RESEARCH

Thyroxine promotes lung cancer growth in an orthotopic mouse model

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Abstract

Thyroid hormones are important for physiology and homeostasis. In addition to nuclear thyroid hormone receptors, the plasma membrane protein integrin $\alpha_v\beta_3$ has been recognized as a receptor for both thyroxine (T₄) and triiodothyronine (T₃). Here, we studied whether thyroid hormone promotes growth of murine lung cancer via $\alpha_v\beta_3$ *in vivo*. Murine Lewis lung carcinoma cells (3LL), stably transfected with luciferase, were injected into mouse lungs. Tumor growth in untreated mice was compared to hypothyroid mice and hypothyroid mice treated with T₃ or T₄ with or without the $\alpha_v\beta_3$ inhibitor 3,5,3',5'-tetraiodothyroacetic acid (Tetrac). Tumor progression was determined by serial *in vivo* imaging of bioluminescence emitted from the tumor. Tumor weight was recorded at the end of the experiment. Neoangiogenesis was determined by immunohistochemistry for CD31. Tumor growth was reduced in hypothyroidism and increased by T₄ treatment. Strikingly, only T₄ but not T₃ treatment promoted tumor growth. This T₄ effect was abrogated by the $\alpha_v\beta_3$ inhibitor Tetrac. Tumor weight and neoangiogenesis were also significantly increased only in T₄-treated mice. The T₄ effect on tumor weight and neoangiogenesis was abolished by Tetrac. *In vitro*, T₄ did not stimulate 3LL cell proliferation or signaling pathway activation. We conclude that T₄ promotes lung cancer growth in this orthotopic mouse model. The tumor-promoting effect is mediated via the plasma membrane integrin $\alpha_v\beta_3$ and increased neoangiogenesis rather than direct stimulation of 3LL cells. These data suggest that such effects of levothyroxine may need to be considered in cancer patients on T₄ substitution.

Key Words

- ▶ integrin $\alpha_v\beta_3$
- ▶ thyroid hormone
- ▶ Lewis lung carcinoma
- ▶ Tetrac
- ▶ thyroxine
- ▶ tumor growth

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Introduction

The thyroid hormones (THs) thyroxine (T₄) and triiodothyronine (T₃) are mostly recognized for their roles in normal growth, development and metabolism. These effects are typically mediated by T₃ and the intracellular thyroid hormone receptors (TRs) α and β . THs are transported into target cells. There, T₃ activates the canonical pathway by binding to TRs, which act as a transcription factors and promote the expression of target

genes (type 1 TH signaling) (Flamant *et al.* 2017). Another mechanism of T₃ action is rapid activation of the PI3K pathway via TRs (TR-dependent signaling of TH without DNA binding, type 3 TH signaling) (Cao *et al.* 2009, Flamant *et al.* 2017, Hönes *et al.* 2017).

In addition to these intracellular TH actions, T₃ and T₄ bind to the plasma membrane protein integrin $\alpha_v\beta_3$, which mediates the signal across the membrane

(TR-independent TH signaling, type 4 TH signaling) (Bergh *et al.* 2005, Flamant *et al.* 2017). $\alpha_v\beta_3$ is expressed on malignant cells and in inflammatory sites, especially in activated macrophages, monocytes, neutrophils, natural killer cells, naive T lymphocytes and endothelial cells (Piali *et al.* 1995, Eliceiri & Cheresh 1998, Wilder 2002, Niu *et al.* 2007). This integrin $\alpha_v\beta_3$ contains two TH binding sites, S1 and S2, which activate different downstream pathways (Davis *et al.* 2011, Freindorf *et al.* 2012): S1 binds T₃ and activates the PI3K/AKT pathway, whereas S2 binds T₄ and, with lower affinity, T₃ and activates PI3K/AKT pathway and MAPK pathway (Lin *et al.* 2009). TH action at $\alpha_v\beta_3$ is inhibited by the deaminated and decarboxylated T₄ derivative Tetrac (3,3,5',5'-tetraiodothyroacetic acid) (Bergh *et al.* 2005). Thus, THs influence cells via several distinct pathways initiated by intracellular and membrane receptors.

