Thyroid hormone inhibits biliary growth in bile duct-ligated rats by PLC/IP3/Ca2+-dependent downregulation of SRC/ERK1/2

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Fava G, Ueno Y, Glaser S, Francis H, DeMorrow S, Marucci L, Marzioni M, Benedetti A, Venter J, Vaculin B, Vaculin S, Alpini G. Thyroid hormone inhibits biliary growth in bile duct-ligated rats by PLC/IP3/Ca2+-dependent downregulation of SRC/ERK1/2. Am J Physiol Cell Physiol 292: C1467–C1475, 2007. First published December 27, 2006; doi:10.1152/ajpcell.00575.2006.—The role of thyroid hormone isoforms: tri-iodothyronine (T3) and thyroxine (T4) on bile duct-ligated (BDL) cholangiocytes is unknown. We evaluated the in vivo and in vitro effects of T3 on cholangiocyte proliferation of bile duct-ligated (BDL) rats. We assessed the expression of α1-, α2-, β1-, and β2-thyroid hormone receptors (THRs) by immunohistochemistry in liver sections from normal and BDL rats. BDL rats were treated with T3 (38.4 μg/day) or vehicle for 1 wk. We evaluated 1) biliary mass and apoptosis in liver sections and 2) proliferation in cholangiocytes. Serum-free T3 levels were measured by chemiluminescence. Purified BDL cholangiocytes were treated with 0.2% BSA or T3 (1 μM) in the absence/presence of U-73122 (PLC inhibitor) or BAPTA/AM (intra-cellular Ca2+ chelator) before measurement of PCNA protein expression by immunoblotting. The in vitro effects of T3 (1 μM) on l) cAMP; IP3, and Ca2+ levels and 2) the phosphorylation of Src Tyr139 and Tyr530 (that, together, regulate Src activity) and ERK1/2 of BDL cholangiocytes were also evaluated. α1-, α2-, β1-, and β2-THRs were expressed by bile ducts of normal and BDL rats. In vivo, T3 decreased cholangiocyte proliferation of BDL rats. In vitro, T3 inhibition of PCNA protein expression was blocked by U-73122 and BAPTA/AM. Furthermore, T3 l) increased IP3 and Ca2+ levels and 2) decreased Src and ERK1/2 phosphorylation of BDL cholangiocytes. T3 inhibits cholangiocyte proliferation of BDL rats by PLC/IP3/Ca2+-dependent decreased phosphorylation of Src/ERK1/2. Activation of the intracellular signals triggered by T3 may modulate the excess of cholangiocyte proliferation in liver diseases.

cholestasis; cholangiopathies; hyperplasia; intrahepatic biliary epithelium; mitosis

NORMAL CHOLANGIOCYTES ARE MITOTICALLY DORMANT BUT PROLIFERATE UNDER CERTAIN PATHOLOGICAL CONDITIONS (3, 4). THERE IS GROWING INFORMATION REGARDING THE REGULATION OF CHOLANGIOCYTE PROLIFERATION (4), WHICH IS MODULATED BY A COMPLEX SYSTEM OF GROWTH FACTORS, HORMONES, AND NEUROPEPTIDES (4, 30, 35). CONDITIONS SUCH AS EXTRAHEPATIC BILIARY OBSTRUCTIONS (2, 4) AND CHOLANGIOPATHIES (4) ARE ASSOCIATED WITH CHANGES IN CHOLANGIOCYTE PROLIFERATION (3, 30). HYPERPLASIC CHOLANGIOCYTE PROLIFERATION CAN BE REPRODUCED IN EXPERIMENTAL MODELS SUCH AS PARALLEL HEPATECTOMY (36) OR BILE DUCT LIGATION (BDL) (2).

The thyroid hormone is a trophic factor for several tissues and is fundamental for the development and the function of the whole organism (54). The thyroid gland produces two thyroid hormone isoforms: tri-iodothyronine (T3), the more active, and thyroxine (T4), which is transformed into T3 in the peripheral tissues by deiodinases (39). These hormones interact with target cells generally by stimulating a direct (genomic) pathway, which depends on the action of nuclear hormone receptors (56). Four subtypes of thyroid hormone nuclear receptors, α1, α2, β1, and β2, have been described (53). The binding of thyroid hormone with its nuclear receptors is accompanied by dissociation of coactivators (e.g., silencing mediator of thyroid hormone and retinoid action, and nuclear corepressor), recruitment of coactivators, and stimulation of transcription and translation of target genes (56).

Alternatively, nongenomic pathways can be activated by thyroid hormone in several tissues and cells, including brown adipose tissue, heart, and pituitary cells (6, 21). Thyroid hormone stimulates the hydrolysis of polyphosphoinositides by phospholipase C (PLC) in liver cells with the production of inositol triphosphate, as well as diacylglycerol (DAG), and consequent protein kinase C (PKC) activation (29).

