Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: December 31, 2023

Citation: Hercbergs A, Lin H-Y, at al., 2023. Overlapping Actions of L-Thyroxine (T4) and Steroids in Breast Cancer Cells: Mediation by Cell Surface Integrin αvβ3, Medical Research Archives, [online] 11(12). https://doi.org/10.18103/mra.v11i12.4963

DOI
https://doi.org/10.18103/mra.v11i12.4963

ISSN: 2375-1924

RESEARCH ARTICLE

Overlapping Actions of L-Thyroxine (T4) and Steroids in Breast Cancer Cells: Mediation by Cell Surface Integrin αvβ3

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ABSTRACT

The overlap of actions of nonpeptide small endocrine molecules—thyroid hormone and steroids—include two panels of actions. One set is initiated at the nuclear receptors for these hormones and a second set of actions for both hormones is initiated at the extracellular domain of plasma membrane integrin αvβ3. This brief review is concerned with integrin-based receptors on breast cancer cells. On such cells, thyroid hormone as L-thyroxine (T4) at physiological concentrations can stimulate proliferation of breast cancer cells via the thyroid hormone analogue receptor on αvβ3 and, in the absence of estrogen, via the nuclear estrogen receptor-α (ER α). Such observations emphasize the postmenopausal relevance of nuclear estrogen receptor. The deaminated T4 derivative, tetraiodothyroacetic acid (tetrac), blocks T4 actions at the integrin. An androgen receptor on the integrin mediates stimulation of breast cancer cell proliferation by dihydrotestosterone (DHT). T4 controls the activation state of the integrin, a factor that may determine the accessibility of the androgen receptor on αvβ3 to DHT and thus to DHT-driven cell proliferation. An estrogen receptor appears to be present on the integrin, but its functions have not been defined. It is not yet known whether tetrac alters function of the steroid receptors that are adjacent to the T4 binding site on αvβ3. The overlap of T4 and steroid functions in breast cancer cells may offer additional options for clinical management of this type of cancer.

Keywords: integrin αvβ3, L-thyroxine (T4), dihydrotestosterone (DHT), estrogen receptor-α (ER α), breast cancer cell proliferation

Introduction

Thyroid hormone as L-thyroxine (T4) was shown to specifically phosphorylate (activate) nuclear estrogen receptor-α (ERA in human breast cancer cells almost twenty years ago.[1] This mitogen-activated protein kinase (MAPK)-dependent process stimulated cell proliferation, mimicking the effects of estradiol (E2). At that time, T4 was regarded only as a prohormone for 3,5,3'-triiodo-L-thyronine (T3), the primary ligand of the nuclear thyroid hormone receptors (TRs) and generator of the genomic effects of thyroid hormone.[2] The T4 action on MAPK was subsequently shown to be mediated by a novel cell surface thyroid hormone receptor on integrin αvβ3.[3] and at which T4 is now recognized as the principal functional ligand.[4] Unexpected in these early breast cancer/thyroid hormone studies were a) the reproduction by a thyroid hormone of a critical estrogen action, b) biological activity of T4 at physiological concentration and c) the possibility that in the absence of estrogen, e.g., the post menopause, a nuclear estrogen receptor in a cancer cell might be functionally regulated by a thyroid hormone. Among integrins, it is only the αvβ3 moiety that contains the receptor for thyroid hormone analogues.[4]

Integrin αvβ3 has subsequently been shown to contain binding sites for steroids—androgen and, possibly, estrogens—and for the polyphenol, resveratrol.[5] Breast cancer cells, like other cancer cells, overexpress this integrin.[4] The liganding of T4 by the integrin apparently activates the protein,[6] i.e., converts it to an extended physical state that facilitates integrin-to-integrin binding and integrin binding to extracellular matrix proteins, such as fiobronectin.[7] We would raise the possibility that activation/unfolding of the integrin improve exposure of other small molecule binding sites on the integrin to their specific ligands.

In this review, we discuss documented and projected overlapping effects of T4 and steroids on the behavior of breast cancer cells. A clinical rationale for the discussion is that there may be therapeutic opportunities in the small molecule-binding by the integrin in breast carcinoma cells.

Thyroid hormones and clinical behavior of breast cancer

Certain clinical and preclinical observations[8-10] have suggested that breast cancer cells proliferate in response to circulating levels of thyroid hormone as T3. Normal and abnormal cell energetic may be imposed by T3.[2] On the other hand, T3 has been shown to induce apoptosis in breast cancer cells[11] and Hercbergs et al.[12] have shown clinically that maintenance of euthyroidism with T3 in conjunction with pharmacological elimination of host T4 slows growth of a variety of tumors, including breast cancer. This treatment state is designated euthyroid hypothyroxinemia.[12]

The Hercbergs clinical experience[12] and an extensive series of preclinical studies have shown that T4 at physiological concentrations supports tumor cell proliferation. In addition to cell proliferation, effects of T4 on cancer cells include contributions to anti-apoptosis,[13] radioresistance,[14] chemoresistance [15] and to increased metastasis.[16] The repor ts cited here either directly assessed the effects of T4 on cancer cells or used chemically-modified tetrac that disables the thyroid hormone receptor on integrin αvβ3 to infer effects of T4.[3,4,17]

