SERPINA3: A key player in glioblastoma malignancy and a potential biomarker for the disease

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Overview

Introduction
  GBM facts and stats
  GBM and SVZ

CSF effects
  Proliferation
  Migration
  Micro array
  Mass spectometry

SERPINA3
  Clinical implications
  Expression changes modulated by CSF
  In vitro effects
  In vivo effects
Glioblastoma (GBM) is the most common and aggressive form of primary brain cancer in adults

Accounting for 54% of new gliomas and 45% of primary malignant tumors

Incidence: ~2-3 per 100,000 people

More common in men than in women (1.3:1)

Surgery, chemo and radiotherapy (Stupp, R., et al, 2005)

Survival expectancy of a patient averages 14 months

Recurrence is close to a 100%
GBM and lateral ventricles

Decreased median survival in patients with tumors associated with the lateral ventricles
Human Brain and neurogenesis

https://beyondthedish.wordpress.com/tag/subventricular-zone/
Results
Effect of CSF on the migration and proliferation of human GBM cells

**Introduction**

**Proliferation**

**Migration**

**Transwell Migrating cells (Normalized to Control)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Migrating Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A + Control</td>
<td>0.8</td>
</tr>
<tr>
<td>1A + Non cancer CSF</td>
<td>1.2</td>
</tr>
<tr>
<td>1A + GBM CSF</td>
<td>2.0</td>
</tr>
<tr>
<td>965 + Control</td>
<td>0.9</td>
</tr>
<tr>
<td>965 + Non cancer CSF</td>
<td>1.1</td>
</tr>
<tr>
<td>965 + GBM CSF</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**% cell growth**

- Control
- cCSF
- ncCSF

**p<0.01, *p<0.05**

**Introduction**

**CSF effects**

**SERPINA3**
Effect of CSF on human GBM cells transcriptome

About 21000 probes

800 genes in WGCNA module

ALDOC

CD44

OPN

SERPINA3

GBM CSF vs NC-CSF

GBM CSF vs vehicle

GBM CSF vs NC-CSF

GBM CSF vs vehicle

Introduction

CSF effects

SERPINA3
1. Centrifugation at 2000RPM for 5 minutes to precipitate cell debris
2. Collect supernatant and filtrate through a 0.4µm membrane
3. Aliquot and ship for Mass Spectrometry analysis
Human CSF proteomics

Introduction

SERPINA3
SERPINA3
(Alpha 1-antichymotrypsin (symbol: $\alpha_1$AC, A1AC, o a1ACT))

- Serine protease inhibitor
- Inhibits cathepsin G and mast cells chymases
- Synthetized by liver, astrocytes and endometrial cells
- Increased levels of protein in inflammation, neurodegenerative diseases and cancer
- Overexpressed in endometrial, melanoma, gastric cancer and glioma
Serpin peptidase inhibitor, clade A member 3 (SERPINA3), is overexpressed in glioma and associated with poor prognosis in glioma patients.
SERPINA3 is overexpressed in GBM and is associated with poor survival

**p<0.01, *p<0.05
Cancer-CSF increases expression of SERPINA3

**p<0.01, *p<0.05**
Silencing of SERPINA3 decreases GBM cell migration, invasion and stem cell characteristics.

**p<0.01, *p<0.05**
Overexpression of SERPINA3 increases GBM cell migration and invasion

**p<0.01, *p<0.05

**p<0.01, *p<0.05
Extracellular SERPINA3 increases GBM cells migration

**p<0.01, *p<0.05**
In vivo experiments
Silencing of SERPINA3 increases survival in mice and decreases proliferation index.
Can SERPINA3 be used as biomarker for GBM?
SERPINA3 levels in CSF

- Non cancer
- Low grade
- High grade

SERPINA3 ng/ml

p=0.0027
Volumetric analysis of brain tumors

Clinical variables

Tumor location (Lim et al, 2007):
+ contacting – non contacting
1. Subventricular zone + Cortex +
2. Subventricular zone + Cortex –
3. Subventricular zone – Cortex +
4. Subventricular zone – Cortex –
SERPINA3 and genetic mutations in GBM
General Summary

1. Pro-migratory effect of cancer- CSF on GBM cells.
2. Higher presence of SERPINA3 in GBM samples.
3. SERPINA3 cellular expression is increased by CSF.
4. Silencing of SERPINA3 decreases GBM migration, proliferation and cellular stemness.
5. Overexpression levels of endogenous SERPINA3 increases GBM migration.
6. Silencing of SERPINA3 increases survival in mice.
7. Higher levels of SERPINA3 in CSF from Cancer patients.
Thank you!

**Neurogenesis and Brain Tumors Laboratory**

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