Immune elements and cancer stemness

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Presenter disclosure information

I have no financial relationships to disclose.

I will not discuss off-label use and/or investigational use in my presentation.
A. Balance, Key to life: Immune imbalances and suppressive networks in the tumor microenvironment.

B. Immune impact, Key to cancer progression

Immune elements and oncogenesis model: MDSCs, Th22
A. Immune imbalances and suppressive networks in the human tumor microenvironment

I. Inhibitory and stimulatory B7 imbalance
II. T cell subset (Treg and effector T) imbalance
III. APC subset (DC and suppressive APC) imbalance


W Zou, L Chen. Inhibitory B7 family molecules in the tumor microenvironment. 8:467-477, Nature Reviews Immunology, 2008

W Zou and N Restifo. Th17 cells, tumor immunity and immune therapy. 10:248-256, Nature Reviews Immunology, 2010
I. Inhibitory and stimulatory B7 imbalance

APC or tumor cell

- B7-1 (CD80)
- B7-2 (CD86)
- B7-H1 (PD-L-1)
- B7-DC (PD-L2)
- B7-H2 (ICOSL)
- B7-H3
- B7-H4 (B7S1, B7x)

MHC

T cell

- MYPPY
- CD28
- CTLA-4
- CD80
- PD-1
- V
- C
- ICOS
- FDPPPF
- ?

Mechanisms of inhibitory B7-H1 (PD-L1) in the evasion of T cell-mediated immunity
Clinical response: it is REAL

2013 Science Breakthrough

June 2, 2012
New England Journal of Medicine

A. Topalian et al: anti-PD-1 treatment
18-36% patients with B7-H1+ tumors had an objective response.

B. Brahmer et al: anti-PD-L1 (B7-H1) treatment
Anti- B7-H1 induced durable tumor regression (objective response rate of 6 to 17%) and prolonged stabilization of disease (rates of 12 to 41% at 24 weeks) in patients with advanced cancers
II. T cell subset imbalance

Effector T cells
- Effector CTL
- Effector CD4+ T cells including Th17

Regulatory T cells
- IL10+CD8+ suppressive T cells
- CD4+CD25+ suppressive T cells

Tumor microenvironment

Tumor immunity

Chemokines/receptors and Treg site/organ trafficking

Thymus

Periphery

Treg

Treg

Bone marrow

Tumor, graft

Lymph node

Inflammatory tissue

CXCL-12

CCL22

CCL19

CCL2, 3, 4, 5, 7, 13

CXCR4

CCR4

CCR7

CD62L

CCR2, CCR5

CD103, CD62P, CD62E

Bone marrow    Tumor, graft     Lymph node    Inflammatory tissue

CXCR4

CCR4

CCR7

CD62L

CCR2, CCR5

CD103, CD62P, CD62E
III. APC subset imbalance

MDC

Potent IL-12, Th-1 polarization, TAA-specific effector memory CTL

Tumor microenvironment

PDC, MDSC, immature DC

No IL-12, Th-2 polarization, TAA-specific IL10+ central memory CD8+ T cells?

Tumor immunity

### Boost tumor immunity

<table>
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<tr>
<th>Immune elements</th>
<th>Enhancing “enhancer”</th>
<th>Hypothesis: missing, insufficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>T cell therapy</td>
<td>Strategy: supplementation</td>
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<tr>
<td>Dendritic cells (DC)</td>
<td>DC vaccine</td>
<td>Purpose: boosting immunity</td>
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<tr>
<td>NK cells</td>
<td>LAK cell injection</td>
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<tr>
<td>Tumor antigen (TAA)</td>
<td>Peptide, TAA-Vectors</td>
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<tr>
<td>Cytokines</td>
<td>IL2, IFNα, IL12</td>
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### Recover tumor immunity

**Target immune suppressive networks**

<table>
<thead>
<tr>
<th>Suppressive elements</th>
<th>Inhibiting “inhibitor”</th>
<th>Hypothesis: suppressive, dysfunctional</th>
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<tr>
<td>Regulatory T cells</td>
<td>Control Tregs</td>
<td>Strategy: subversion</td>
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<tr>
<td>Regulatory DC, MDSC</td>
<td>Blocking suppressive signal</td>
<td>Purpose: recovering immunity</td>
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<tr>
<td>Self, Dominant Ag</td>
<td>Ag release and priming</td>
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<tr>
<td>IL6, IL10, M-CSF, VEGF</td>
<td>Blocking common pathways</td>
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B. Immune impact, Key to cancer progression

Immune elements and oncogenesis model

1. MDSCs and ovarian cancer stemness
2. Th22 and colon cancer stemness
Important concepts:

Stem cells
Shinya Yamanaka
Kyoto University

John B. Gurdon
University of Cambridge

Cancer stem cells?

