ANTI-APOPTOTIC AND PRO-ANGIOGENIC PROPERTIES OF ENDOGENOUS THYROID HORMONE AND ITS ACTION ON P-GLYCOPOPROTEIN MAY BLUNT RESPONSE TO CONVENTIONAL CHEMOTHERAPY

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MECHANISMS OF THYROID HORMONE ACTION

- Thyroid hormone (L-thyroxine, T<sub>4</sub>; 3,5,3’-triiodo-L-thyronine, T<sub>3</sub>) acts via genomic and nongenomic mechanisms. The genomic pathway depends primarily upon formation of intranuclear complexes of T3 with the nuclear thyroid hormone receptor proteins (TRs).
- The nongenomic pathways include actions initiated at a cell surface receptor for T<sub>4</sub> and T<sub>3</sub> on the extracellular domain of integrin ανβ3.
Thyroxine ($T_4$)

3,5,3'-Triiodothyronine ($T_3$)
Nongenomic Actions of Thyroid Hormone Initiated at the Cell Surface

Protein trafficking
Serine phosphorylation of nuclear proteins
Specific gene transcription
Activity of ion transporters
Integrin $\alpha v\beta 3$ is primarily expressed by dividing cells and thus concentrated and activated in cancer cells and rapidly-dividing endothelial cells that serve cancers. Usually viewed as a sensing mechanism for extracellular matrix (ECM) proteins and critical to cell-matrix and cell-cell interactions that underlie tissue organization, $\alpha v\beta 3$ has recently been appreciated to bind small molecules and to contain the cell surface receptor for thyroid hormone.
As a prototypic small molecule ligand of integrin $\alpha v \beta 3$, thyroid hormone importantly alters transcription of differentially-regulable genes important to cancer cell function and angiogenesis—and a) regulates integrin vascular growth factor receptor function, b) alters the state of the actin cytoskeleton, c) changes intracellular protein trafficking and d) controls the function of plasma membrane NHE1 and Na,K-ATPase.
Thyroid hormone and cancer cell proliferation
Thyroid hormone and breast cancer cell proliferation

ICI = ICI 182,780, fulvestrant
OVCAR-3 cells

**IP: anti-integrin αv**
Blot: anti-NCoR
- NCoR

Blot: anti-SMRT
- SMRT

Blot: anti-p300
- p300

Blot: anti-pSTAT1
- pSTAT1

Blot: anti-pERK1/2
- pERK1
- pERK2

- Lamin B

\[ T_4 (10^{-7} \text{ M}) \quad - \quad + \quad - \quad + \quad - \quad + \]

**Fig. 3**
H510A, small cell lung carcinoma cells
H522, NSCLC cells
A  
**NCI-H510A cells**

<table>
<thead>
<tr>
<th>ICI (nM)</th>
<th>20</th>
<th>2</th>
<th>20</th>
<th>2</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; (10&lt;sup&gt;-7&lt;/sup&gt; M)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (10&lt;sup&gt;-7&lt;/sup&gt; M)</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

**Blot: anti-ER-α**

- 62 kDa - ER-α

**Blot: anti-pERK1/2**

- 37 kDa - pERK1
- 37 kDa - pERK2

**Blot: anti-PCNA**

- 37 kDa - PCNA

**Blot: anti-phosphoERK1/2**

- 61 kDa - Lamin-B

**IP: anti-ER-α**

- 62 kDa - pER-α

B  
**Thymidine Incorporation**

ICI = ICI 182,780,
In vitro stimulation of cell proliferation (PCNA), activation of ERKs, PI3K by thyroid hormone analogues

Fig. 1
Hypothyroid
Median Survival: 10.1 mos

Non-hypothyroid
Median Survival: 3.1 mos

Spontaneous or medically-induced hypothyroidism has been shown to favorably affect the clinical courses of GBM (Cleveland Clinic), breast cancer (MD Anderson Cancer Center), renal cell carcinoma (TKI therapy at multiple centers) and head-and-neck cancers (Cleveland Clinic).
Thyroid hormone has anti-apoptotic activity in cancer cells
RV = resveratrol
Thyroxine (T₄)

3,5,3’-Triiodothyronine (T₃)

Tetrac

Low-grade thyromimetic within cells
TH antagonist at integrin αvβ3 TH receptor
Thyroid hormone inhibits induction by RV of Ser-15 phosphorylation of p53
Thyroid hormone is pro-angiogenic by a variety of mechanisms. This is relevant to support by the hormone of cancer-related vascularization and to vascularity of nonmalignant conditions, such as skin diseases.
Angiogenesis in the CAM

Summary of effects of T<sub>4</sub>, T<sub>4</sub>-agarose and tetrac on angiogenesis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Angiogenesis Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>67 ± 9</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (0.1 nM)</td>
<td>156 ± 16**</td>
</tr>
<tr>
<td>Tetrac (0.1 μM)</td>
<td>76 ± 9</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; + tetrac</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;-agarose (total, 0.1 μM)</td>
<td>194 ± 28**</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;-agarose + tetrac</td>
<td>74 ± 7</td>
</tr>
</tbody>
</table>
Data represent mean ± SD, n=8; *p<0.01, indicating significant stimulation of angiogenesis. PBS, phosphate-buffered saline.
Inhibitory Effect of αvβ3 MAB (LM609) on T4-stimulated Angiogenesis in the CAM Model