A PHYSIOLOGICAL DOSE OF THYROID HORMONE HAS TROPHIC EFFECTS ON HEPATIC TISSUE (48). A SINGLE DOSE OF T3 ENHANCES LIVER REGENERATION FOLLOWING PARTIAL HEPATECTOMY (48). CONVERSELY, THYROID HORMONE INHIBITS DNA SYNTHESIS IN OTHER CELL LINES (45). THYROID HORMONE HAS A BIPHASIC EFFECT ON TISSUES DEPENDING ON THE CONCENTRATION USED (50). LOWER CONCENTRATIONS STIMULATE GROWTH AND CALCIFICATION, WHEREAS HIGH CONCENTRATIONS OF THYROID HORMONE INHIBIT GROWTH AND STIMULATE REABSORPTION OF BONE TISSUE (50). IN THIS STUDY, WE DEMONSTRATED THE INHIBITORY EFFECT OF HIGH DOSES OF THYROID HORMONE ON CHOLANGIOCYTE GROWTH OF BDL RATS.

METHODS

Animal model. Male Fischer 344 rats (150–175 g) were purchased from Charles River (Wilmington, MA) and kept in a temperature-
controlled environment (20–22°C) with a 12:12-h light-dark cycle. Animals had free access to water and chow. The in vivo experiments were performed in rats that, immediately after BDL (2), were treated by intraperitoneal implanted Alzet osmotic minipumps (Alzet, Palo Alto, CA) releasing vehicle solution or T3 (38.4 μg/day) in 1% BSA for 1 wk. T3 was solubilized in 1N NaOH. 1% BSA was used to maintain the solubility of the compound without binding so tightly as to sequester the T3 (19). Before each experiment, animals were anesthetized with pentobarbital sodium (50 mg/kg ip). Before liver perfusion, 5 ml of blood were withdrawn from the abdominal aorta for the measurement of serum T3 levels. Study protocols were performed in compliance with the institution guidelines.

Materials. Reagents were purchased from Sigma (St. Louis, MO). The antibodies for immunohistochemistry and immunoblots were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) unless differently indicated. The phospholipase C inhibitor, U-73122 (40), was purchased from Calbiochem (La Jolla, CA). The substrate for γ-glutamyl transeptidase (γ-GT), N-(γ-γ-glutamyl)-4-methoxy-2-naphthylamide, was purchased from Polysciences (Warrington, PA). RIA kits used for the determination of intracellular cAMP and β-myo-inositol 1,4,5-trisphosphate (IP₃) levels were purchased from Amersham (Arlington Heights, IL).

Purification of cholangiocytes. The in vitro experiments were performed in BDL cholangiocytes isolated by immunomagnetic separation (1, 16, 23) using a monoclonal antibody (IgM, kindly provided by Dr. R. Faris, Brown University, Providence, RI) against an unidentified antigen expressed by all intrahepatic cholangiocytes (23). Cell number and viability (greater than 97%) were assessed by 0.2% BSA or T3 (1 μM) with 0.2% BSA. For the measurement of IP₃ levels, cholangiocytes (4 × 10⁵) were stimulated for 5 min at room temperature with 0.2% BSA (basal) or T3 (1 μM) with 0.2% BSA. For the measurement of IP₃ levels, cholangiocytes (4 × 10⁵) were stimulated for 5 min at room temperature with 0.2% BSA or T3 (1 μM) with 0.2% BSA in the absence or presence of the Src inhibitor, PP2 (1 μM) (13). Intracellular cAMP and IP₃ levels were measured by RIA (33, 34, 36).

Intracellular Ca²⁺ fluorescence measurements in BDL cholangiocytes, treated with 0.2% BSA or T3 (1 μM) with 0.2% BSA, were performed using fluo-3 AM (Molecular Probes, Eugene, OR) and a Fluoroscan Ascent FL (Thermobasystems, Helsinki, Finland) microplate reader equipped with three injectors (41). Purified cholangiocytes were loaded for 1 h at room temperature with 5 μM fluo-3 AM in 1× Hanks’ balanced salt solution (HBS) with 0.1% Pluronic F-127 (Molecular Probes). After being washed, cells were resuspended in 1× HBS and incubated for 30 min at room temperature. The loaded cells were pelletted and reconstituted at 4 × 10⁶ cells per 100 μl of 1× HBS and then added to the well of a 96-well black microplate. The baseline fluorescence was measured 50 times at 2-s intervals. T3 dissolved in HBS buffer was injected sequentially into separate wells, and the fluorescence intensity was measured at 538 nm for 3 min at 1-s intervals. The excitation wavelength was 485 nm. Intracellular Ca²⁺ concentration was calculated as follows: intracellular Ca²⁺ = Kd(Fmax−F)/F+Fmax (Fmax refers to fluorescence intensity measured after permeabilization of the cells with 1% Nonidet P-40. Then, 0.1 M EGTA was added to chelate Ca²⁺ and minimum fluorescence intensity (Fmin) was obtained. The Kd of fluo-3 AM was 390 nm (25).

