Immune impact on cancer stemness and metastasis

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Immune impact on cancer stemness and metastasis

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I have no financial relationships to disclose.

I will not discuss off label use and/or investigational use in my presentation.
Think Big

1. Balance is the key to life: Immune imbalance in the tumor microenvironment.
2. Oncogenesis model

Use the simplest method and technology to address the most complex cross-functional issues
Impact of immune imbalances in the human tumor microenvironment

Immune imbalances:
- Inhibitory and stimulatory B7 family members
- APC subsets
- Effector and regulatory T cell subsets


APC subset imbalance

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

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

Tumor microenvironment

Tumor immunity

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

Imbalance between Treg and Th17: Treg suppressed Th17 via adenosinergic pathway.

ARL67156 - a structural analogue of ATP and an ectonucleotidase inhibitor.
I. Th17 stemness and cancer

II. MDSC, microRNA and cancer stemness
I. Th17 stemness and mechanisms

- Th17 cells: a minor population
- GVHD, autoimmunity, tumor immunity

*Forever Seventeen*

It is that quality possessed by some which draws all others with its magnetic force ---- Elinor Glyn
Polyfunctionality of Th17 cells in human cancer
High apoptotic resistance capacity

TCR engagement

Cisplatin treatment

Annexin V (%) vs. Day

- Th1
- Th2
- Th17

Control
Th17
High proliferative renewal capacity

Colon cancer

SSC

Th0 4%
Th1 8%
Th17 15%

Ki67

IL-17

CFSE

IL-17+

IL-17−
Lasting persistence

[Graph showing percentage change in HLA-A2+ expression in Blood, Spleen, and Liver for Th1, Th2, and Th17 cells]
Long-term immunity

Tumor volume (mm$^3$) vs. Days after tumor inoculation

- Tumor only
- CD8$^+$ T
- Th17
- CD8$^+$ T + Th17

* indicates statistical significance.
Stem cell, anti-apoptosis, and Notch genes

- **NANOG expression**
  - Control: 0
  - Th17: 2
  - * indicates significance

- **SOX2 expression**
  - Control: 0
  - Th17: 3
  - * indicates significance

- **OCT3/4 expression**
  - Control: 0
  - Th17: 3
  - * indicates significance

- **HEY2 relative expression**
  - Control: 5
  - Th17: 30
  - * indicates significance

- **HEY1 relative expression**
  - Control: 1
  - Th17: 15
  - * indicates significance

- **BCLXL relative expression**
  - Control: 0.1
  - Th17: 0.2

- **BCL2 relative expression**
  - Control: 0.1
  - Th17: 0.2

- **CHERP relative expression**
  - Control: 2
  - Th17: 4
  - * indicates significance

- **HEY1 relative expression**
  - Control: 1
  - Th17: 15
  - * indicates significance
Th17 cells may have stem cell character

Proliferative self-renewal
Apoptosis resistance
Long-lived and lasting persistence
Stem cell associated genes

Mechanism?
Th17 cells express HIF-1α

HIF1A relative expression

Control
Th17

HIF1A relative expression

Th1
Th2
Th17
HIF-1α controls Bcl expression in Th17 cells

BCL2 relative expression

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BCLXL relative expression

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HIF-1α controls Th17 cell survival

Annexin V (%)

Scramble  
shHIF-1α

Control

HIF-1 inhibitor

7%
32%

Annexin V
Notch, not HIF directly regulates Bcl expression in Th17 cells

![Diagram showing hBcl-2 promoter activity](#)

- **Control**
- **HIF-1α**
- **Myc**
- **Notch-IC**

**hBcl-2 promoter activity (fold of increase)**

- 0
- 0.5
- 1.0
- 1.5
- 2.0
- 2.5
Notch directly regulates Bcl and Th17 survival
Notch directly regulates Bcl and Th17 survival

Annexin V+ cells (fold of increase)

