Identifying Novel Combination Therapeutic Targets for Pancreatic Cancer: Real-Time Label-Free Monitoring Cancer Cell Survival & Migration

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Overview

• Synthetic Lethality

• APE1’s regulation of STAT3 DNA binding
  – Genetic approaches: overexpression and knockdown
  – Pharmacologic approach: small molecule inhibitors

• Effect on Cancer Cell Survival after Dual targeting of APE1 and STAT3
  – Pancreatic cancer

• Tumor-stroma interactions
  – Conditioned media
  – Co-cultures
**Synthetic Lethality:** the pairing of two hits is sufficient to kill cancer cells

Non-tumor cells

- DNA Repair Pathways intact
- Live cells

Tumor cells

- Other DNA Repair Pathways compensate
- Live cells

Tumor cells

- Loss of two DNA Repair Pathways
- Tumor cells die

**Symbols:**
- **X** = BRCA1/2 deficiency
- **Green circle** = DNA repair active
- **Red circle** = DNA repair compromised

**Additional Notes:**
- **PARP inhibitor OR APE1 inhibitor**
- **Synthetic Lethality:** the pairing of two hits is sufficient to kill cancer cells
Redox Control of Transcription Factors by APE1/Ref-1

**ACTIVE TRANSCRIPTION FACTOR**

Ape1/Ref-1: **OFF**

Transcription Factor Oxidized

No DNA Binding → No Activation of Target Genes

**ACTIVE TRANSCRIPTION FACTOR**

Ape1/Ref-1: **ON**

Transcription Factor Reduced

Activation of Target Genes → Protein Expression
New project in collaboration with Angelo Cardoso, Mark Kelley (IU) and Patrick Gunning (Univ. Of Toronto): STAT3 inhibitors

• Two reports of STAT3 & APE1/Ref-1 interacting:
  – HIF1α, STAT3, APE1/Ref-1, p300 all found on VEGF promoter under hypoxic conditions
  – IL-6-induced STAT3 DNA binding is stimulated in the presence of APE1/Ref-1 in HepG2 cells

• One report that STAT3 DNA binding is redox-sensitive; APE1/Ref-1 redox activity?

• STAT3 inhibitors
  (commercially available)
  – STATTIC
  – S3I-201
  From Univ. of Toronto
  – BP-1-102
  – SF-1066
Contribution of STAT3 signaling pathway to cancer metastasis.

Huang S Clin Cancer Res 2007;13:1362-1366
Block APE1/Ref-1 and/or STAT3 in tumor

Block APE1/Ref-1 and/or STAT3 in microenvironment

APE1/Ref-1 in patient stroma

APE1/Ref-1 in patient tumor

Blood-borne/Lymphatic Dissemination (vasculature)

Stromal Fibroblast

ECM

Tumor mass

APE1/Ref-1

STAT3

p

p
Treatment of Pancreatic Cancer

Resectable Disease

- Surgery

- Adjuvant Gemcitabine

Unresectable Disease

- Gemcitabine as a single agent better than 5-FU
  Burris et al, J Clin Oncol, 15:2403-2413

Why APE1/Ref-1 and STAT3 in PDAC?

APE1/Ref-1 redox control of TFs involved in hypoxia response and signaling

\[ \text{APE1/Ref-1} \xrightarrow{\text{(reduced)}} \text{TF} \xrightarrow{\text{(oxidized)}} \text{APE-1, NF\kappa B, HIF-1\alpha, STAT3}} \]

Target genes

\[ \downarrow O_2 \]
Pancreatic cancer patient tumors express APE1 and STAT3 in both tumor and stroma.

A

Patient # 1

B

<table>
<thead>
<tr>
<th>Ref-1</th>
<th>Histone H3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panc-1</td>
<td>PaCa2</td>
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</table>

B

p-STAT3 | T-STAT3 | APE1/Ref-1 | Tubulin

p-STAT3 IHC
PDAC stable cells expressing pGF-STAT3-Luciferase

- STAT3

+ STAT3

Imaging Core, Dr. Paul Territo

![Graph showing emission (Photons/s*m^2) for PaCa-2 STAT3 for weeks 3, 4, 5, and 6.](image-url)
Inhibition of APE1/Ref-1 via E3330 or overexpression of C65A-APE1/Ref-1

STAT3 DNA binding is redox sensitive and can be stimulated by APE1/Ref-1.

EMSA assays

<table>
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<tr>
<th>No NE</th>
<th>IL-6</th>
<th>0</th>
<th>1.0</th>
<th>2.0</th>
<th>0.25 mM</th>
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<tr>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>-</td>
<td>+</td>
<td></td>
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[Diagram showing EMSA results with different conditions]
Proliferation monitored by xCELLigence system from Acea Biosciences

- allows us to monitor cells in real time
- measures electrical impedance across interdigitated micro-electrodes integrated on the bottom of tissue culture E-Plates.
- The impedance measurement provides quantitative information about the biological status of the cells, including cell number, viability, and morphology

+ Cytotoxic agent
Blocking the redox function of Ape1/Ref-1 via overexpression of Redox-dead mutant (C65A) slows down cellular proliferation and cannot transactivate STAT3.

