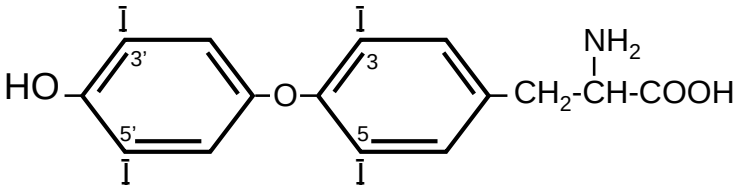
ANTI-APOPTOTIC AND PRO-ANGIOGENIC PROPERTIES OF ENDOGENOUS THYROID HORMONE MAY OPPOSE RESPONSE TO CONVENTIONAL CHEMOTHERAPY

Paul J. Davis, MD

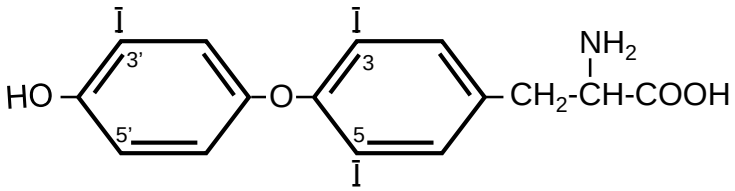
Albany Medical College and Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Albany, NY USA

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 T3 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 $\alpha v \beta 3$.

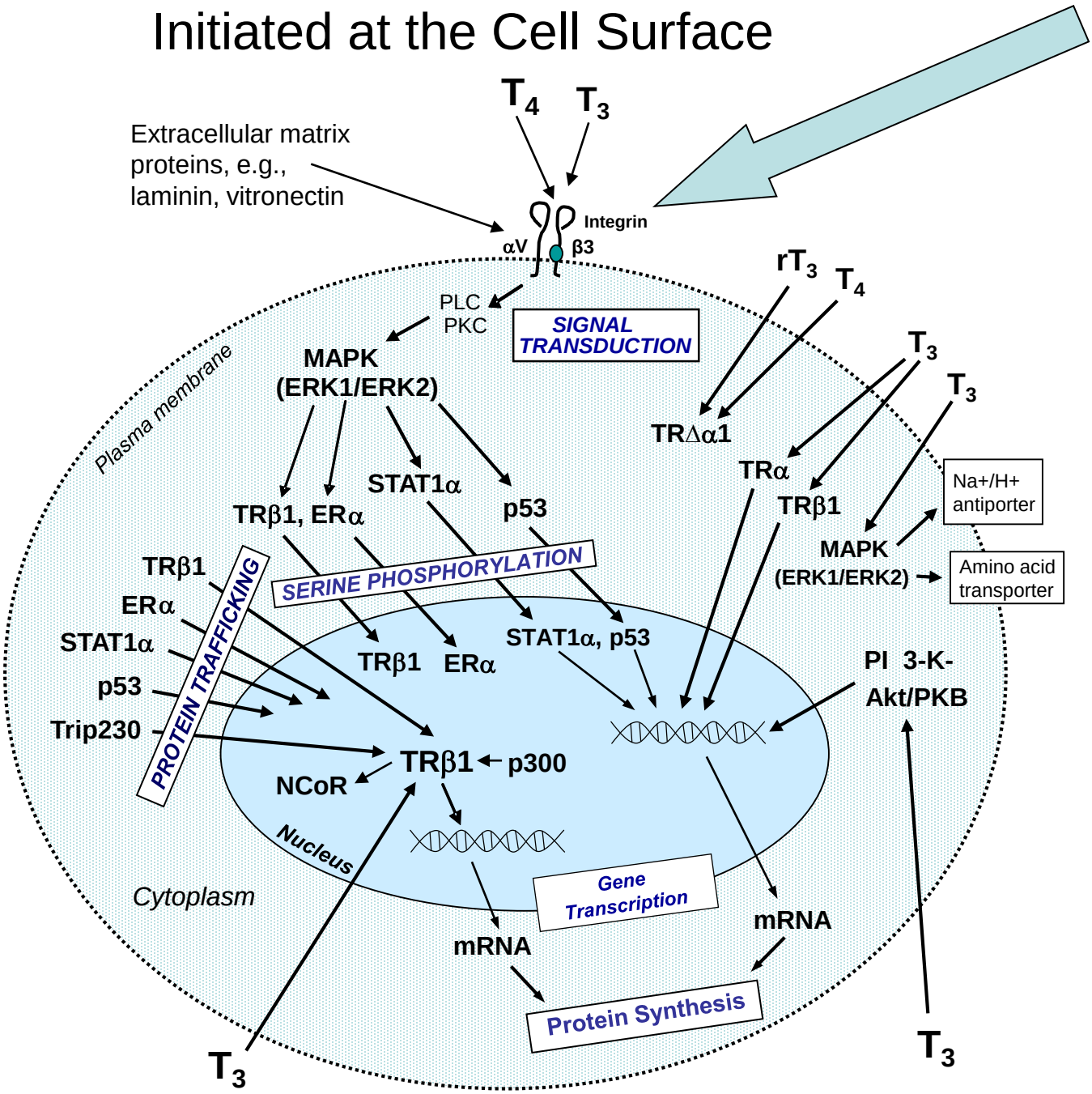


Thyroxine (T_4)



3,5,3'-Triiodothyronine (T_3)

Nongenomic Actions of Thyroid Hormone Initiated at the Cell Surface

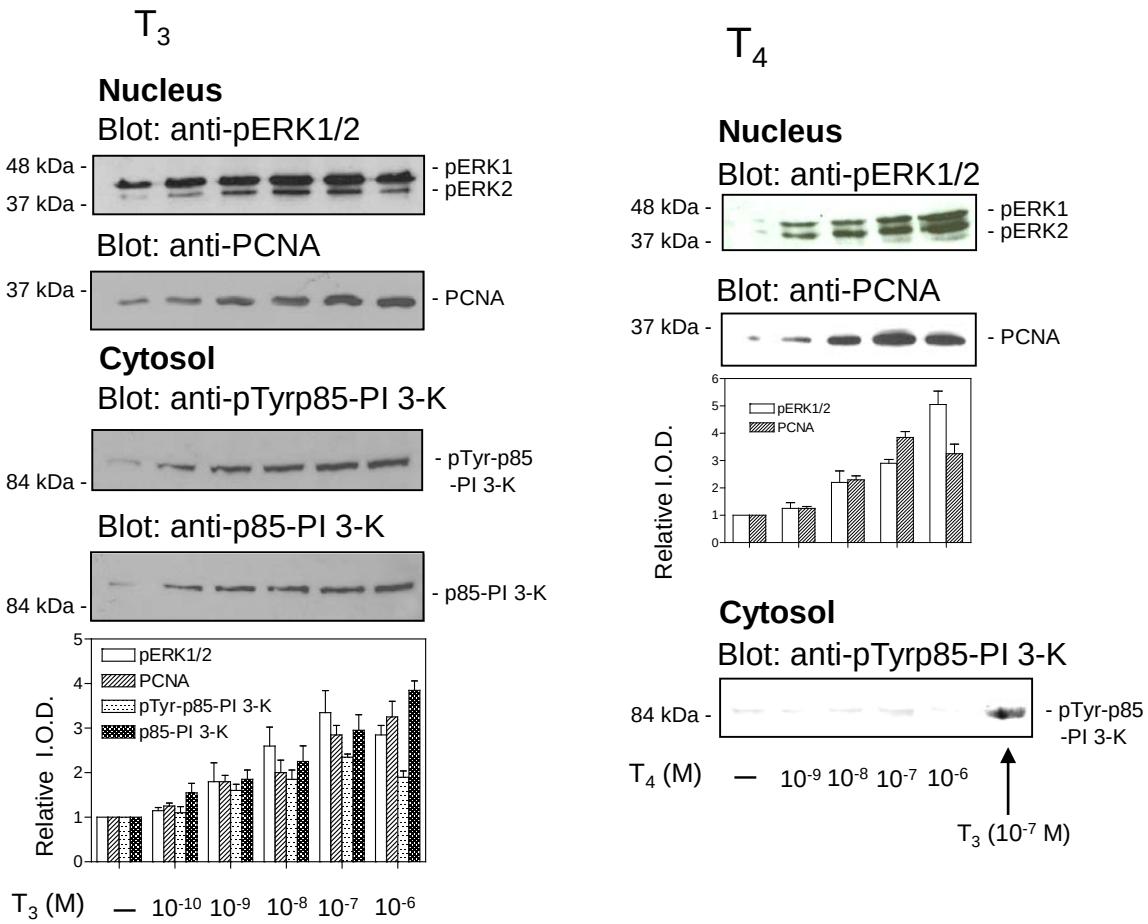


Protein trafficking
Serine phosphorylation of nuclear proteins
Specific gene transcription
Activity of ion transporters

Integrin αvβ3 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, αvβ3 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 αvβ3, thyroid hormone importantly alters transcription of differentially-regulable genes important to cancer cell survival pathways 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.

