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# (Thyroid) Hormonal regulation of breast cancer cells

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Thyroid hormone as L-thyroxine (T4) acts nongenomically at physiological concentrations at its cancer cell surface receptor on integrin  $\alpha\beta3$  ('thyrointegrin') to cause cancer cell proliferation. In the case of estrogen receptor (ER $\alpha$ )-positive breast cancer cells, T4 via the integrin promotes ER $\alpha$ -dependent cancer growth in the absence of estrogen. Thus, tumor growth in the post-menopausal patient with ER $\alpha$ -positive cancer may again be ER-dependent because of T4. Additional mechanisms by which T4 may contribute uniquely to aggressive breast cancer behavior—independently of ER—are stimulation of immune checkpoint inhibitor gene expression and of several anti-apoptosis mechanisms. These observations may call for consideration of elimination of host T4 production in breast cancer patients whose response is suboptimal to standard chemotherapy regimens. Euthyroidism in such a setting may be maintained with exogenous 3,3',5-triiodo-L-thyronine (T3).

## KEYWORDS

L-thyroxine (T4), 3, 5, 3'-triiodo-L-thyronine(T3), integrin  $\alpha\beta3$ , breast cancer, estrogen receptor- $\alpha$  (ER $\alpha$ ), euthyroid hypothyroxinemia

## Introduction

Steroid hormones have genomic and nongenomic actions that overlap, as do the genomic and nongenomic mechanisms of action of thyroid hormone (1). In the case of thyroid hormones, for example, L-thyroxine (T4) at the cell surface receptor for thyroid hormone analogues on plasma membrane integrin  $\alpha\beta3$  nongenomically causes phosphorylation/activation of nuclear thyroid hormone receptor TR $\alpha$  (2, 3). Thus, T4 that is thought to function primarily as a prohormone for nuclear 3,5, 3'-triiodo-L-thyronine (T3) can alter the activation state of the nuclear thyroid hormone receptor.

We briefly emphasize here that nongenomic action of T4 at  $\alpha v\beta 3$  may overlap with the genomic mechanism of action of estrogen at the nuclear estrogen receptor (ER $\alpha$ ) (3). This appears to have implications for breast carcinoma cell proliferation (4) in the menopausal woman and for cancers of other tissues that may be estrogen-responsive. We also emphasize that the thyroid hormone receptor on the integrin ('thyrointegrin') in breast cancer cells may contribute to other clinically undesirable actions of these cancer cells, including immune checkpoint desensitization/anti-apoptosis (5).

## Thyrointegrin function in ER $\alpha$ -positive and -negative breast cancer cells

The proliferative effects of physiological concentrations of T4 and estradiol on ER $\alpha$  in human breast cancer cells are comparable and are similarly reduced by pharmacologic inhibition of mitogen-activated protein kinase (MAPK) activity (4). Activation of MAPK by T4 is thyrointegrin-dependent (6). Interestingly, integrin-dependent stimulation by T4 of proliferation of triple-negative ER cells also occurs (7). Thus, more than one mechanism exists for the stimulation by T4 of breast cancer cell proliferation. The apparent clinical implications of these observations are that 1) in post-menopausal women with ER-positive breast cancer, T4 may serve an estrogen-like function on tumor cell proliferation and 2) in pre-menopausal patients with breast cancer, T4 may be a stimulatory factor, regardless of estrogen receptor status of the disease.

## Immune checkpoint regulation via thyrointegrin in breast cancer cells

The programmed death-1 (PD-1)/PD-ligand 1 (PD-L1) immune checkpoint is among a set of such checkpoints that are critical cancer cell defenses against host immune system destruction by T cells (8, 9). Lin and co-workers have shown that T4 causes the accumulation in breast cancer cells—and other tumor cells—of PD-L1 and PD-1 (5) and thus may protect the cancer cells against destruction by host T cells. This pharmacologic action of T4 *via* the thyrointegrin is anti-apoptotic, serving to prevent specific phosphorylation and activation of p53.

