

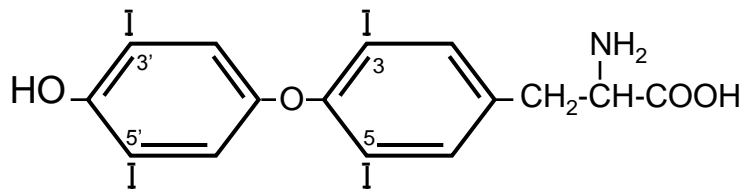
ANTI-APOPTOTIC AND PRO-  
ANGIOGENIC PROPERTIES OF  
ENDOGENOUS THYROID  
HORMONE AND ITS ACTION ON  
P-GLYCOPROTEIN MAY BLUNT  
RESPONSE TO CONVENTIONAL  
CHEMOTHERAPY

Paul J. Davis, MD

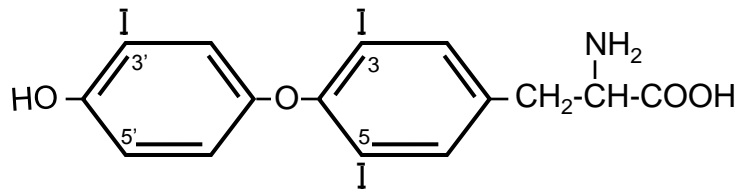
Albany Medical College and  
Pharmaceutical Research  
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Pharmacy and Health  
Sciences, Albany, NY USA

# MECHANISMS OF THYROID HORMONE ACTION

- Thyroid hormone (L-thyroxine,  $T_4$ ; 3,5,3'-triiodo-L-thyronine,  $T_3$ ) acts via genomic and nongenomic mechanisms. The genomic pathway depends primarily upon formation of intranuclear complexes of  $T_3$  with the nuclear thyroid hormone receptor proteins (TRs).
- The nongenomic pathways include actions initiated at a cell surface receptor for  $T_4$  and  $T_3$  on the extracellular domain of integrin  $\alpha v \beta 3$ .

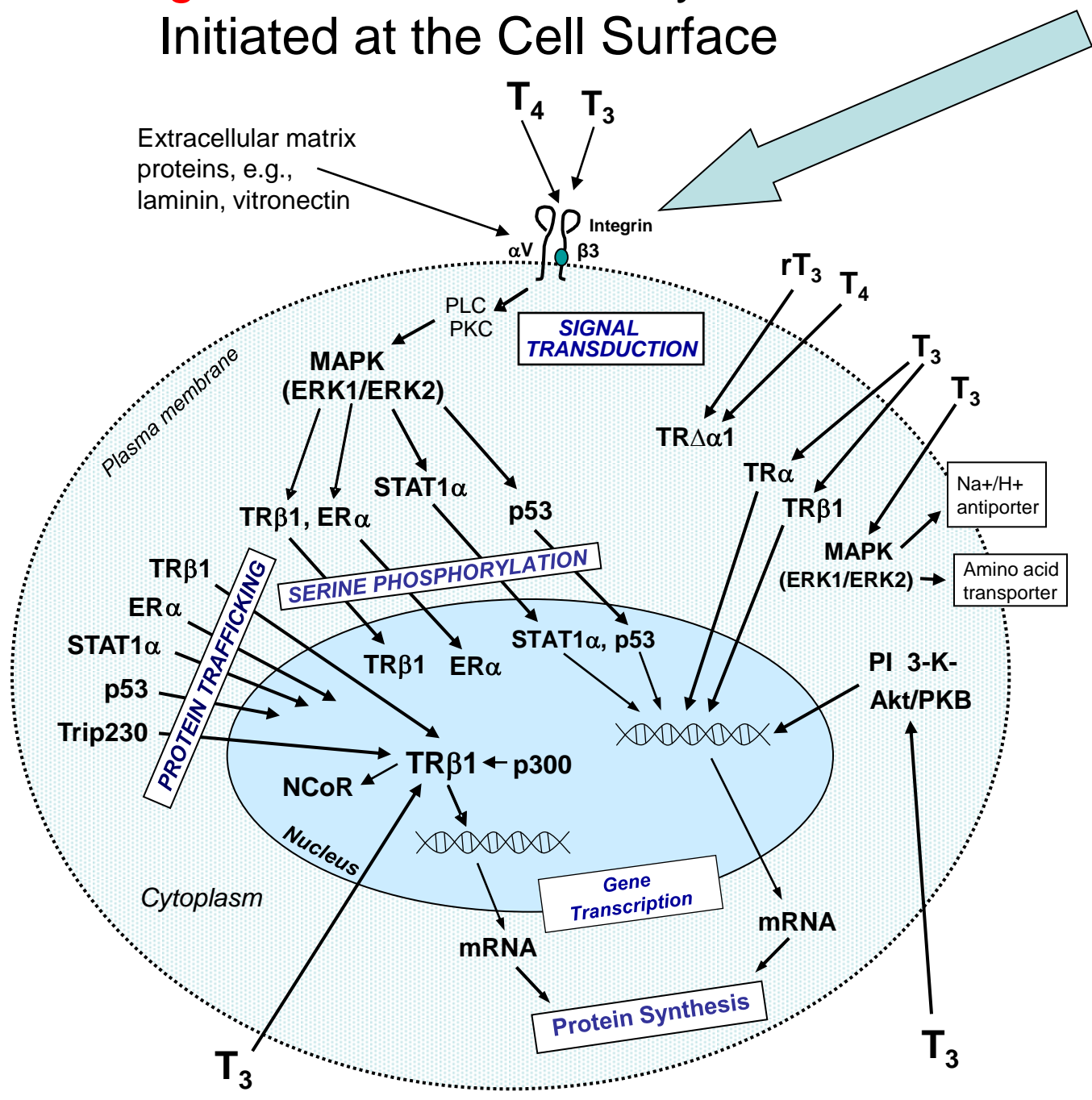


Thyroxine (T<sub>4</sub>)



3,5,3'-Triiodothyronine (T<sub>3</sub>)

# Nongenomic Actions of Thyroid Hormone Initiated at the Cell Surface



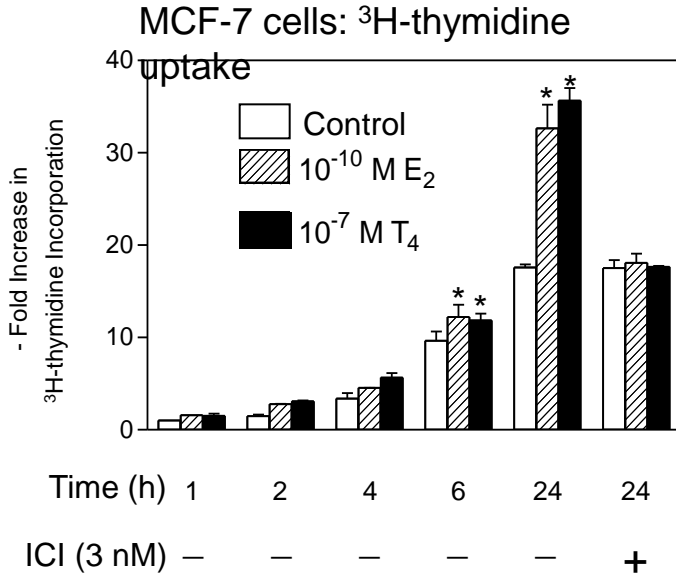
- Protein trafficking
- Serine phosphorylation of nuclear proteins
- Specific gene transcription
- Activity of ion transporters

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.