U87MG (GBM) cells

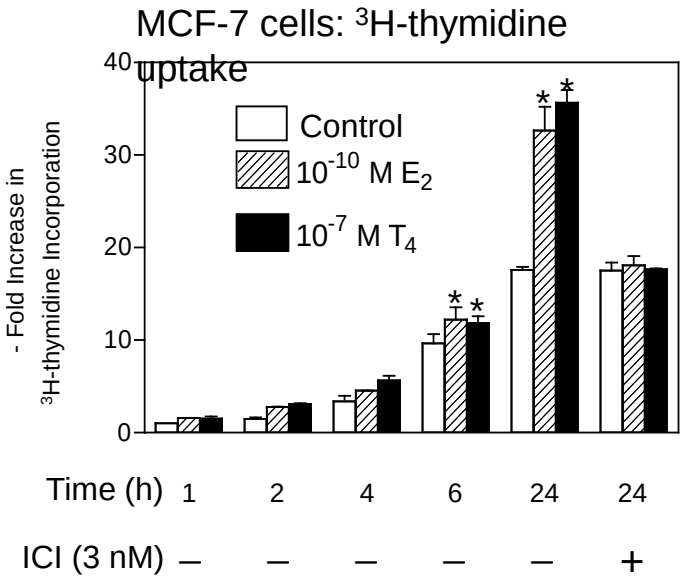


In vitro stimulation of cell proliferation (PCNA), activation of ERKs, PI3K by thyroid hormone analogues

