Chemosphere 308 (2022) 136354

Contents lists available at ScienceDirect

Chemosphere

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

The influence of sunitinib and sorafenib, two tyrosine kinase inhibitors, on development and thyroid system in zebrafish larvae

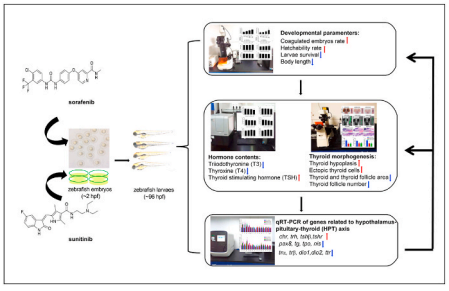
Gang Wei [a,b,c,1,*], Cao-xu Zhang [a,1], Yu Jing [a], Xia Chen [d], Huai-dong Song [a], Liu Yang [a,**]

[a] The Core Laboratory in Medical Center of Clinical Research, Department of Molecular Diagnostics & Endocrinology, Shanghai Ninth People's Hospital, State Key Laboratory of Medical Genomics, Shanghai Jiao Tong University School of Medicine, Shanghai, 200011, China
[b] Key Laboratory of Pollution Exposure and Health Intervention of Zhejiang Province, Hangzhou, 310015, China
[c] Beijing Key Laboratory of Diabetes Research and Care, Department of Endocrinology, Beijing Diabetes Institute, Beijing Tongren Hospital, Capital Medical University, Beijing, 100730, China
[d] Department of Endocrinology, Shanghai Gongli Hospital, Shanghai, 200135, China

HIGHLIGHTS

- Sunitinib and sorafenib exerted remarkably toxicities effects on the survival and development of zebrafish embryos/larvae.
- Sunitinib and sorafenib significantly decreased T3 and T4 content, but increased TSH content.
- Sunitinib and sorafenib displayed a strikingly abnormal thyroid gland organogenesis in zebrafish larvae.
- Sunitinib and sorafenib altered genes in HPT axis and promoted genes regulating THs levels in a negative feedback mechanism.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Kamala-Kannan Seralathan

Keywords:
Anti-tumor drug
Danio rerio
Thyroid disruption
Hypothalamus-pituitary-thyroid axis

ABSTRACT

Recently, the potential toxic effects of various pharmaceuticals on the thyroid endocrine system have raised considerable concerns. In this study, we evaluated the adverse effects of sorafenib and sunitinib, two widely used anti-tumor drugs, on the developmental toxicities and thyroid endocrine disruption by using zebrafish (*Danio rerio*) model. Zebrafish embryos/larvae were exposed to different contentions (0, 10, 50 and 100 nM) of sorafenib and sunitinib for 96 hpf. The results revealed that waterborne exposure to sorafenib and sunitinib exhibited remarkable toxic effects on the survival and development in zebrafish embryos/larvae, which was accompanied by obvious disturbances of thyroid endocrine system (e.g., decreased T3 and T4 content, increased TSH content) and genes' transcription changes within the hypothalamus-pituitary-thyroid (HPT) axis. In addition, we verified a strikingly abnormal thyroid gland organogenesis in zebrafish larvae in response to sorafenib and sunitinib, by assessing the development of thyroid follicles using the WISH staining of *tg*, the Tg (*tg*:GFP) zebrafish transgenic line, and histopathological analysis. Taken together, our results indicated sorafenib and sunitinib exposure could induce obvious developmental toxicities and thyroid function disruption in zebrafish embryos/larvae, which

* Corresponding author. The Core Laboratory in Medical Center of Clinical Research, Department of Molecular Diagnostics & Endocrinology, Shanghai Ninth People's Hospital, State Key Laboratory of Medical Genomics, Shanghai Jiao Tong University School of Medicine, Shanghai, 200011, China.
** Corresponding author.
E-mail addresses: gangwei_2013@163.com (G. Wei), 18210655830@sina.cn (L. Yang).
[1] These authors contributed equally.

https://doi.org/10.1016/j.chemosphere.2022.136354
Received 26 May 2022; Received in revised form 12 August 2022; Accepted 2 September 2022
Available online 7 September 2022

might involve a regulatory mechanism, at least in part, by destroying the thyroid follicle structure, and by disturbing the balance of the HPT axis.

1. Introduction

Pharmaceuticals are a unique set of emerging environmental pollutants and their special characteristics (e.g, their fate and behavior) cannot be simulated by other organic chemical contaminants (Fatta--Kassinos et al., 2011). But, so far, the importance or potential presence of emissions from households and hospitals is still unknown. After administration, many medications (e.g., anti-tumor drugs, antibiotics, hormones and lipid regulators, etc.) are not (bio)degraded and excreted by patients into wastewater (Hu et al., 2008; de Jongh et al., 2012; Oosterhuis et al., 2013; Hussain et al., 2021; Kokilaramani et al., 2021). Besides, often expired or unused drugs are disposed of directly by flushing down the drains/toilet (Verlicchi et al., 2012). These pharmaceuticals could enter aquatic environment and threaten the health of humans and environmental organisms. Importantly, the detection of various pharmaceuticals within aquatic environment has recently raised considerable concerns, especially for their possible toxic effects on the thyroid axis of aquatic organisms (Olalla et al., 2018).

Currently, tyrosine kinase inhibitors (TKIs) are characterized as an important type of molecular multi-targeted agents, which have been registered for the treatment of cancers with considerable efficacy. These new drugs are generally considered less toxic than classical cytotoxic chemotherapy, but still elicit adverse side effects including hypertension and fatigue (Fallahi et al., 2014). In particular, thyroid dysfunction such as hypothyroidism is the most frequent side effect induced by TKIs (Torino et al., 2009; Basolo et al., 2022). Although the molecular mechanisms underlying this correlation are not yet clear, several speculative theories have been proposed, including VEGF inhibition, inhibition of radioiodine thyroid uptake, and autoimmune mechanism (Baffert et al., 2006; Mannavola et al., 2007; Wong et al., 2007; Abdel-Rahman and Fouad, 2014). It is well known that hypothalamic-pituitary-thyroid (HPT) axis controls thyroid gland function via regulating the synthesis and metabolism of thyroid hormones (THs) (Kang et al., 2017; Yue et al., 2017). During hypothyroidism, the HPT axis, in turn, is regulated by a negative feedback loop to maintain THs homeostasis. E.g. the decreased THs concentrations in serum stimulate an increase release of thyrotropin-releasing hormone (TRH, from hypothalamus) and thyrotropin (TSH, from pituitary) to restore the levels of THs (Przybyla et al., 2018). However, it remains elusive whether the TKIs-triggered toxic effects on thyroid gland are linked to disordered HPT axis activity. In this regard, sunitinib (Sutent) and sorafenib (Nexavar), as approved for the first-line and second-line therapy, respectively, are widely used in clinic for the treatment of metastatic renal cell carcinoma (mRCC) in China (Fallahi et al., 2014; Wei et al., 2021). Whereas, several studies had reported that thyroid dysfunction was frequently observed in mRCC patients receiving sunitinib or sorafenib, as evidenced by decreased serum T3 and T4 levels, increased serum TSH levels, as well as reduced thyroid size, etc (Clemons et al., 2012; Feldt et al., 2012; Kitajima et al., 2012; Wu and Huang, 2020). The incidence of hypothyroidism induced by sunitinib was slightly higher than sorafenib as evaluated in different studies, ranging from 36 to 85% and 20–36%, respectively (Clemons et al., 2012; Fallahi et al., 2014). Therefore, the potential biological mechanisms by which sunitinib and sorafenib induce hypothyroidism require further experimental researches.

In the endocrine system, the thyroid gland is the one of the largest endocrine glands in the human body (Zoeller et al., 2007). Thyroid gland plays a critical role in the early development stage of organisms by the regulation of biological effects of THs (Zoeller et al., 2007). Consequently, the potential toxic effects of environmental pollutants including the active compounds of pharmaceuticals on thyroid disruption has received increasing attention in the field of biomedical applications in recent years. In zebrafish, the thyroid endocrine system shows conserved mechanisms as compared with humans (Noyes et al., 2016). Thyroid hormone (TH) for T4 production in thyroid follicle begins about 72 h post-fertilization (hpf) (Elsalini and Rohr, 2003). Moreover, numerous reports have proved that the toxicity profiles of various chemical compounds and drugs in zebrafish are strikingly consistent with mammalian models (Aspatwar et al., 2020). For these reasons, zebrafish is highly suitable for studying thyroid gland disruption, often serving as the intermediated step between *in vitro* (cell-based testing) and *in vivo* (conventional animal evaluation) assessment of drug toxicity (Malhab et al., 2016; Reinwald et al., 2021).

Previous studies had demonstrated that exposure of various pharmaceuticals can cause the thyroid axis imbalance and induced apparent developmental toxicity in zebrafish (Williams et al., 2005; Vigone et al., 2015; Leonard et al., 2016; Skarha et al., 2019). However, limited information is currently available on sorafenib - or sunitinib - induced alteration in the HPT axis of zebrafish and whether this is associated with the underlying mechanism involved in thyroid disorders and developmental abnormalities is still far from being elucidated. In this study, we applied the zebrafish embryos/larvae model to evaluate the developmental toxicities of sunitinib and sorafenib, two widely used TKIs in mRCC patients, and further explore the underlying mechanisms by evaluating the thyroid function disturbances (e.g., its THs levels, thyroid morphogenesis, as well as HPT axis-related genes' expression) due to sunitinib and sorafenib exposure. The findings achieved in the present study would provide valuable and novel information of sunitinib and (or) sorafenib about the hormone-, molecular- and organism-effects, particularly with reference to the thyroid disruption.