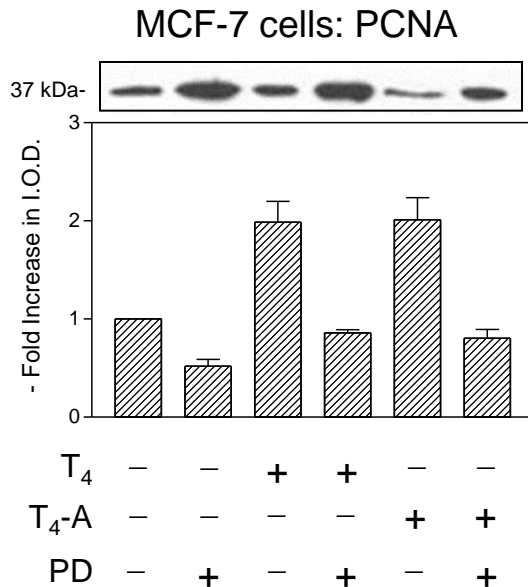
As a prototypic small molecule ligand of integrin  $\alpha v \beta 3$ , thyroid hormone importantly alters transcription of differentially-regulable genes important to cancer cell function and angiogenesis—and a) regulates integrin vascular growth factor receptor function, b) alters the state of the 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

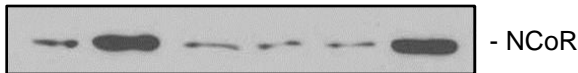




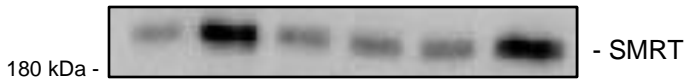
# OVCAR-3 cells

## IP: anti-integrin $\alpha$ v

Blot: anti-NCoR



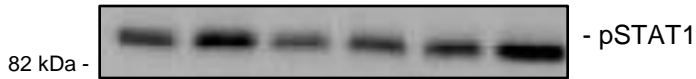
Blot: anti-SMRT



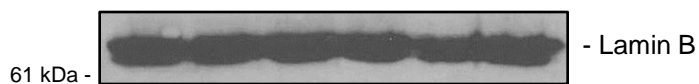
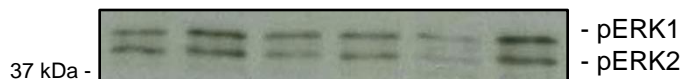
Blot: anti-p300



Blot: anti-pSTAT1

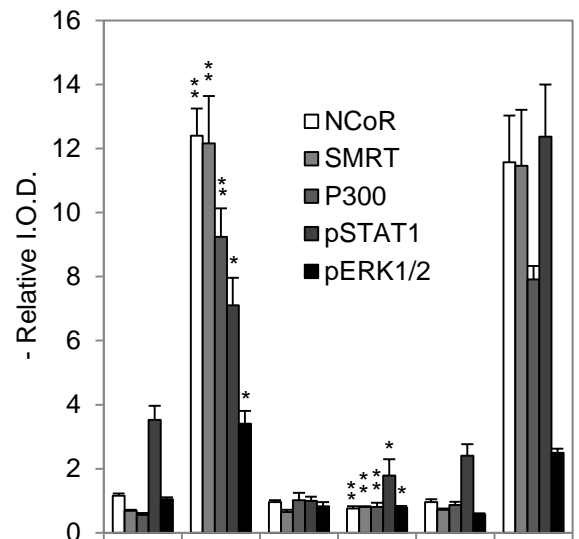


Blot: anti-pERK1/2



← C → | ← siRNA → | ← scRNA →

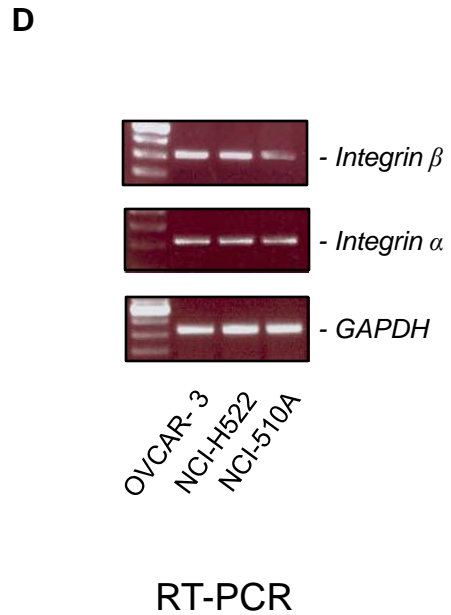
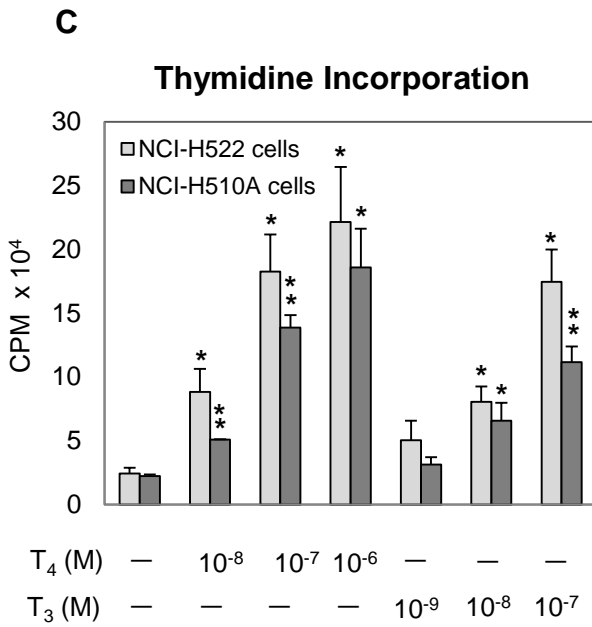
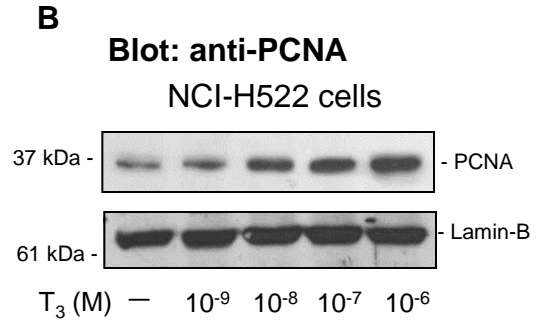
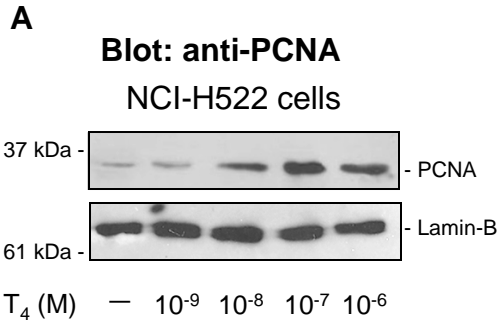
$T_4$  ( $10^{-7}$  M)    -    +    -    +    -    +