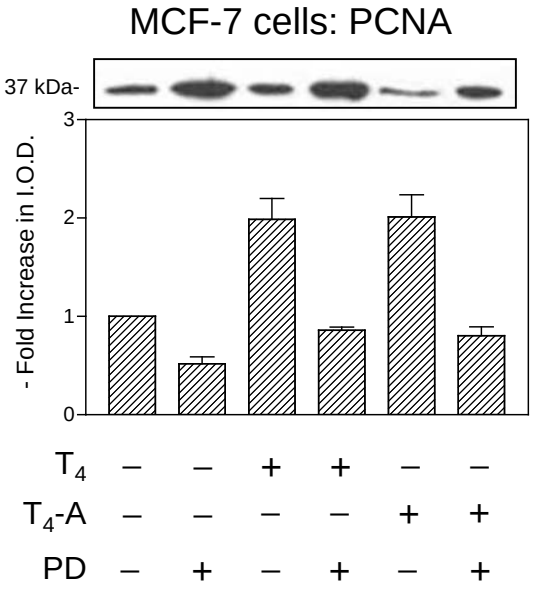
Fig. 1

Thyroid hormone and cancer cell proliferation

Thyroid hormone and breast cancer cell proliferation



ICI = ICI 182,780, fulvestrant



OVCAR-3 cells

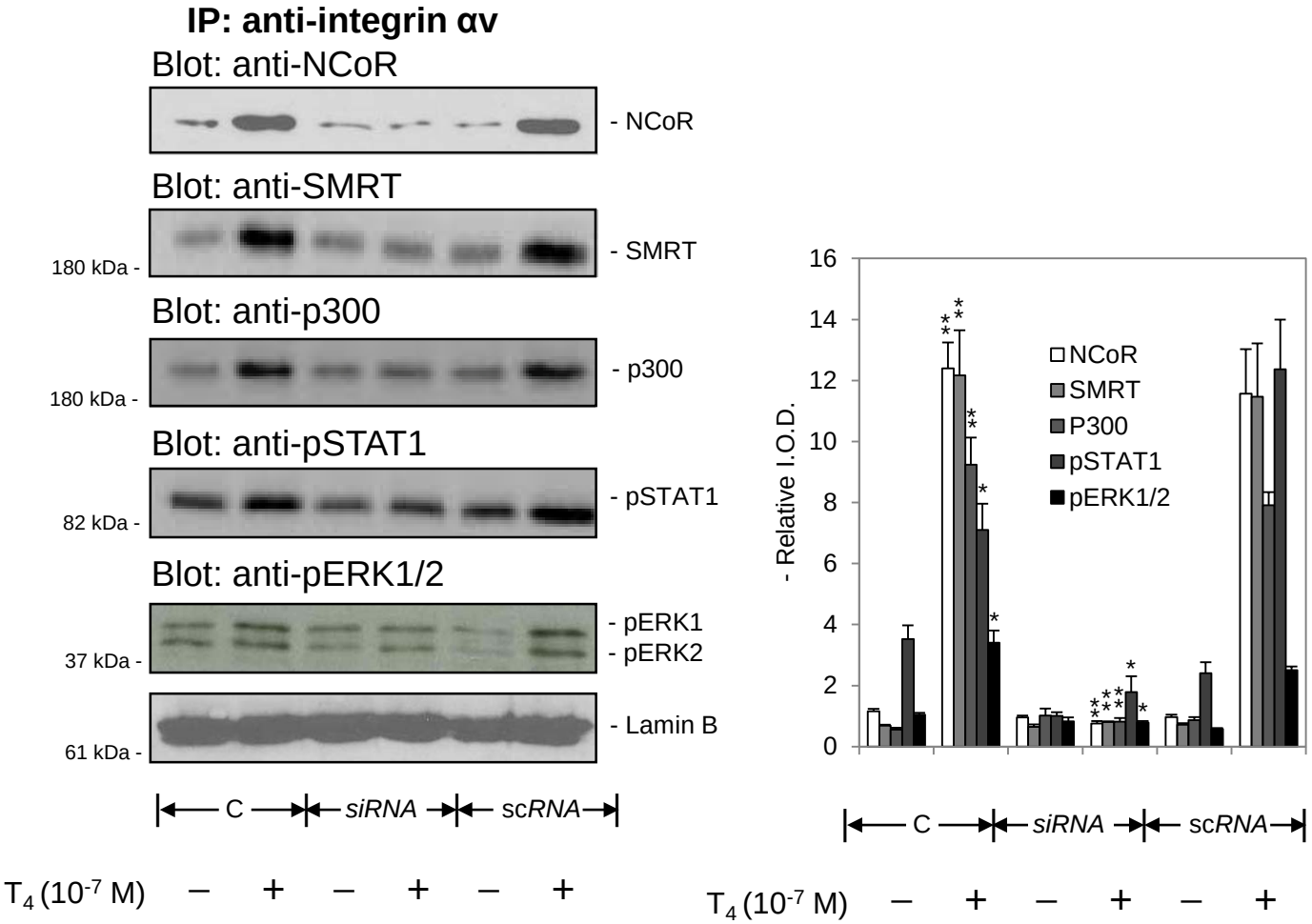
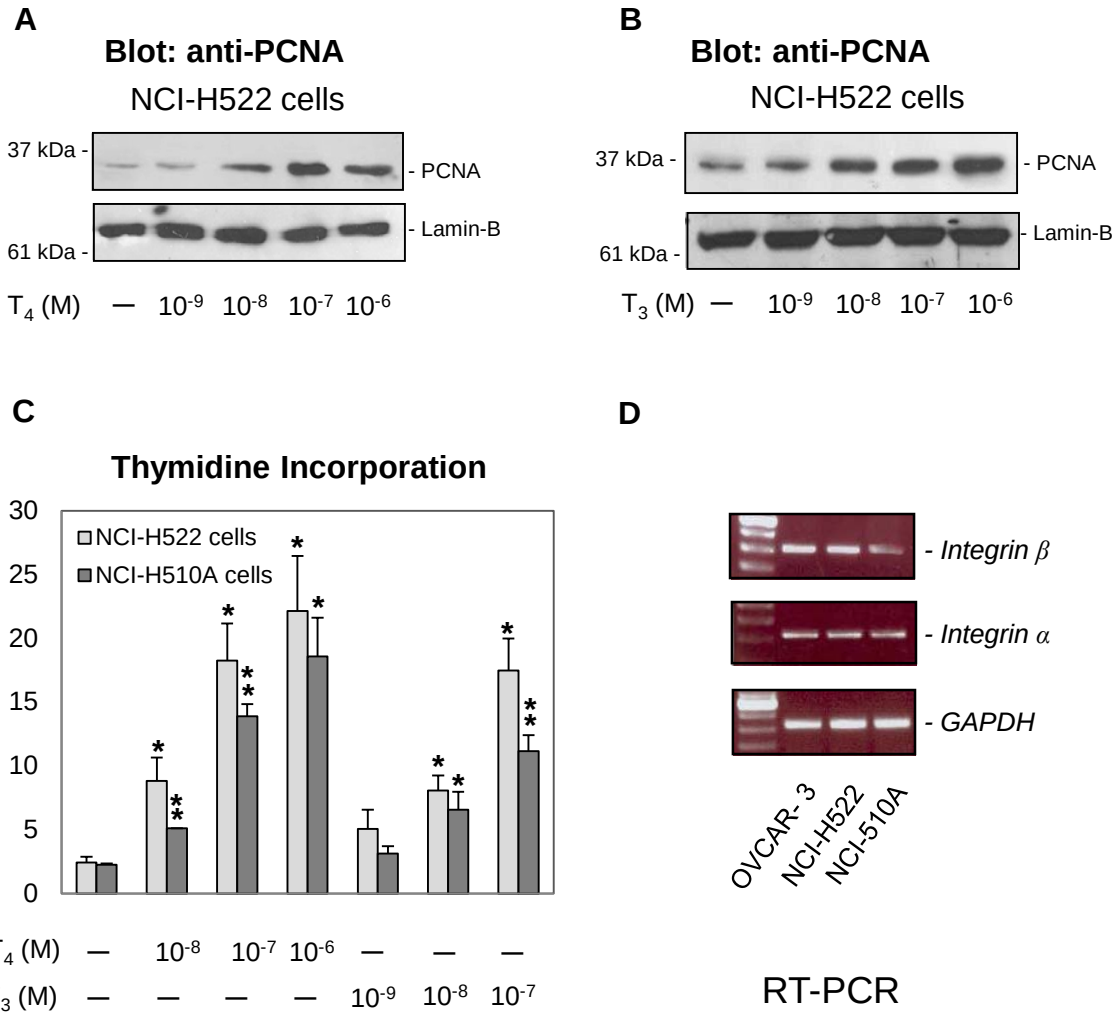
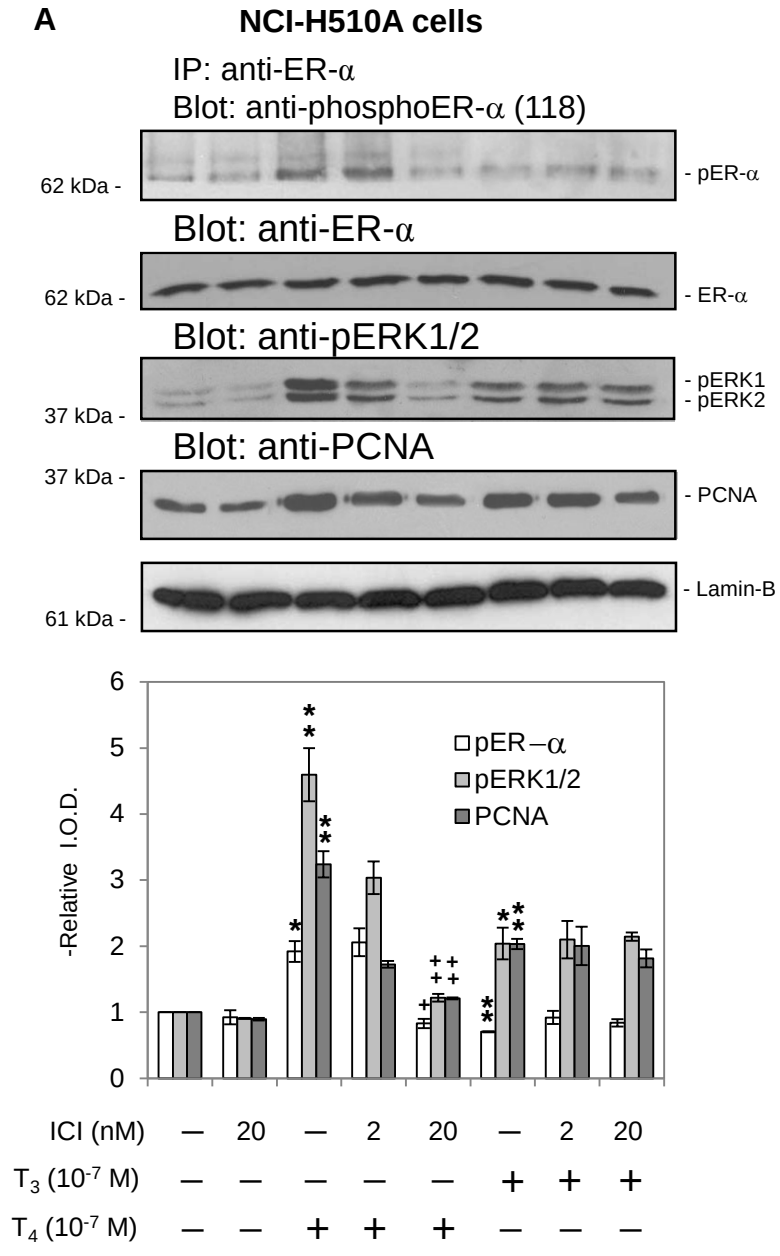
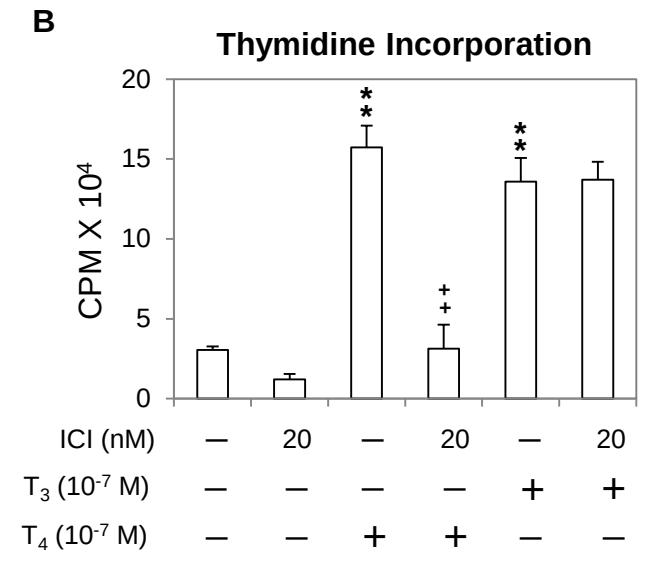


Fig. 3



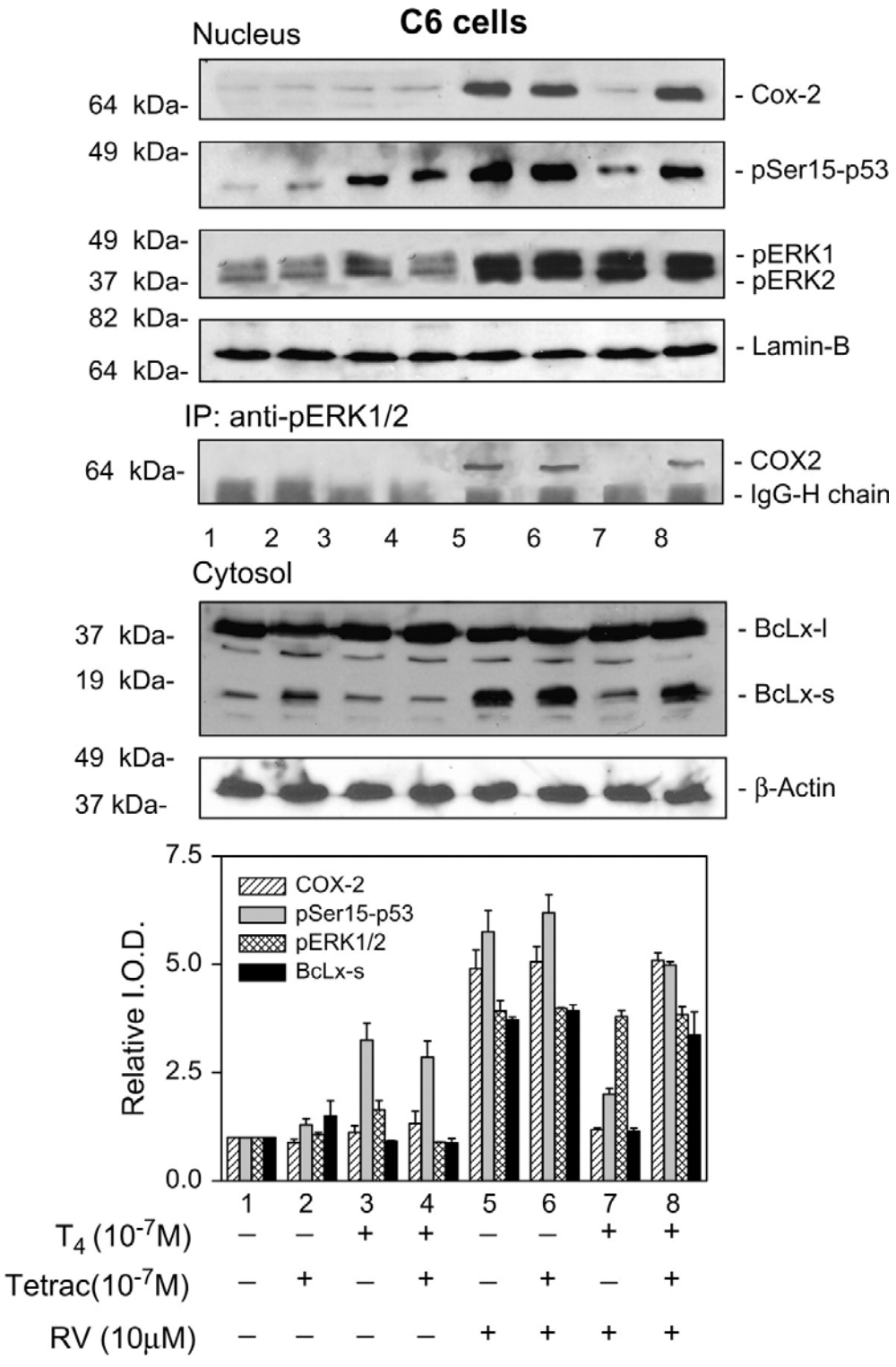
H510A, small cell lung carcinoma cells
H522, NSCLC cells



