flow cytometry.

Flow cytometry

Four antibody panels

1. CD3/CD45/CD4 or CD8
2. CD3/CD45/CD4/CD25
3. CD33/CD45/HLA-DR/CD14
4. CD33/CD45/HLA-DR/CD14/CTLA4 or PDL1
Myeloid populations

Antibodies: CD33/CD14/HLA-DR

Dendritic cells
CD14-DR+

Monocytes
CD14+DR+

Granulocytic MDSC
(gMDSC) CD14-DR-

Monocytic MDSC
(mMDSC) CD14+DR-
**Results**

Pancreatic cancer cell conditioned media effects on **Lymphocyte** and **Immature myeloid cell** subsets.

Median, minimum, maximum values and statistical analysis of data

<table>
<thead>
<tr>
<th></th>
<th>Control (n=17) Median (IQR)</th>
<th>BxPC3 (n=6) Median (IQR)</th>
<th>Capan 1 (n=11) Median (IQR)</th>
<th>MiaPaCa2 (n=6) Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD4⁺</strong></td>
<td>50 (44-57)</td>
<td>58 (39-61)</td>
<td>50 (41-51)</td>
<td>56 (41-61)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.674</td>
<td>p=0.386</td>
<td>p=0.917</td>
<td></td>
</tr>
<tr>
<td><strong>CD8⁺</strong></td>
<td>23 (15-27)</td>
<td>19 (15-31)</td>
<td>22 (16-27)</td>
<td>19 (15-32)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.715</td>
<td>p=0.625</td>
<td>p=0.917</td>
<td></td>
</tr>
<tr>
<td><strong>CD4⁺CD25⁺</strong></td>
<td>10.0 (8.8-11.4)</td>
<td>8.9 (8.0-10.8)</td>
<td><strong>11.9 (10.4-13.0)</strong></td>
<td>9.0 (7.7-10.8)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.269</td>
<td>p=0.014*</td>
<td>p=0.599</td>
<td></td>
</tr>
<tr>
<td><strong>CD14⁺HLA-DR⁺</strong></td>
<td>71 (67-86)</td>
<td>59 (36-74)</td>
<td>81 (76-88)</td>
<td>59 (40-77)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.075</td>
<td>p=0.062</td>
<td>p=0.463</td>
<td></td>
</tr>
<tr>
<td><strong>CD14⁺HLA-DR⁻</strong></td>
<td>0.0 (0.0-0.4)</td>
<td>0.2 (0.0-0.3)</td>
<td>0.1 (0.0-0.4)</td>
<td>0.0 (0.0-0.3)</td>
</tr>
<tr>
<td>P value</td>
<td>p=0.465</td>
<td>p=0.391</td>
<td>p=0.715</td>
<td></td>
</tr>
<tr>
<td><strong>CD14⁺HLA-DR⁺</strong></td>
<td>16.5 (7.9-20.7)</td>
<td>26.9 (15.0-46.8)</td>
<td><strong>10.8 (9.7-14.4)</strong></td>
<td>29.7 (11.8-46.3)</td>
</tr>
<tr>
<td>P value</td>
<td>p=0.116</td>
<td>p=0.033*</td>
<td>p=0.249</td>
<td></td>
</tr>
<tr>
<td><strong>CD14⁺HLA-DR⁻</strong></td>
<td>9.3 (3.0-13.2)</td>
<td><strong>14.3 (12.3-16.2)</strong></td>
<td>6.7 (1.2-11.2)</td>
<td>9.7 (8.1-17.3)</td>
</tr>
<tr>
<td>P value</td>
<td>p=0.028*</td>
<td>p=0.285</td>
<td>p=1.00</td>
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</tr>
</tbody>
</table>

**Immature CD3³⁺ myeloid cells (%)**

- **gMDSC cells**
- **Treg**
- **Dendritic cells**
- **Ineffective**
### Results

Pancreatic cancer cell conditioned media effects on **PDL1** and **CTLA4** in immature myeloid cell subsets

<table>
<thead>
<tr>
<th></th>
<th>Control (n=14) Median (IQR)</th>
<th>BxPC3 (n=6) Median (IQR)</th>
<th>Capan 1 (n=8) Median (IQR)</th>
<th>MiaPaCa2 (n=6) Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD14</strong>+<strong>HLA</strong>-DR**+** PDL**+**</td>
<td>56 (37-73)</td>
<td>61 (23-82)</td>
<td>76 (73-92)</td>
<td>50 (21-76)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.249</td>
<td>p=0.036</td>
<td>p=0.686</td>
<td></td>
</tr>
<tr>
<td><strong>CD14</strong>+<strong>HLA</strong>-DR**+** PDL**+**</td>
<td>31 (22-36)</td>
<td>26 (10-50)</td>
<td><strong>46 (39-53)</strong></td>
<td>33 (28-45)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.753</td>
<td>p=0.017*</td>
<td>p=0.173</td>
<td></td>
</tr>
<tr>
<td><strong>CD14</strong>-<strong>HLA</strong>-DR**-** PDL**+**</td>
<td>2.7 (0.4-4.8)</td>
<td>3.3 (0.3-6.3)</td>
<td>3.8 (1.3-10.6)</td>
<td>1.7 (1.3-5.8)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.753</td>
<td>p=0.108</td>
<td>p=0.345</td>
<td></td>
</tr>
</tbody>
</table>

**PDL1 expression among CD33+ cells**

**CTLA4 expression among CD33+ cells**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=14) Median (IQR)</th>
<th>BxPC3 (n=6) Median (IQR)</th>
<th>Capan 1 (n=8) Median (IQR)</th>
<th>MiaPaCa2 (n=6) Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD14</strong>+<strong>HLA</strong>-DR**+** CTLA4**+**</td>
<td>16 (12-23)</td>
<td>28 (9-54)</td>
<td><strong>6 (1-8)</strong></td>
<td>15 (10-20)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.345</td>
<td>p=0.028*</td>
<td>p=0.500</td>
<td></td>
</tr>
<tr>
<td><strong>CD14</strong>+<strong>HLA</strong>-DR**+** CTLA4**+**</td>
<td>13 (4-21)</td>
<td>4 (2-16)</td>
<td><strong>4 (1-7)</strong></td>
<td>10-2-57</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.116</td>
<td>p=0.043*</td>
<td>p=0.917</td>
<td></td>
</tr>
<tr>
<td><strong>CD14</strong>-<strong>HLA</strong>-DR**-** CTLA4**+**</td>
<td>12.3 (10.0-13.2)</td>
<td><strong>5.7 (0.5-10.7)</strong></td>
<td><strong>4.3 (2.9-5.8)</strong></td>
<td>8.3 (2.9-19.5)</td>
</tr>
<tr>
<td>p-value</td>
<td>p=0.028*</td>
<td>p=0.046*</td>
<td>p=0.753</td>
<td></td>
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</tbody>
</table>
Is the downregulation of CTLA4 implicated in immunosuppression?

Yes

The downregulation of CTLA4 expression in myeloid cells is associated with an immunosuppressive phenotype.
Results 2

S100A8/A9 heterocomplex cause:

CD14+HLA-DR- mMDSC (p=0.043)

CD14-HLA-DR+ DC (p=0.017)

PDL1+ cells among DC (p=0.018)

CTLA4+ cells among mMDSC (p=0.028)
Conclusions

SOLUBLE FACTORS

S100A8/A9 heterocomplex

MDSC

PANCREATIC CANCER CELLS

gMDSC

CTLA4-

mMDSC

CTLA4-

IMMUNOSUPPRESSION

Anti-CTLA4 therapies = To be cautious