Besides their role in development and physiology, THs also play a role in pathological conditions. THs can directly stimulate cancer cell proliferation *in vitro*, for example, lung adenocarcinoma (Kinoshita *et al.* 1991), breast carcinoma (Hall *et al.* 2008) and prostate cancer cells (Tsui *et al.* 2008). These TH effects are thought to be mediated by integrin $\alpha_v\beta_3$ and not the canonical TRs α and β , because the stimulatory effect can be inhibited by pretreatment with Tetrac (Lin *et al.* 2008, 2009, Meng *et al.* 2011). Furthermore, Tetrac suppressed the expression of EGFR, VEGF, multiple cyclins, catenins and cytokines in human tumor cells (Davis *et al.* 2014).

As thyroxine is one of the most widely prescribed drugs and cancer is the second leading cause of death, a considerable number of cancer patients will be on thyroxine substitution. It is therefore important to study the effects of T₃ and T₄ on cancer progress *in vivo*. Here, we demonstrate in an orthotopic mouse model that T₄ promotes growth of murine non-small-cell lung cancer (NSCLC) cells.

Materials and methods

Cell culture

Lewis lung carcinoma cells (3LL) were kindly provided by Raphael Nemenoff (University of Colorado, Denver, CO, USA) and stably transfected with pCCL-MNDU3-LUC plasmid containing the firefly luciferase gene as previously described (Supplementary Fig. 1, see section on [supplementary data](#) given at the end of this article) (Christoph *et al.* 2013). The cell line was validated before start of the experiments by short tandem repeats

(Microsynth Seqlab, Göttingen, Germany). Cells were cultured in RPMI 1640 medium (Hyclone Laboratories, Logan, UT, USA) supplemented with 10% fetal bovine serum in 5% CO₂ at 37°C to 65–70% confluence. For the proliferation assay, cells were grown in RPMI 1640 medium with 10% fetal bovine serum (FBS) depleted of TH by treatment of FCS with anion exchange resin (Aldrich Amberlite IRA-400 Cl, Sigma) and charcoal. Thyroid hormone concentration was below the limit of detection after treatment. T₄, T₃ and Tetrac were dissolved in DMSO, which was also used as control.

Proliferation assay

To study the influence of T₄ on growth of 3LL cells *in vitro*, 10⁵ cells were seeded on day one in triplicates in TH-depleted FBS. 3LL cells were cultured in the absence of T₄ or supplemented with 100nM, 1μM, 10μM and 100μM T₄. Medium was renewed every 2–3 days. On day 3, 5 and 8 all 3LL cells were harvested and counted in a Neubauer chamber. The experiment was repeated three times.

Western blot

0.4 × 10⁶ 3LL cells were seeded in a six-well plate. Twenty-four hours later, medium was changed to RPMI 1640 medium supplemented with 2.5% TH-depleted FBS for 16 hours. The following day cells were stimulated with different concentration of T₄ or Tetrac (dissolved in 0.01 M NaOH and 0.1% BSA) for 30 min as previously described (Bergh *et al.* 2005). As an internal positive control, 3LL cells were also stimulated with 50 ng/mL EGF (Sigma Aldrich) for 5 min. After the stimulation, whole-protein lysates were obtained using RIPA buffer (150 mM NaCl, 50 mM HCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 2 mM EDTA, 50 mM NaF) supplemented with complete protease and PhosSTOP inhibitor (Roche). Thirty micrograms of protein were separated on a 10% SDS-polyacrylamide gel, transferred onto PVDF membrane overnight at 4°C. PVDF membranes were blocked with 5% milk powder for 1 h. The following primary antibodies were used: pAKT (S473) (Cell Signaling, No. 4060), pERK1/2 (T202,Y204) (Cell Signaling, No.4370) and α -Tubulin (Merck Millipore, 05-829).

