Life Sciences 154 (2016) 52–57

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Triiodothyronine (T_3) induces *HIF1A* and *TGFA* expression in MCF7 cells by activating PI3K

Fernanda Cristina Fontes Moretto [a,*], Maria Teresa De Sibio [a], Aline Carbonera Luvizon [b], Regiane Marques Castro Olimpio [a], Miriane de Oliveira [a], Carlos Augusto Barnabe Alves [c], Sandro José Conde [a], Célia Regina Nogueira [a]

[a] Department of Internal Medicine, Botucatu Medical School, Univ Estadual Paulista - UNESP, Botucatu, SP 18618-970, Brazil
[b] Department of Pathology, Botucatu Medical School, Univ Estadual Paulista - UNESP, Botucatu, SP 18618-970, Brazil
[c] Department of Morphology, Bioscience Institute, Univ Estadual Paulista - UNESP, Botucatu, SP 18618-970, Brazil

ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form 4 April 2016
Accepted 15 April 2016
Available online 17 April 2016

Keywords:
Thyroid hormone
Breast cancer
Gene expression
Extra-nuclear pathway

ABSTRACT

High expression levels of hypoxia inducing factor 1 alpha are related to mammary carcinogenesis. In previous studies, we demonstrated that expression of transforming growth factor alpha increases upon treatment with triiodothyronine, but this expression does not occur in cellular models that do not express the estrogen receptor, or when cells are co-treated with the anti-estrogen, tamoxifen. The aim of this study was to determine the effect of the hormone triiodothyronine on the expression of the genes *HIF1A* and *TGFA* in the breast cancer cell line MCF7. The cell line was subjected to treatment with triiodothyronine at the supraphysiological dose of 10^{-8} M for 10 min, 30 min, 1 h, and 4 h in the presence or absence of actinomycin D, the gene expression inhibitor, cycloheximide, the protein synthesis inhibitor, and LY294002, the phosphoinositide 3 kinase inhibitor. *HIF1A* and *TGFA* mRNA expression was analyzed by reverse transcription polymerase chain reaction. For data analysis, we used analysis of variance complemented by Tukey test and an adopted minimum of 5% significance. We found that *HIF1A* and *TGFA* expression increased in the presence of triiodothyronine at all times studied. *HIF1A* expression decreased in triiodothyronine-treated cells when gene transcription was also inhibited; however, *TGFA* expression decreased after 10 and 30 min of treatment even when transcription was not inhibited. We found that activation of PI3K was necessary for triiodothyronine to modulate *HIF1A* and *TGFA* expression.

1. Introduction

Despite the significant advances in diagnosis and treatment, breast cancer (BC) remains an important and ongoing challenge for those responsible for public health [1]. The hormone triiodothyronine (T_3) stimulates the proliferation of multiple types of non-malignant cells [2–4], including breast [5] and thyroid [6] cells. Several studies on the influence of thyroid hormone (TH) in breast carcinomas, including work by our group, have reported a higher frequency of hyperthyroidism in postmenopausal patients with breast cancer [7–9].

Many tumors induce transcription of angiogenesis-related genes, including the hypoxia inducing factor (*HIF1*) and transforming growth factor alpha (*TGFA*). *HIF1* induces the translation of angiogenesis-related genes favoring this route of carcinogenesis [10]. Previous studies demonstrated that overexpression of *HIF1* and *TGFA* is positively correlated with tumor aggressiveness and malignant progression of human neoplasms [11–13]. Zhong et al. [14] showed that the alpha subunit of *HIF1*, *HIF1A*, is overexpressed in various human tumors (colon, breast, stomach, lung, skin, ovary, prostate, kidney, and pancreatic carcinomas), and that *HIF1A* is more pronounced in pre-malignant lesions than in benign lesions [14].

TGFA, which encodes a mammalian growth factor that can be affected by diet and hormones, is located on chromosome 2p13, comprises 80 kb of genomic DNA, and has six exons [15–16]. *TGFA* is produced by tumor cells and secreted by many tumors [17]. Overexpression of *TGFA* may occur during malignant progression [11].

In previous work, we demonstrated that *TGFA* expression increases after treatment with triiodothyronine (T_3) in MCF7 cells the same effect was observed in primary cultures [18,19].

Although T_3 often acts through the classic genomic regulation of gene transcription, a number of effects of T_3 occur rapidly and are not affected by inhibitors of transcription or by protein synthesis [20–22]. In these instances, T_3 may act via a mechanism that requires sites on the plasma membrane. For example, it may act through the activation of integrin αvβ3, which activates ERK1/2 and culminates in local membrane events, such as Na^+/H^+ ion transport systems, or through complex cellular events, such as cell proliferation. Alternatively, T_3 may act through the cytoplasm. The TH can activate the mitogen-activated

* Corresponding author at: Botucatu Medical School, Univ Estadual Paulista – UNESP, Distrito de Rubião Jr s/n, Botucatu, SP 18618-000, Brazil.
E-mail address: fer_fontesbtu@hotmail.com (F.C.F. Moretto).

http://dx.doi.org/10.1016/j.lfs.2016.04.024
0024-3205/

protein kinase (MAPK) and phosphoinositide 3 kinase (PI3K). PI3K activation subsequently can result in T_3–dependent transcription of specific genes, insertion of the Na^+ K^+-ATPase into the plasma membrane, and modulation of the ATPase activity. Studies using cell lines have shown changes in the expression of several genes after treatment with T_3-dependent PI3K [23–25]. The objective of this study was to determine the effect of T_3 hormone at supraphysiological doses on the activation of the PI3K pathway by measuring the expression of *HIF1A* and *TGFA* in MCF7 breast cancer cells.