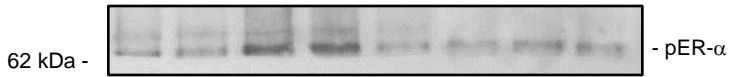
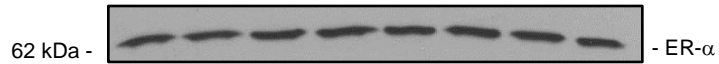
← C → | ← siRNA → | ← scRNA →

$T_4$  ( $10^{-7}$  M)    -    +    -    +    -    +

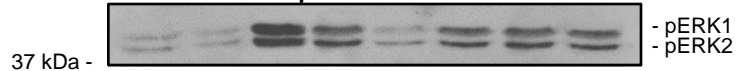
Fig. 3



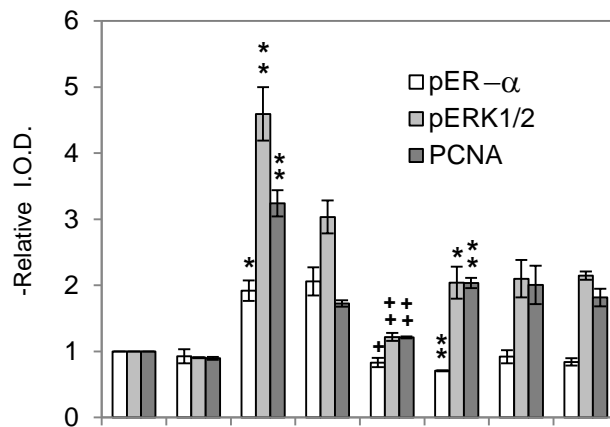
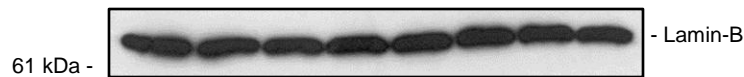
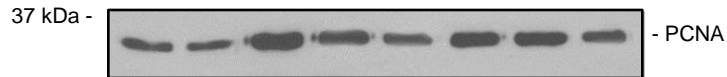
H510A, small cell lung carcinoma cells  
H522, NSCLC cells

**A****NCI-H510A cells**IP: anti-ER- $\alpha$ Blot: anti-phosphoER- $\alpha$  (118)Blot: anti-ER- $\alpha$ 

Blot: anti-pERK1/2



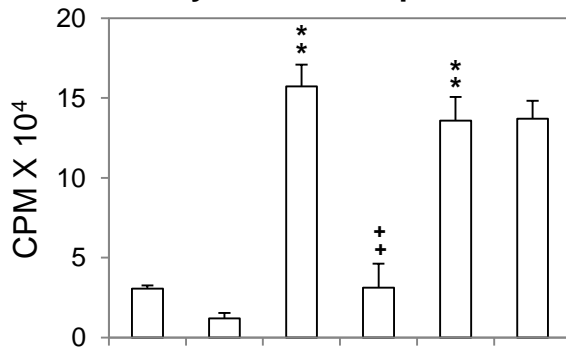
Blot: anti-PCNA



ICI (nM) - 20 - 2 20 - 2 20

T<sub>3</sub> (10<sup>-7</sup> M) - - - - + + +T<sub>4</sub> (10<sup>-7</sup> M) - - + + + - - -**B****Thymidine Incorporation**