Western blot

Tumor mass

Luciferase assay with STAT3 reporter

Transactivation activity (fold)

0 1 2 3 4

pcDNA  wtAPE1/Ref-1  C65A

APE1/Ref-1

APE1/Ref-1

APE1/Ref-1

APE1/Ref-1

APE1/Ref-1

APE1/Ref-1

APE1-C65A

APE1-C65A

APE1-C65A

APE1-C65A

APE1-C65A

APE1-C65A

APE1-C65A
Inhibition of APE1/Ref-1 via knockdown decreases STAT3 reporter activity.

When APE1 protein levels are reduced, STAT3 activity is decreased.

STAT3 DNA binding is redox sensitive and can be stimulated by APE1/Ref-1... and inhibited by E3330.

Inhibition of STAT3 with small molecule inhibitors

Growth factor or cytokine signaling

STAT3

Transcription

STATTIC / S3I-201 / BP-1-102

Inhibition of STAT3 with small molecule inhibitors.
STAT3 inhibitors are specific to STAT3 and inhibit proliferation of PDAC cells.

Dual targeting of APE1 and STAT3 dramatically affects proliferation.

Dual targeting of APE1/Ref-1 and STAT3 induces apoptosis and activates Caspase 3.

Chou-Talalay method used to further show strong synergy between APE1 and STAT3 inhibitors.

<table>
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<th>Cells</th>
<th>Drugs</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt;</th>
<th>ED&lt;sub&gt;75&lt;/sub&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Synergy</th>
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<tr>
<td>Panc-1</td>
<td>STATTIC + E3330</td>
<td>0.0008</td>
<td>0.007</td>
<td>0.92</td>
<td>++++</td>
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<tr>
<td>PaCa-2</td>
<td>STATTIC + E3330</td>
<td>0.4</td>
<td>0.65</td>
<td>0.81</td>
<td>+++</td>
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<tr>
<td>Panc-1</td>
<td>S3I-201 + E3330</td>
<td>0.14</td>
<td>0.18</td>
<td>0.99</td>
<td>++++</td>
</tr>
<tr>
<td>PaCa-2</td>
<td>S3I-201 + E3330</td>
<td>0.006</td>
<td>0.018</td>
<td>0.91</td>
<td>++++</td>
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</table>
Probing interactions between tumor and stroma and APE1’s role using conditioned media experiments

Cancer-associated fibroblasts (CAFs) → Conditioned media from CAFs → Monitor tumor cell proliferation and signaling via xCELLigence and qPCR

To address role of APE1 in CAFs signaling to tumor cells:
- Knock APE1 down
- Treat with APE1 redox inhibitor (E3330)
And then prepare the conditioned media
Tumor cell proliferation is blocked when APE1 is inhibited in the stromal cells, M2B1.

Patient-derived tumor cell proliferation

Proliferation with Acea’s xCELLigence system
Probing interactions between tumor and stroma using co-culture experiments with eInserts

1500 CAF cells
3780 CAF cells

Media only
+ CAFs

Tumor cells
Inhibition of either STAT3 or APE1/Ref-1 inhibits the migration of Panc-1 cells.

**xCELLigence Migration Assay**

- Upper Chamber
- Lower Chamber
- Porous polyester membrane
- Pre-treated cells in serum-free media
- Media with chemoattractant

After migration:

*increased electrical impedance = higher combinational index (CI) value*
Dual targeting of STAT3 and APE1/Ref-1 dramatically inhibits migration of Panc-1 cells.
Growth factor or cytokine signaling (IL-6)

**APE/Ref-1**

STAT3 dimers dissociate from receptor and dimerize via SH2 domain

Transcription

Cyclin D1, Survivin, BCL-XL
Downstream targets of STAT3 are reduced following dual targeting strategy.
Both APE1/Ref-1 inhibitor, E3330 and STAT3 inhibitor, S3I-201 delay the growth of ectopic xenografts in PDAC cell line, PaCa-2.

Ready to do combination experiments.....
Future Directions

• Combination treatments in Orthotopic & GEM models of Pancreatic cancer
• Continue to dissect the mechanism of the dramatic synergy between STAT3 inhibitor and APE1/Ref-1 inhibitor
• Tumor – stroma interactions following APE1/Ref-1 and STAT3 inhibition
• Interrogate signaling between tumor and stroma and activation of STAT3, HIF1, NFκB, and/or AP-1
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