2. Materials and methods

2.1. Chemicals and reagents

Sorafenib (Cat. # HY-10255A) and sunitinib (Cat. # HY-10201) were purchased from MedChemExpress (Monmouth Junction, NJ). The chemical structure and CAS number of sorafenib and sunitinib, respectively, are shown in Fig. S1. Sorafenib and sunitinib (each 10 mM) were dissolved in DMSO (purity of 99.9%, Sigma) as stock solutions and stored at −20 °C. During the experimental period, the test chemicals were diluted into corresponding concentrations.

2.2. Maintenance and toxicity test of zebrafish embryo/larvae

Parental zebrafish of wild type AB strain and Transgenic Tg (*tg*:GFPP) line were obtained from the Zebrafish Resource Center of China, which were maintained according to our previous descriptions (Yang et al., 2021). The methods were conducted following the approved guidelines of Shanghai Jiao Tong University School of Medicine. Briefly, acclimation of adult zebrafish (male and female) were maintained in a recirculating aquarium tank, at a constant temperature (28 ± 1% °C) and with a photoperiod 14 h light:10 h dark. According to the experimental purposes, we designed and performed a series of experiments, separately; fertilized embryos at ~2 hpf (blastula stage) were randomly assigned to several 24-well plates (NEST Biotechnology, China) with 1 mL of different concentrations (0, 10, 50 and 100 nM) of sorafenib and sunitinib exposure solution in each well. The rang of experimental concentrations for sorafenib and sunitinib were chosen based on clinically observed serum concentrations and preliminary range-finding experiments (Molhoek et al., 2009; Walraven et al., 2018). As the maximum and the trough concentrations of sorafenib in plasma were 8.5

and 6.4 μM, and the tissue levels of sunitinib were predicted to be in the 1–3 μM. Therefore, in this study we chose a conservative concentration of 100 nM or less. For the development toxicity analysis, twenty-four embryos per concentration, with three replicates, were exposed to sorafenib and sunitinib, and half of the exposure solutions with the indicated chemical of the same concentration were renewed daily. The developmental parameters (e.g., larval survival rates, time to hatch, etc.) of zebrafish were recorded each day. After 96 h exposure, the surviving zebrafish larvae in each groups were anesthetized with 0.016% tricaine (Sigma) solution, collected in clean 1.5 mL EP tubes, and body lengths and weights were measured followed by hormones measurement and gene expression.

2.3. Quantification of sorafenib and sunitinib in exposure solutions

Quantification of sorafenib and sunitinib after 0, 24, and 48 hpf exposure were performed on an Agilent1260 Infinity HPLC system (Agilent Technologies, USA). Chromatographic separation was obtained on a Zorbax sb-c18 (150 × 4.6 mm, 5 μm, Agilent) analytical column. The mobile phase was consisted of acetonitrile (MeCN) - pure water (H2O) and the detection wavelength was 203 nm. Samples with a volume of 70 μL were injected into the column at 25 °C. The gradient elution program was set as follows: 0.0–10.0 min, 5.0–95.0% MeCN and at a flow rate of 0.5 mL min^{-1}. The internal standard approach was used for quantification analysis and a signal-to noise ratio of 10 was defined as the limits of quantification (Yang et al., 2020).

2.4. Hormone isolation and measurement

Total T3 and total T4, and TSH were extracted and analyzed by using commercial kit for fish (CAMILO, Nanjing, China) according to the manufacture's protocols. In brief, 600 larvae (200 larvae with triplicates) for each treatment and control groups were collected at 96 hpf, weighted, and homogenized in 400 μl PBS buffer (Procell Life Science & Technology Co., Ltd.) in a clean 1.5 mL Eppendorf (EP) tube. After the larvae were sonicated on ice, vortexed and centrifuged, the supernatants (approximately 350 μl each group, with triplicates) were collected and transferred into another clean 1.5 mL EP tube, respectively. The indicated hormones were measured by using a SynergyH1 microplate reader (Bio Tek, USA) and the contents of THs were normalized to body weight of zebrafish larvae.

2.5. RNA extraction and RT-qPCR analysis

Total RNA was isolated and collected from sixty larvae in each group (with triplicates) by using Fast Pure Cell/Tiss Total RNA isolation kit V2 (Vazyme Biotech, Nanjing, China). Then, the total RNAs (1000 ng) in each sample were subjected to reverse transcriptions with the Vazyme HiScript II Q RT SuperMix kit and RT-qPCR analysis were conducted (with duplicate) using Vazyme AceQ qPCR SYBR Green Master Mix on a QuantStudio 6 Flex Real-Time PCR System (ABI). Glyceraldehyde phosphate dehydrogenase (Gapdh) was performed as a control and relative mRNA expression was normalized to Gapdh using $2^{-\Delta\Delta Ct}$ method (Wei et al., 2020). The abbreviation and full name of all the investigated genes in zebrafish are listed in Supplementary Table 1 (Table S1). The sequences of gene-specific primers are obtained as previously described (Fu et al., 2020; Jin et al., 2021), which are listed in Table S2.

2.6. Whole-mount in situ hybridization (WISH)

The WISH for Zebrafish was conducted as our previous study (Yang et al., 2021). Briefly, the embryos/larvae in each groups were fixed in 4% PFA (Elabscience) at 4 °C overnight and immersed in methanol at −20 °C. The dehydrated larvae were washed with PBST and subjected to Proteinase K (final concentration: 5 mg mL^{-1}) treatment of at room temperature for 30 min. Then the larvae were re-fixed for 1 h in 4% PFA at 4 °C and were then washed with PBST three times. Anti-sense RNA probes for *tg* and *tshβ* were transcribed with the plasmid containing zebrafish thyroglobulin and thyroid-stimulating hormone subunit beta a by Digoxigenin (DIG) -RNA labeling kit (Roche) and was purified with Quick Spin Columns (Roche). NBT/BCIP chromogenic substrates (Wako) were used for the colorimetric reaction. Processed larvae were mounted in 100% glycerol (Cat. # abs47048166, Absin Bioscience, Inc., Shanghai, China) on glass slides (Cat. # abs7049, Absin) and images were taken using Nikon SMZ25 microscope. The integrated optical density (IOD) values of WISH were measured using Image J software.

2.7. Confocal microscopy

Live transgenic Tg (*tg*:GFP) larvae were anesthetized with 0.016% tricaine (Sigma) following sorafenib and sunitinib exposure, and were then mounted on glass-bottom dishes and embedded with 1% low-melting point agarose. The photographic images were taken using a confocal laser microscope (Nikon A, Nikon). The fluorescence images were conducted and analyzed by application of Imaris software and Image J software.

2.8. Histopathological examination

The zebrafish larvae were fixed in 4% paraformaldehyde after exposure to sorafenib and sunitinib for 96 hpf. Then the larvae were embedded in paraffin, serially sectioned (5 μm-thickness) along the sagittal plane with a microtome (Thermo Scientific, MICRO HM325, USA) in the lateral-to-medial direction. Every nine section (three sections on a single slide) was stained with hematoxylin and eosin (H&E, Cat.# G1080, Solabio, Beijing, China) followed by the morphological examination using Olympus camera under a microscope. Measurements and evaluation of thyroid follicles (number and area) were performed by two independent technicians who were blinded to the treatment conditions.

2.9. Statistical analysis

Data are expressed as mean ± SD and carried out with Microsoft Excel (Microsoft Office) and (or) Graphpad Prism 8 (GraphPad Software). Sharpiro-Wilk test were used for the normal data distribution, and kruskal-Wallis test was used for non-parametric analysis. To analyse normal distribution values, data were evaluated using either or a one-way analysis of variance (ANOVA) for three (or more) groups followed by Tukey's multiple comparison post hoc tests or an unpaired two-tailed Student's *t*-test for two groups. The statistical details of experiments can be found in the figure legends. $P < 0.05$ was considered to be a statistically significant difference.

3. Results

3.1. Measurement of sorafenib and sunitinib treatments

The measured concentrations of sorafenib and sunitinib at 0, 24 and 48 hpf after exposure remained relatively stable, as evidenced by HPLC analyses, which were shown in Table S2. The result of determination exhibited that these two compounds were great thermostability after 48h exposure at 28 °C, which showed similar tendencies throughout the experiments. Since a good agreement was displayed between the actual and nominal concentrations, all results presenting in subsequent analyses were based on the nominal concentrations.