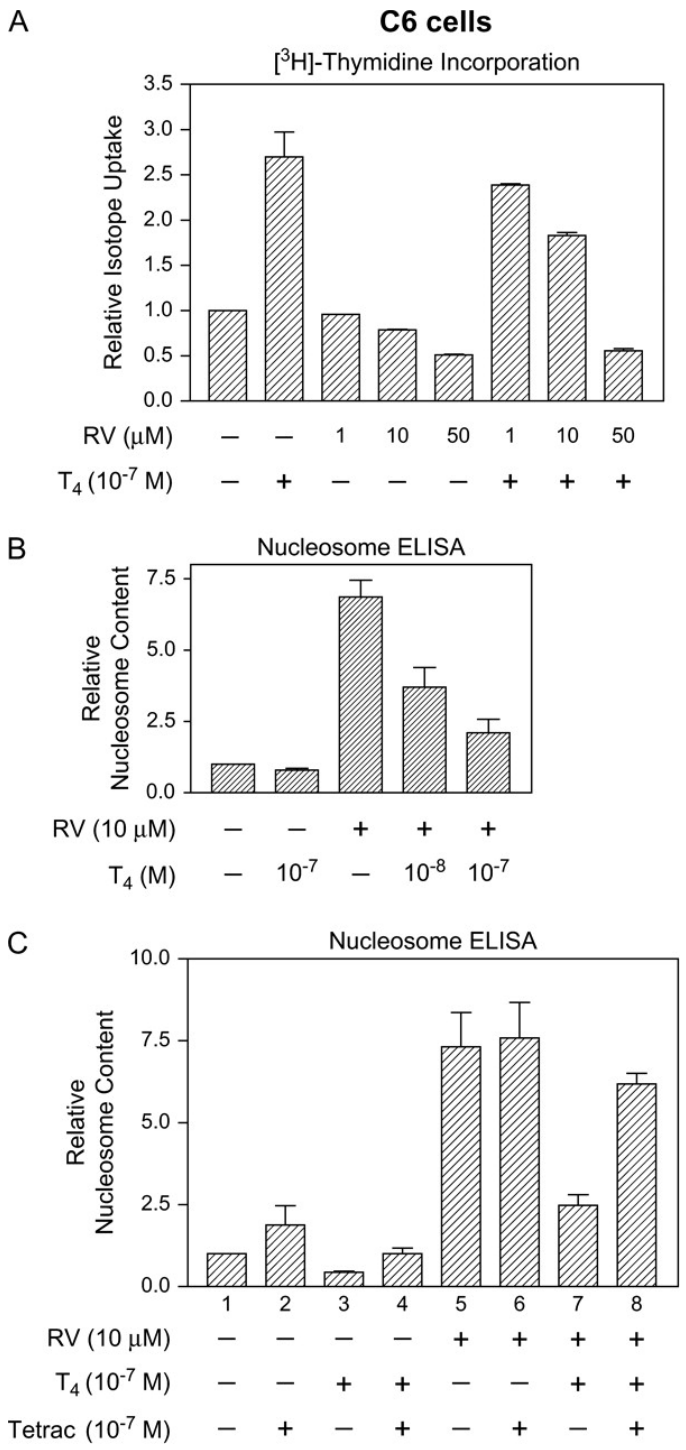


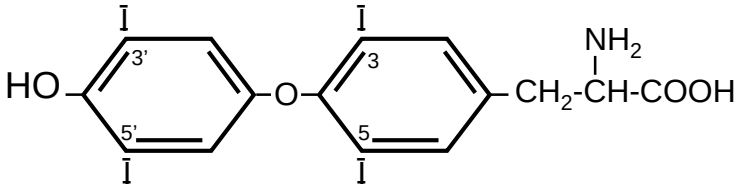
ICI = ICI 182,780,

Thyroid hormone has anti-apoptotic activity in cancer cells

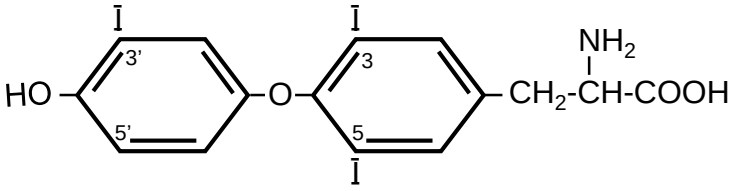


RV = resveratrol

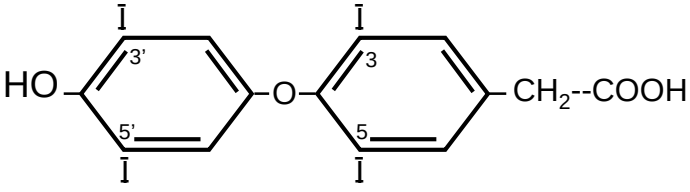

A
C6 cells
[3H]-Thymidine Incorporation
Relative Isotope Uptake
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
RV (μM) – – 1 10 50 1 10 50
T4 (10-7 M) – + – – – + + +
B
Nucleosome ELISA
Relative Nucleosome Content
7.5
5.0
2.5
0.0
RV (10 μM) – – + + +
T4 (M) – 10-7 – 10-8 10-7
C
Nucleosome ELISA
Relative Nucleosome Content
10.0
7.5
5.0
2.5
0.0
1 2 3 4 5 6 7 8
RV (10 μM) – – – – + + + +
T4 (10-7 M) – – + + – – + +
Tetrac (10-7 M) – + – + – + – +



Thyroxine (T_4)

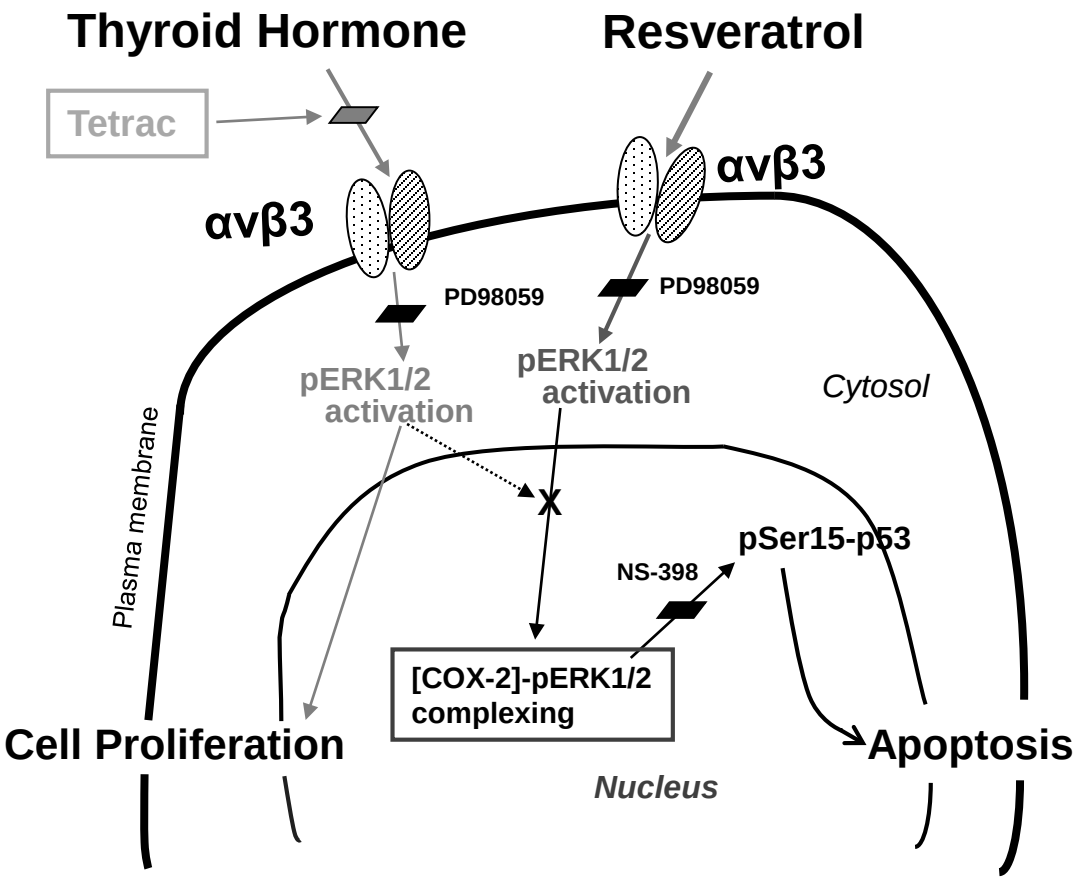


3,5,3'-Triiodothyronine (T_3)