<table>
<thead>
<tr>
<th>CAM Treatment</th>
<th># of branch pts ± SEM</th>
<th>% Inhibition ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>73 ± 8</td>
<td></td>
</tr>
<tr>
<td>T4(0.1μM)</td>
<td>170 ± 16</td>
<td>0</td>
</tr>
<tr>
<td>T4+LM609(10μg)</td>
<td>109 ± 9</td>
<td>64 ± 9</td>
</tr>
</tbody>
</table>

PBS

T₄ (total, 0.1μM)

T₄+ LM609(10μg)
By a variety of mechanisms, thyroid hormone, specifically $T_4$, has been shown to influence the activity and abundance of P-glycoprotein (P-gp; MDR1; ABCB1), a plasma membrane efflux pump that serves to shorten intracellular retention time of traditional chemotherapeutic agents that are ligands for the protein (doxorubicin, etoposide, paclitaxel, etc.). Thus, thyroid hormone may support chemoresistance.
Results of $T_4$ action

Increased EGF input to $P$-gp

$\psi_{[Na^+]_i}$

$\uparrow$ Calmodulin

$\uparrow$ MDRI transcription

$\uparrow$ EGFR transcription

Bold blue arrows indicate stimulatory action of tetrac/Nanotetrac on Na$^+$, K$^+$ - ATPase, Na$^+$/H$^+$ antiporter and EGF receptor
SUMMARY

There are multiple implications of all of these functions of thyroid hormone recently recognized to occur on cancer cells and on angiogenesis.

• Normal thyroid function may support tumor cell proliferation and limit effectiveness of chemotherapy.

• Induced or spontaneous hypothyroidism may impede cancer cell function.
SUMMARY 2

- It is desirable to have a specific pharmacologic antagonist of thyroid hormone actions at integrin $\alpha v \beta 3$ (= tetrac, Nanotetrac).
- The affinity of the thyroid hormone receptor on $\alpha v \beta 3$ is higher for $T_4$ than $T_3$; at physiological concentrations, free $T_4$ supports tumor cell proliferation and cancer cell survival pathway gene transcription.
- Nonmalignant, hypervascular skin disorders, such as rosacea, may be thyroid hormone-supported.
COLLABORATORS

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Osnat Ashur-Fabian, PhD  Israel
Effect of tetrac on tube formation by human dermal microvascular endothelial cells (HDMEC).

In this 3-dimensional human microvascular endothelial cell sprouting assay, cells were mixed with gelatin-coated Cytodex-3 beads and the mixture suspended in endothelial basal medium (EBM) with 15% normal human serum, mixed and cultured overnight in a CO\textsubscript{2} incubator. The “EC-beads” were then placed in a fibrinogen solution and thrombin added. After polymerization of the fibrin, EBM and 20% human serum were added and samples incubated for 24-48 h.
**A**

*ER-α gene expression*

- Green: phosphoER-α
- Red: Nucleoprotein

NCI-H510A  NCI-H522

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**B**

**NCI-H522 cells**

- Green: phosphoER-α
- Red: Nucleoprotein

<table>
<thead>
<tr>
<th>Condition</th>
<th>Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><img src="image" alt="Control" /></td>
</tr>
<tr>
<td>L-T_{4}</td>
<td><img src="image" alt="L-T_{4}" /></td>
</tr>
<tr>
<td>Tetrac</td>
<td><img src="image" alt="Tetrac" /></td>
</tr>
<tr>
<td>Tetrac + T_{4}</td>
<td><img src="image" alt="Tetrac + T_{4}" /></td>
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</table>

**C**

**IP: anti-integrin-αv**

- ER-α Promoter
- Input Control

<table>
<thead>
<tr>
<th>Condition</th>
<th>Images</th>
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<tbody>
<tr>
<td>T_{4} -</td>
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<tr>
<td>T_{4} +</td>
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</tr>
<tr>
<td>IgG</td>
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</tbody>
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**NCI-H-522 cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Relative Bound DNA</th>
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<tbody>
<tr>
<td>Con</td>
<td><img src="image" alt="Con" /></td>
</tr>
<tr>
<td>ICI</td>
<td><img src="image" alt="ICI" /></td>
</tr>
<tr>
<td>T_{4}</td>
<td><img src="image" alt="T_{4}" /></td>
</tr>
<tr>
<td>ICI + T_{4}</td>
<td><img src="image" alt="ICI + T_{4}" /></td>
</tr>
</tbody>
</table>
A

Nucleosome ELISA

- Fold Increase in nucleosome content

RV (μM) 0 1 10 1 10
T₄ (10⁻⁷ M) 0 + 0 + ++

B

Nucleosome ELISA

- Fold Increase in nucleosome content

Tetrac (10⁻⁷ M) 0 + 0 + ++
RV (10 μM) 0 0 + + + +
T₄ (10⁻⁷ M) 0 + + − − + +

BHP 18-21 cells
FTC 236 cells

BHP 2-7 cells
BHP 18-21 cells
Effects of L-T₄ or T₄ Nanoparticles (NP) on Endothelial Cell Migration toward Vitronectin (VN)

Boyden apparatus