3.2. Survival and developmental toxicity of zebrafish embryos/larvae

The effects of three different doses (10, 50, 100 nM) of sorafenib and sunitinib on zebrafish embryo/larvae were evaluated at 96 hpf. Our

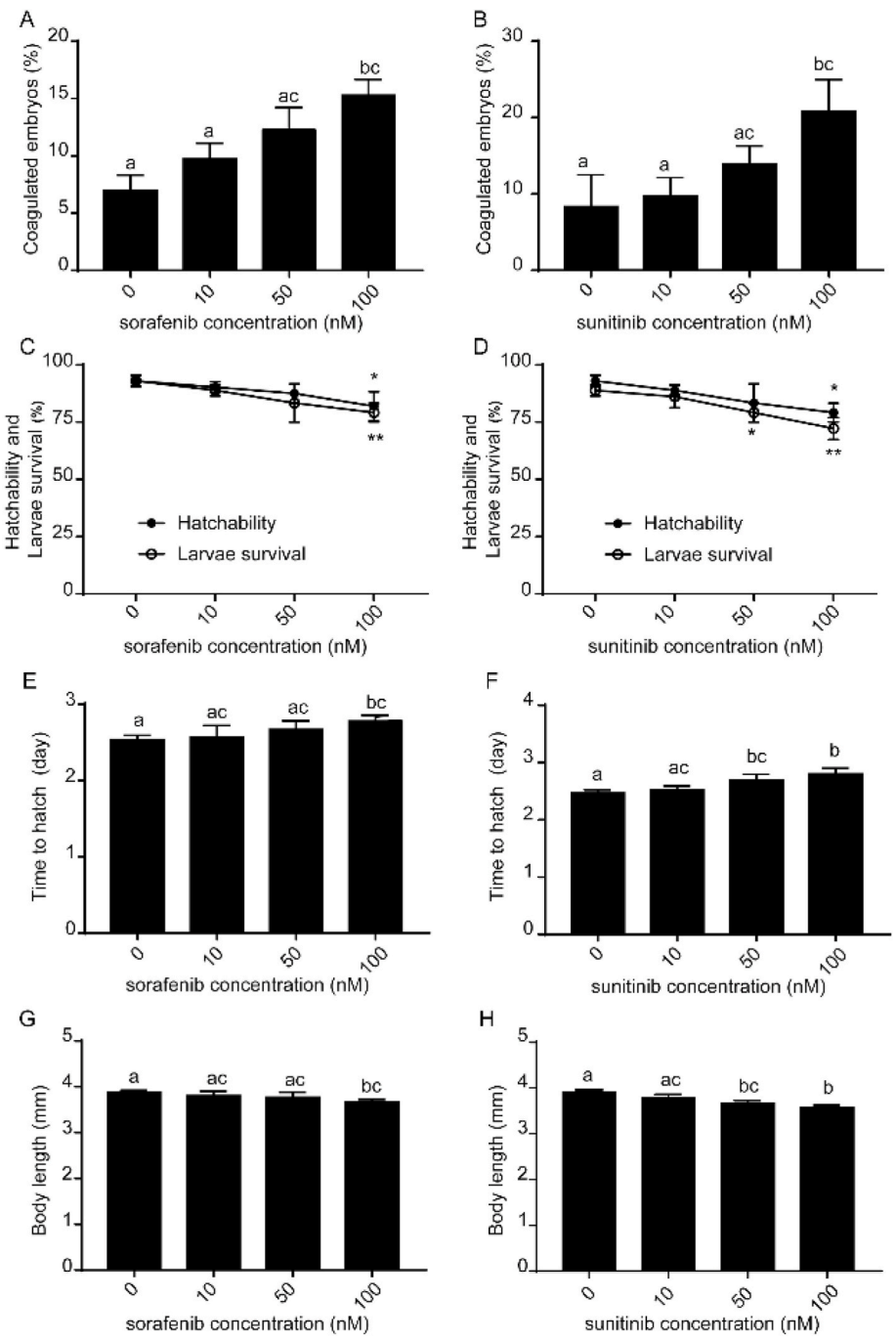


Fig. 1. Survival and developmental toxicities of zebrafish embryos/larvae following exposure to different concentrations (0, 10, 50 and 100 nM) of sorafenib and sunitinib during 96 hpf. (A, B) Coagulated embryos rates, (C, D) hatchability and larval survival rates, (E, F) time to hatch (day), and body length (mm) were shown. A, C, E and G for sorafenib, B, D, F and H for sunitinib. $^{*}P < 0.05$; $^{**}P < 0.01$ as compared with the control group. (One-way ANOVA test followed by a Tukey's multiple comparison).

results showed that waterborne exposure to 100 nM of sorafenib and sunitinib both significantly increased the coagulation rates of zebrafish embryos as compared with the control group (Fig. 1A and B), which furthermore caused a remarkable decrease in hatchability rates and larvae survival rates (Fig. 1C and D). Consistent with these results, the hatching times of zebrafish embryos were obviously delayed after exposure to 100 nM of sorafenib and sunitinib (Fig. 1E and F). Moreover, the body lengths of zebrafish larvae in 100 nM of sorafenib and sunitinib were markedly shorter than those of the control group (Fig. 1G and H). Notably, compared with the control group, the developmental toxicity to zebrafish embryos/larvae were promoted gradually as concentrations of the sorafenib- and sunitinib-treated groups increased, although obviously worse effects were observed in a dose–dependent manner only following exposure to sunitinib (Fig. 1B, D, 1F and 1H).

3.3. Concentration of hormones in zebrafish larvae

Thyroid disruption after exposure to sorafenib and sunitinib in zebrafish larvae for 96 hpf was evaluated by ELISA assay for detecting the whole-body T3 and T4 contents and shown in Fig. 2. Compared with the control group, the whole-body levels of T3 and T4 were both significantly decreased in zebrafish larvae exposed to 100 nM sorafenib and sunitinib (Fig. 2A–D). Interestingly, the concentration of TSH was obviously increased in zebrafish larvae exposed to 50 nM and 100 nM sorafenib and sunitinib (Fig. 2E and F).

3.4. Expression changes in HPT axis in zebrafish larvae

Exposure of zebrafish embryos/larvae to sorafenib and sunitinib for

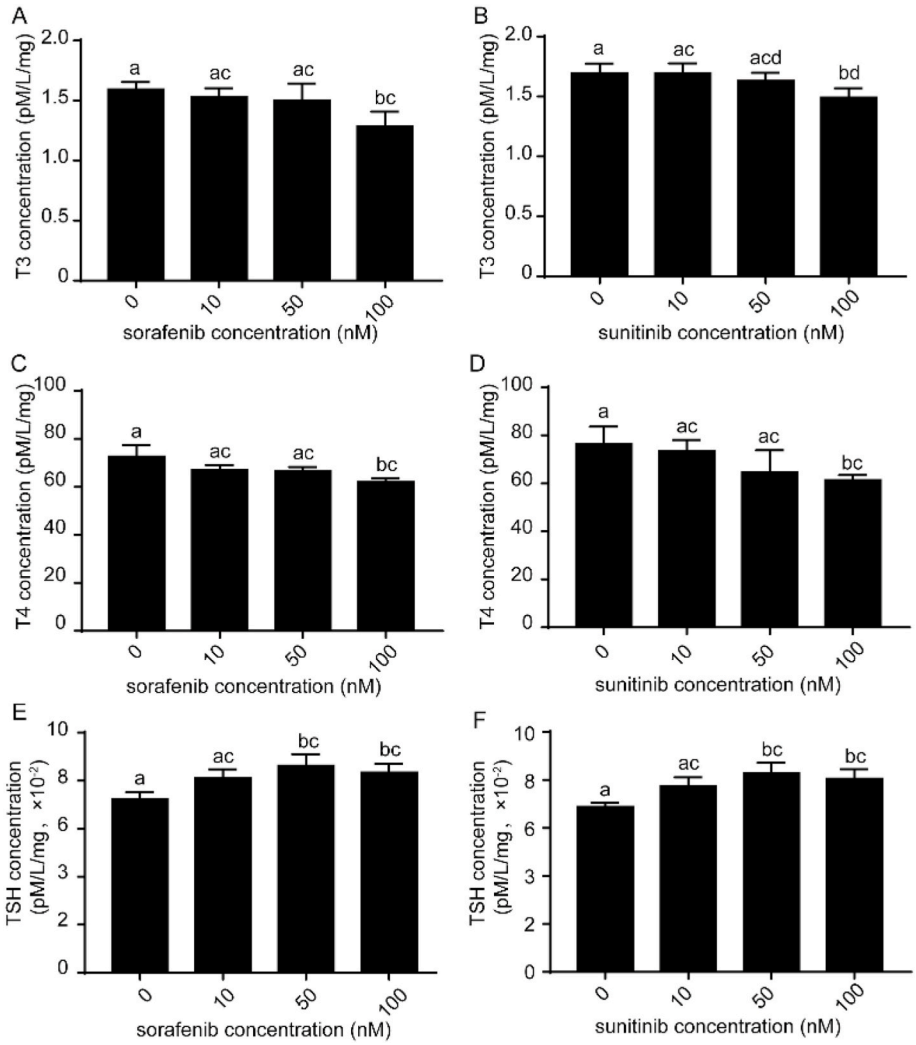


Fig. 2. Levels of T3, T4, and TSH in zebrafish larvae following exposure to different concentrations (0, 10, 50 and 100 nM) of sorafenib and sunitinib for 96 hpf. (A, B) T3 levels, (C, D) T4 levels, (E, F) TSH levels. A, C and E for sorafenib, B, D and for sunitinib. (One-way ANOVA test followed by a Tukey's multiple comparison).