2. Materials and methods

2.1. Reagents

RPMI 1640 medium, fetal bovine serum (FBS), and antibiotic solution with 1:100 dilution were purchased from Gibco BRL (Grand Island, NY, USA). Actinomycin D (ACT D), cycloheximide (CHX), triiodothyronine (T_3), LY294002 (LY), dimethylsulfoxide (DMSO), sodium hydroxide (NaOH), and charcoal-stripped FBS were purchased from Sigma Aldrich (St Louis, MO, USA).

2.2. Cell culture

Project approved by the Research Ethics Committee of Botucatu Medical School, protocol 3367-2009.

The MCF7 cell line (BC immortalized cells) was initially obtained from a primary culture of breast cancer cells originating from the pleural effusion of a female patient with metastasis [26]. These cells express ER α and β, as well as TR α and β [18,27–28]. The human breast cancer cell line MCF7 [kindly provided by Prof. Dr. Maria Mitzi Brentani, Laboratory of Oncology, FMUSP, and initially purchased from the American Type Culture Collection (ATCC), Manassas, Virginia, USA] were expanded, grown, and maintained in the cell bank of the Clinical Medicine Experimental Laboratory, UNESP, Botucatu. All experiments were repeated in triplicate for all treatments and times, between the second and third passages. The MCF7 cells were cultivated in RPMI 1640 medium supplemented with 1.2 g/L $NaHCO_3$, 10 mM Hepes, pH 7.4, and 10% FBS. The cell lines were maintained at 37 °C in 5% CO_2. The medium was changed every 2 d. Before starting the inhibitor and hormone treatments, the medium was replaced with a phenol red-free medium to remove all hormones in the medium. The cells then were incubated with 10% charcoal-stripped FBS. After incubation, the cells were treated with 10 nM T_3 for 10 min, 30 min, 1 h and 4 h; all treatments were initiated together and, subsequently, the cells were collected at the end of the respective treatment durations. The inhibitors and the T_3 hormone were added in the following amounts: actinomycin D (ACTD, 5.0 μg/mL, T_3 associate ACTD group); cycloheximide (CHX, 50 μM, T_3 associate CHX group); and LY294002 (50 μm, T_3 associate LY group). ACTD was used as a transcription inhibitor; CHX was used as a translation inhibitor; and LY294002 to verify the involvement of the PI3K pathway. The untreated, control group received only 0.1% NaOH (diluent: T_3). The inhibitors LY294002 and CHX were added to the medium 1 h prior to T_3 treatment.

2.3. Gene expression

Total RNA was extracted from the MCF7 cells using Trizol (Invitrogen, San Paulo, Brazil), according to the manufacturer's instructions. The High Capacity cDNA reverse transcription kit for RT-PCR® (Invitrogen, São Paulo, Brazil) was used for the synthesis of 20 μL of complementary DNA (cDNA) from 1000 ng of the total RNA. *HIF1A* expression levels ((Hs00153153_m1, Applied Biosystems) and *TGFA* expression levels (Hs00608187_m1, Applied Biosystems) were determined using qRT-PCR. Quantitative analysis was performed using the Applied Biosystems StepOne Plus system with the TaqMan qPCR commercial kit (Applied Biosystems), according to the manufacturer's instructions. The amplification conditions were as follows: activation of the enzyme at 50 °C for 2 min, denaturation at 95 °C for 10 min, and cDNA amplification during the 40 cycles comprising denaturation at 95 °C for 15 s and extension at 60 °C for 1 min. All experiments were performed in duplicate. Gene expression was quantified, relative to the data obtained from the control group, after normalization with an internal control (GAPDH, Hs02758991_g1), using the $2^{-\Delta\Delta Ct}$ method, as described previously [29].

2.4. Statistical analysis

For the statistical analysis, we used ANOVA in conjunction with the Tukey test and the Student's *t*-test, with a minimum significance of 5%. The data are expressed as mean ± standard deviation.

3. Results

Treatment with T3 increases *HIF1A* and *TGFA* expression. Figs. 1 and 2 represent *HIF1A* and *TGFA* expression, respectively, in MCF7 cells treated with T_3 for 10 min, 30 min, 1 h, and 4 h. The results demonstrate that treatment with T_3 promotes a significant, time-dependent increase in *HIF1A* and *TGFA* expression in breast cancer cells.

Treatment with inhibitors actinomycin D, cycloheximide and LY294002 had no effect on *HIF1A* and *TGFA* expression in breast cancer cells. Figs. 3 and 4 represent *HIF1A* and *TGFA* expression, respectively, in MCF7 cells treated with inhibitors actinomycin D (ACTD), cycloheximide (CHX) and LY294002 (LY) for 10 min, 30 min, 1 h and 4 h. The results showed that there was no statistical difference in the control group compared to inhibitors. C, Control; actinomycin D (ACTD), cycloheximide (CHX) and LY294002 (LY). Comparison com C × ACTD; C × CHX, C × LY.