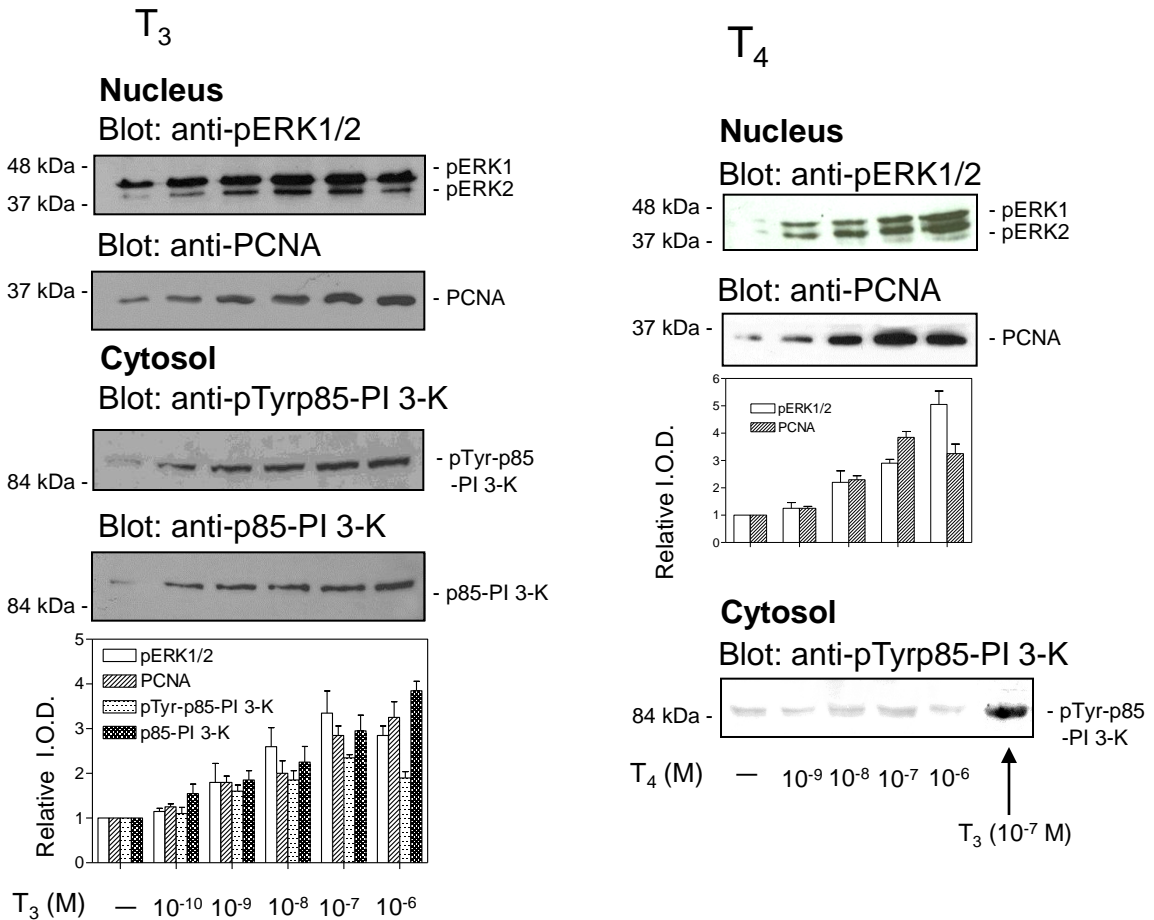
ICI = ICI 182,780,



ICI (nM) - 20 - 20 - 20

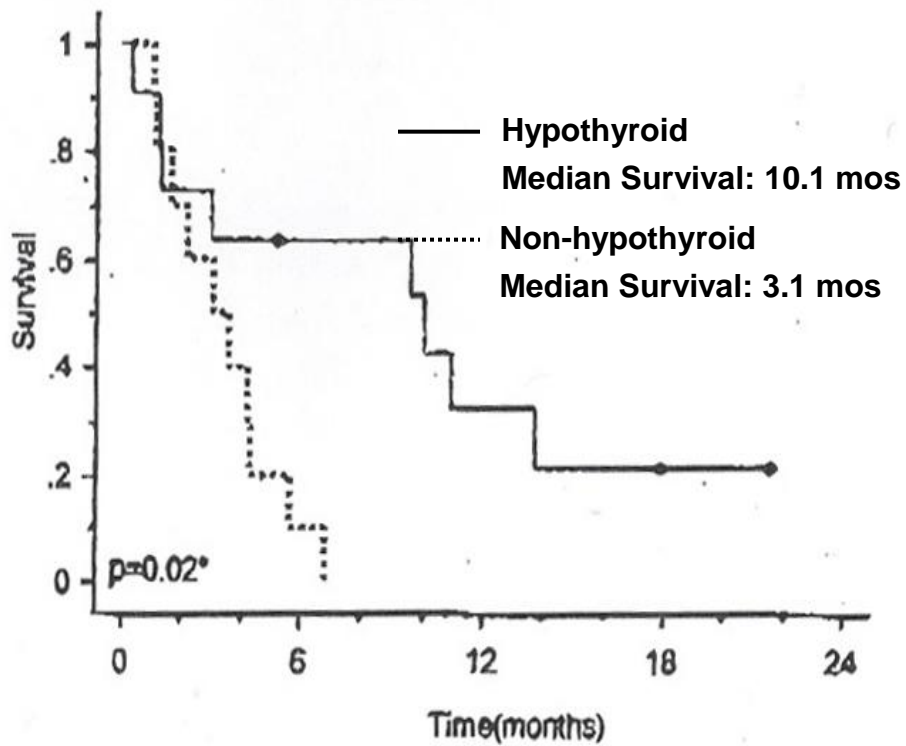
T<sub>3</sub> (10<sup>-7</sup> M) - - - + +T<sub>4</sub> (10<sup>-7</sup> M) - - + + - -

## U87MG (GBM) cells



*In vitro* stimulation of cell proliferation (PCNA), activation of ERKs, PI3K by thyroid hormone analogues

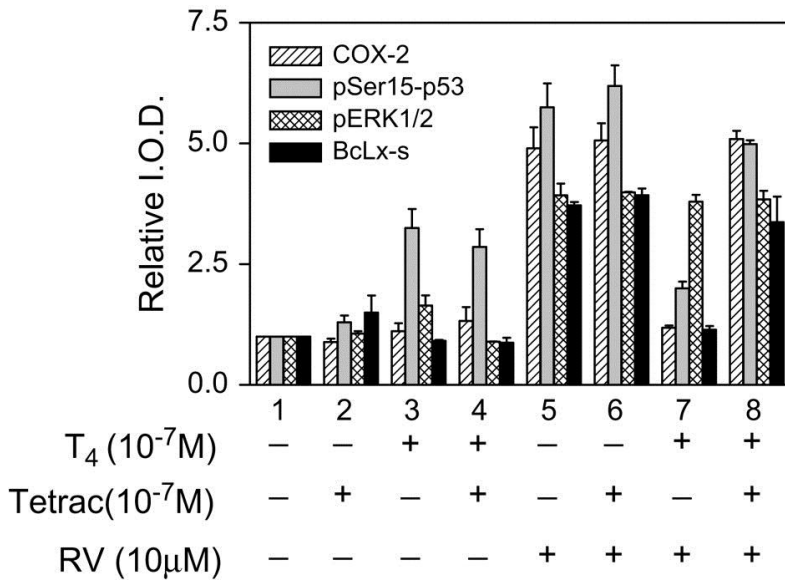
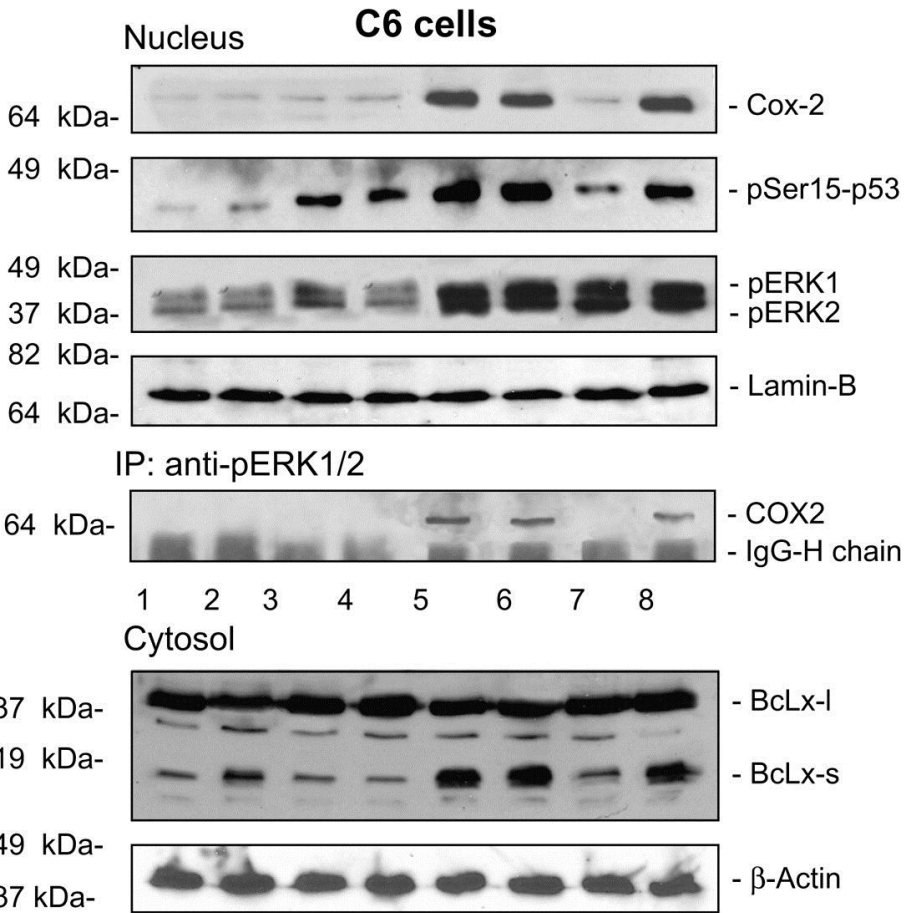
Fig. 1