96 hpf promoted variations in mRNA expression of key genes related to HPT axis (Fig. 3 and S2). Transcription levels of *crh*, *trh*, *tshβ* and *tshr* were significantly increased in sorafenib-treated and sunitinib-treated groups at the concentration of 50 and (or) 100 nM (Fig. 3A, B, S2A and S2B). Expressions of *pax8*, *tg*, *tpo*, *nis*, *dio1*, *dio2*, *trα*, *trβ* and *ttr* were significantly decreased (Fig. 3A, B, S2A and S2B). However, the gene level of *ugt1ab* displayed no significant alterations following 96 hpf exposure up to 100 nM sorafenib and sunitinib (Fig. 3A, B, S2A and S2B).

3.5. A negative feedback regulation of THs levels

In order to directly evaluate the abnormalities of thyroid function, by WISH staining of *tg* expression, we observed an obvious alteration of thyroid morphogenesis in zebrafish larvae following exposure to 100 nM of sorafenib and sunitinib for 96 hpf compared to those exposed to vehicle, which noticeably reflected the thyroid dysgenesis-triggered athyresis (Fig. 4A). Notably, shorter single-band structure with "empty" thyroid area, ectopic *tg*-possive thyroid cells and thyroid hypoplasia were detected in zebrafish larvae after exposure to 100 nM of sorafenib and sunitinib, but not in those of control group (Fig. 4A). Moreover, the thyroid area in sorafenib- and sunitinib-treated groups were strikingly decreased by 53.24% and 41.75%, respectively, as compared to control group (Fig. 4A and B). Consistently, compared to the control groups, the expression levels (base on IOD) of *tg* were remarkably lower in sorafenib- and sunitinib-treated groups (each $P < 0.001$).

In addition, compared with control groups, we also observed an striking change of pituitary morphogenesis (e.g, larger size with darker staining color) by WISH staining of *tsh* expression in zebrafish larvae after exposure to 100 nM of sorafenib and sunitinib for 96 hpf (Fig. 5A). Furthermore, consistent with the raised whole-body levels of TSH content measured by ELISA and qPCR assay (Fig. 2E, D, 3A, 3B, S2A and S2B), the expression levels (base on IOD) of *tsh* by WISH staining were strikingly higher in sorafenib- and sunitinib-treated groups (each $P < 0.001$) (Fig. 5B). These results together strongly indicated a negative feedback loop with TSH for compensating the decreased of THs levels caused by thyroid dysfunction.

3.6. Thyroid development and pathological analysis

To better understand the development and pathological changes of thyroid gland in zebrafish larvae after exposure to 100 nM of sorafenib and sunitinib for 96 hpf, we further performed confocal living imaging and histological and morphometric analysis of thyroid gland by using Tg (*tg*:GFP, labeling thyroid follicular cells) zebrafish transgenic line (Fig. 6A) and by H&E staining (Fig. 6B), respectively. As shown in Fig. 6A and B, confocal living imaging showed that sorafenib and sunitinib induced a strikingly developmental retardation in Tg (*tg*:GFP)

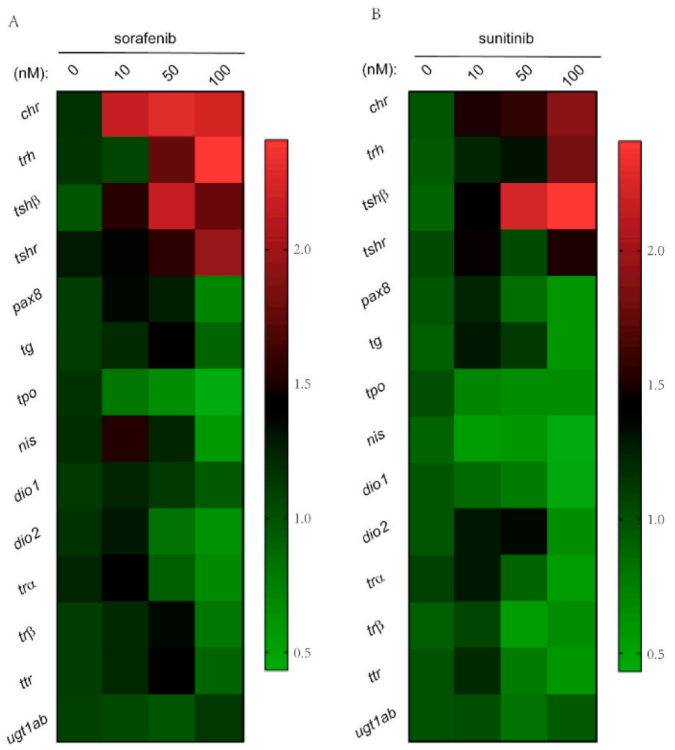


Fig. 3. Relative mRNA expression of HPT axis-related genes in zebrafish larvae following exposure to different concentrations (0, 10, 50 and 100 nM) of sorafenib and sunitinib for 96 hpf. A and B for sorafenib and sunitinib, respectively.

zebrafish larvae (Fig. 6A); Histological analysis showed smaller follicle colloid lumen, the hypotrophy of follicular epithelium and hypoplasia of follicle cells (Fig. 6B). Moreover, compared with the control groups, the thyroid follicle areas were strikingly decreased by 53.37% and 41.04%, respectively (Fig. 6C), and the thyroid follicle numbers were remarkably reduced by 2.2 and 2.4, respectively, following sorafenib and sunitnib exposure at the concentration of 100 nM (Fig. 6D).

4. Discussion

The results in the present study revealed that waterborne exposure to sorafenib and sunitinib had a remarkable influence on the survival and development of zebrafish embryos/larvae, which was accompanied by the marked disturbances of thyroid endocrine system such as the THs levels and gene's transcription levels within the HPT axis. Additionally, we verified a strikingly abnormal thyroid gland organogenesis in zebrafish larvae exposed to sorafenib and sunitinib, by assessing the development of thyroid follicles using the WISH staining of tg, the Tg (*tg*: GFP) zebrafish transgenic line and histology analysis, all clearly showing thyroid dysgenesis. The results of this study would complement the understanding of environmental risks of targeted anti-cancer drugs, such as TKIs, to the health of human and aquatic organisms.

Similar to humans, the thyroid endocrinology system in zebrafish plays a crucial role in development, somatic growth, differentiation and metabolism, especially during the larval development stage (de Souza Anselmo et al., 2018; Han et al., 2021). Previous papers had proved that the toxic effects on developmental impairment caused by environmental factors (e.g, pharmaceuticals) were closely associated with thyroid homeostasis disturbances (Williams et al., 2005; Vigone et al., 2015; Weiss et al., 2015; Leonard et al., 2016; Skarha et al., 2019). In the present study, we first examined the survival and development toxicity of zebrafish embryos/larvae exposure to 0, 10, 50 and 100 nM sorafenib and sunitinib for 96 hpf. We found that waterborne exposure to sorafenib and sunitinib both significantly increased mortality and promoted developmental toxicities in zebrafish embryos/larvae, though only sunitinib exposure showed in a dose-dependent manner. These results implied that sorafenib and sunitinib exposure triggered endocrine disrupting toxicity could result in organism-level effects.

Next, we measured the whole-body THs (total T3 and total T4) in zebrafish larvae to investigate the possibility of thyroid endocrine system disturbances due to sorafenib and sunitinib exposure. We found that sorafenib and sunitinib exposure resulted in a markedly decreased T3 and T4 content and caused hypothyroidism phenotype in zebrafish larvae. Thus, it is reasonable that noteworthy hatching delay and

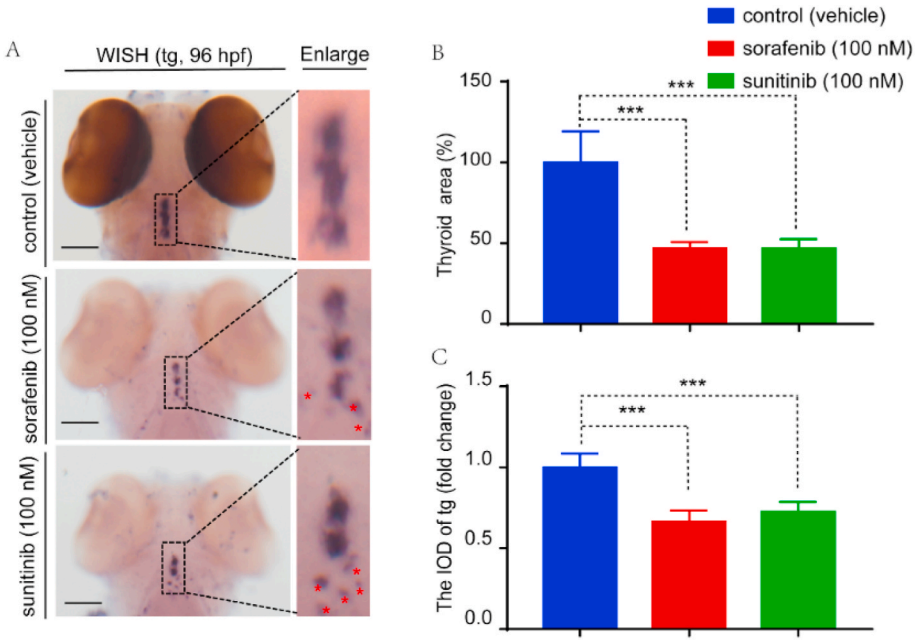


Fig. 4. Whole-mount *in situ* hybridization (WISH) of *tg* in zebrafish larvae following exposure to 100 nM of sorafenib and sunitinib for 96 hpf. (A) WISH of *tg*, (B) the relative decreased thyroid area (%), (C) the IOD of *tg* based on WISH. Red asterisks in A indicate the ectopic thyroid cells. ***$P < 0.001$ as compared with the control (two-tailed t-student test).