Gene expression analysis

Total RNA was isolated from 3LL cells (RNeasy Kit, Qiagen) and stored at –80 °C. Two micrograms of total RNA were reverse transcribed into cDNA with

SuperScript III (Invitrogen) and random hexamer primers. qRT-PCR was performed using Roche SYBR Green I Master Mix on a LightCycler LC480 (Roche). Primer sequences will be provided at request. Expression was normalized on that of 18S RNA. Ct values <35 were used for analysis and calculation of the fold change in gene expression by the efficiency-corrected method (Pfaffl 2001).

Animals and treatments

B6(Cg)-*Tyr^{c-2J}* (C57BL/6J) were obtained from The Jackson Laboratory. All animal studies were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV, Germany) and were performed according to the German Regulations for Laboratory Animal Science (GVSOLAS) and the European Health Law of the Federation of Laboratory Animal Science Associations (FELASA). Animals were housed under standard laboratory conditions (temperature 22±1°C and 12h light, 12h dark light cycle. Food and water were provided *ad libitum*. At the age of 7 weeks, male mice were randomized into treatment groups (*n*=6 per group). The euthyroid group received control diet (Harlan Laboratories, Roßdorf, Germany) and tap water. In all other groups, hypothyroidism was induced 21 days prior to injection of 3LL cells by feeding low iodine diet (Harlan Laboratories, Roßdorf, Germany) and 0.02% methimazole (Sigma Aldrich), 0.5% sodium perchlorate monohydrate (Sigma Aldrich) and 0.3% saccharin (Sigma Aldrich) in the drinking water. Hypothyroidism condition was maintained until the end of experiment.

Overall, 500,000 luciferase-tagged 3LL cells were resuspended in 40µL HBSS containing 10% Matrigel (Matrix Growth Factor Reduced, BD Biosciences, San Jose, CA, USA) for orthotopic injection (Poczobutt *et al.* 2013). Cell viability was determined by trypan blue dye staining, and cells were used if viability was >95%. Mice were deeply anesthetized with isoflurane inhalation. Local anesthesia was administered subcutaneously with 50µL 0.5% lidocaine (2.5mg/kg). The right chest wall was disinfected and a small skin incision (~3mm) was made along the right lateral axillary line at the level of the xiphoidal process. Cells were directly injected into the lung parenchyma of mice and the wound was closed with a liquid topical tissue adhesive surgibond (SMI AG Steinerberg, St. Virth, Belgium).

Starting 7 days after surgery, treatment group animals received intraperitoneal injections of T₄ 1µg/g body weight, T₃ 45ng/g body weight and/or

Tetrac 1µg/g body weight (Supplementary Fig. 2). T₃ and T₄ were injected i.p. 5times/week and Tetrac i.p. 3times/week. All reagents were purchased from Sigma Aldrich and dissolved in 0.01M NaOH, 0.1% BSA and diluted in phosphate buffered saline (PBS) 1:10. Solutions were prepared freshly every week and were kept protected from light at 4°C. Control groups received 0.01M NaOH and 0.1% BSA diluted in PBS 1:10 i.p. Mice were euthanized when predefined criteria according to GVSOLAS were reached, for example, gasping or severe body weight loss. After mice were euthanized, blood was collected from heart, incubated on ice for 30min and centrifuged for 20min with 17,000g at 4°C. Serum was collected and stored at -80°C until measurement. Before tissue collection, mice were perfused with heparinized saline through a needle placed in the right ventricle. Tumor tissue was removed, weighed and used for either flow cytometry or immunohistochemistry.