Hercbergs AA et al, *Anticancer Res*, 2003

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



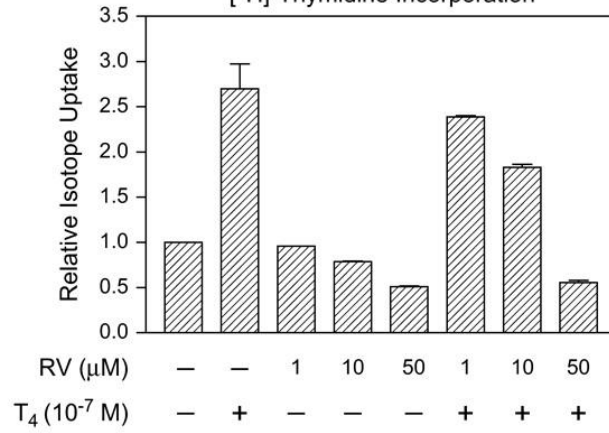
RV = resveratrol



A

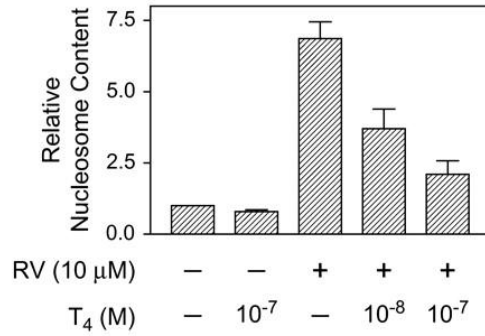
**C6 cells**

[<sup>3</sup>H]-Thymidine Incorporation



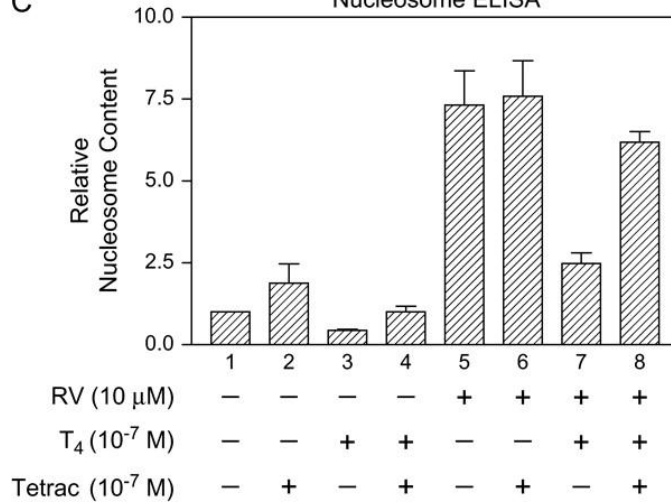
B

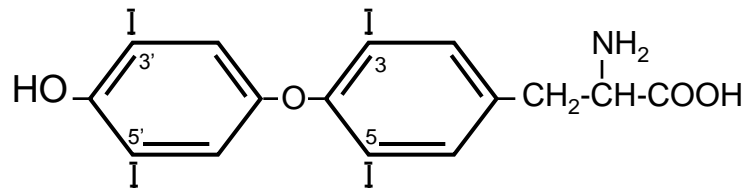
**Nucleosome ELISA**



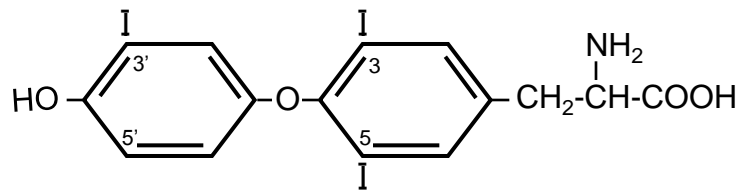
C

**Nucleosome ELISA**

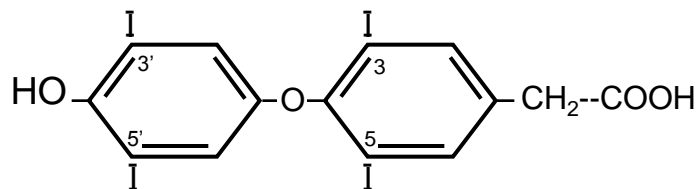




Thyroxine (T<sub>4</sub>)

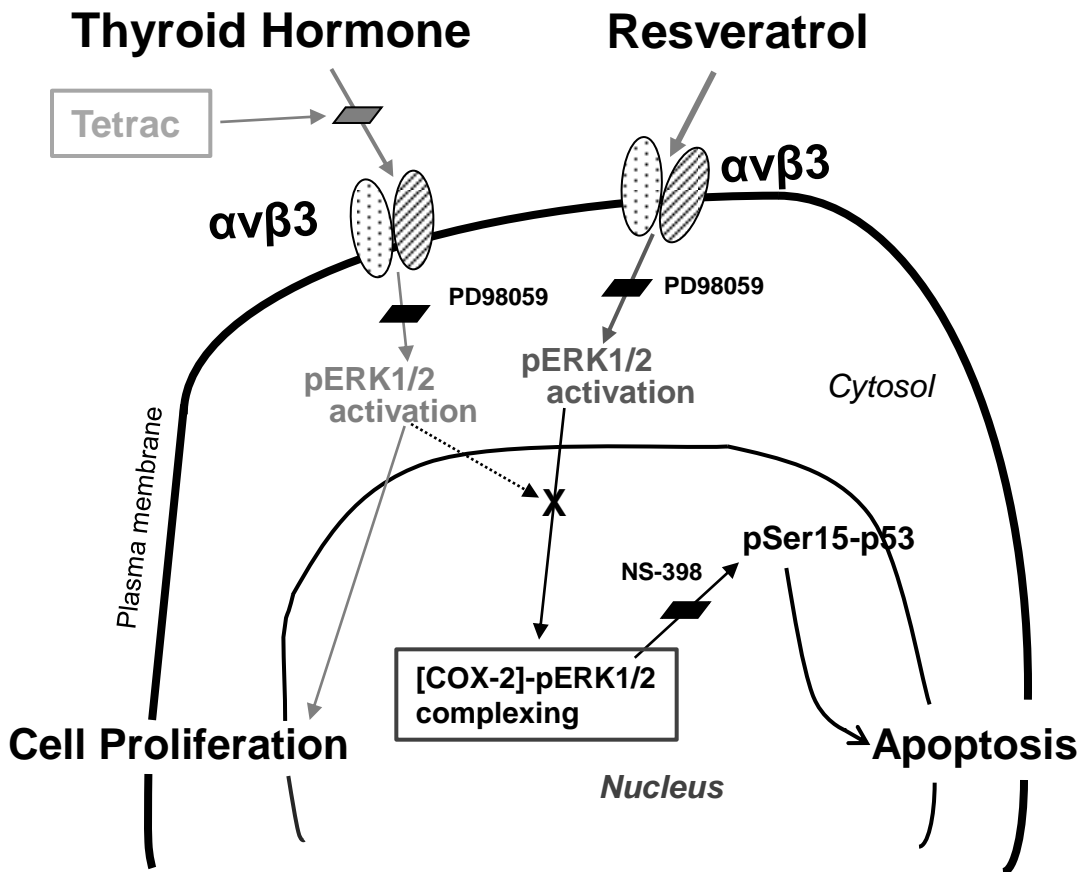


3,5,3'-Triiodothyronine (T<sub>3</sub>)



Tetrac

Low-grade thyromimetic within cells  
 TH antagonist at integrin  $\alpha v \beta 3$  TH  
 receptor