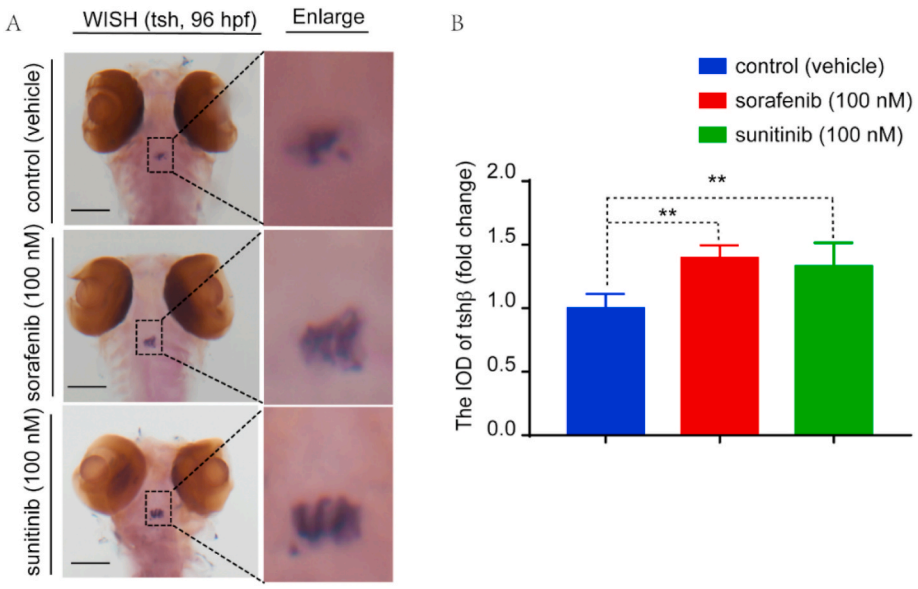


Fig. 5. Whole-mount *in situ* hybridization (WISH) of *tsh* in zebrafish larvae following exposure to 100 nM of sorafenib and sunitinib for 96 hpf. (A) WISH of *tsh*, (B) the IOD of *tsh* based on WISH. Data are expressed as mean ± SD. $^{**}P < 0.01$ as compared with the control (two-tailed t-student test).

decreased body lengths in zebrafish larvae of sorafenib and sunitinib exposure could be attributed to decreased levels of THs, which were probably caused by thyroid function impairment. Likewise, the abnormality of thyroid function and a reduction in body length in zebrafish larvae have been reported following exposure to other environmental contaminants, accompanied by marked alterations in T3 and T4 level (Jin et al., 2021; Di Paola et al., 2022; Lee et al., 2022; Zhang et al., 2022). On the other hand, TSH, secreted by pituitary gland, regulates the synthesis and secretion of THs from thyroid gland. In this study, the elevated TSH contents in zebrafish larvae after exposure to sofafenib and sunitinib could involve in a negative feedback mechanism for compensating the decreased THs caused by thyroid function impairment (Beck-Peccoz and Persani, 1994). Collectedly, lower levels of whole-body THs (T3 and T4) and increased TSH concentration supported the concept that sorafenib and sunitinib exposure caused the thyroid endocrine disruption and further resulted in developmental retardation in zebrafish embryos/larvae.

Thyroglobulin (Tg) is a glycoprotein specific to differentiated thyroid tissue. In zebrafish, the mRNA expression of *tg* is induced at 32–36 hpf, which is indispensable to thyroid hormone formation (Mincione et al., 2011). Since its expression is confined to thyroid cells, *tg* is always used as the specific and powerful marker of thyroid cells. Thus, we next conducted WISH staining of *tg* expression in zebrafish larvae to precise identification of thyroid tissue. We found a remarkably arrested development of thyroid morphogenesis (e.g, shorter single-strand structure with "empty" thyroid area, increased ectopic *tg*-possive thyroid cells and thyroid hypoplasia), demonstrating thyroid dysgenesis-induced athyresis after sorafenib and sunitinib exposure. Given the possible negative feedback mechanism for regulating THs as aforementioned, we performed WISH staining of *tsh* expression in in zebrafish larvae. As expected, we proved that the strikingly higher expression levels (base on IOD) of *tsh* by WISH staining in pituitary gland of sorafenib- and sunitinib-treated groups relative to control groups, accompanied with an obvious pituitary morphogenesis changes, e.g, larger size with darker staining color, apparently implying the hyperplasia in TSH-positive cell (thyrotroph). These results together strongly demonstrated that the up-regulated *tsh* expression and increased TSH secretion attributed to the negative feedback mechanism of HPT axis for keeping THs at normal levels.

Next, we also applied transgenic zebrafish expression fluorescent reporter (Tg (*tg*:GFP) line: labeling follicular cells) for dynamic phenotypic analyses of thyroid morphogenesis. Confocal living imaging further demonstrated that sorafenib and sunitinib exposure led to a strikingly developmental delay of thyroid status normalization, especially exerting a notably decreased thyroid follicular areas and number. Further, we conducted histological and morphometric examination of thyroid gland to determine whether sorafenib and sunitinib exposure has an adverse influence on thyroid structure (i.e, structural thyroid damage) that further contributes to thyroid function impairment. We confirmed that sorafenib and sunitinib exposure both resulted in an apparent thyroid toxicity, which were accompanied by abnormal thyroid histological changes, including smaller follicle colloid lumen, the hypotrophy of follicular epithelium and hypoplasia of follicle cells. Moreover, this histopathological evidence clearly shown a thyroid atrophy, which might be attributed to the dysfunction of thyroid follicular epithelial cells (Sciarrillo et al., 2021). Notably, the sorafenib- and sunitinib-induced hypothyroidism in zebrafish is also well aligned with previous reports in human clinical trials (Clemons et al., 2012; Del Fabbro et al., 2012; Kitajima et al., 2012; Wu and Huang, 2020). For example, several prospective and retrospective studies had documented that TKIs drug such as sorafenib and sunitinib frequently induced a thyroid dysfunction (e.g., hypothyroidism such as decreased levels of T3 and T4, but increased TSH content) in mRCC patients (Clemons et al., 2012; Fallahi et al., 2014). All of the evidences together with former analysis of hormonal biomarkers in this study demonstrated that sorafenib and sunitinib triggered a hypostimulation of the thyroid follicles (and caused hypothyroidism), leading to a further THs synthesis and secretion impairment.

The thyroid-disrupting mechanisms are complex, which may impact thyroid function at many levels. Apart from direct effects (e.g., the synthesis and release of THs, the changes of metabolic state, the THs function via its receptor or binding proteins of blood, etc.), indirect effects through hypothalamus and pituitary are mainly manners involved in thyroid homeostasis. It should be noticed that the HPT axis plays a crucial role in the development, growth, and metabolism of zebrafish (Liu et al., 2019b). In this regard, we finally examined the thyroid function status by examining the gene transcript profile involved in HPT axis. Among them, *trh*, *tshβ* and *tshr* are key regulators of the HPT axis and are closely involved in the THs secretion pathway. In addition, corticotrophin (*crh)* also function as a regulator of HPT axis by stimulating TSH secretion, which further promotes the thyroid to release THs. Hence, the transcription changes of these factors along the HPT axis are