Tetrac

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

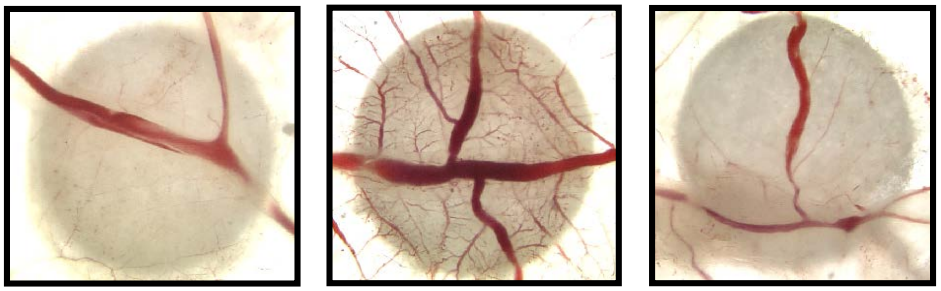


Thyroid hormone inhibits induction by RV of Ser-15 phosphorylation of p53

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.

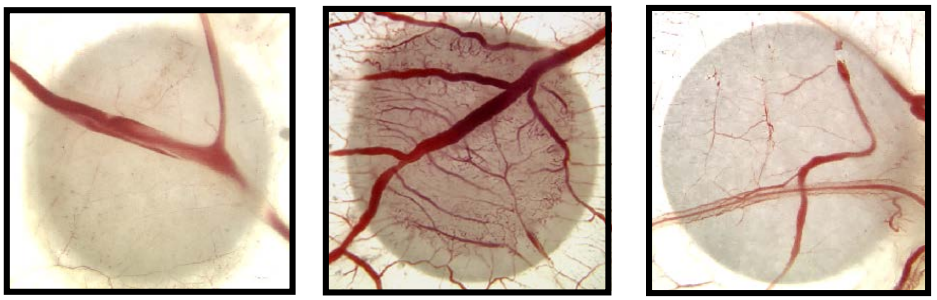
Angiogenesis in the CAM

A



PBS T_4 T_4 + Tetrac

B



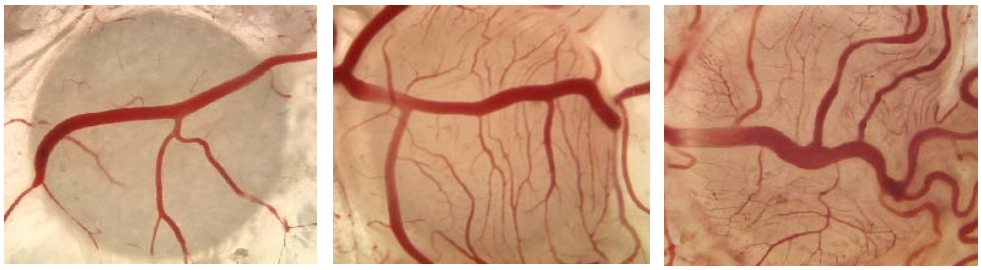
PBS T_4-ag T_4-ag + Tetrac

C

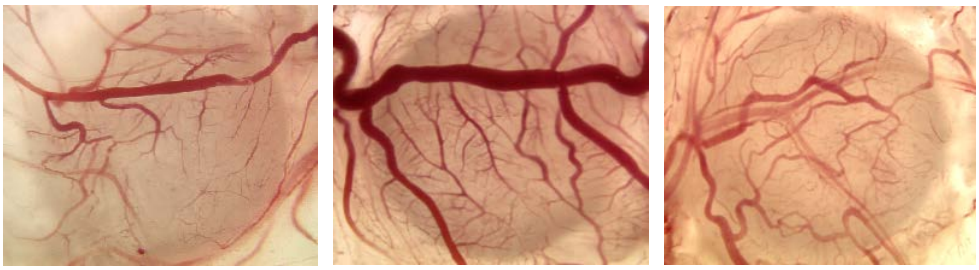
Summary of effects of T_4, T_4-agarose and tetrac on angiogenesis

Treatment	Angiogenesis Index
PBS	67 ± 9
T_4 (0.1 nM)	156 ± 16**
Tetrac (0.1 μM)	76 ± 9
T_4 + tetrac	66 ± 6
T_4-agarose (total, 0.1 μM)	194 ± 28**
T_4-agarose + tetrac	74 ± 7

A



PBS (Control) | T_4 (0.1 µM) | T_4-agarose (0.1 µM)



DITPA (0.01 µM) | VEGF (2.0 µg/ml) | b-FGF (1.0 µg/ml)

B

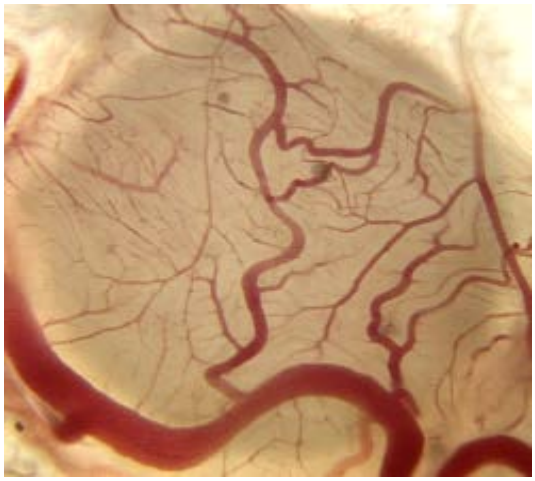
Treatment	Mean Vessel Branch Points ± SD
PBS (Control)	87 ± 9
T_4 (0.1 µM)	148 ± 7*
T_4-agarose (0.1 µM)	167 ± 8*
DITPA (0.01 µM)	134 ± 11*
DITPA (0.1 µM)	170 ± 9*
VEGF (2.0 µg/ml)	168 ± 10*
b-FGF (1.0 µg/ml)	174 ± 8*

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

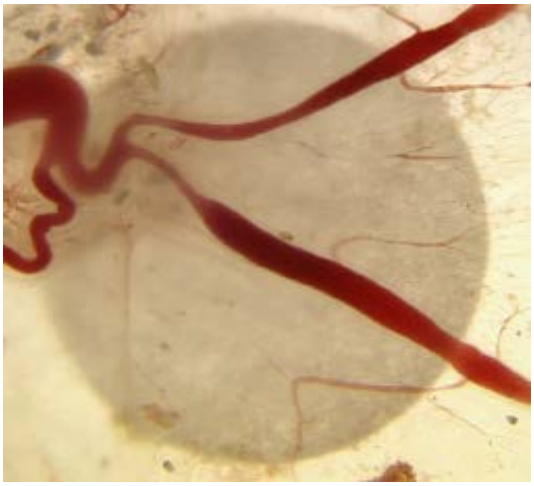
Inhibitory Effect of αvβ3 MAB (LM609) on T4-stimulated Angiogenesis in the CAM Model