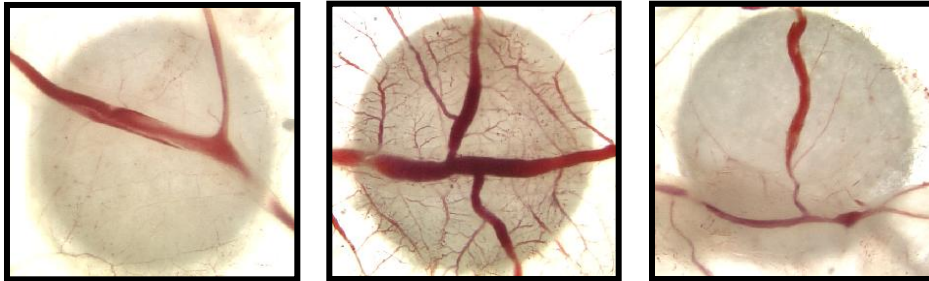


Thyroid hormone inhibits induction by RV of Ser-15 phosphorylation of p53

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

A



PBS

T<sub>4</sub>

T<sub>4</sub> + Tetrac

B



PBS

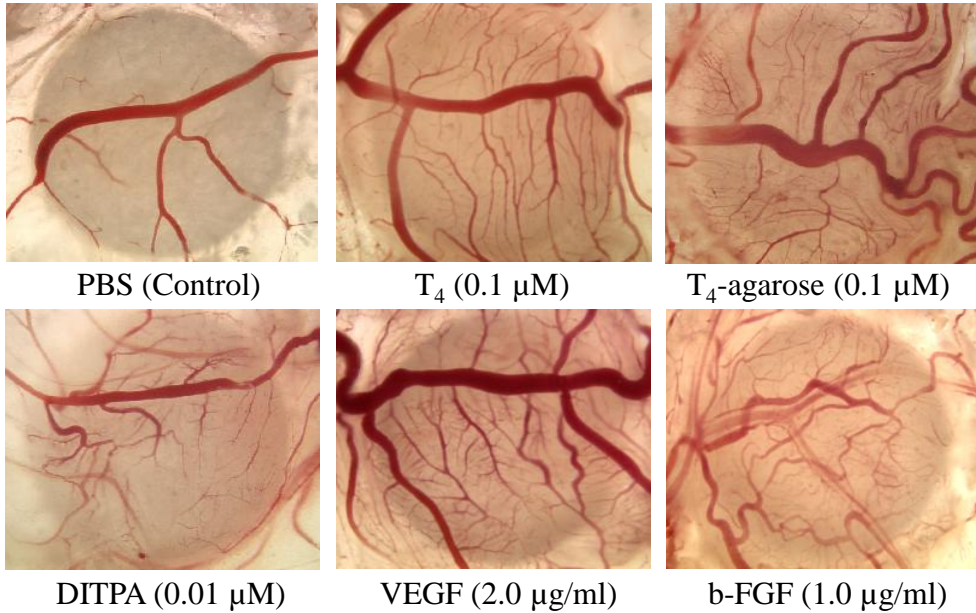
T<sub>4</sub>-ag

T<sub>4</sub>-ag + Tetrac

C

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

<u>Treatment</u>	<u>Angiogenesis Index</u>
PBS	67 ± 9
T <sub>4</sub> (0.1 nM)	156 ± 16**
Tetrac (0.1 μM)	76 ± 9
T <sub>4</sub> + tetrac	66 ± 6
T <sub>4</sub> -agarose (total, 0.1 μM)	194 ± 28**
T <sub>4</sub> -agarose + tetrac	74 ± 7

**A****B**

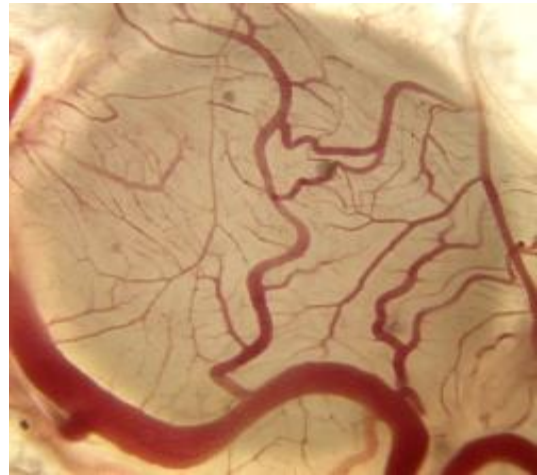
Treatment	Mean Vessel Branch Points $\pm$ SD
PBS (Control)	87 $\pm$ 9
T <sub>4</sub> (0.1 μM)	148 $\pm$ 7*
T <sub>4</sub> -agarose (0.1 μM)	167 $\pm$ 8*
DITPA (0.01 μM)	134 $\pm$ 11*
DITPA (0.1 μM)	170 $\pm$ 9*
VEGF (2.0 μg/ml)	168 $\pm$ 10*
b-FGF (1.0 μg/ml)	174 $\pm$ 8*

Data represent mean  $\pm$  SD, n=8; \*p<0.01, indicating significant stimulation of angiogenesis. PBS, phosphate-buffered saline.

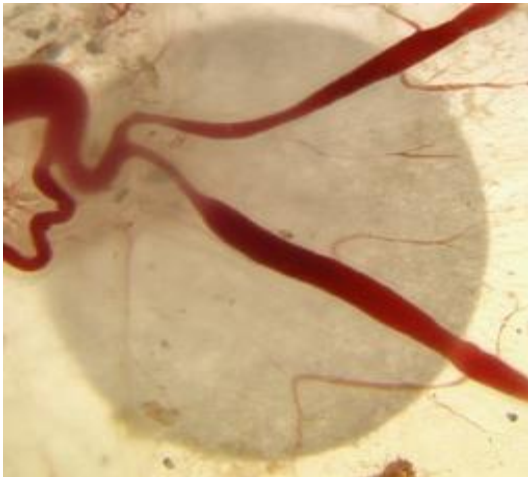
# Inhibitory Effect of $\alpha v\beta 3$ MAB (LM609) on T<sub>4</sub>-stimulated Angiogenesis in the CAM Model