Evaluation of the intracellular mechanisms involved in T3 regulation of cholangiocyte growth. BDL cholangiocytes were incubated at 37°C with T3 (0.2% BSA for 2 h or T3 (1 μM) with 0.2% BSA for 2 h in the absence/presence of preincubation (30 min) with U-73122 (a PLC inhibitor, 1 μM) (40) or BAPTA/AM (an intracellular Ca²⁺ chelator, 5 μM) (24). We evaluated, by immunoblots (16) of protein (10 μg) from whole cholangiocyte lysate, the expression of PCNA (normalized to β-actin) (15). Furthermore, we determined by immunoblots whether T3 decreases Src Tyr139 and increases Src Tyr530 phosphorylation (changes that, together, indicate a decreased Src activity) (27, 42) and 2 decreases the phosphorylation of extracellular signal-regulated kinase (ERK1/2) by treating purified BDL cholangiocytes with 0.2% BSA or T3 (1 μM) with 0.2% BSA at 37°C for 2 h. After the membranes were stripped, the expression of total Src and ERK1/2 was evaluated. Proteins were visualized using chemiluminescence (ECL Plus kit, Amersham Life Science). The intensity of the bands was determined by scanning video densitometry using the phospho-imager, Storm 860, Amersham Biosciences (Piscataway, NJ) using the ImageQuant TLV 2003.02 (Little Chalfont, Buckinghamshire, UK). Paraffin-embedded liver sections (5 μm, 3 sections analyzed per group) were stained with hematoxylin and eosin before determining lobular damage, necrosis, and portal inflammation. Inflammatory cells were counted in a coded fashion in all the portal tracts present in the section under evaluation. We assigned a score of zero when we found inflammatory cells in only one to two portal tracts; a score of 1 when less than 25% of the total portal tract presented inflammatory cells; a score of 2 if less than 50% of the portal tracts was infiltrated by inflammation; and a score of 3 when we found inflammation in more than 50% of the total portal tract. In each slide, we counted 10 different portal tracts. Following the selected staining, liver sections were examined in a coded fashion by light microscopy with an Olympus BX-40 (Tokyo, Japan) microscope equipped with a camera. Apoptosis was evaluated by terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) analysis, using a commercially available kit (Wako Chemicals, Tokyo, Japan) (37).

Effect of T3 on intracellular cAMP, IP₃, and Ca²⁺ levels. After isolation, BDL cholangiocytes were incubated for 1 h at 37°C to restore membrane proteins damaged by proteolytic enzyme treatment (26). For the measurement of cAMP levels, cholangiocytes (5 × 10⁵) were stimulated for 5 min at room temperature with 0.2% BSA (basal) or T3 (1 μM) with 0.2% BSA. For the measurement of IP₃ levels, cholangiocytes (4 × 10⁵) were stimulated for 5 min at room temperature with 0.2% BSA or T3 (1 μM) with 0.2% BSA in the absence or presence of the Src inhibitor, PP2 (1 μM) (13). Intracellular cAMP and IP₃ levels were measured by RIA (33, 34, 36).

Cholangiocytes express THRs. Immunohistochemistry of liver sections shows positive staining for α₁-, α₂-, β₁-, and
β2-THRs in cholangiocytes of normal, BDL, and T3-treated BDL rats (Fig. 1). α1- and α2-THRs are localized in the cytoplasm of normal cholangiocytes, whereas after BDL these receptors are expressed mostly in the nucleus of these cells (Fig. 1). β1- and β2-THRs are expressed mostly in the cytoplasm of cholangiocytes in normal and BDL liver sections (Fig. 1). A substantial difference in the localization of THRs in BDL cholangiocytes was not evident after T3 chronic administration. A positive staining was present in hepatocytes of the three groups of animals (Fig. 1).