In vivo bioluminescence imaging

Mice were anesthetized with 1.5–2% isoflurane during imaging procedures. Bioluminescence was detected with an IVIS Lumina II imaging system (Perkin Elmer) 5min after intraperitoneal injection of 150µg/g D-luciferin (Perkin Elmer) dissolved in PBS. Mice were imaged with sequential 30, 60, 90 and 120s of exposure. Changes in bioluminescence intensity over time were measured and are presented as total flux values in photons per second. The total flux was calculated in regions of interest (ROI) on each mouse with Living Image 4.1 acquisition and analysis software (Perkin Elmer).

Flow cytometry

Lung tumor tissue was digested with collagenase type IV (150U/ml, Worthington Biochemical, Lakewood, NJ, USA) and DNase (10U/ml, Sigma Aldrich) for 1h at 37°C and minced through cell strainers to dissociate cells. Single cell suspensions of 1×10⁶ cells were incubated with anti-CD16/32 antibody for 10min, washed and then incubated 20min for extracellular receptors (anti-CD3, anti-CD4, anti-CD8, anti-CD11, anti-CD51, anti-CD61, anti-NK1.1, anti-Ly-6G/Ly-6C and anti-F4/80). After the incubation, cells were fixed and flow cytometry was performed on a BD FACS Aria III (BD Biosciences). For intranuclear staining of Foxp3 (Biolegend, San Diego, CA, USA) samples were additionally incubated for 30min. Data were analyzed with FlowJo V10 (FlowJo, Ashland, OR, USA).

Figure 1

T_4 treatment promotes tumor growth. (A) Exemplary illustration of bioluminescence intensity in one mouse per treatment group over time. (B) Tumor weight relative to euthyroid mice. a = $P < 0.0001$ for T_4 -treated mice vs hypothyroid, T_3 , Tetrac and T_3 + Tetrac-treated mice; b = $P < 0.01$ for T_4 treated mice vs euthyroid mice, $n = 6$. Mice were euthanized when predefined criteria were reached.

Immunohistochemistry

All tissue sections were deparaffinized and rehydrated through graded alcohol baths (70% – 96% – 100% ethanol, Sigma Aldrich). Following pretreatment in EDTA buffer (pH 9.0) at 95°C for 20 min, the primary anti-CD31 antibody (D8V9E, Cell Signaling) was incubated for 30 min at room temperature. Immunoreactivity was detected with a classical polymer system (HRP) (Zytomed, Berlin, Germany). Cell nuclei were stained with haematoxylin (1:8; Roth, Karlsruhe, Germany) for 5 min (Dako Autostainer, Dako) and analyzed on an Olympus BX51 Upright microscope with Cell Sens Dimension software.

Serum measurements

TSH concentration was measured as previously described (Pohlenz *et al.* 1999). Serum total T₄ (TT₄) and free T₃ (FT₃) concentrations were measured using commercial ELISA kits according to the manufacturer's instructions (DRG Instruments GmbH, Marburg, Germany; FT₃: EIA-2385; TT₄: EIA-1781) with a detection limit of 0.5 µg/dL for TT₄ and 0.05 pg/mL for FT₃.

Statistical analysis

Data were analyzed with one-way ANOVA (GraphPad Prism 6, GraphPad Software).

Results

Induction of hypothyroidism

Mice were rendered hypothyroid and treated with either T₄, T₃, Tetrac or a combination of TH and Tetrac (Supplementary Fig. 2). Modulation of thyroid state was confirmed by body weight measurements and measurement of serum concentration of TH in final serum. Hypothyroidism led to a body weight plateau in hypothyroid mice, whereas euthyroid mice gained weight (Supplementary Fig. 3) (Engels *et al.* 2016). Induction of hypothyroidism was successful with significantly reduced TH concentration and elevated TSH (TSH >8000 vs 51 mU/L; FT₃ 1.4 vs 2.6 pg/mL and TT₄ 0.34 vs 2.7 µg/dL). Treatment of hypothyroid mice with T₃ or T₄ resulted in increased FT₃ and increased TT₄ concentration, respectively (Supplementary Fig. 4). The ELISA could not distinguish between T₄ and its derivative Tetrac and these groups are not displayed.