0 0.5 1.0 1.5 2.0 2.5 3.0

Scramble

shHIF-1α

shHIF-1α + Vector

shHIF-1α + Notch-IC
Th17 cell stemness and HIF/Notch/Bcl-2

Signaling regulation

Survival, apoptosis, expansion and plasticity

--- Stem cell genes ---

---

Th17

Bcl-2

HIF-1α

Notc

Th17 cell stemness and HIF/Notch/Bcl-2

Survival, apoptosis, expansion and plasticity

Chronic environment

Plasticity

long lived effector memory cells

Long-term anti-tumor immunity

Long-term pathogen immunity

Chronic inflammation

Allergy
Th17 cell stemness and HIF/Notch/Bcl-2


1. Th17 dynamics and tumor immunity
Th17 cells are polyfunctional, and mediate protective tumor immunity

Th17 and Th1 cells collaboratively impact tumor immunity

2. Th17 stemness and mechanisms
Human Th17 cells have stem cell properties
II. Myeloid derived suppressor cells (MDSCs), microRNA and cancer stemness
MDSCs and primary ovarian cancer progression

Primary ovarian cancer

Survival Probability

Time to death (weeks)

MDSC\textsuperscript{LOW}

MDSC\textsuperscript{HIGH}

Primary ovarian cancer

DFS Probability

Time to progression (weeks)

MDSC\textsuperscript{LOW}

MDSC\textsuperscript{HIGH}
MDSCs and ovarian cancer metastasis

Metastatic ovarian cancer

Survival Probability

Time to death (weeks)

MDSC_{LOW}  MDSC_{HIGH}

DFS Probability

Time to progression (weeks)

MDSC_{LOW}  MDSC_{HIGH}
MDSCs promote ovarian cancer metastasis

- Tumor incidence (%)
- Liver weight (g)
- Lung metastasis

Days after inoculation

Control
MDSC

* Indicates significant difference
MDSCs promote ovarian cancer stemness

**Diagram:**
- **Sphere increase (fold):**
  - MDSC Donors: No, 1, 2, 3, 4, 5, 6
  - Values range from 0 to 2.5

- **Relative expression:**
  - Genes: OCT3/4, SOX2, NANOG, NOTCH2, NOTCH3, CHERP, HEY1, HEY2
  - Values range from 0 to 3.5
MDSCs stimulate microRNA101 and promote ovarian cancer stemness

MDSC Donor

microRNA101 increase

Sphere increase

Control

microRNA101 inhibitor
MicroRNA101 targets CtBP2 and promote ovarian cancer stemness

WT 3’UTR-CtBP2
5’ ... AGUGUGAGUUACCGU - UACUGUAU
3’ AAGUCAAUAGUGUCAUGACAU

Mutant 3’UTR-CtBP2
5’ ... AGUGUGAGUUACCGU - UACUGUAU
3’ AAGUCAAUAGUGUCAUGACAU

has-microRNA101
MicroRNA101 targets CtBP2 and promote ovarian cancer stemness.
MicroRNA101 represses CtBP2 and promote ovarian cancer stemness

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NANOG  OCT4/3  SOX2
MicroRNA101 promote ovarian cancer incidence and metastasis.
Low CtBP2 is associated with poor survival

Survival Probability

Time to death (weeks)

DFS Probability

Time to progression (weeks)
MDSCs cancer stemness via microRNA101/CtBP2

Ovarian cancer associated MDSCs

Tumor cell

miR101 upregulation

miR101

miR101

cTBP2 downregulation

CtBP2 target genes

Derepression

Increased stemness
MDSCs, microRNA and cancer stemness

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

2. MDSCs: Stem niche
   Promoting and sustaining cancer stem cell pool
Oncogenesis model

Tumor initiation: Genetic mutations and instability

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

Extrinsic stemness signal (MDSCs, macrophages, fibroblasts), signal 2:
Environmental stem cell niche (Signals for stemness maintenance).
Mutation + extrinsic signals \(>10^{-8}\)

Immune suppressive signal (MDSCs, Tregs), signal 3:
Mutation + environmental niche + suppressed immunity \(>> 10^{-8}\)

Three signal oncogenesis model
Controversial?

Inequalities in evaluating Th17 and tumor

- Th17 ≠ IL-17+ cell
- Th17 ≠ IL-17
- Th17 ≠ IL-23
- Exogenous IL-17 ≠ Endogenous IL-17
- ROR gene expression ≠ Th17
- Mouse ≠ Human
- Immunodeficient ≠ Immune Competent
- Early Cancer Stage ≠ Advanced Cancer Stage
- Chemical Carcinogen-induced Cancer ≠ Chronic Infection-Associated Cancer ≠ Spontaneous Cancer

Th17 cells in human spontaneous epithelial carcinoma