Glioma cancer stem cells mediate immune suppression that can be reversed with STAT3 blockade

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Immune suppression in Malignant Glioma Patients

**Mechanisms**
- Immune suppressive cytokines (TGF-β, IL-10, PGE2)
- Loss of antigen/MHC expression
- Lack of co-stimulation in the tumor microenvironment (induction of anergy)
- Induction of T cell apoptosis
- Expression of inhibitory co-stimulation (B7-H1)
- Enhanced Tregs
- Immune suppressive microglia/macrophages
- Cancer stem cells/cancer initiating cells?

**Manifestations**
- Decreased delayed type hypersensitivity responses to recall antigens
- Diminished antibody responses
- Impaired T cell proliferation and responses to IL-2
- Impaired cytotoxic/effector T cell responses
- T cell anergy/unresponsiveness

Is there a common pathway?
The STAT3 pathway is active in many cancers including gliomas.

The STAT3 pathway is a key regulatory pathway in immune suppression

Yu et al. Nature Reviews Immunology 7, 41–51 (January 2007)
Glioma cancer stem cells (gCSCs)

• gCSCs possesses marked capacity for proliferation, self-renewal and differentiation. (Singh S, et al. Cancer Res. 2003, 63: 5821-5828)

• gCSCs can establish tumors with all of the classical features of human GBM. (Galli R, et al. Cancer Res. 2004, 64: 7011-7021)

Hypothesis: gCSCs contribute to the immune suppression evident in glioma patients and STAT-3 pathway blockade can reverse this immunosuppression.
Properties of GBM Cancer Stem Cells (gCSC)/cancer initiating cells

- Form neurospheres
- Form infiltrating tumors
- Are induced to differentiate into various glia populations
- Express CD133

DMEM-F-12 supplemented with EGF (20ng/mL), bFGF and B27 (1:50)
The immune phenotype of gCSCs are immune inhibitory.

**MHC I**
- 99.6%

**MHC II**
- 1.6%

**CD40**
- 0.8%

**CD80**
- 0.6%

**CD86**
- 6.3%

**B7-H1**
- 34.9%
gCSCs inhibit T cell proliferation

- **Medium (control)**
  - Anti-CD3/CD28: 58.9
  - PHA: 49.9

- **gCSC 1**
  - Anti-CD3/CD28: 31.9
  - PHA: 29.4

- **gCSC 2**
  - Anti-CD3/CD28: 12.2
  - PHA: 3.0

- **gCSC 3**
  - Anti-CD3/CD28: 20.2
  - PHA: 7.4

**Flow cytometry analysis**

- CFSE: 0.1, 0.3, 0.3, 0.1
- CD133: 0.4, 0.6, 0.6, 0.4
gCSCs induce FoxP3+ Tregs
gCSCs induce T cell apoptosis

Medium (control)  gCSC 1  gCSC 2  gCSC 3

Annexin V  7-AAD
The degree of gCSC-mediated immune suppression correlates with *in vivo* tumorigenicity.
Cytokines elaboration by gCSC screened by RayBio Cytokine Antibody Array and verified by ELISA

TGF-β 76 (39-118) pg/mL
PGE₂ 169 (79-277) pg/mL
CCL-2 210 (0-391) pg/mL
Galectin-3 2,600 (500-4,500) pg/mL
IL-10 0 pg/mL
Soluble Fas 0 pg/mL
IL-6 0 pg/mL
NO 0 pg/mL
CCL-2 210 (0-391) pg/mL
B7-H1 contributes to cell-contact dependent immune suppression
Inhibition of p-STAT3 reverses immune suppression
WP1066 restores immunological responses
Conclusions

gCSC contribute to malignant glioma patient immune suppression by:
  – Inhibition of T cell proliferation, activation and effector function
  – Induction of T cell apoptosis (via Galectin-3, B7-H1)
  – Stimulation of Tregs (via TGF-β, B7-H1)

CD133 expression does not correlate with the degree of immune suppression.
The degree of gCSC mediated immune suppression inversely correlates with *in vivo* survival.
The immune suppressive properties of gCSCs on T cell effector function and proliferation can be reversed with inhibitors of the p-STAT3 pathway.
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Conclusions

1. GBM cancer stem-like cells (gCSCs) possess intrinsic immunosuppressive properties;

2. Immunosuppressive properties of the gCSCs are reduced upon differentiation;

3. p-STAT-3 inhibitor, WP1066, can partially reverse immunosuppressive properties of the gCSCs.
Future Direction

1. Identify the secreted product(s) from cancer stem cells mediating their immune suppressive properties;

2. Clarify mechanisms by which gCSCs induce regulatory T cells;

3. Ascertain whether immunosuppressive properties of gCSCs mainly depend on STAT-3 pathway.
Galectin-3 can induce T cell apoptosis

[Graph showing flow cytometry results for different conditions with Annexin V and 7-AAD staining]
The degree of gCSC mediated immune suppression does not correlate with CD133 expression.