Inhibition of RNA synthesis with actinomycin D affects T_3-mediated increases in *HIF1A* expression and *TGFA* expression. To determine if the effect of T_3 on the expression of *HIF1A* and *TGFA* requires mRNA synthesis, we inhibited mRNA synthesis in breast tumor cells with actinomycin D (ACTD). When comparing *HIF1A* expression in cells treated with T_3 alone to expression in cells treated with T_3 and ACTD, we found that *HIF1A* mRNA levels higher at all time points (Fig. 5). This result suggests that gene transcription is required for the action of T_3. *TGFA* expression was significantly higher in T_3 treated cells than in cells treated with T_3 and ACTD for 10 min; however, after 30 min, there was no statistical difference between the two treatments (Fig. 6).

Inhibition of protein synthesis with cycloheximide affects T_3-mediated increases in *HIF1A* expression and *TGFA* expression. To determine if T_3-dependent regulation of *HIF1A* and *TGFA* mRNA levels requires protein synthesis, breast cancer cells were incubated in the presence of T_3, and with or without cycloheximide (CHX). Figs. 7 and 8 show the combined effect of T_3 and CHX on *HIF1A* and *TGFA* expression, respectively, when compared to the effect of T_3 alone. In relation

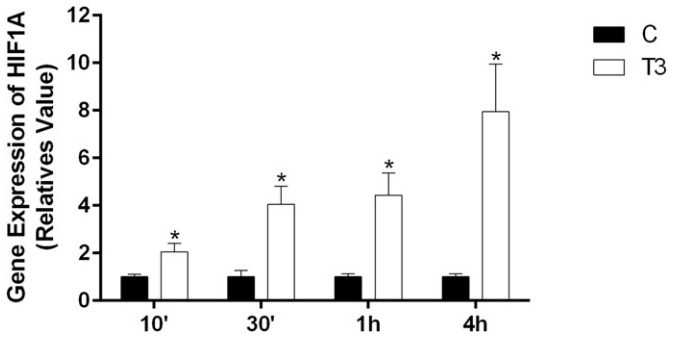

Fig. 1. Time-dependent effect of T_3 on *HIF1A* mRNA at 10 min, 30 min, 1 h, and 4 h in MCF7 cells. T_3 = 10 nM T_3, C = no T_3. Data are expressed as mean ± standard deviation values and were analyzed using the Student's *t*-test. *$p < 0.05$ in the C associate T3 comparison. n = 3 for each treatment.

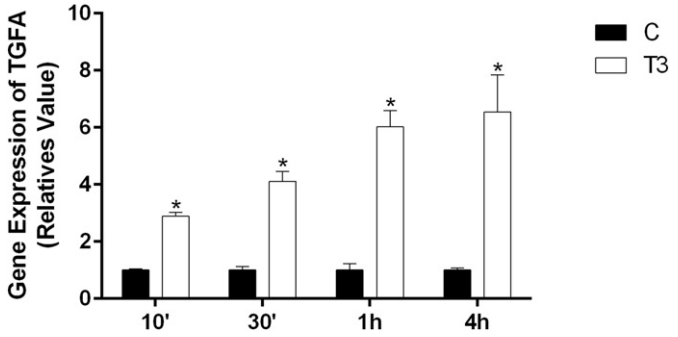

Fig. 2. Time-dependent effect of T_3 on *TGFA* mRNA at 10 min, 30 min, 1 h, and 4 h in MCF7 cells. T_3 = 10 nM T_3, C = no T_3. Data are expressed as mean ± standard deviation values and were analyzed using the Student's *t*-test. *$p < 0.05$ in the CxT3 comparison. n = 3 for each treatment.

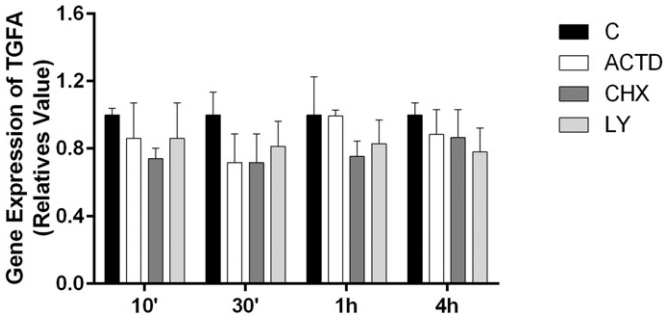

Fig. 4. Treated with inhibitors ACTD, CHX and LY were compared to control cells on the modulation *TGFA* mRNA at 10 min, 30 min, 1 h and 4 h in MCF7 cells. ANOVA, supplemented by the Tukey test, in the CxACTD; CxCHX and CxLY comparison. n = 3 for each treatment.

the group T_3 associate CHX compared to T_3, was significantly higher in T_3 treated cells than in cells treated with T_3 and CHX in *HIF1A* and *TGFA* expression at all time points studied.

Inhibition of P13K signaling affects T_3-mediated increases in *HIF1A* expression and *TGFA* expression. To determine if the activation of the PI3K pathway is required for the regulation of *HIF1A* and *TGFA* expression by T_3, breast cancer cells were incubated in the presence of T_3 and with or without the P13K inhibitor, LY294002 (LY). As shown in Figs. 9 and 10, there was significantly higher in T_3 treated cells than in cells treated with T3 and LY in *HIF1A* and *TGFA* expression at all time points studied.

