Dissecting the mechanism responsible for the anti-cancer stem cell properties of gamma-tocotrienol
Localized disease

Cured

Prostatectomy (e.g. Surgery)

Hormone ablation (e.g. chemical castration)

Castration resistant

Chemotherapy (e.g. Docetaxel)

Death

- Late onset (99.5% are diagnosed at age of 45 or older).
- Early stage with locally confined tumor
  - Prostatectomy produces a five-year survival rate of >90%
  - Around 15% of patients will have disease relapse
- When diagnosed at advanced stage
  - Hormone ablation produces immediate tumor regression
  - Most of patients will progress to castration resistant (terminal) stage
  - Currently incurable (Docetaxel, the best ever chemodrug, can only extend survival for ~ 2 months)

Roxburgh et al., The Internet Journal of Urology. 2007
Laboratory of Cancer Therapeutic Development

Anti-cancer drug screening with >200,000 natural product extracts
Creating a favourable environment:

- Highly tumorigenic and resistant to conventional treatments
- Actively socializing with the neighbours such as osteoblasts, adipocytes or tumor-associated macrophage
- Blocking out “enemies” through inhibiting lymphocyte infiltration, suppressing T-cell activation or accelerating T-cell exhaustion
- Identifying the signalling pathways that help CSCs to grow and manipulates the surrounding microenvironment may open up new opportunities for novel therapeutic development

Modified from Weilbaecher KN et al. Nature Reviews Cancer 2011

Modified from Benjamin B et al. Nature Reviews Cancer 2013

Current Research Programme: Targeting cancer stem cells (CSCs) and their niche
Mice injected with vehicle pretreated cancer cells

Mice injected with γ-T3 pretreated cancer cells

Mice orally fed with γ-T3

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of mice</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mice with visible tumour at week 4</td>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Percentage of tumour initiation</td>
<td>100%</td>
<td>38%</td>
<td>25%</td>
</tr>
<tr>
<td>Average size of tumors (mm³)</td>
<td>421 ± 31</td>
<td>&lt;25</td>
<td>&lt;25</td>
</tr>
</tbody>
</table>

Vehicles

- Vehicle
- Docetaxel (40ng/ml)
- γ-T3 (10µg/ml)

p=0.006

Luk et al 2010. *Int J Cancer.*
Identification of Ang-1 as a novel T3-downstream target

180K Agilent cDNA array

- Ang-1 mRNA relative expression (Fold Change relative to vehicle)
  - Vehicle: +
  - γ-T3 (10 µg/ml): −

- Ang-1 secretion relative expression (Fold Change relative to vehicle)
  - Vehicle: +
  - γ-T3 (10 µg/ml): −

- Ang-1 (600 ng/ml)

Arai F et al. Stembook.

CD49f
Bmi-1
p27
Beta-actin

APCRC-Q
Australian Prostate Cancer Research Centre
Queensland
Ang-1/Tie-2 promotes prostate tumor bone metastasis

PC-3 (Tie-2 –) cells: n=8

PC-3 (Tie-2 +) cells: n=8

3,000 cells per mice

Weeks 0  8

Incidence of metastasis

<table>
<thead>
<tr>
<th>Cells injected (3,000 cells per mouse)</th>
<th>No. of mice</th>
<th>Bone metastasis</th>
<th>Soft tissue metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tie-2Low</td>
<td>n=8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tie-2High</td>
<td>n=8</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

P value < 0.05
T3 synergize the anti-cancer effect of Tie-2 inhibitor

**PC-3**

**C42B**
T3 synergize the anti-cancer effect of Tie-2 inhibitor

PC-3

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<td>120</td>
</tr>
<tr>
<td>Gamma-T3</td>
<td>80</td>
</tr>
<tr>
<td>0.05 μM Tie-2 I</td>
<td>80</td>
</tr>
<tr>
<td>0.1 μM Tie-2 I</td>
<td>80</td>
</tr>
<tr>
<td>0.2 μM Tie-2 I</td>
<td>80</td>
</tr>
<tr>
<td>γ-T3 + 0.05 μM Tie-2 I</td>
<td>60</td>
</tr>
<tr>
<td>γ-T3 + 0.1 μM Tie-2 I</td>
<td>60</td>
</tr>
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γ-T3
Tie-2 I (5 μM)

Cleaved PARP
PARP
Beta-actin

C42B

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γ-T3
Tie-2 I (5 μM)

Cleaved PARP
PARP
Beta-actin

APCRC-Q
Australian Prostate Cancer Research Centre Queensland
Tie-2 inhibitor promotes T3-induced autophagy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>β-actin</th>
<th>Phospho AMPK</th>
<th>Total AMPK</th>
<th>LCB II</th>
<th>p62/SQSTM1</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tie-2 I (5 μM)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>γ-T3 + Tie-2 I</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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mRFP (autolysosomes) | EGFP (autophagosomes) | Merge

Vehicle

Tie-2 I

γ-T3

γ-T3 + Tie-2 I
Therapeutic potential of T3 against metastatic prostate cancer
Australian Prostate Cancer Research Centre-Queensland
Prof Colleen Nelson
Prof Judith Clements
Prof Pamela Russell

Princess Alexandra Hospital
Dr. Simon Wood (Head of Urology Department)

Queensland Institute of Medical Research
Prof Kum Kum Khanna

The University of Hong Kong
Dr. Terence Lee (Department of Pathology)
Dr. Kok Wah Chan (Department of Pathology)
Dr. Yick Pang Ching (Department of Anatomy)

The Chinese University of Hong Kong
Prof Franky Chan (Dept of Anatomy)