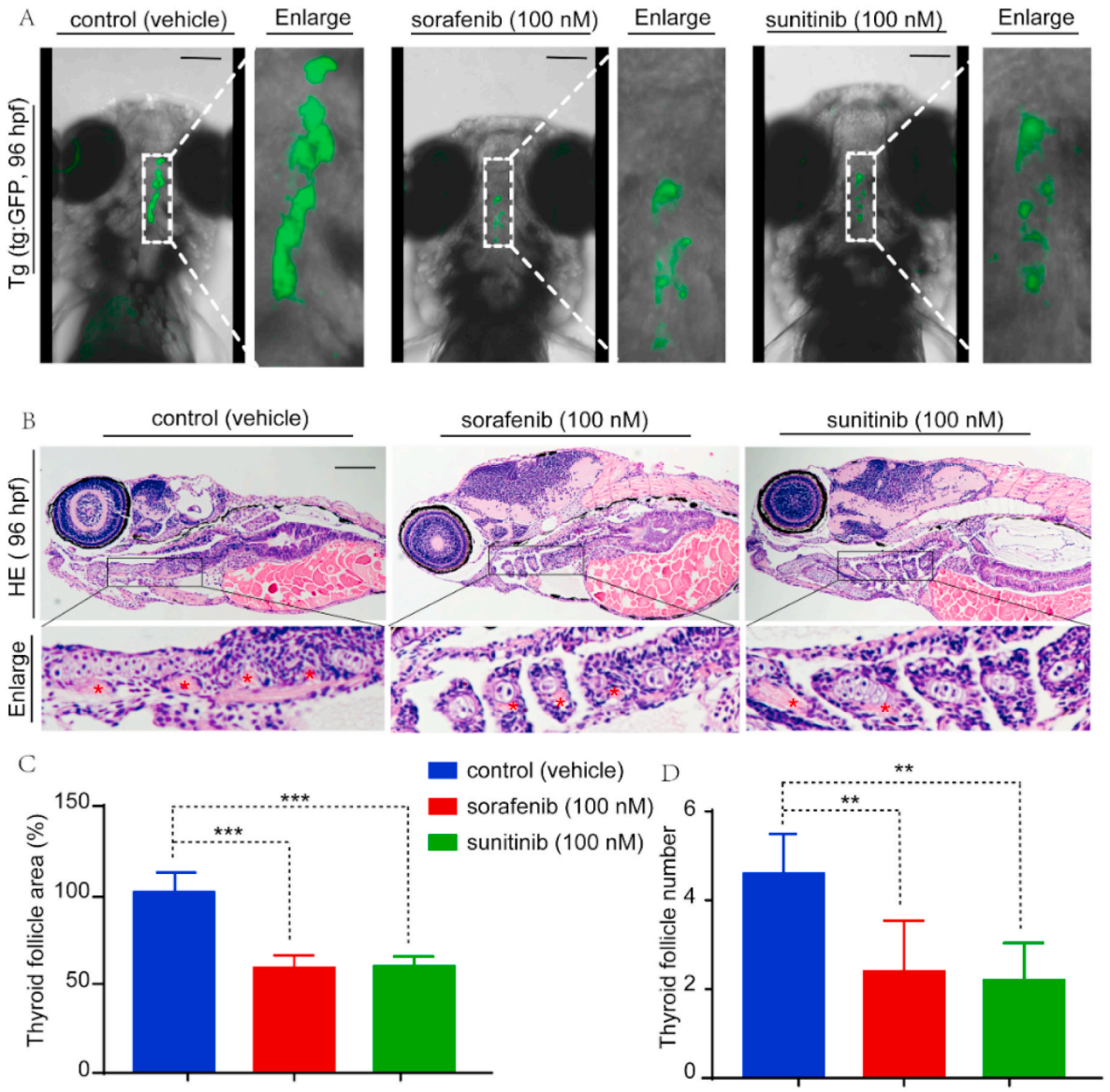


Fig. 6. Morphological and histopathological analysis of thyroid in zebrafish larvae following exposure to 100 nM of sorafenib and sunitinib for 96 hpf. (A) Confocal imaging, and (B) histological analysis of thyroid were examined. (C) Relative decreased thyroid follicle area (%), and (D) thyroid follicle number were analyzed. Red asterisks in B indicate the thyroid follicles. $^{**}P < 0.01$; $^{**}P < 0.001$ as compared with the control (two-tailed t-student test).

often applied for assessment of endocrine disorders caused by pharmaceuticals in the environment. In this study, the mRNA levels of *crh*, *trh*, *tshβ* and *tshr* were remarkably up-regulated following exposure to sorafenib and sunitinib. Therefore, we speculated that, in line with increased TSH content and WISH of *tsh*, the up-regulated genes' transcription could promote the synthesis and release of THs as a negative feedback loop to compensate for the reduced levels of T4 and T3. Interestingly, similar responses caused by exposing to various pharmaceuticals have been documented in zebrafish larvae (Xu et al., 2019; Lee et al., 2022).

The thyroid follicle is the functional unit of thyroid gland (Moroni et al., 2020). *Pax8*, as an important thyroid transcription, regulates the expression of thyroid-specific gene (e.g., *tg*), which also play an pivotal role in the maturation of follicular cells, especially at the late differentiation period (Zannini et al., 1992). During THs formation, *nis* is required for sodium and iodine transport from basolateral plasma to follicular cells (Dai et al., 1996). *Tg* is the protein precursor of THs, and is catalyzed by tpo into the iodination of tg (Kim et al., 2021). The iodinated tg, which is stored in thyroid colloid lumen, undergoes the sequential proteolytic cleavage by proteases and were further secreted into blood as active THs (Rousset et al., 1989). In this study, the down-regulated expression of *pax8*, *tg*, *tpo* and *nis* were observed in zebrafish larvae in response to sorafenib and sunitinib, which might contribute to the decreased THs levels and histological changes of thyroid dysgenesis, further leading to thyroid function impairment. The biological activity of THs is mediated by binding to its specific receptors. *Trα* and *trβ* are two mainly isoforms of thyroid receptors in zebrafish. In this study, the decreased mRNA expression of *trα* and (or) *trβ* was observed in 96 hpf zebrafish larvae exposed to sorafenib and (or) sunitinib, respectively, which may influence the other genes' transcription and further regulate the thyroid functioning. *Ttr* is considered to be a critical transporter, which can bind with THs and transfer them to various target tissues in zebrafish (Jia et al., 2016; Hamers et al., 2020). In the present study, the decreased *ttr* expression was observed in zebfafish larvae probably due to the diminished THs contents after exposure to sorafenib and sunitnib.

For THs metabolism, *dio1* and *dio2* are two trace deiodinases that regulate the peripheral and circulating THs content. Specify, *dio1*

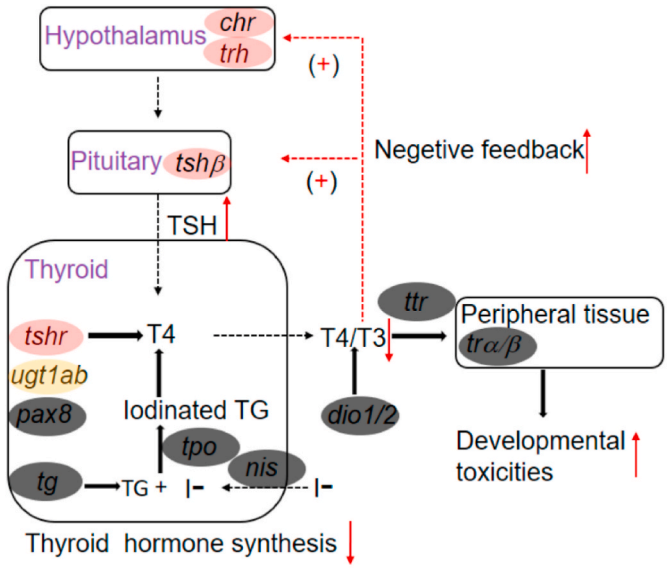


Fig. 7. The influence of sorefenib and sunitinib exposure on the developmental toxicities and thyroid endocrine disruption in zebrafish larvae (*Danio rerio*). Genes with light pink or shallow black backgrounds display that the gene's transcription levels are strikingly higher or lower than those in control, respectively. Gene with pale yellow shows that the gene's transcription levels are no obviously changes as compared with those in control.

mediates the recovery of iodine and degradation of THs, and *dio2* confers the conversion of T4 into active T3 (Liu et al., 2019a). Hence, we proposed that the decreased dio1/2 transcription levels in this study may be another reason for the reduced T4 and T3 content in zebrafish larvae. In addition, *ugt1ab* plays an essential function in THs homeostasis (Liu et al., 2019a). However, we didn't find notable changes of *ugt1ab* expression, which also show the complexity of HPT axis dysregulation in thyroid function. Collectively, as the schematic diagram shown in Fig. 7, the combined influence of sofafenib and sunitinib exposure on the developmental toxicities and thyroid endocrine disruption in zebrafish embryos/larvae (*Danio rerio*) was summarized.

Congenital hypothyroidism (CH) is one of most common inherited diseases which is accompanied by disruption of thyroid endocrine system, with a 1/2000–1/4000 incidence in newborns worldwide (Crank et al., 2020). In recent years, the incidence of CH in China has apparent increased due to improved screening strategies and novel genetic approach (Fan et al., 2015; Andrianou et al., 2016). Nevertheless, the environmental factors have been documented to promote an increased prevalence of hypothyroidism, including drugs interfering with thyroid function and thyroid disrupting chemicals, etc (Vigone et al., 2015). Consequently, the findings in this study are important for public health, which help to develop higher standards of screening programmes, especially for special at-risk categories such as newborns with specific drug exposure. More importantly, the findings in this study also advance the risk factors of congenital hypothyroidism, and the accurate evaluation and prediction of endocrine toxicity caused by specific drug exposure should be considered, especially with a focus on thyroid dysfunction.

5. Conclusion

In this study, we found that sorafenib and sunitinib exposure could induce obvious toxic effects on survival and development in zebrafish embryos/larvae, which was closely associated with the thyroid disruption. Moreover, transcription changes of HPT axis-related genes and destroyed thyroid follicle structure at the early life stage could play pivotal roles in these processes. Our findings added novel evidence to the thyroid disrupting effects that caused by exposure to targeted drugs, indicating clinical application and scientific evaluation of TKIs such as sorafenib and sunitinib for guaranteeing their safety and efficacy are needed.

Credit author statement

Gang Wei: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing-original draft, Writing-review & editing, Project administration, Funding acquisition. **Cao-xu Zhang**: Formal analysis, Data curation, Resources, Software, Writing-review & editing. **Yu Jing**: Investigation, Writing - review & editing. **Xia Chen**: Funding acquisition. **Huai-dong Song**: Supervision. **Liu Yang**: Project administration, Investigation, Formal analysis, Visualization, Resources, Writing-review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No.8200032), Key Laboratory of Pollution Exposure and Health Intervention of Zhejiang Province (No. 20220208), Project funded by China Postdoctoral Science Foundation (Nos. 2021M702178 and 2022T150424), and Special Clinical Research Project of Shanghai Health Commission (No. 202040315).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.136354.