4. Discussion and conclusions

Cellular hypoxia and metabolic stress occurs in many cancers. *HIF1A* is a central regulator of oxygen homeostasis. Previous studies indicated that *HIF1A* is overexpressed in many types of cancer, primarily BC, whereas mediate *HIF* proteins the adaptation to cellular hypoxia [14, 30–31]. High *HIF1A* expression in human breast cancer cells are related to breast cancer and the molecular changes that result from tumor vascularization. Like *HIF1A*, *TGFA* is also secreted by many tumors and possesses angiogenic properties [17]. The involvement of *TGFA* in the interaction between neoplastic epithelial cells and stromal cells has been intensively studied in recent years [17,32–33]. *TGFA* recognizes binds to the epithelial growth factor (EGF) receptor and, like EGF, is a potent inducer of mitosis during breast development [34].

Studies have described the antiproliferative effect of TH [5] on BC in ER positive cells and have demonstrated the ability of T_3 to increase cell proliferation in the presence of the TH receptor by inducing the expression of genes normally stimulated by E_2, including the progesterone receptor (*PgR*), *TGFB1*, and *TGFA* [35–36].

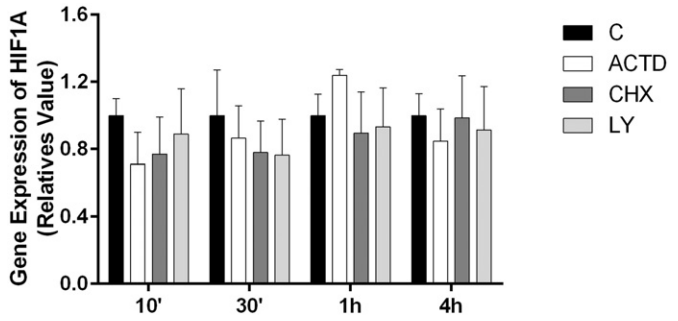

Fig. 3. Treated with inhibitors ACTD, CHX and LY were compared to control cells on the modulation *HIF1A* mRNA at 10 min, 30 min, 1 h and 4 h in MCF7 cells. ANOVA, supplemented by the Tukey test, in the CxACTD; CxCHX and CxLY comparison. n = 3 for each treatment.

Previous studies have indicated that T_3 promotes gene expression by binding to the nuclear TH receptor [37]. Recent studies also have suggested that cytosolic cascades are the underlying mechanism of T_3 action [38–39]. The present study confirms the action of T_3 on breast cancer cells (MCF7), showing an increase in *HIF1A* and *TGFA* expression as a result of treatment with T_3 for different times (Figs. 1 and 2). These results correlate with studies that demonstrated increased expression of a number of genes, such as amphiregulin (*AREG*) in MCF7 cells [40], leptin in adipocytes 3T3-L1 cells [41], and *HIF1A* in human fibroblast cells [39], upon treatment with T_3. Taken together, these results show that T_3 affects the transcription of *HIF1A* and *TGFA* at both short periods of time.

Our results show that, as compared to the results for control-treated cells, treatment with inhibitors alone had no effect on *HIF1A* and *TGFA* expression in breast cancer cell lines.

Our next step was to determine if T_3 acts to stabilize *HIF1A* and *TGFA* mRNA using ACTD. ACTD is a general transcriptional inhibitor with anticancer activity, and is approved for the treatment of sarcomas, Wilms' tumor trophoblasts, and germ cells [42–43]. As shown in Fig. 4, after 10 min of treatment with ACTD, *TGFA* expression decreases. After 30 min of treatment, there was no difference in *TGFA* expression, demonstrating stability of the mRNA. Our results are in agreement with the study of Volpato et al. [44] who tested whether the increase in *GLUT4* expression after treatment of 3T3-L1 cells with T_3 for 30 min is maintained in the presence of ACTD. The authors found that ACTD did not prevent the action of T_3 [44].

Extra-nuclear mechanisms can potentially influence gene expression. Although the onset of action is via an extra-nuclear mechanism that relies on alternative routes, the consequences of that action can include increased transcription [25]. This suggests that there might be an interrelationship between nuclear and extra-nuclear events of TH. With regard to extra-nuclear events, we can distinguish two classes of target genes by the actions of T_3 that occur using the PI3K pathway: 1) a class of genes induced directly by the action of this pathway in which protein

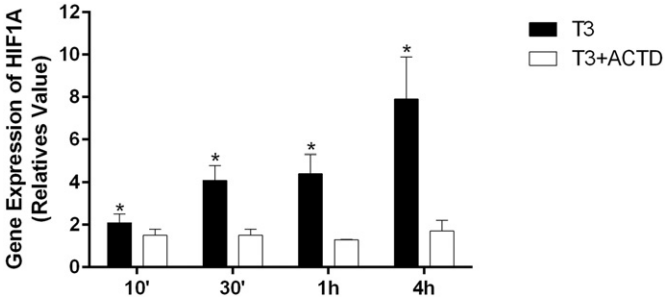

Fig. 5. Effect of T_3 and T_3 + ACTD on the modulation of *HIF1A* mRNA at 10 min, 30 min, 1 h and 4 h. ANOVA, supplemented by the Tukey test, *$p < 0.05$; n = 3 for each treatment.