Oncogenesis model
Robert A. Weinberg
Massachusetts Institute of Technology
B. Immune impact, Key to cancer progression

Immune elements and oncogenesis model

1. MDSCs and ovarian cancer stemness
MDSCs and primary ovarian cancer progression

Primary ovarian cancer

Survival Probability

Time to death (weeks)

0 100 200 300 400 500 600 700

MDSC\textsuperscript{LOW}

--- MDSC\textsuperscript{HIGH}

Primary ovarian cancer

DFS Probability

Time to progression (weeks)

0 100 200 300 400 500 600 700

MDSC\textsuperscript{LOW}

--- MDSC\textsuperscript{HIGH}
MDSCs and ovarian cancer metastasis
MDSCs promote ovarian cancer metastasis
MDSCs promote ovarian cancer stemness

**Sphere increase (fold)**

<table>
<thead>
<tr>
<th>MDSC Donors</th>
<th>No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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**Relative expression**

- OCT3/4
- SOX2
- NANOG
- NOTCH2
- NOTCH3
- CHERP
- HEY1
- HEY2

The diagrams show the increased sphere formation and relative expression levels for different MDSC donors compared to the control (No).
MDSCs promote ovarian cancer stemness

Mechanism? Genetics? Epigenetics?
MDSCs stimulate microRNA101 and promote ovarian cancer stemness
MDSCs stimulate microRNA101 and promote cancer stemness

Mechanism?

Eight repressor complexes: SWI-SNF, PRC1, NURD, CoREST (CtBP2), NCoR, PRC2(EZH2), SIN3, TLE
MicroRNA101 targets CtBP2 and promotes ovarian cancer stemness

**WT 3’UTR-CtBP2**

5' ... AGUGUGAGUUACC - - UACUGUAU

3' AAAGUCAAUAGUUGUCAGACAU

**Mutant 3’UTR-CtBP2**

5' ... AGUGUGAGUUACC - - AUCAGAAU

3' AAAGUCAAUAGUUGUCAGACAU

has-microRNA101
MicroRNA101 targets CtBP2 and promotes ovarian cancer stemness
MicroRNA101 represses CtBP2 and targets core stemness genes
MDSCs support stemness via microRNA101/CtBP2

Ovarian cancer associated MDSCs

Tumor cell

miR101 upregulation

CtBP2 downregulation

Derepression CtBP2 target genes

Increased stemness

CtBP2 mRNA

Stem cell genes repressing

CtBP2

Transcription

Increased stemness
MDSCs, microRNA101 and cancer stemness

1. MDSCs: Immune suppression
   Creating and maintaining immune suppressive environment

2. MDSCs: Stem niche
   Promoting and sustaining cancer stem cell pool
B. Immune impact, Key to cancer progression

Immune elements and oncogenesis model

2. Th22 and colon cancer stemness
2. Th22 and human colon cancer stemnes
Th22 and IL-22 in the colon cancer environment

**IL22 expression (x 10^-3)**

Cancer

Blood

Blood plot:
- CD3 vs. IL-22
- Colony cancer plot:
  - CD3 vs. IL-22
  - IL-22 expression levels: 65, 71
Th22-derived IL-22 promotes colon carcinogenesis

**Tumor incidence (%)**

**Tumor volume (mm³)**

Days after inoculation

Days after inoculation

Control Anti-IL-22

Control Anti-IL-22

* Significant difference

** Significant difference
IL-22 promotes cancer spheres

Sphere number

Patient 1 vs Patient 2

Control
T cells
T cells + anti-IL-22

Patient 1
Patient 2

IL-22 (ng/ml)

0
5
20
50
100
IL-22 targets core stem cell genes

Gene expression

- **NANOG**
- **SOX2**
- **POU5F1**

Control  
T cells  
T cells + anti-IL-22

Time (h) 0 24 48 72

Sox2

β-actin

Time (h) 0 6 12 24 48

OCT3/4

Nanog

NICD

β-actin
Th22 cells stimulate core stem cell genes and promote cancer stemness

Mechanism?