Figure 2

T₄ does not stimulate Lewis lung carcinoma cell proliferation *in vitro*. Lewis lung cancer cells were grown in thyroid hormone-depleted medium or in the presence of 0.1, 1, 10 and 100 µM T₄. None of the T₄ doses increased cell proliferation.

T₄ treatment promotes tumor growth, which is abolished by Tetrac

Tumor progression was determined by serial *in vivo* bioluminescence imaging of light emission from the luciferase-expressing 3LL cells. Compared to euthyroid mice, hypothyroidism in mice led to reduced bioluminescence signal intensity (Fig. 1A and Supplementary Table 1). Signal intensity was increased in T₄-treated mice, but not in T₃-treated mice (Fig. 1A). Bioluminescence doubling time as a measure of tumor progress was shortest in T₄ treated mice (Supplementary Table 1). This effect was abrogated by Tetrac. Similarly, lung tumor weight was significantly increased only in T₄-treated mice, but not in T₃-treated mice (Fig. 1B, Supplementary Table 2). This T₄-mediated effect was abolished by Tetrac treatment, resulting in diminished bioluminescence signal intensity and smaller tumor weight of T₄/Tetrac-treated animals compared to T₄-treated mice. These data demonstrate

Figure 3

Signaling pathway activation. 3LL cells were treated with T₄ or Tetrac. Compared to vehicle control, T₄ did not increase Akt or Erk phosphorylation.

Figure 4

Increased CD31+ staining after T₄ treatment. Immunohistochemistry staining of CD31+ from formalin-fixed paraffin-embedded tumor of (A) euthyroid mice, (B) T₃-treated mice, (C) T₄-treated mice and (D) T₄ + Tetrac-treated mice. (E) T₄-treated mice showed a significant increase of CD31+ vessels in thyroxine-treated mice compared to all other groups indicating neoangiogenesis. This effect was abolished in the presence of Tetrac. Bars represent 50 μ M; a = $P < 0.0001$ for T₄-treated mice vs each other treatment group.

that T₄ and not T₃ promotes tumor growth. Overall survival, however, was not different between the treatment groups (Supplementary Fig. 5).

T₄ does not increase 3LL cell proliferation or signaling pathway activation

To determine if T₄ exerts a direct effect on proliferation of Lewis lung cells, we grew 3LL cells in T₄-depleted medium and added T₄ in doses from 0.1 to 100 μ M. The cells showed the sigmoid proliferation curve. There was no difference in proliferation rate with any of the T₄ doses (Fig. 2), indicating that T₄ does not directly stimulate 3LL cell growth. Furthermore, neither Akt nor Erk phosphorylation was increased by T₄ treatment (Fig. 3). Together, these results suggest that the tumor-promoting effect may not originate in the 3LL cells themselves but may rather be due to increased neoangiogenesis.

T₄ promotes tumor neoangiogenesis

As it was shown previously that T₄ increases neoangiogenesis in chick chorioallantoic membrane assays, we determined the extent of neoangiogenesis in the tumors by staining for platelet endothelial cell adhesion molecule,

CD31 (Mousa *et al.* 2008). We observed significantly increased vascularization in the tumors of T₄-treated mice, which again was abrogated by co-treatment with Tetrac (Fig. 4A, B, C, D and E). T₃ treatment alone had no effect. Of note, Tetrac alone did not reduce vascularization compared to tumors from untreated mice.

Thyroid hormone does not influence AlphaV or Beta3 expression

Next, we tested gene induction by TH in 3LL cells. *AlphaV* and *Beta3* mRNA expression was not changed by T₃ or T₄ (Fig. 5A and B), neither with short (6 h) nor long (24 h) term treatment. However, expression of known TH-responsive genes *Klf9* and *Vegf* was induced in 3LL cells by T₃ and T₄ (Fig. 5C and D). Induction of both genes was not affected by pretreatment with the $\alpha_v\beta_3$ inhibitor Tetrac, which suggests that their induction does not depend on $\alpha_v\beta_3$ but is rather mediated by the canonical TRs.