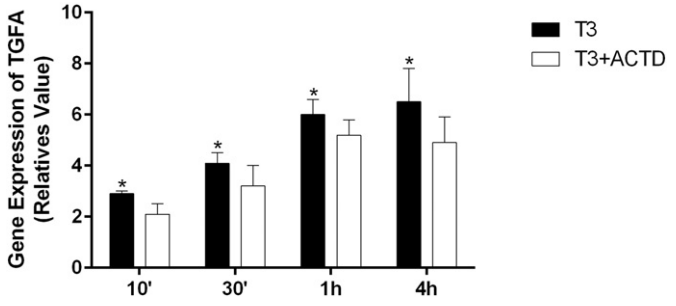

Fig. 6. Effect of T_3 and T_3 associate ACTD on the modulation of *TGFA* mRNA levels at 10 min, 30 min, 1 h and 4 h. ANOVA, supplemented by the Tukey test, *p < 0.05; n = 3 for each treatment.

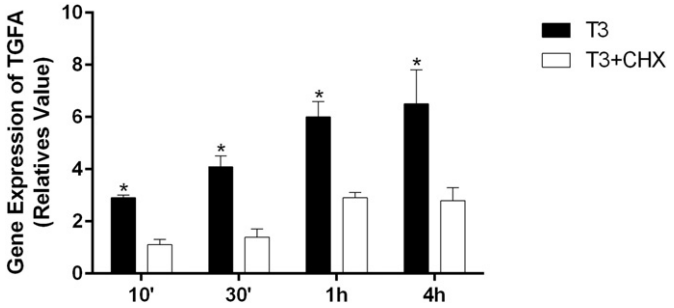

Fig. 8. Effect of T_3 and T_3 associate CHX on the modulation of *TGFA* mRNA levels at 10 min, 30 min, 1 h and 4 h. ANOVA, supplemented by the Tukey test, *p < 0.05; n = 3 for each treatment.

synthesis is not required "a priori" for the treatment to influence the action of the hormone, and 2) a class of genes whose expression is altered in the presence of the hormone, but is dependent on the synthesis of a specific protein previously known to be indirectly affected by extra-nuclear T_3 [39].

In order to determine whether T_3 directly or indirectly regulates *HIF1A* and *TGFA*, we used CHX to inhibit protein synthesis. We found that CHX reduces the action of T_3, leading to decreased levels of *HIF1A* and *TGFA*, at all treatment times, (Figs. 5 and 6, respectively), indicating that the effects of T_3 on these genes requires prior protein synthesis and that T_3 acts on these genes indirectly. Studies by Moeller et al. [39] with human fibroblasts show that the action of T_3 on *HIF1A* is direct because expression was not affected by the presence of CHX, suggesting there was no need for a precursor protein to stimulate the expression of *HIF1A.* However, the expression of *Glut1*, *PFKP*, and *MCT4* decreased in the presence of CHX, suggesting a precursor protein was needed in order for T_3 to indirectly regulate these genes [39].

The PI3K pathway functions in a wide variety of cellular processes, including intracellular pathways, cytoskeletal organization, cell growth and transformation, and the prevention of apoptosis [45–46]. The P13K pathway also affects differentiation of multiple cell lineages [47–48]. The concept of the effect of TH was diverse, including extra-nuclear actions of T_3 and T_4. T_3 activates the PI3K pathway, which leads to an increase in the expression of several genes, including *HIF1A* and the calcineurin inhibitor, *ZAKI-4α* [25,39].

Several studies using cell lines highlight changes in gene expression after treatment with T_3 that are dependent on the PI3K pathway [23–25, 39,41]. Among the genes stimulated by treatment with T_3 via the PI3K pathway, we focused on genes related to energy metabolism, including *HIF1A*, *GLUT1*, *PFKP*, and *MCT4*. Studies from our group recently suggested that leptin is modulated by T_3 in a PI3K-dependent manner in 3T3-L1 adipocytes [41]. In all of these studies, alteration in gene expression occurred through a non-nuclear mechanism of action of the thyroid hormone that was dependent on PI3K signaling, because all expression was inhibited by concomitant treatment with the PI3K – specific inhibitor, LY294002.

In this study, we used LY29400 to determine if activation of the PI3K pathway is required for T_3-mediated regulation of *HIF1A* and *TGFA* expression. As shown in our results, *HIF1A* and *TGFA* expression increased upon treatment with T_3, but not upon treatment with T_3 and LY294002 (Fig.7 and Fig.8). The use of PI3K inhibitors like LY demonstrates that this effect is dependent on a TH signaling cascade that is initiated by PI3K. This result suggests that the increase in transcriptional regulation of *HIF1A* and *TGFA* is possibly mediated by the PI3K pathway, which is activated by T_3. Moeller et al. [39] showed that T_3-dependent activation of *HIF1A* in a human fibroblast cell line is likely independent of PI3K activation. Cao et al. [38] reported that T_3 interacts with the p85A subunit of PI3K by binding to TRβ in the cytosol. This binding leads to activation of PI3K and the subsequent activation of a signaling cascade comprising sequential phosphorylation of protein kinase b (Akt), protein target of rapamycin in mammals (mTOR), and its substrate, ribosomal protein S6 kinase 70 (p70S6K). This signaling occurs very rapidly, with the mTOR phosphorylation occurring within 10 min after treatment with T_3 [49]. Other studies indicate that the extra-nuclear activity of TH occurs through the activation of PI3K, with the phosphorylation of proteins that are already present in the cytoplasm [50–52].