A review of the M.D. Anderson Cancer Center's extensive experience with spontaneous hypothyroidism and development of breast cancer showed that primary hypothyroidism was associated with a decreased risk of breast cancer and a less aggressive clinical course when breast tumor complicated hypothyroidism.[18] Among the indicators of less aggressive course was decreased metastatic disease. Other clinical studies have also found that decreased thyroid function may protect against breast cancer[19,20] and that endogenous trends toward increased thyroid function enhances breast cancer risk.[21,22] Of additional interest is that thyroid hormone replacement therapy may be a breast cancer risk factor.[23]

The definition of the plasma membrane integrin αvβ3 receptor for thyroid hormone and appreciation that T4 was the primary ligand for this site permitted the projection of clinical implications for T4 actions on tumor cells. First, circulating T4 may restore clinical importance of the estrogen receptor in ER-positive breast cancers in postmenopausal women. *In vitro* studies that would be helpful here would be comparison of free T4 levels in the upper tertile and lower tertile of the normal range on proliferation in ER-positive breast cancer cells cultured in postmenopausal sera. Second, elevated free T4 levels that are achieved in some patients with the non-thyroidal illness syndrome (NTIS)[24] should be examined for additive effects on breast cancer cell proliferation *in vitro* in pre-menopausal patient sera and postmenopausal sera in which ER-positive cancer cells are cultured. This information may also be relevant to the occasional postmenopausal patient

with a history of thyroid cancer who is on TSH suppressive dosage of T4. Third, although the view of the authors of the present paper is that T3 has little or no stimulatory effect on ER- positive breast cancer, we have in the Introduction cited references that describe trophic effects of this thyroid hormone analogue on tumor cells. Thus, *in vitro* studies of proliferation of ER-positive breast cancer cells should also test a range of concentrations of T3 for breast cancer cell proliferation effects in postmenopausal sera-containing culture media.

Possible contributions of αvβ3 integrin to effects of steroid hormones on breast cancer cell proliferation

As noted above, a testosterone-binding site has been described on the neck of integrin αvβ3, close to the thyroid hormone receptor.[5] X-ray crystallographic analysis of this integrin has also identified a possible estrogen receptor site that is proximal to the androgen site.[5] Integrin protein structure is wholly different from the structures of the nuclear steroid hormone receptors. In a human breast cancer cell line that lacks ERαα, dihydrotestosterone (DHT) stimulates cell proliferation that is inhibited by antibody to integrin αvβ3. Antibody to the integrin does not affect DHT action on proliferation in breast cancer cells that express ER, indicating that DHT can affect breast cancer cell proliferation by multiple complex mechanisms.[5]

We also suggest that mechanisms exist for the possibility that thyroid hormone as T4 might affect the DHT-breast cancer proliferation model. That is, T4 affects the activation state of integrin αvβ3[4] and this may regulate the accessibility of the androgen receptor on the integrin. The extended (activated) state of the integrin induced by T4 may control access of the steroid ligand to its integrin receptor site.

Documented and projected roles of tetrac in overlapping functions of thyroid hormone and certain steroids

As mentioned above, tetrac is a thyroid hormone analogue that disables the thyroid hormone receptor on cancer cell integrin αvβ3. T4 effects at the integrin on activation of the protein and on cell proliferation are blocked by tetrac or chemically-modified tetrac.[4,6] Because rapidly-dividing blood vessel cells overexpress the integrin and activate the latter, tetrac also inhibits cancer-related angiogenesis.[4]

Against this background, we raise several tetrac-related clinical issues related to the overlapping actions of thyroid hormone and steroids. First, low endogenous tetrac levels are measurable in the circulation.[4,25] Are lower or upper quartile levels in the normal range of any importance to steroid-dependent or T4-dependent breast cancer behavior? Needed are *in vitro* studies of the action on breast cancer cell proliferation of tetrac at several physiological levels. Second, should tetrac or derivatized tetrac be examined for utility in reducing recurrence of breast cancer in patients already treated with conventional stragtegies?

Conclusions

Preclinical observations support the existence of overlapping thyroid hormone-based and steroid-based contributions to breast cancer cell proliferation. The mechanisms of such contributions discussed here relate to a T4 receptor and steroid receptors that exist on the extracellular domain of plasma membrane integrin αvβ3 expressed by and activated in breast cancer and other tumor cells. The integrin androgen receptor has been shown to be functional in breast cancer cells, whereas the possible functions of an integrin estrogen receptor remain to be determined. It is clear that T4 and testosterone can act via discrete receptors on the integrin to stimulate cell proliferation and that the nuclear estrogen receptor and the integrin T4 receptor have overlapping actions. It is clinically desirable to eliminate these effects. T4 can also act at αvβ3 to stimulate angiogenesis in a variety of cancers and to foster radioresistance.[4,14] It has not yet been determined whether androgens or estrogen can affect angiogenesis and radiosensitivity in breast cancer cells via the integrin.

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