PBS



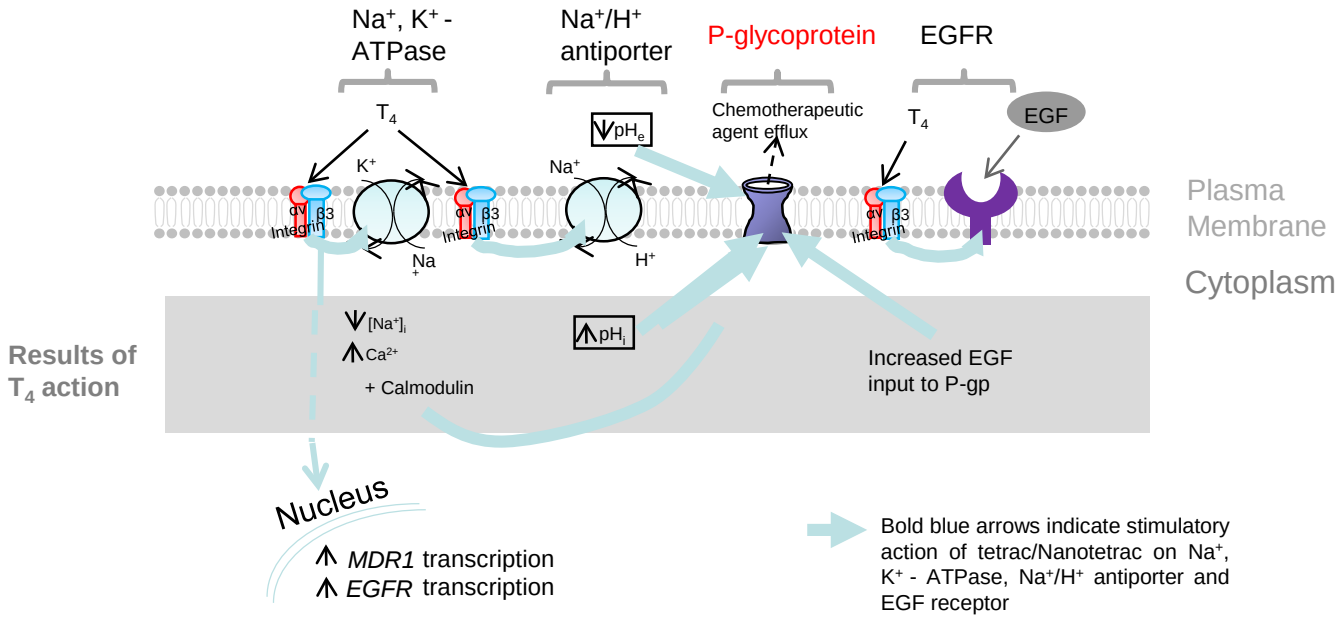
T_4 (total, 0.1μM)



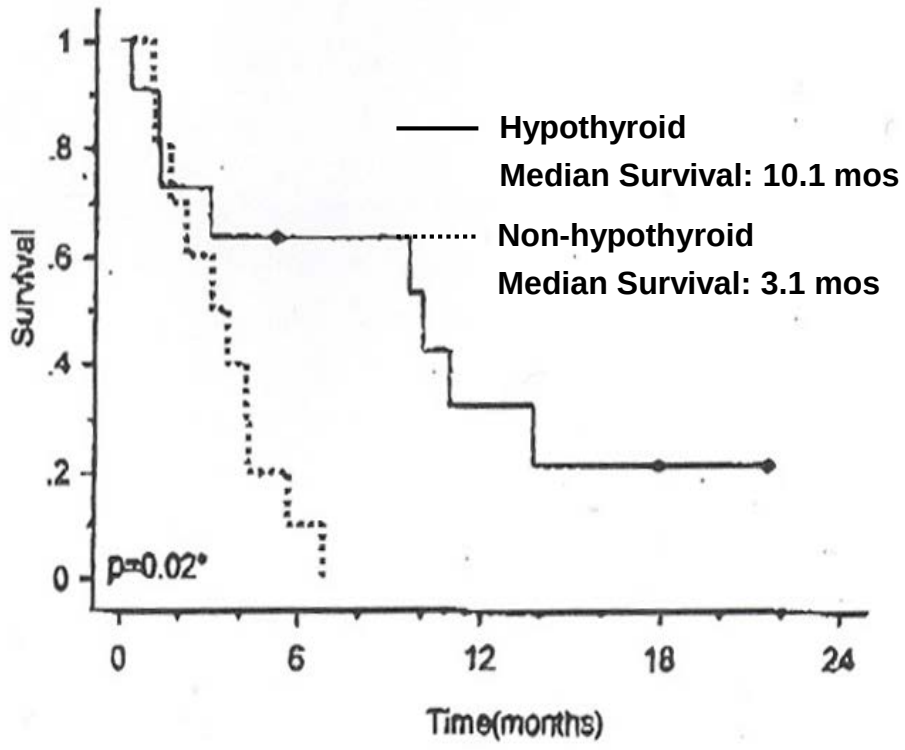
T_4+ LM609(10μg)

CAM Treatment	# of branch pts ± SEM	% Inhibition ± SEM
PBS	73 ± 8	
T4(0.1uM)	170 ± 16	0
T4+LM609(10ug)	109 ± 9	64 ± 9

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.


Na+, K+ - ATPase
Na+/H+ antiporter
P-glycoprotein
EGFR
T4
pHe
Chemotherapeutic agent efflux
T4
EGF
K+
Na+
αV β3 Integrin
αV β3 Integrin
αV β3 Integrin
Na+
H+
Plasma Membrane
Cytoplasm
[Na+]i
Ca2+
+ Calmodulin
pHi
Increased EGF input to P-gp
Results of T4 action
Nucleus
MDR1 transcription
EGFR transcription
Bold blue arrows indicate stimulatory action of tetrac/Nanotetrac on Na+, K+ - ATPase, Na+/H+ antiporter and EGF receptor

Glioblastoma multiforme patients



AA Hercbergs, Anticancer Res 23:617-626, 2003

M. Cristofanilli et al. (MD Anderson Cancer Center, Houston) showed spontaneous hypothyroidism delayed development of human breast cancer, reduced tumor invasiveness and incidence of metastasis.

Cancer 103:1122-1128, 2005

M. Nelson and co-workers (Cleveland Clinic) have shown that when hypothyroidism complicates the post-treatment course—following surgery, radiation—of head-and-neck cancer, there is improved survival and longer recurrence-free survival.

Arch Otolaryngol Head Neck Surg 132:1041-1046, 2006

M. Schmidinger (Vienna) and two other European cancer center research groups have shown that tyrosine kinase inhibitor (TKI) therapy of kidney cancer (renal cell carcinoma) was effective when the TKI induced the side effect of hypothyroidism.

Cancer 117:534-544, 2011

The Oncologist®

Symptom Management and Supportive Care

Medically Induced Euthyroid Hypothyroxinemia May Extend Survival in Compassionate Need Cancer Patients: An Observational Study

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Hypothyroxinemia • Triiodothyronine • Cancer • Survival

ABSTRACT

Background. Clinical studies have shown that interventional lowering of serum free thyroxine (FT4) may be associated with extended survival in patients with some terminal cancers. The report of success with this approach in glioblastoma multiforme caused involvement of the author (A.H.) in the prospective consultative management of 23 end-stage solid tumor patients in whom hypothyroxinemia was induced to prolong life.

Patients and Methods. Patients were self-referred or recommended by attending physicians to the author (A.H.) and had advanced cancers of the brain, ovary, lung, pancreas, salivary gland, and breast or had mesothelioma or soft-tissue sarcoma. Hypothyroxinemia was achieved in euthyroid patients by using methimazole, with the addition of 3,3′,5-triiodo-L-thyronine (L-T3) to prevent hypothyroidism and suppress endogenous thyrotropin (TSH). In patients with pre-existent primary hypothyroidism, T3 administration was substituted for T4 replacement. Serum FT4 and TSH concentrations were serially monitored to enable adjustments to drug therapy and prevent clinical hypothyroidism. Survival was measured from the date of hypothyroxinemia induction with T3 or methimazole plus T3. Outcomes were compared with the odds of death based on the Surveillance Epidemiology and End Results and American Joint Committee on Cancer databases and literature reports.

Results. The survival time of 83% (19 of 23) of patients exceeded the 20% expected 1-year survival for this hypothyroxinemic, end-stage cancer group. The difference between actual and expected survival was significant.

Conclusion. Although this is an uncontrolled observational experience with frank limitations, compassionate medical induction of hypothyroxinemia should be considered for patients with advanced cancers to whom other avenues of treatment are closed. *The Oncologist* 2015;20:1–5

Implications for Practice: There is increasing documentation, but still limited general appreciation, of the proliferative effect of circulating L-thyroxine (L-T4) on solid tumors. The novelty of the current set of case histories is that endogenous T4 may be safely and effectively reduced or depleted in end-stage solid tumor patients and replaced with exogenous triiodothyronine (T3). This change served to stabilize or improve the clinical status of patients with advanced cancers. In the 23 patient cases collected in this report, therapeutic induction of euthyroid hypothyroxinemia was safe and appeared to be compatible with conventional end-stage anticancer measures or used as sole therapy. This novel treatment paradigm, which features generic T3, usually with generic methimazole, is immediately available to medical practitioners involved in palliative cancer care.