Studies demonstrating the mechanism by which TH can alter the expression of genes linked to breast cancer proliferation or to other cancer therapies offer alternative approaches for the treatment of this disease. Our study confirms that T_3 hormone acts through the activation of the extra-nuclear pathway for the expression of *HIF1A* and *TGFA* in the MCF7 cell lines, involving the PI3K signaling pathway.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

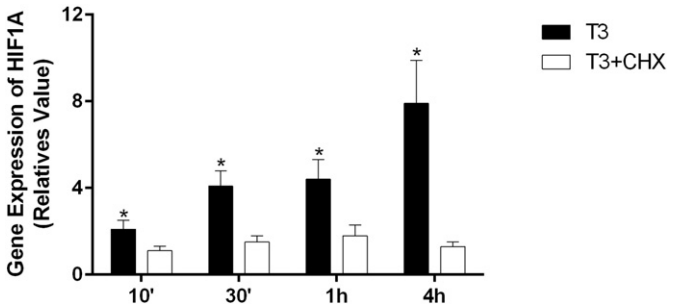

Fig. 7. Effect of T_3 and T_3 associate CHX on the modulation of *HIF1A* mRNA levels at 10 min, 30 min, 1 h and 4 h. ANOVA, supplemented by the Tukey test, *p < 0.05; n = 3 for each treatment.

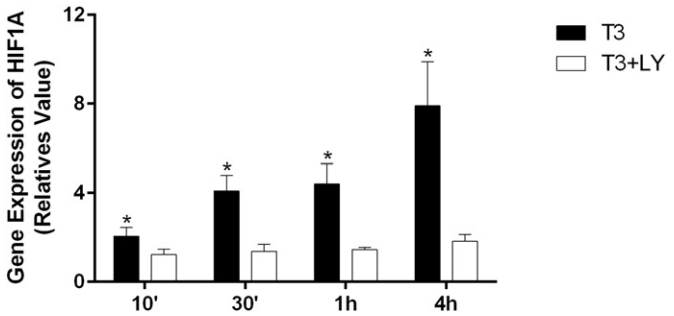

Fig. 9. Effect of T_3 and T_3 associate LY on the modulation of *HIF1A* mRNA levels at 10 min, 30 min, 1 h and 4 h. ANOVA, supplemented by the Tukey test, *p < 0.05; n = 3 for each treatment.

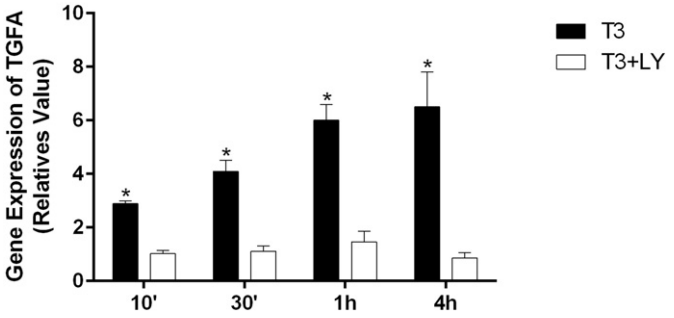

Fig. 10. Effect of T_3 and T_3 associate LY on the modulation of *TGFA* mRNA levels at 10 min, 30 min, 1 h and 4 h. ANOVA, supplemented by the Tukey test, $^*p < 0.05$; $n = 3$ for each treatment.

Funding

This research work was funded by CAPES and FAPESP (process No. 2012/04684-9 and 2014/15209-5).

Acknowledgments

We are grateful to Dra. Denise Fecchio for providing your cell culture lab, Professor Dr. Carlos Roberto Padovani by statistical analysis and Meire librarian for the correction of the references. We would also like to thank Editage for its high-quality translation of the manuscript into English.