**PBS**



**T<sub>4</sub> (total, 0.1 $\mu$ M)**

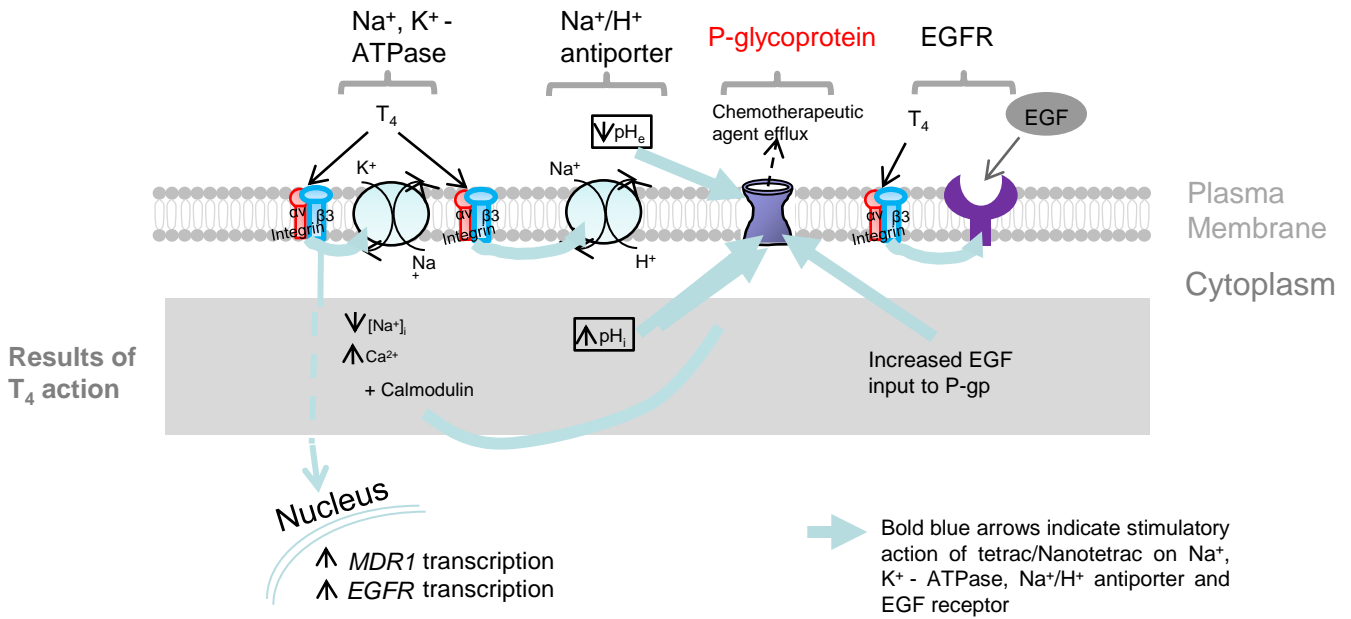


**T<sub>4</sub>+ LM609(10 $\mu$ g)**

<b>CAM Treatment</b>	<b># of branch pts <math>\pm</math> SEM</b>	<b>%Inhibition <math>\pm</math> SEM</b>
<b>PBS</b>	<b>73 <math>\pm</math> 8</b>	
<b>T4(0.1<math>\mu</math>M)</b>	<b>170 <math>\pm</math> 16</b>	<b>0</b>
<b>T4+LM609(10ug)</b>	<b>109 <math>\pm</math> 9</b>	<b>64 <math>\pm</math> 9</b>

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.





# 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

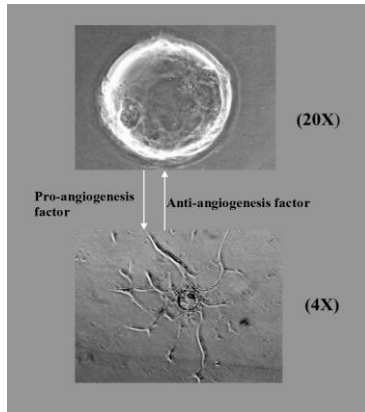
- It is desirable to have a specific pharmacologic antagonist of thyroid hormone actions at integrin  $\alpha v \beta 3$  (= tetrac, Nanotetrac).
- The affinity of the thyroid hormone receptor on  $\alpha v \beta 3$  is higher for  $T_4$  than  $T_3$ ; at physiological concentrations, free  $T_4$  supports 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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Hung-Yun Lin, PhD	Albany
Heng-Yuan Tang, MA	Albany
Thangirala Sudha, PhD	Albany
Faith B. Davis, MD	Albany
Murat Yalcin, DVM, PhD	Turkey
Sandra Incerpi, PhD	Italy
Osnat Ashur-Fabian, PhD	Israel

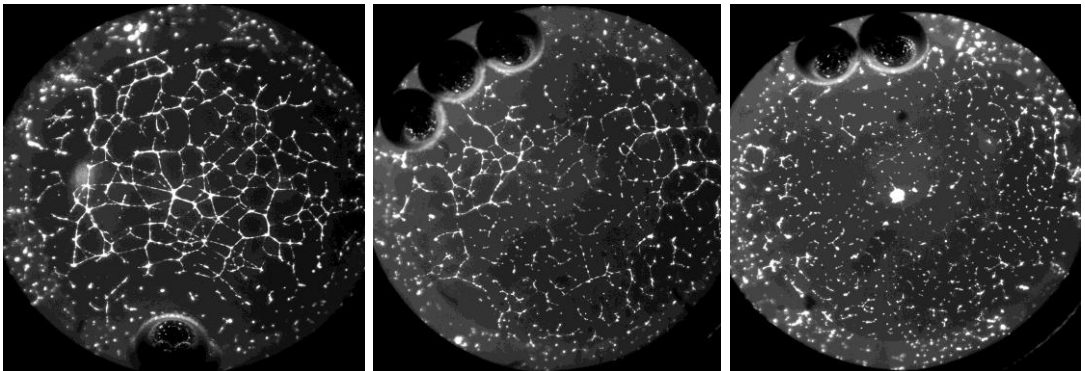


**A** Effect of tetrac on tube formation by human dermal microvascular endothelial cells (HDMEC).

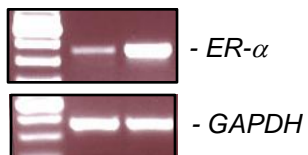


In this 3-dimensional human microvascular endothelial cell sprouting assay, cells were mixed with gelatin-coated Cytodex-3 beads and the mixture suspended in endothelial basal medium (EBM) with 15% normal human serum, mixed and cultured overnight in a CO<sub>2</sub> incubator. The “EC-beads” were then 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.

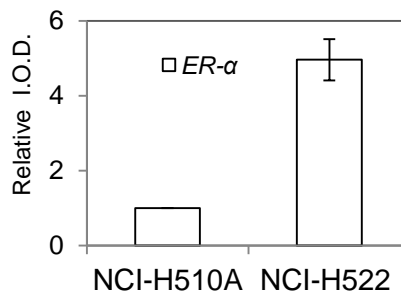
**B**



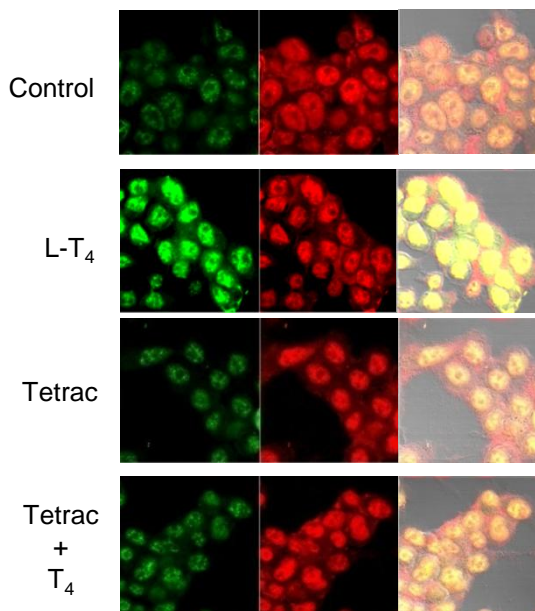
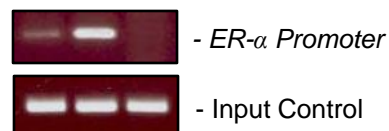
VEGF	+	+	+
Tetrac	—	1.0	2.5

