Therapeutic Potential of Targeting Sphingosine Kinase 1 in Epithelial Ovarian Cancer

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Introduction (1) – Sphingolipids & SK

Sphingolipids (bioactive signaling molecules)

Ceramide
- Apoptosis
- Senescence
- Differentiation

Sphingosine

S1P (Sphingosine-1-Phosphate)
- Proliferation
- Survival
- Migration
- Angiogenesis

Sphingosine Kinases (SK1 / SK2)

- maintain the balance between “stop” or “go” signals.
- substantial recent attention as potential therapeutic targets for cancer.
Introduction (2) – **SK1 in cancer**

**↑ SK1 expression**
- Lung, breast, colon, stomach, prostate, thyroid & brain cancer. (Johnson et al., 2005, Guan et al., 2011)

**SK1 as oncogene**
- contributes to **cancer progression** leading to ↑
  oncogenic transformation, tumor growth, chemoresistance, angiogenesis & metastasis. (Pchejetski., 2011)
- correlates with **cancer stage**, chemotherapy response, & **tumor aggressiveness** in prostate, breast, glioma, gastric & lung cancer. (Alshaker et al., 2013)
Introduction (3) – SK inhibitors

- Various SK inhibitors decreased cell viability in hematologic malignancy (Zhang. 2008) as well as solid tumor cell lines including prostate, breast, leukemia, lung, liver, pancreatic, bladder, renal cancer. (Alshaker. 2013) (French. 2003)

- In mouse model, SK inhibitor decreased tumor growth & metastasis in prostate cancer (Pchejetski. 2008) & melanoma (LaMontagne. 2006) models.

- Phase I clinical trial with Safingol in combination with cisplatin in advanced solid tumors (n=43): a significant regression of liver & lung metastasis and had prolonged stable disease. (Dickson. 2011)
Objective


• To evaluate the therapeutic potential of targeting SK1 with siRNA or inhibitors in epithelial ovarian cancer.

SK inhibitor
2-(p-Hydroxyanilino)-4-(p-chlorophenyl) thiazole, HCl, (Calbiochem)

For just SK1

SK1 inhibitor
(FTY720, Fingolimod)
- a potent immunosuppressive drugs
- FDA approved, for MS
Methods & Results (1) – **SK1 expression / siRNA**

**SK1 expression**

<table>
<thead>
<tr>
<th>ES2</th>
<th>RMG2</th>
<th>A2780-par</th>
<th>A2780-CP20</th>
<th>SKOV3i3p1</th>
<th>SKOV3-TR</th>
<th>HeyA8</th>
<th>HeyA8-MDR</th>
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<th>SK1</th>
<th>β-actin</th>
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**SK1 siRNA transfection**

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<tr>
<th>Cell</th>
<th>Agent</th>
<th>Negative control</th>
<th>siRNA-SK1 #1</th>
<th>siRNA-SK1 #2</th>
<th>siRNA-SK1 #3</th>
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Methods & Results (2) — *In Vitro (siRNA)*

- SK1 siRNA transfection

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**MTT assay**

- **A2780-par**
  - Cell viability (%)
  - 48h: Control, 18; siRNA-SK1#3, 15
  - 72h: Control, 22; siRNA-SK1#3, 17
  - 96h: Control, 25; siRNA-SK1#3, 20

- **SKOV3ip1**
  - Cell viability (%)
  - 48h: Control, 15; siRNA-SK1#3, 12
  - 72h: Control, 20; siRNA-SK1#3, 17
  - 96h: Control, 25; siRNA-SK1#3, 22

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**Apoptosis assay (FACS)**

- **A2780par, 48h**
  - Annexin V Positive cell (%)
  - Control, 18; siRNA-SK1#3, 25

- **SKOV3-ip1, 48h**
  - Annexin V Positive cell (%)
  - Control, 18; siRNA-SK1#3, 28
Methods & Results (3) – *In Vitro (SK inhibitor)*

**SK inhibitor**

**MTT assay**

48HR

- **A2780par**

72HR

- **SKOV3ip1**

**Apoptosis assay (FACS)**

- **A2780par**

- **SKOV3ip1**

**SK1 inhibitor (FTY720)**

48HR

- **A2780par**

72HR

- **SKOV3ip1**

**P-values**

- 0.01
- 0.01
- 0.01
- 0.01
- 0.01
- 0.05
Methods & Results (4) — *In Vitro*

**Bax: apoptosis marker**

- Bax antibody (Epitomics, Cat # 1c063-1)

**SK enzyme activity (ELISA)**

- Sphingosine kinase activity assay kit (Echelon Bio-sciences, Cat # k-3500)

**Graphs**

- A2780-par, 24h
  - *P* < 0.01
  - *P* < 0.01

- SKOV3ip1, 24h
  - *P* = 0.02
  - *P* < 0.01
Methods & Results (5) – *In Vivo (SK1 inhibitor)*

**In Vivo Therapy for FTY720 in Orthotopic Mouse Model**

- **Murine Species:** Female athymic nude mice, 10 mice/group

0 day

A2780 cell injection

7 day

FTY720 10mg/kg ip injection

Every 2 days

Sacrifice

35 day

- **Tumor weight**
- **SK enzyme activity**

Graphs showing:
- A2780PAR
- Tumor wt. (g)
- Sphingosine kinase activity

*P = 0.025*   
*A2780PAR*

*P = 0.02*   
*A2780PAR*
Methods & Results (6) – Clear cell ca. & drug-resistant

- **RMG2** (Clear cell ca) 48HR
  
- **A2780-CP20** (Cisplatin-resistant)

- **ES2** (Clear cell ca)
  
- **SKOV3-TR** (Paclitaxel-resistant)
Methods & Results (7) – Clear cell ca. & drug-resistant

ES2 (Clear cell ca.) 72HR A2780-CP20 (Cisplatin-resistant)
Summary

- **SK inhibition** by siRNA or inhibitors significantly affected cell survival & apoptosis in EOC.

- **SK1 inhibitor** significantly decreased the tumor weight in **orthotopic mouse model** for EOC.

- Overall these results show a significant potential for **SK1 as a molecular target** for ovarian cancer therapy.

- Further evaluations for **combination therapy with SK1 inhibitors & conventional CTx** is required for preclinical & clinical studies in EOC.
Thank you for your attention

Translational Research Team of Gynecologic Cancer, SMC
Supplementary data – SK1 expression

Immunohistochemistry: Pending
SK2 enzyme

- SK1 almost universally promotes cell survival and proliferation but the function of SK2 appears to be more complex.

- Early studies demonstrated that over-expression of SK2 suppresses cell growth and enhances apoptosis, suggesting that although the two enzymes use the same physiologic substrate and generate the same product, SK2 might have a role opposite to that of SK1.

Trends in Biochemical Sciences February 2011, Vol. 36, No. 2