References

Abdel-Rahman, O., Fouad, M., 2014. Risk of thyroid dysfunction in patients with solid tumors treated with VEGF receptor tyrosine kinase inhibitors: a critical literature review and meta analysis. Expet Rev. Anticancer Ther. 14, 1063–1073.
Andrianou, X.D., Gängler, S., Piciu, A., Charisiadis, P., Zira, C., Aristidou, K., Piciu, D., Hauser, R., Makris, K.C., 2016. Human exposures to bisphenol A, bisphenol F and chlorinated bisphenol A derivatives and thyroid function. PLoS One 11, e0155237.
Aspatwar, A., Berrino, E., Bua, S., Carta, F., Capasso, C., Parkkila, S., Supuran, C.T., 2020. Toxicity evaluation of sulfamides and coumarins that efficiently inhibit human carbonic anhydrases. J. Enzym. Inhib. Med. Chem. 35, 1765–1772.
Baffert, F., Le, T., Sennino, B., Thurston, G., Kuo, C.J., Hu-Lowe, D., McDonald, D.M., 2006. Cellular changes in normal blood capillaries undergoing regression after inhibition of VEGF signaling. Am. J. Physiol. Heart Circ. Physiol. 290, H547–H559.
Basolo, A., Matrone, A., Elisei, R., Santini, F., 2022. Effects of tyrosine kinase inhibitors on thyroid function and thyroid hormone metabolism. Semin. Cancer Biol. 79, 197–202.
Beck-Peccoz, P., Persani, L., 1994. Variable biological activity of thyroid-stimulating hormone. Eur. J. Endocrinol. 131, 331–340.
Clemons, J., Gao, D., Naam, M., Breaker, K., Garfield, D., Flaig, T.W., 2012. Thyroid dysfunction in patients treated with sunitinib or sorafenib. Clin. Genitourin. Cancer 10, 225–231.
Crank, M.N., Cottrell, J.N., Mitchell, B.L., Valentovic, M.A., 2020. Characterization of fetal thyroid levels at delivery among appalachian infants. J. Clin. Med. 9.
Dai, G., Levy, O., Carrasco, N., 1996. Cloning and characterization of the thyroid iodide transporter. Nature 379, 458–460.
de Jongh, C.M., Kooij, P.J., de Voogt, P., ter Laak, T.L., 2012. Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water. Sci. Total Environ. 427–428, 70–77.
de Souza Anselmo, C., Sardela, V.F., de Sousa, V.P., Pereira, H.M.G., 2018. Zebrafish (Danio rerio): a valuable tool for predicting the metabolism of xenobiotics in humans? Comparative biochemistry and physiology. Toxicol. Pharmacol.: CBP 212, 34–46.