**A****ER- $\alpha$  gene expression**

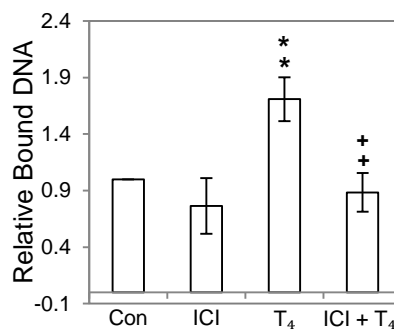
NCI-H510A  
NCI-H522

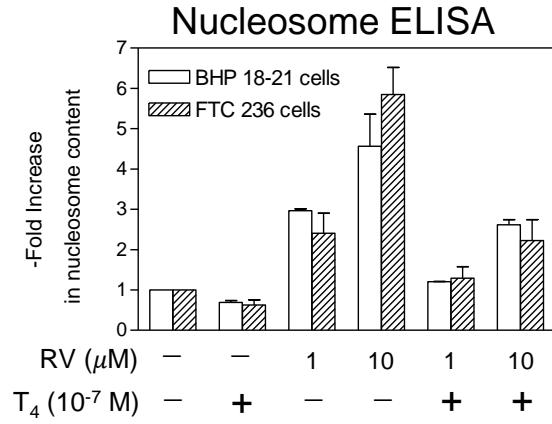
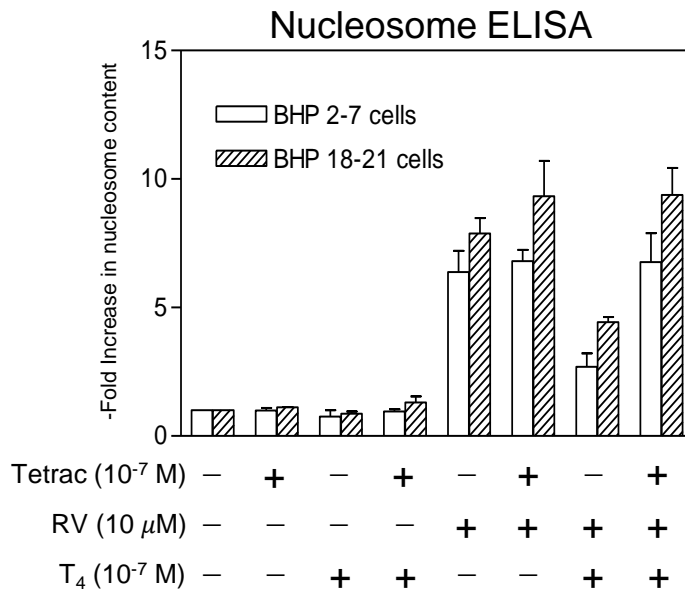
**ER- $\alpha$  gene expression****B****NCI-H522 cells**Green: phosphoER- $\alpha$ 

Red: Nucleoprotein

**C****IP: anti-integrin- $\alpha$ v**

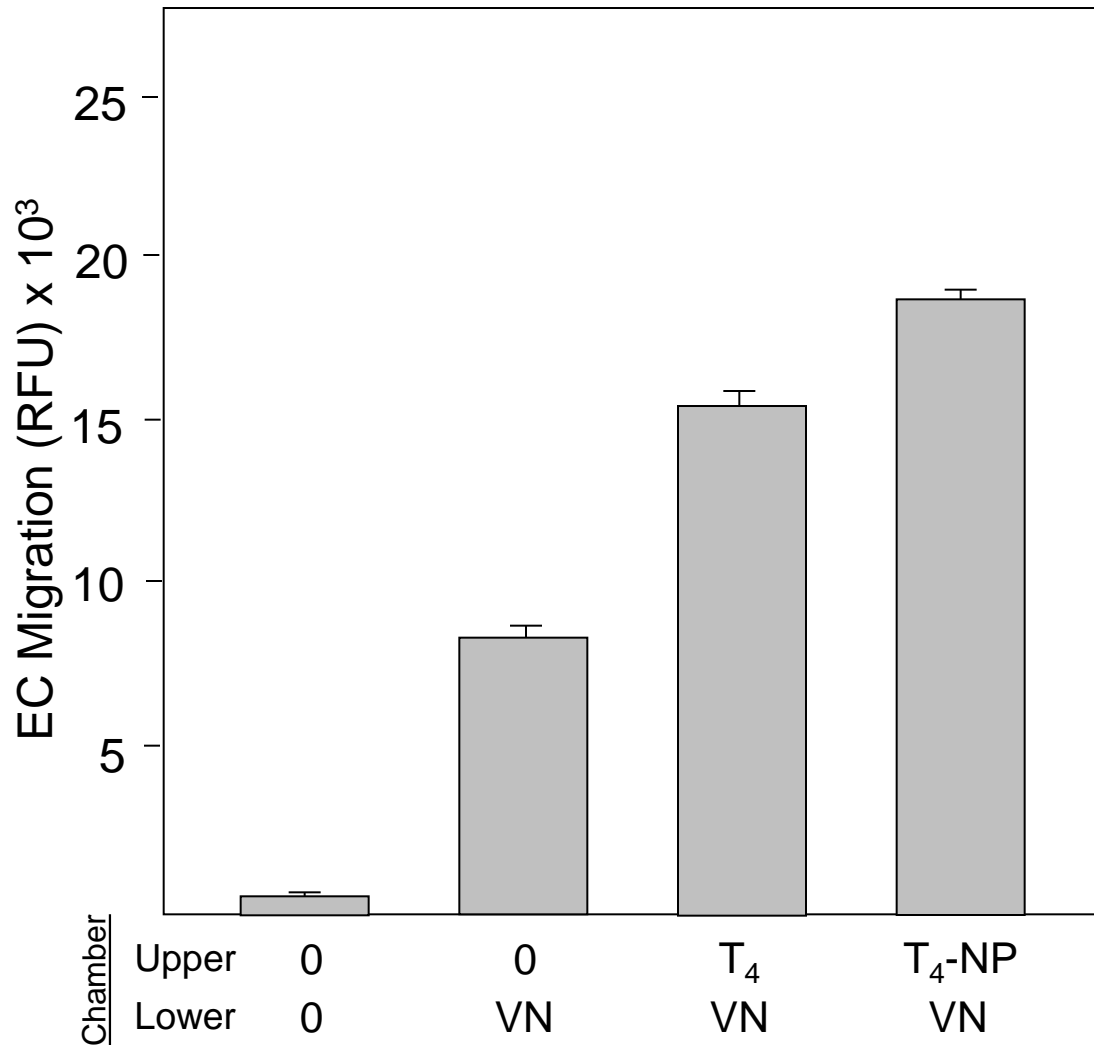
T<sub>4</sub> - +  $\uparrow$   
IgG

**NCI-H-522 cells**

**A****B**



**Effects of L-T<sub>4</sub> or T<sub>4</sub> Nanoparticles (NP) on Endothelial Cell Migration toward Vitronectin (VN)**



Boyden apparatus