References

[1] P. Porter, "Westernizing" women's risks? Breast cancer in lower-income countries, N. Engl. J. Med. 358 (2008) 213–216.
[2] A. Francavilla, B.I. Carr, A. Azzarone, L. Polimeno, Z. Wang, D.H. Van Thiel, et al., Hepatocyte proliferation and gene expression induced by triiodothyronine in vivo and in vitro, Hepatology 20 (1994) 1237–1241.
[3] M. Di Fulvio, A.H. Coleoni, C.G. Pellizas, A.M. Masini-Repiso, Tri-iodothyronine induces proliferation in cultured bovine thyroid cells: evidence for the involvement of epidermal growth factor-associated tyrosine kinase activity, J. Endocrinol. 166 (2000) 173–182.
[4] R. Malik, N. Mellor, C. Selden, H. Hodgson, Triiodothyronine enhances the regenerative capacity of the liver following partial hepatectomy, Hepatology 37 (2003) 79–86.
[5] H.Y. Tang, H.Y. Lin, S. Zhang, F.B. Davis, P.J. Davis, Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor, Endocrinology 145 (2004) 3265–3272.
[6] H.Y. Lin, H.Y. Tang, A. Shih, T. Keating, G. Cao, P.J. Davis, et al., Thyroid hormone is a MAPK-dependent growth factor for thyroid cancer cells and is anti-apoptotic, Steroids 72 (2007) 180–187.
[7] P.P. Saraiva, N.B. Figueiredo, C.R. Padovani, M.M. Bertani, C.R. Nogueira, Profile of thyroid hormones in breast cancer patients, Braz. J. Med. Biol. Res. 38 (2005) 761–765.
[8] S.J. Conde, R.A.M. Luvizotto, M.T. Sibio, C.R. Nogueira, Thyroid hormone profile in breast cancer patients in postmenopause, Arq Bras Endocrinol Metabol. 56 (2012) 238–243.
[9] S.J. Conde, R.A.M. Luvizotto, M.T. Síbio, C.R. Nogueira, Thyroid hormone status interferes with estrogen target gene expression in breast cancer samples in menopausal women, ISRN Endocrinol. 317398 (2014), http://dx.doi.org/10.1155/2014/317398 eCollection 2014.
[10] W.M. Linehan, M.M. Walther, B. Zbar, The genetic basis of cancer the kidney, J. Urol. 170 (2003) 2163–2172.
[11] K. Pavelic, R. Hrascan, S. Kapitanovic, N. Karapandza, Z. Vranes, M. Belicza, et al., Multiple genetic alterations in malignant metastatic insulinomas, J. Pathol. 177 (1995) 395–400.
[12] P. Vaupel, O. Thews, M. Hoeckel, Treatment resistance of solid tumors: role of hypoxia and anemia, Med. Oncol. 18 (2001) 243–259.
[13] A. Unruh, A. Ressel, H.G. Mohamed, R.S. Johnson, R. Nadrowitz, E. Richter, et al., The hypoxia inducible factor-1 alpha is negative factor for tumor therapy, Oncogene 22 (2003) 3213–3220.
[14] H. Zhong, A.M. DeMarzo, E. Laughner, M. Lim, D.A. Hilton, D. Zagzag, et al., Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases, Cancer Res. 59 (1999) 5830–5835.
[15] H. Marquardt, M.W. Hunkapiller, L.E. Hood, D.R. Twardzik, J.E. De Larco, J.R. Stephenson, et al., Transforming growth factors produced by retrovirus-transformed rodent fibroblasts and human melanoma cells: amino acid sequence homology with epidermal growth factor, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 4684–4688.
[16] A.R. Vieira, Association between the transforming growth factor alpha gene and nonsyndromic oral clefts: a HuGE review, Am. J. Epidemiol. 163 (2006) 790–810.
[17] H.M. Verheul, E.E. Voest, R.O. Schlingemann, Are tumours angiogenesis-dependent? J. Pathol. 202 (2004) 5–13.
[18] C.R. Nogueira, M.M. Brentani, Triiodothyronine mimics the effects of estrogen in breast cancer cell lines, J. Steroid Biochem. Mol. Biol. 59 (1996) 271–279.
[19] S.J. Conde, R.A. Luvizotto, M.T. Sibio, M.L. Katayama, M.M. Brentani, C.R. Nogueira, Tamoxifen inhibits transforming growth factor-alpha gene expression in human breast carcinoma samples treated with triiodothyronine, J. Endocrinol. Investig. 31 (2008) 1047–1051.
[20] P.J. Davis, F.B. Davis, W.D. Lawrence, Thyroid hormone regulation of membrane CA2(+)-ATPase activity, Endocr. Res. 15 (1989) 651–682.
[21] P.J. Davis, A. Shih, H.Y. Lin, L.J. Martino, F.B. Davis, Thyroxine promotes association of mitogen-activated protein kinase and nuclear thyroid hormone receptor (TR) and causes serine phosphorylation of TR, J. Biol. Chem. 275 (2000) 38032–38039.
[22] C. Wrutniak-Cabello, F. Casas, G. Cabello, Thyroid hormone action in mitochondria, J. Mol. Endocrinol. 26 (2001) 67–77.
[23] S.Y. Cheng, Thyroid hormone receptor mutations and disease: beyond thyroid hormone resistance, Trends Endocrinol. Metab. 16 (2005) 176–182.
[24] P.J. Davis, J.L. Leonard, F.B. Davis, Mechanisms of nongenomic actions of thyroid hormone, Front. Neuroendocrinol. 29 (2008) 211–218.
[25] L.C. Moeller, M. Broecker-Preuss, Transcriptional regulation by nonclassical action of thyroid hormone, Thyroid. Res. 4 (2011) Suppl 1:S6.
[26] S.C. Brooks, E.R. Locke, H.D. Soule, Estrogen receptor in a human cell line (MCF-7) from breast carcinoma, J. Biol. Chem. 248 (1973) 6251–6253.
[27] W.L. McGuire, G.C. Chamness, M.E. Costlow, R.E. Shepherd, Hormone dependence in breast cancer, Metabolism 23 (1974) 75–100.
[28] M. Razandi, A. Pedram, I. Merchenthaler, G.L. Greene, E.R. Levin, Plasma membrane estrogen receptors exist and functions as dimers, Mol. Endocrinol. 18 (2004) 2854–2865.
[29] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method, Methods 25 (2001) 402–408.
[30] P. Birner, M. Schind, A. Obermair, C. Plank, G. Breitenecker, G. Oberhuber, Overexpression of hypoxia-inducible factor 1α is a marker for an unfavorable prognosis in early-stage invasive cervical cancer, Cancer Res. 60 (2000) 4693-90.
[31] K.L. Talks, H. Turley, K.C. Gatter, P.H. Maxwell, C.W. Pugh, P.J. Ratcliffe, et al., The expression and distribution of the hypoxia-inducible factors HIF-1α and HIF-2α in normal human tissues, cancers, and tumor associated macrophages, Am. J. Pathol. 157 (2000) 411–421.
[32] T.D. Tlsty, P.W. Hein, Know thy neighbor: Stromal cells can contribute oncogenic signals, Curr. Opin. Genet. Dev. 11 (2001) 54–59.
[33] J.E. Fata, Z. Werb, M.J. Bissell, Regulation of mammary gland branching morphogenesis by the extracellular matrix and its remodeling enzymes, Breast Cancer Res. 6 (2004) 1–11.
[34] K. Plaut, Role of epidermal growth factor and transforming growth factors in mammary development and lactation, J. Dairy Sci. 76 (1993) 1526–1538.
[35] S.H. Cestari, N.B. Figueiredo, S.J. Conde, S. Clara, M.L. Katayama, C.R. Padovani, et al., Influence of estradiol and triiodothyronine on breast cancer cell lines proliferation and expression of estrogen and thyroid hormone receptors, Arq. Bras. Endocrinol. Metabol. 53 (2009) 859–864.
[36] S.J. Conde, R.D. Luvizotto, M.T. de Sibio, C.R. Nogueira, Human breast tumor slices as an alternative approach to cell lines to individualize research for each patient, Eur. J. Cancer Prev. 21 (2012) 333–335.
[37] E.D. Hoopfer, L. Huang, R.J. Denver, Basic transcription element binding protein is a thyroid hormone-regulated transcription factor expressed during metamorphosis in *Xenopus laevis*, Develop. Growth Differ. 44 (2002) 365–381.
[38] X. Cao, F. Kambe, L.C. Moeller, S. Refetoff, H. Seo, Thyroid hormone induces rapid activation of Akt/protein kinase B-mammalian target of rapamycin-p70S6K cascade through phosphatidylinositol 3-kinasein human fibroblasts, Mol. Endocrinol. 19 (2005) 102–112.
[39] L.C. Moeller, A.M. Dumitrescu, S. Refetoff, Cytosolic action of thyroid hormone leads to induction of hypoxia-inducible factor-1alpha and glycolytic genes, Mol. Endocrinol. 19 (2005) 2955–2963.
[40] N.B. Figueiredo, S.H. Cestari, S.J. Conde, R.A. Luvizotto, M.T. De Sibio, D. Perone, et al., Estrogen-responsive genes overlap with triiodothyronine-responsive genes in a breast 1 carcinoma cell line, Sci. World J. 969404 (2014), http://dx.doi.org/10.1155/2014/969404 eCollection 2014.
[41] M. de Oliveira, R.A.M. Luvizotto, R.M. Olimpio, M.T. De Sibio, S.J. Conde, C.B.R. Silva, et al., Triiodothyronine increases mRNA and protein leptin levels in short time in 3T3-L1 adipocytes by PI3K pathway activation, PLoS One 8 (2012), e74856.
[42] M.H. Tattersall, J.E. Sodergren, S.K. Dengupta, D.H. Trites, E.J. Modest, E. Frei, Pharmacokinetics of actinoymcin D in patients with malignant melanoma, Clin. Pharmacol. Ther. 17 (1975) 701–708.
[43] E.J. Estlin, G.J. Veal, Clinical and cellular pharmacology in relation to solid tumours of childhood, Cancer Treat. Rev. 29 (2003) 253–273.
[44] C.B. Volpato, E.M. Shiraishi, A.C. Latronico, M.T. Nunes, Thyroid hormone modulates the polyadenylation of growth hormone mRNA in rats, Endocr. J. 47 (2000) 169.
[45] A. Toker, L.C. Cantley, Signaling through the lipid products of phosphoinositide-3-OH kinase, Nature 387 (1997) 673–676.
[46] B. Vanhaesebroeck, S.J. Leevers, G. Panayotou, M.D. Waterfield, Phosphoinositide 3-kinases: a conserved family of signal transducers, Trends Biochem. Sci. 22 (1997) 267–272.
[47] D. Aubin, A. Gagnon, A. Sorisky, Phosphoinositide 3-kinase is required for human adipocyte differentiation in culture, Int. J. Obes. 29 (2005) 1006–1009.