INTRODUCTION

Thyroid hormone plays a major role in physiologic processes crucial to growth, maturation, and metabolism [1]. It has been hypothesized that thyroid hormone is an endogenous modulator of malignant tumors that may affect disease prognosis [2, 3], and supportive clinical literature has recently been reviewed by Moeller and Fuhrer [4]. In a phase II clinical trial of recurrent glioblastoma multiforme (GBM) conducted by the author (A.H.), medically induced hypothyroidism was associated with significantly longer progression-free survival and overall survival rates [5, 6]. In that study, it was found that prolongation of survival correlated significantly and independently with circulating free thyroxine (FT4) levels that had declined to values below the lower limit of the reference range. The drawbacks in this GBM study were delay in depletion of blood FT4—50% of patients died prior to reaching that FT4 target—and clinical morbidity, principally fatigue, in those who

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A. Hercbergs et al. (Cleveland Clinic) induced euthyroid hypothyroxinemia in 23 end-stage tumor patients (brain, ovary, lung, breast, pancreas). Survival duration in the treated patients was 3.1-fold (29 months vs. 9.3 months) that predicted by standard databases.

Oncologist 20:72-76, 2015

Synthroid® (L-T4) is the third most commonly prescribed trademarked drug in the U.S. because hypothyroidism is very common.

In the writing of each prescription for L-T4, what do we need to consider?

CLINICAL QUESTIONS ABOUT THYROID HORMONE AND CANCER

- What are the effects of incidental hypothyroidism on behavior of cancers?
- How aggressively to replace thyroid hormone in hypothy-roidism induced by anti-cancer agents, e.g., TKI's?
- Is there value of induction of hypothy-roidism in cancer patients?
- Is there a difference in T4 vs. T3 replacement in the setting of hypothyroidism and cancer?

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.

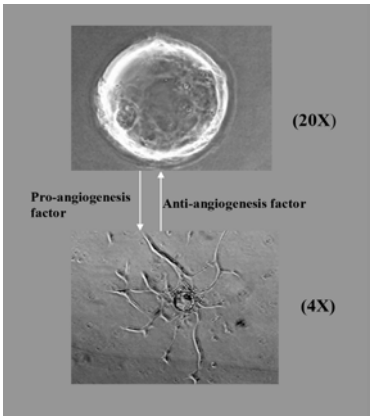
SUMMARY 2

- It is desirable to have a specific pharmacologic antagonist of thyroid hormone actions at integrin $\alpha v\beta 3$ (= tetrac, Nanotetrac [triac]).
- 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 rosacea, may be thyroid hormone-supported.

COLLABORATORS

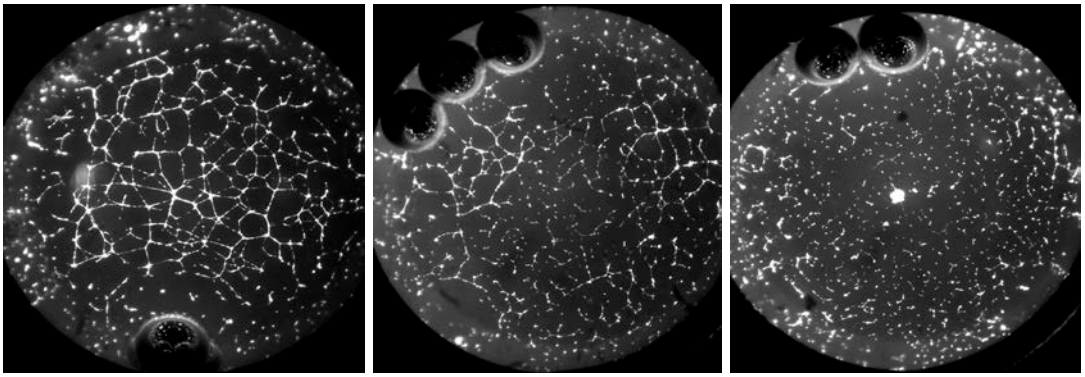
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Heng-Yuan Tang, MA	Albany
Thangirala Sudha, PhD	Albany
Faith B. Davis, MD	Albany
Murat Yalcin, DVM, PhD	Turkey
Sandra Incerpi, PhD	Italy
Osnat Ashur-Fabian, PhD	Israel

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_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.

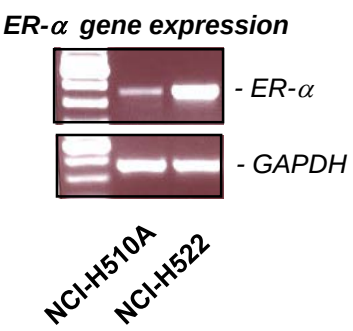
B

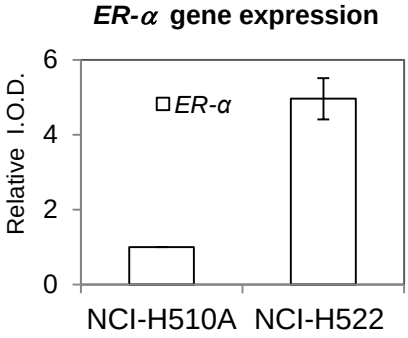


VEGF	+	+	+
Tetrac	–	1.0	2.5

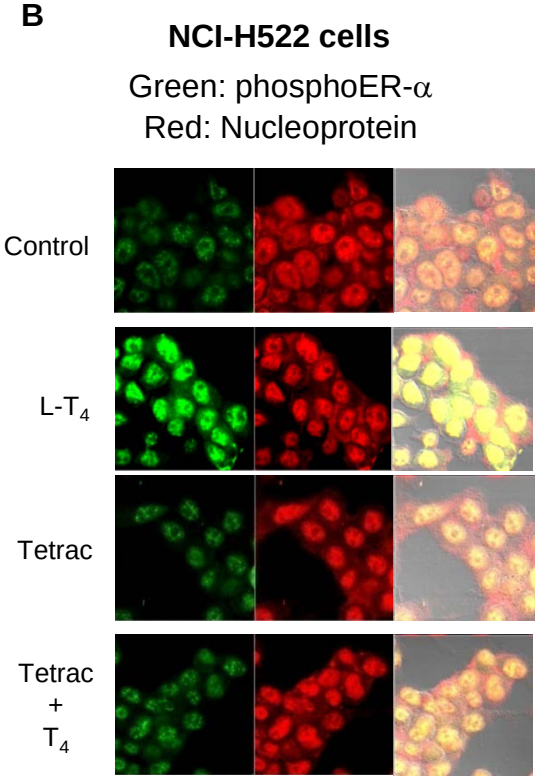
Int Angiol, 2006

A

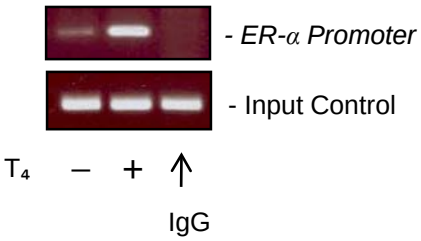

ER-α gene expression
- ER-α
- GAPDH
NCI-H510A
NCI-H522

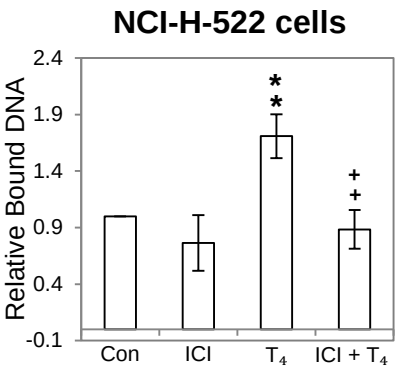

ER-α gene expression
Relative I.O.D.
6
4
2
0
ER-α
NCI-H510A
NCI-H522

B

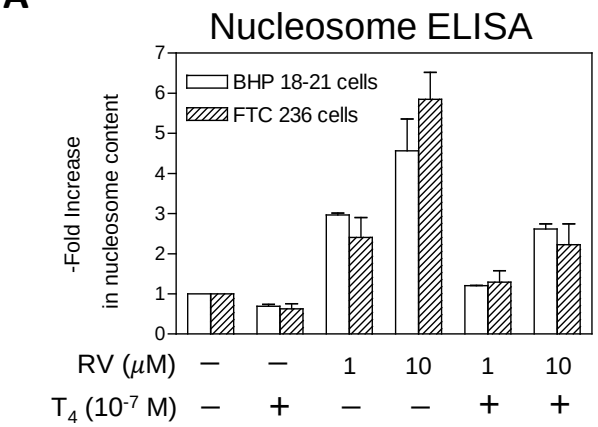

NCI-H522 cells
Green: phosphoER-α
Red: Nucleoprotein
Control
L-T4
Tetrac
Tetrac + T4