Treatment with thyroid hormone does not alter the composition of infiltrating immune cells

To investigate if the composition of infiltrating immune cells, which also express $\alpha_v\beta_3$, is altered in tumors we

characterized the tumor infiltrate by flow cytometry. No significant differences were found between tumors from euthyroid mice or any of the treatment groups for the composition of infiltrating T-regs (CD4+, Foxp3+), cytotoxic T-cells (CD3+, CD8+), tumor-associated macrophages (F4/80+, Gr-1+), macrophages (F4/80+), natural killer cells (NK 1.1+), T-helper cells (CD3+, CD4+) or dendritic cells (CD11c+) (Table 1).

Discussion

TH can bind to the plasma membrane receptor integrin $\alpha_v\beta_3$, which mediates signaling across the membrane (Bergh *et al.* 2005). $\alpha_v\beta_3$ is expressed in invasive tumors, endothelial cells and inflammatory sites, especially in

activated macrophages, monocytes, neutrophils, natural killer cells and naive T lymphocytes (Piali *et al.* 1995, Eliceiri & Cheresch 1998, Wilder 2002). Here, we show that growth of orthotopically implanted Lewis lung cell carcinoma was promoted by T₄.

Importantly, it was only T₄ and not T₃ treatment that showed a growth-stimulating effect on the 3LL tumors. This is supported by the fact that, due to conversion, the T₃ concentration was similar in T₄ and T₃-treated mice and, thus, T₃ cannot be responsible for the observed difference in tumor growth. The tumor-promoting effect of T₄ on 3LL cells implanted in murine lungs was abrogated by co-treatment with the $\alpha_v\beta_3$ inhibitor Tetrac, which demonstrates that $\alpha_v\beta_3$ mediates this effect and not the nuclear TRs. This effect could either be mediated by a direct effect of T₄ on 3LL cells,

Figure 5

Expression of *AlphaV* (A) and *Beta3* (B) in LLC determined after incubation with increasing doses of T₃ (1, 10 and 100 nM) and T₄ (1, 10, 100 nM and 1 μM) for 6 h and 24 h (mean ± s.e.m.). An increased expression of thyroid hormone responsive gene *Klf9* (Krüppel-like factor 9) (C) and *Vegf* (vascular endothelial growth factor) (D) was detected after treatment with TH (T₃, 100 nM and T₄, 1 μM) or Tetrac (100 nM) for 6 h and 24 h (mean ± s.e.m.; one-way ANOVA with Tukey's *post hoc* test; ns = not significant).

Table 1 Flow cytometry analysis revealed no treatment-induced change in the subgroups of infiltrating immune cells.

	CD4+, Foxp3+	S.E.M.	CD3+, CD8+	S.E.M.	F4/80+, Gr-1+	S.E.M.	F4/80+	S.E.M.	NK1.1+	S.E.M.	CD3+, CD4+	S.E.M.	CD11c+	S.E.M.
Euthyroid	1.00	±0.04	1.00	±0.16	1.00	±0.13	1.00	±0.13	1.00	±0.17	1.00	±0.16	1.00	±0.14
Hypothyroid	0.85	±0.15	1.29	±0.19	1.02	±0.13	0.81	±0.13	1.00	±0.15	1.19	±0.12	1.23	±0.14
Hypo + T3	1.06	±0.17	1.31	±0.20	0.75	±0.12	0.82	±0.12	1.13	±0.19	1.15	±0.19	0.76	±0.17
Hypo + T4	0.86	±0.13	1.07	±0.18	0.88	±0.16	0.87	±0.16	1.13	±0.07	1.28	±0.12	1.09	±0.1
Hypo + T3 + Tetra	0.94	±0.12	0.84	±0.15	0.96	±0.08	0.86	±0.08	1.18	±0.14	1.22	±0.15	1.13	±0.19
Hypo + T4 + Tetrac	0.90	±0.10	0.84	±0.19	0.96	±0.14	0.81	±0.14	1.18	±0.2	1.17	±0.18	0.78	±0.15
Hypo + Tetrac	0.94	±0.17	0.81	±0.19	0.91	±0.25	0.83	±0.25	0.82	±0.16	1.08	±0.14	0.81	±0.17