## Apoptosis inhibition by T4 *via* thyrointegrin in breast cancer cells

The preceding section introduced the topic of T4-induced anti-apoptosis *via* an immune checkpoint, but without primarily involving an immune system mechanism. In addition to disrupting pro-apoptotic activation of p53 in the intrinsic apoptotic pathway in cancer cells, T4 may call on other mechanisms to block apoptosis. For example, T4 interferes with the Fas-dependent extrinsic pathway of promoting apoptosis (5) and also enhances expression of gene coding for the X-linked inhibitor of apoptosis (XIAP) protein (5). These effects are initiated at the thyroid hormone receptor on plasma membrane integrin  $\alpha v\beta 3$ . Zyla et al. have recently confirmed that T4 is anti-apoptotic in breast cancer cells (10). Thus, T4 confers on breast cancer and other tumor cells a multifactorial, anti-apoptotic behavior pattern. It is important to emphasize that it is physiological concentrations of T4 that achieve this clinically undesirable effect.

## Support by T4 of metastasis of breast cancer

A number of actions of physiological concentrations of T4 at its receptor on integrin  $\alpha v\beta 3$  on tumor cells and rapidly-dividing blood vessel cells are relevant to cancer metastasis, as we have pointed out (11). These nongenomically initiated actions culminate in regulation of expression of genes linked to angiogenesis, matrix metalloproteinases and receptor tyrosine kinases. That  $\alpha v\beta 3$  is essential to the metastasis of breast cancer to bone was shown by Sloane et al. (12) and we have reported in preclinical studies that pharmacologic blockade of this thyrointegrin eliminates established breast cancer metastases in bone and other tissues (11).

## MDR-1/P-glycoprotein gene expression, chemoresistance of breast cancer and T4

Multidrug resistance (MDR) of cancer cells is conferred by hyperexpression of single or multiple ATP binding cassettes (ABC) transporters that, located in the cell nucleus, export chemotherapeutic agents that target the nucleus (13). MDR in breast cancer cells is particularly a function of expression of *ABCB1* (MDR-1/p-glycoprotein, P-gp) (13, 14). Thyroid

hormone causes transcription of P-glycoprotein (15, 16) and also stimulates activity of the transcript (17). We have shown that tetrac can restore chemosensitivity of tumor cells (18) and thus we propose that the action of T4 to stimulate transcription of the *ABCB1* gene—and support chemoresistance—is mediated by integrin  $\alpha\text{v}\beta3$ .

## Discussion: Clinical implications of actions of T4 on breast cancer cells

The purpose of this brief review is to suggest that thyroid hormone as T4 has actions that may be sources of difficulties in management of breast cancer. Examples presented here are T4 acting like an estrogen at ER in the postmenopausal patient, induced failure by T4 of immune checkpoint inhibitor drugs in management of breast cancer patients and T4-induced suboptimal effectiveness of pro-apoptotic regimens.

We know that the risk of developing breast cancer is increased in hyperthyroidism (19, 20) and somewhat decreased in hypothyroidism (10), that levothyroxine increases the clinical risk of breast cancer (21) and that hypothyroidism can alter the clinical course of breast cancer for the better (22). In addition, the substitution of T3 for T4 in maintaining euthyroidism (euthyroid hypothyroxinemia) can improve the course of management of advanced cancers, including breast cancer (23). Given such information and the ability of T4 to promote breast cancer cell proliferation *in vitro*, we propose that 1) euthyroid hypothyroxinemia be considered a therapeutic option in euthyroid breast cancer patients whose response to conventional anti-breast cancer therapy is suboptimal and 2) this stratagem be prospectively studied.

The reported actions of thyroid hormone on P-gp gene expression, whether genomic (15) or nongenomic (24), raise the possibility that the hormone in the cancer patient may foster tumor cell chemoresistance. This possibility has not yet been examined in breast cancer cells, but integrin  $\alpha\text{v}\beta3$ —which bears

the cell surface receptor for T4—has been shown to be an important contributor to the doxorubicin resistance of metastatic breast cancer (14). Breast cancer cell chemoresistance may also be a consequence of XIAP gene expression (25) and we have noted above that T4, acting *via* its plasma membrane receptor, upregulates expression of the gene for this factor.

## Author contributions

The initial draft of the manuscript was written by PD, but all co-authors edited the drafts. This is a review manuscript and all authors have published multiple pre-clinical or clinical observations that are cited in the References section. All authors contributed to the article and approved the submitted version.

## Conflict of interest

PD and SM hold stock in a small pharmaceutical company, NanoPharmaceuticals LLC, that is developing anticancer drugs.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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