C

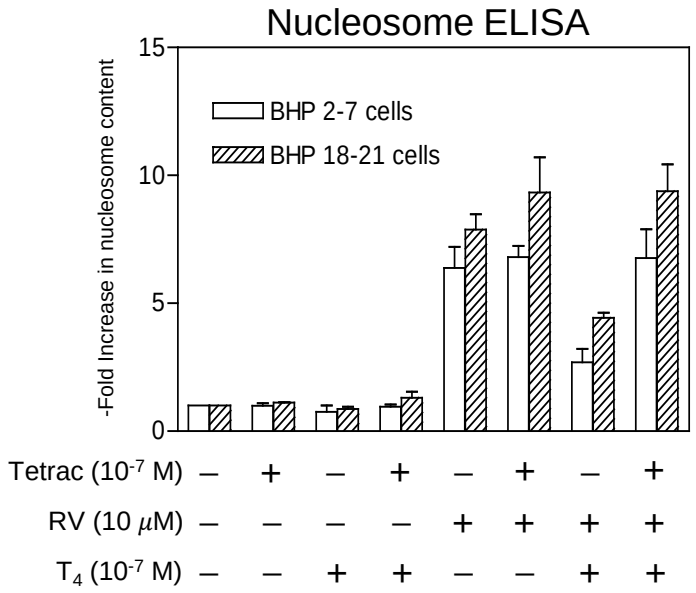

IP: anti-integrin-αv
- ER-α Promoter
- Input Control
T4 – + ↑
IgG


NCI-H-522 cells
Relative Bound DNA
2.4
1.9
1.4
0.9
0.4
-0.1
**
++
Con
ICI
T4
ICI + T4

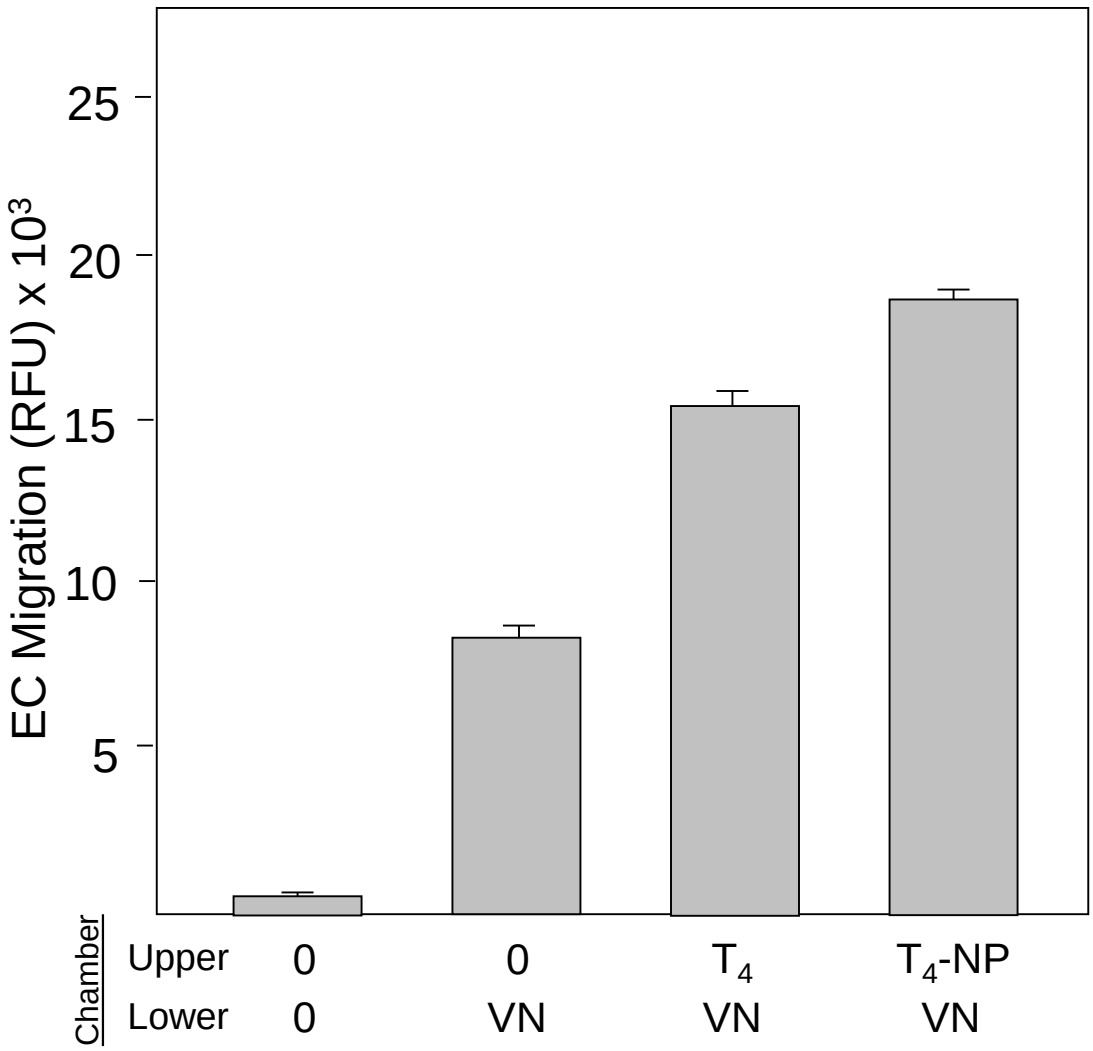
A


Nucleosome ELISA
BHP 18-21 cells
FTC 236 cells
-Fold Increase in nucleosome content
0
1
2
3
4
5
6
7
RV (μM) – – 1 10 1 10
T4 (10-7 M) – + – – + +

B


Nucleosome ELISA
BHP 2-7 cells
BHP 18-21 cells
-Fold Increase in nucleosome content
0
5
10
15
Tetrac (10-7 M) – + – + – + – +
RV (10 μM) – – – – + + + +
T4 (10-7 M) – – + + – – + +

Effects of L-T_4 or T_4 Nanoparticles (NP) on Endothelial Cell Migration toward Vitronectin (VN)



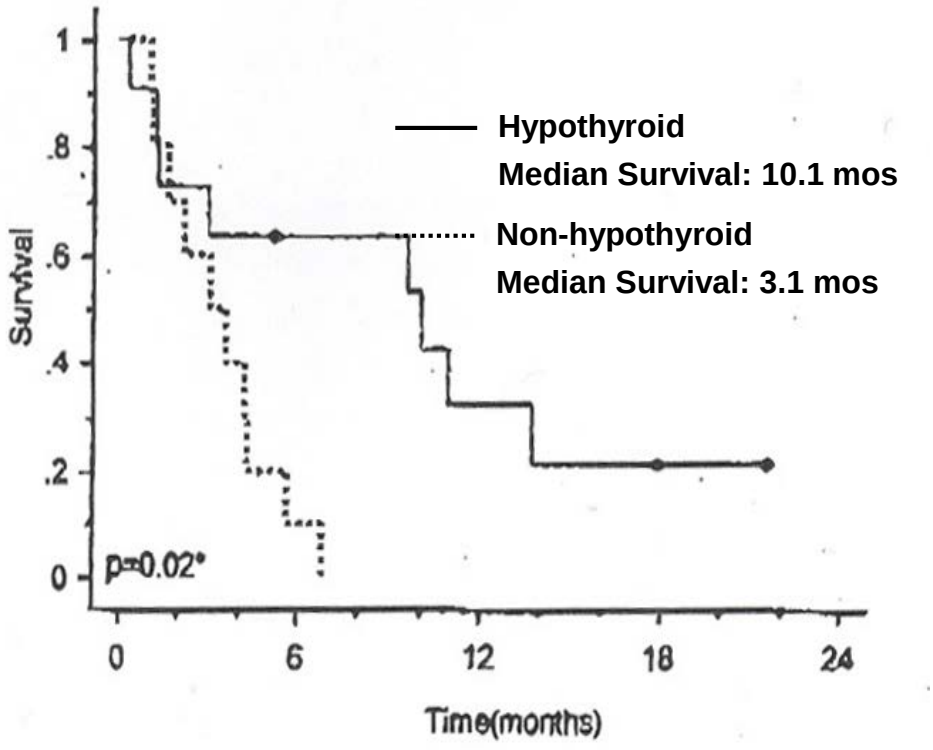
Boyden apparatus

Summary 1

- Acting genomically at TRs, thyroid hormone (T3) is critical to normal embryonic development, to basal activities of a variety of enzymes, critical protein gene expression and regulation of protein turnover.
- Acting nongenomiclly at mitochondria, T3 regulates cell respiration/metabolic rate.

- Integrin avb3 contains a cell surface receptor for nongenomic actions of T4 and T3 relevant to cancer and blood vessels.
- Thyroid hormone, primarily T4, acts experimentally at the integrin receptor via ERK1/2 to support tumor cell proliferation (breast, glioma, head-and-neck, *thyroid*, ovary, pancreas, kidney, lung, prostate), anti-apoptosis and angiogenesis.
- Inhibiting actions of T4 at the integrin experimentally or reducing circulating T4 levels clinically in small series of patients slows progression of a variety of human tumors.

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) an head-and neck cancers (Cleveland Clinic).



Hercbergs AA *et al, Anticancer Res*, 2003