Genetic?
Major transcription factor (STAT3)

Epigenetic?
Major histone marks
IL-22 induces global H3K79 di-methylation
H3K79 methylation

Histone 3 Lysine 79 di-methylation (H3K79me2):
- DOT1L mediated methyltransferase
- Generally a mark for activation

EPZ004777 is a specific DOT1L inhibitor
IL-22 induced-H3K79 di-methylation controls colon cancer stemness

H3K79 inhibitor inhibited IL-22 dependent colon cancer stemness

Dot1L knockdown inhibited IL-22 dependent colon cancer stemness
IL-22 induces H3K79 di-methylation on stem cell core gene promoters

**NANOG**

- IgG
- H3K79m2 Control
- H3K79m2 IL-22

**SOX2**

- IgG
- H3K79m2 Control
- H3K79m2 IL-22

**OCT3/4**

- IgG
- H3K79m2 Control
- H3K79m2 IL-22

STAT3: TGGCATTCCAGCAATGAT
STAT3: ggggtccc

STAT3: CCGGGAACCTCCCCGAGATGC
STAT3: ATCCCGGAA

STAT3: AGGGAAAGC
STAT3: TGCCACCTCCAGGAATTG
STAT3: TCCCATTCCCTGGATTTGA
IL-22 induces H3K79 di-methylation on stem cell core gene promoters

![Graph showing IL-22 induction of H3K79 di-methylation](image)

*Indicates statistical significance.
IL-22 induced-H3K79me2 on stem cell core gene promoters depends on STAT3.
DOT1L and H3K79me2

Clinical relevance?
Expression of DOT1L and H3K79me2 is associated with poor cancer survival

Cumulative Proportion survival

Low DOT1L

High DOT1L

Low H3K79me2

High H3K79me2

Time (months)
Th22 cells and cancer stemness: STAT3/DOT1L/H3K79me2/stem genes

Th22 cells activate STAT3 in tumor cells

STAT3 directly activates Dot1L expression

Dot1L activates stem cell core genes

Th22 cells and cancer stemness: STAT3/DOT1L/H3K79me2/stem genes

Th22 cells activate STAT3 in tumor cells

STAT3 directly activates Dot1L expression

Dot1L activates stem cell core genes

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Th22 cells activate STAT3 in tumor cells

STAT3 directly activates Dot1L expression

Dot1L activates stem cell core genes
Oncogenesis model

Potential cancer initiation: Genetic mutations/instability

Genetic signal, signal 1 (Knudson hypothesis):
Intraclonal genetic alternation: $10^{-8} \times 10^{-8} = 10^{-16}$
Interclonal genetic alternation: $10^{-8} + 10^{-8} = 2 \times 10^{-8}$

Extrinsic stemness signal (MDSC, macrophage, Th22), signal 2:
Environmental inflammatory stimuli (Signals for stemness maintenance).
Mutation + extrinsic signals $>10^{-8}$

Immune suppressive signal (MDSCs, Tregs), signal 3:
Mutation + environmental stimuli + suppressed immunity $>> 10^{-8}$
Three signal T cell activation model

**Signal 1**
- MHC
- TCR

**Signal 2**
- B7-1 (CD80)
- B7-2 (CD86)
- MYPPY
- CD28
- CTLA-4

**Signal 3**
- B7-H1 (PD-L1)
- B7-DC (PD-L2)
- B7-H2 (ICOSL)
- B7-H3
- B7-H4 (B7S1, B7x)
- FDPPPF
- ICOS
- ICOS

**Signal 1**
- V
- C

**Signal 2**
- MYPPY

**Signal 3**
- V
- C

**Signal 2**
- CD80
- PD-1
- ?

**Signal 3**
- ?
Take home message: Three signal oncogenesis model

Oncogenesis: Cancer initiation, establishment and progression

Oncogenesis $\Downarrow$ Initiation

Signal 1: Genetic signal
Signal 2: Stemness (inflammatory) signal
Signal 3: Immune suppressive signal