increasing their proliferation, or by an indirect effect, for example, increased neovascularization of the tumors, or a combination of both. However, treatment of 3LL with T₄ did not increase cell proliferation or signaling pathway activation. Tumors depend on an increased blood supply for rapid growth and $\alpha_v\beta_3$ acts proangiogenically. $\alpha_v\beta_3$ is expressed on endothelial cells and interacts with proangiogenic agents like FGF-2 (Sahni & Francis 2004) and VEGFR2 (Masson-Gadais *et al.* 2003). Indeed, we found a significant increase of new vessels in T₄-treated mice, which was completely abrogated by pretreatment with Tetrac. Together, these results suggest that T₄/ $\alpha_v\beta_3$ stimulate tumor growth via increased neovascularization and not by direct 3LL cell stimulation.

Another set of T₄ target cells within the tumors might be infiltrating immune cells that also express $\alpha_v\beta_3$ such as macrophages, natural killer cells and T lymphocytes. However, we did not detect any treatment-dependent changes in the immune cell profile.

Our findings correspond to clinical observations. Patients with NSCLC treated with the PD-1 blockers nivolumab and pembrolizumab frequently experience thyroid dysfunction, predominantly hypothyroidism which may be preceded by a short episode of hyperthyroidism due to destructive thyroiditis. According to two recent reports, progression-free and overall survival was significantly longer in hypothyroid patients with NSCLC and PD-1 blockade compared to euthyroid patients (Kim *et al.* 2017, Osorio *et al.* 2017). While it cannot be ruled out that hypothyroidism is only a surrogate marker of antitumor treatment efficacy due to higher drug concentrations or different susceptibility of the immune system in a subset of patients, our results suggest that thyroid hormone itself can indeed influence NSCLC progression by enhancing neovascularization via $\alpha_v\beta_3$.

Because T₄ effects similar to those observed in this NSCLC mouse model have been previously reported in non-orthotopic approaches with 3LL, mammary adenocarcinoma (C3HBA) and fibrosarcoma (T241) cells (Shoemaker *et al.* 1976, Kumar *et al.* 1979, Kinoshita *et al.* 1991), promotion of tumor growth by T₄ appears to be a more general phenomenon with implications for cancer patients. Hypothyroidism may develop during tyrosine kinase inhibitor treatment (Illouz *et al.* 2014, Schweizer *et al.* 2014) or due to autoimmune thyroiditis as a frequent side effect of checkpoint inhibitor treatment in advanced malignancies including lung cancer (Illouz *et al.* 2014). In these situations, substitution with thyroxine is considered. But there is reasonable concern that

substituting these cancer patients with T₄ could be harmful. Our results support the idea that in cancer patients, thyroxine may have more consequences than only normalizing TSH, and it seems necessary to determine which cancer entities are sensitive to TH. Future cancer treatment studies, especially with substances that can induce hypothyroidism, should be designed in a way that allows for an analysis of thyroid function status and its contribution on treatment outcome.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/ERC-18-0353>.

Declaration of interest

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

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Author contribution statement

SL and LCM designed the studies; SL, SC and GSH conducted experiments; SL, SC, ST, GSH, KWS, DF, LCM analyzed data; SL, SC and LCM wrote, and all authors contributed to the manuscript.

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