[48] J. Fang, M. Ding, L. Yang, L.Z. Liu, B.H. Jiang, PI3K/PTEN/AKT signaling regulates prostate tumor angiogenesis, Cell. Signal. 19 (2007) 2487–2497.
[49] L.C. Moeller, X. Cao, A.M. Dumitrescu, H. Seo, S. Refetoff, Thyroid hormone mediated changes in gene expression can be initiated by cytosolic action of the thyroid hormone receptor beta through the phosphatidylinositol 3-kinase pathway, Nucl. Recept. Signal. 4 (2006), e020.
[50] J. Lei, C.N. Mariash, D.H. Ingbar, 3,3′,5-Triiodo-L-thyronine up-regulation of Na,K-ATPase activity and cell surface expression in alveolar epithelial cells is Src kinase- and phosphoinositide 3-kinase-dependent, J. Biol. Chem. 279 (2004) 47589–47600.
[51] P.J. Davis, F.B. Davis, H.Y. Lin, S.A. Mousa, M. Zhou, M.K. Luidens, Translational implications of nongenomic actions of thyroid hormone initiated at its integrin receptor, Am. J. Physiol. Endocrinol. Metab. 297 (2009) 1238-6.
[52] H.Y. Lin, M. Sun, H.Y. Tang, C. Lin, M.K. Luidens, S.A. Mousa, et al., L-Thyroxine vs. 3,5,3′-triiodo-L-thyronine and cell proliferation: activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase, Am. J. Phys. Cell Physiol. 296 (2009) 980–991.