Del Fabbro, E., Dev, R., Cabanillas, M.E., Busaidy, N.L., Rodriguez, E.C., Bruera, E., 2012. Extreme hypothyroidism associated with sunitinib treatment for metastatic renal cancer. J. Chemother. 24, 221–225.
Di Paola, D., Natale, S., Iaria, C., Crupi, R., Cuzzocrea, S., Spanò, N., Gugliandolo, E., Peritore, A.F., 2022. Environmental Co-exposure to potassium perchlorate and Cd caused toxicity and thyroid endocrine disruption in zebrafish embryos and larvae (Danio rerio). Toxics 10.
Elsalini, O.A., Rohr, K.B., 2003. Phenylthiourea disrupts thyroid function in developing zebrafish. Dev. Gene. Evol. 212, 593–598.
Fallahi, P., Ferrari, S.M., Vita, R., Di Domenicantonio, A., Corrado, A., Benvenga, S., Antonelli, A., 2014. Thyroid dysfunctions induced by tyrosine kinase inhibitors. Expet Opin. Drug Saf. 13, 723–733.
Fan, X., Chen, S., Qian, J., Sooranna, S., Luo, J., Li, C., Tang, Q., Lin, C., 2015. Incidence and interrelated factors in patients with congenital hypothyroidism as detected by newborn screening in guangxi, China. In: Global pediatric health, 2, 2333794x14567193.
Fatta-Kassinos, D., Meric, S., Nikolaou, A., 2011. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. Anal. Bioanal. Chem. 399, 251–275.
Feldt, S., Schüssel, K., Quinzler, R., Franzmann, A., Czeche, S., Ludwig, W.D., Schulz, M., 2012. Incidence of Thyroid Hormone Therapy in Patients Treated with Sunitinib or Sorafenib: a Cohort Study, vol. 48. European journal of cancer, Oxford, England, pp. 974–981, 1990.
Fu, J., Guo, Y., Wang, M., Yang, L., Han, J., Lee, J.S., Zhou, B., 2020. Bioconcentration of 2,4,6-tribromophenol (TBP) and thyroid endocrine disruption in zebrafish larvae. Ecotoxicol. Environ. Safety 206, 111207.
Hamers, T., Kortenkamp, A., Scholze, M., Molenaar, D., Cenijn, P.H., Weiss, J.M., 2020. Transthyretin-binding activity of complex mixtures representing the composition of thyroid-hormone disrupting contaminants in house dust and human serum. Environ. Health [erspect. 128, 17015.
Han, J., Gu, W., Barrett, H., Yang, D., Tang, S., Sun, J., Liu, J., Krause, H.M., Houck, K.A., Peng, H., 2021. A roadmap to the structure-related metabolism pathways of per- and polyfluoroalkyl substances in the early life stages of zebrafish (Danio rerio). Environ. Health [erspect. 129, 77004.
Hu, J., Chang, H., Wang, L., Wu, S., Shao, B., Zhou, J., Zhao, Y., 2008. Detection, occurrence and fate of indirubin in municipal sewage treatment plants. Environ. Sci. Technol. 42, 8339–8344.
Hussain, A., Mahdi, W.A., Alshehri, S., Bukhari, S.I., Almaniea, M.A., 2021. Application of green nanoemulsion for elimination of rifampicin from a bulk aqueous solution. Int. J. Environ. Res. Publ. Health 18.
Jia, P.P., Ma, Y.B., Lu, C.J., Mirza, Z., Zhang, W., Jia, Y.F., Li, W.G., Pei, D.S., 2016. The effects of disturbance on hypothalamus-pituitary-thyroid (HPT) Axis in zebrafish larvae after exposure to DEHP. PLoS One 11, e0155762.
Jin, M., Dang, J., Paudel, Y.N., Wang, X., Wang, B., Wang, L., Li, P., Sun, C., Liu, K., 2021. The possible hormetic effects of fluorene-9-bisphenol on regulating hypothalamic-pituitary-thyroid axis in zebrafish. Sci. Total Environ. 776, 145963.
Kang, H.M., Lee, Y.H., Kim, B.M., Kim, I.C., Jeong, C.B., Lee, J.S., 2017. Adverse effects of BDE-47 on in vivo developmental parameters, thyroid hormones, and expression of hypothalamus-pituitary-thyroid (HPT) axis genes in larvae of the self-fertilizing fish Kryptolebias marmoratus. Chemosphere 176, 39–46.
Kim, M.J., Kim, H.H., Song, Y.S., Kim, O.H., Choi, K., Kim, S., Oh, B.C., Park, Y.J., 2021. DEHP down-regulates tshr gene expression in rat thyroid tissues and FRTL-5 rat thyrocytes: a potential mechanism of thyroid disruption. Endocrinol. Metab.(Seoul, Korea) 36, 447–454.
Kitajima, K., Takahashi, S., Maeda, T., Yoshikawa, T., Ohno, Y., Fujii, M., Miyake, H., Fujisawa, M., Sugimura, K., 2012. Thyroid size change by CT monitoring after sorafenib or sunitinib treatment in patients with renal cell carcinoma: comparison with thyroid function. Eur. J. Radiol. 81, 2060–2065.
Kokilaramani, S., Al-Ansari, M.M., Rajasekar, A., Al-Khattaf, F.S., Hussain, A., Govarthanan, M., 2021. Microbial influenced corrosion of processing industry by re-circulating waste water and its control measures - a review. Chemosphere 265, 129075.
Lee, S., Lee, J.S., Kho, Y., Ji, K., 2022. Effects of methylisothiazolinone and octylisothiazolinone on development and thyroid endocrine system in zebrafish larvae. J. Hazard Mater. 425, 127994.
Leonard, J.A., Tan, Y.M., Gilbert, M., Isaacs, K., El-Masri, H., 2016. Estimating margin of exposure to thyroid peroxidase inhibitors using high-throughput in vitro data, high-throughput exposure modeling, and physiologically based pharmacokinetic/pharmacodynamic modeling. Toxicol. Sci. : Off. J. Soc. Toxicol. 151, 57–70.
Liu, M., Yi, S., Chen, P., Chen, M., Zhong, W., Yang, J., Sun, B., Zhu, L., 2019a. Thyroid endocrine disruption effects of perfluoroalkyl phosphinic acids on zebrafish at early development. Sci. Total Environ. 676, 290–297.
Liu, X., Cai, Y., Wang, Y., Xu, S., Ji, K., Choi, K., 2019b. Effects of tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and triphenyl phosphate (TPP) on sex-dependent alterations of thyroid hormones in adult zebrafish. Ecotoxicol. Environ. Safety 170, 25–32.
Malhab, L.J., Descamps, S., Delaval, B., Xirodimas, D.P., 2016. The use of the NEDD8 inhibitor MLN4924 (Pevonedistat) in a cyclotherapy approach to protect wild-type p53 cells from MLN4924 induced toxicity. Sci. Rep. 6, 37775.
Mannavola, D., Coco, P., Vannucchi, G., Bertuelli, R., Carletto, M., Casali, P.G., Beck-Peccoz, P., Fugazzola, L., 2007. A novel tyrosine-kinase selective inhibitor, sunitinib, induces transient hypothyroidism by blocking iodine uptake. J. Clin. Endocrinol. Metab. 92, 3531–3534.
Mincione, G., Di Marcantonio, M.C., Tarantelli, C., D'Inzeo, S., Nicolussi, A., Nardi, F., Donini, C.F., Coppa, A., 2011. EGF and TGF-β1 effects on thyroid function. J. Thyroid Res. 2011, 431718.
Molhoek, K.R., McSkimming, C.C., Olson, W.C., Brautigan, D.L., Slingluff Jr., C.L., 2009. Apoptosis of CD4(+)CD25(high) T cells in response to Sirolimus requires activation of T cell receptor and is modulated by IL-2. Cancer immunology, immunotherapy. CII 58, 867–876.
Moroni, L., Barbaro, F., Caiment, F., Coleman, O., Costagliola, S., Conza, G.D., Elviri, L., Giselbrecht, S., Krause, C., Mota, C., Nazzari, M., Pennington, S.R., Ringwald, A., Sandri, M., Thomas, S., Waddington, J., Toni, R., 2020. SCREENED: a multistage model of thyroid gland function for screening endocrine-disrupting chemicals in a biologically sex-specific manner. Int. J. Mol. Sci. 21.
Noyes, P.D., Garcia, G.R., Tanguay, R.L., 2016. Zebrafish as an IN VIVO model for sustainable chemical design. Green Chem. : Int. J. Green Chem. Res. : GC 18, 6410–6430.
Olalla, A., Negreira, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2018. A case study to identify priority cytostatic contaminants in hospital effluents. Chemosphere 190, 417–430.
Oosterhuis, M., Sacher, F., Ter Laak, T.L., 2013. Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. Sci. Total Environ. 442, 380–388.
Przybyla, J., Geldhof, G.J., Smit, E., Kile, M.L., 2018. A cross sectional study of urinary phthalates, phenols and perchlorate on thyroid hormones in US adults using structural equation models (NHANES 2007-2008). Environ. Res. 163, 26–35.
Reinwald, H., König, A., Ayobahan, S.U., Alvincz, J., Sipos, L., Göckener, B., Böhle, G., Shomroni, O., Hollert, H., Salinas, G., Schäfers, C., Eilebrecht, E., Eilebrecht, S., 2021. Toxicogenomic fin(ger)prints for thyroid disruption AOP refinement and biomarker identification in zebrafish embryos. Sci. Total Environ. 760, 143914.
Rousset, B., Selmi, S., Alquier, C., Bourgeat, P., Orelle, B., Audebet, C., Rabilloud, R., Bernier-Valentin, F., Munari-Silem, Y., 1989. In vitro studies of the thyroglobulin degradation pathway: endocytosis and delivery of thyroglobulin to lysosomes, release of thyroglobulin cleavage products–iodotyrosines and iodothyronines. Biochimie 71, 247–262.
Sciarrillo, R., Di Lorenzo, M., Valiante, S., Rosati, L., De Falco, M., 2021. OctylPhenol (OP) alone and in combination with NonylPhenol (NP) alters the structure and the function of thyroid gland of the lizard Podarcis siculus. Arch. Environ. Contam. Toxicol. 80, 567–578.
Skarha, J., Mínguez-Alarcón, L., Williams, P.L., Korevaar, T.I.M., de Poortere, R.A., Broeren, M.A.C., Ford, J.B., Eliot, M., Hauser, R., Braun, J.M., 2019. Cross-sectional associations between urinary triclosan and serum thyroid function biomarker concentrations in women. Environ. Int. 122, 256–262.
Torino, F., Corsello, S.M., Longo, R., Barnabei, A., Gasparini, G., 2009. Hypothyroidism related to tyrosine kinase inhibitors: an emerging toxic effect of targeted therapy. Nat. Rev. Clin. Oncol. 6, 219–228.
Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment–a review. Sci. Total Environ. 429, 123–155.
Vigone, M.C., Di Frenna, M., Weber, G., 2015. Heterogeneous phenotype in children affected by non-autoimmune hypothyroidism: an update. J. Endocrinol. Invest. 38, 835–840.
Walraven, M., Homs, M.Y.V., van der Veldt, A.A.M., Dekker, H., Koldenhof, J., Honeywell, R., Barendrecht, A., Sebastian, S.A.E., Parr, N., Koekman, A.C., Voest, E. E., Roest, M., Korporaal, S.J.A., Verheul, H.M.W., 2018. Platelet function is disturbed by the angiogenesis inhibitors sunitinib and sorafenib, but unaffected by bevacizumab. Angiogenesis 21, 325–334.
Wei, G., Sun, H., Dong, K., Hu, L., Wang, Q., Zhuang, Q., Zhu, Y., Zhang, X., Shao, Y., Tang, H., Li, Z., Chen, S., Lu, J., Wang, Y., Gan, X., Zhong, T.P., Gui, D., Hu, X., Wang, L., Liu, J., 2021. The thermogenic activity of adjacent adipocytes fuels the progression of ccRCC and compromises anti-tumor therapeutic efficacy. Cell Metabol. 33, 2021–2039 e2028.
Wei, G., Sun, H., Liu, J.L., Dong, K., Liu, J., Zhang, M., 2020. Indirubin, a small molecular deriving from connectivity map (CMAP) screening, ameliorates obesity-induced metabolic dysfunction by enhancing brown adipose thermogenesis and white adipose browning. Nutr. Metab. 17, 21.
Weiss, J.M., Andersson, P.L., Zhang, J., Simon, E., Leonards, P.E., Hamers, T., Lamoree, M.H., 2015. Tracing thyroid hormone-disrupting compounds: database compilation and structure-activity evaluation for an effect-directed analysis of sediment. Anal. Bioanal. Chem. 407, 5625–5634.
Williams, F.L., Ogston, S.A., van Toor, H., Visser, T.J., Hume, R., 2005. Serum thyroid hormones in preterm infants: associations with postnatal illnesses and drug usage. J. Clin. Endocrinol. Metab. 90, 5954–5963.
Wong, E., Rosen, L.S., Mulay, M., Vanvugt, A., Dinolfo, M., Tomoda, C., Sugawara, M., Hershman, J.M., 2007. Sunitinib induces hypothyroidism in advanced cancer patients and may inhibit thyroid peroxidase activity. Thyroid : Off. J. Am. Thyroid Ass. 17, 351–355.
Wu, J., Huang, H., 2020. Acquired hypothyroidism in patients with metastatic renal cell carcinoma treated with tyrosine kinase inhibitors. Drug Des. Dev. Ther. 14, 3977–3982.
Xu, C., Sun, X., Niu, L., Yang, W., Tu, W., Lu, L., Song, S., Liu, W., 2019. Enantioselective thyroid disruption in zebrafish embryo-larvae via exposure to environmental concentrations of the chloroacetamide herbicide acetochlor. Sci. Total Environ. 653, 1140–1148.
Yang, H., Lai, H., Huang, J., Sun, L., Mennigen, J.A., Wang, Q., Liu, Y., Jin, Y., Tu, W., 2020. Polystyrene microplastics decrease F-53B bioaccumulation but induce inflammatory stress in larval zebrafish. Chemosphere 255, 127040.
Yang, R.M., Zhan, M., Zhou, Q.Y., Ye, X.P., Wu, F.Y., Dong, M., Sun, F., Fang, Y., Zhang, R.J., Zhang, C.R., Yang, L., Guo, M.M., Zhang, J.X., Liang, J., Cheng, F., Liu, W., Han, B., Zhou, Y., Zhao, S.X., Song, H.D., 2021. Upregulation of GBP1 in

thyroid primordium is required for developmental thyroid morphogenesis. Genet. Med. : Off. J. Am. Coll. Med. Genet. 23, 1944–1951.
Yue, Z., Yu, M., Zhang, X., Dong, Y., Tian, H., Wang, W., Ru, S., 2017. Semicarbazide-induced thyroid disruption in Japanese flounder (Paralichthys olivaceus) and its potential mechanisms. Ecotoxicol. Environ. Safety 140, 131–140.
Zannini, M., Francis-Lang, H., Plachov, D., Di Lauro, R., 1992. Pax-8, a paired domain-containing protein, binds to a sequence overlapping the recognition site of a homeodomain and activates transcription from two thyroid-specific promoters. Mol. Cell Biol. 12, 4230–4241.
Zhang, S., Guo, X., Lu, S., He, J., Wu, Q., Liu, X., Han, Z., Xie, P., 2022. Perfluorohexanoic acid caused disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. Ecotoxicol. Environ. Safety 232, 113283.
Zoeller, R.T., Tan, S.W., Tyl, R.W., 2007. General background on the hypothalamic-pituitary-thyroid (HPT) axis. Crit. Rev. Toxicol. 37, 11–53.