Effect of chronic T3 administration on serum fT3 levels, liver histology, cholangiocyte proliferation and apoptosis. Chronic administration of T3 significantly increased fT3 serum levels in BDL rats compared with controls (Table 1). We found

Fig. 1. Immunohistochemistry for thyroid hormone receptors (THRs) in liver sections from normal, bile duct ligation (BDL), and tri-iodothyronine (T3)-treated BDL rats. A positive reaction for α1-, α2-, β1-, and β2-THRs is seen in cholangiocytes of normal, BDL, and T3-treated BDL rats. Specifically, α1- and α2-THRs appear to be localized in the cytoplasm of normal cholangiocytes (indicated by black arrows), whereas in 1-wk BDL rats these receptors are expressed mostly in the nucleus of these cells (white arrows). β1- and β2-THRs are expressed only in the cytoplasm of cholangiocytes in both normal and BDL rat liver sections, as indicated by black arrows. No difference in the localization of THRs in BDL cholangiocytes was evident after T3 chronic administration with respect to BDL rats treated with vehicle. A positive staining is also present in hepatocytes of the 3 groups of animals. Original magnification ×40.
no difference in inflammatory infiltrate and necrosis in liver parenchyma of T3-treated BDL rats with respect to controls (Table 1). Lobular damage was similar and minimally evident in both groups of animals (Table 1). The evaluation of cholangiocyte apoptosis showed no difference between T3-treated BDL rats and controls (Table 1).

Administering T3 to BDL rats reduced the number of PCNA- and CK-19-positive cholangiocytes and the number of γ-GT-positive ducts in liver sections compared with controls (Fig. 2A). A decrease in PCNA protein expression was seen in purified cholangiocytes from T3-treated BDL rats compared with cholangiocytes from BDL rats treated with vehicle (Fig. 2B).

Evaluation of the intracellular mechanisms involved in T3 regulation of cholangiocyte proliferation. In vitro, T3 did not change intracellular cAMP levels of BDL cholangiocytes compared with purified BDL cholangiocytes treated with 0.2% BSA (basal; Fig. 3A). T3 increased IP3 (Fig. 3B) and Ca2+ (Fig. 3C) levels in BDL cholangiocytes compared with purified cholangiocytes treated with 0.2% BSA (Fig. 3, B and C). T3-induced increases in IP3 levels were not blocked by PP2 (Fig. 3B), a finding that shows that Src is downstream to IP3/Ca2+ pathway (8). We demonstrated that 1) treatment of purified BDL cholangiocytes with T3 decreased PCNA protein expression (Fig. 4 and 2) T3 inhibition of cholangiocyte PCNA protein expression was blocked by U-73122 and BAPTA/AM (Fig. 4). T3 decreased Src Tyr 139 and increased Src Tyr 530 phosphorylation of BDL cholangiocytes, hence decreasing Src activity (Fig. 5A). We demonstrated that T3 decreased phosphorylation of ERK1/2 in purified cholangiocytes from BDL rats (Fig. 5B). The data demonstrate that T3 inhibits cholangiocyte proliferation and Src and ERK1/2 phosphorylation by a transduction pathway involving the activation of PLC/IP3/Ca2+.

**DISCUSSION**

We demonstrated that 1) cholangiocytes from normal and BDL rats express α1-, α2-, β1-, and β2-THRs. We found a difference in the cell localization of TRs in normal and BDL cholangiocytes. In fact, α1- and α2-TRs appeared to be localized in the cytoplasm of normal cholangiocytes, but after 1 wk of BDL, α1 and α2 were expressed mostly in the nucleus. Conversely, β1 and β2 were expressed only in the cytoplasm of cholangiocytes from normal and BDL rats. No evidence was found in the localization of TRs in BDL cholangiocytes after T3 administration. The change in cell localization of TRs in normal mammary epithelial cells with respect to pathological conditions has been described (8). Previous studies described TRα as located in the nuclei of epithelial cells from normal breast ducts and acini, whereas in breast cancer cells such receptors are located in the cytoplasm (8). Moreover, TRβ presented a nuclear location in benign proliferative diseases and carcinomas in situ and a cytoplasmic localization in normal breast and infiltrative carcinomas (8).

In this study, we showed for the first time the presence of α- and β-TRs in cholangiocytes. Moreover, we observed that all TR subtypes appeared to be localized in the cytoplasm of normal cholangiocytes but, in hyperplastic conditions (i.e., following BDL), only α1 and α2 were expressed in the nuclei of these cells. Many mutations of TRs and methylations in the promoter of this receptor have been described in different carcinoma types (38). Thus it is possible that variations of the expression or cellular location of TRs, which may cause changes in their functionality, could increase the tumoral development or promote an advantage in the cellular transformation (8). However, because no data on the expression of TRs in malignant cholangiocytes are available, further studies comparing the expression of TRs both in benign and malignant biliary diseases are required to establish the degree of involvement of these receptors in the development of biliary diseases.

The role of T3 as a primary mitogen for hepatic cells in vivo has been demonstrated (43, 44), but its lack of effects in cultured hepatocytes was described (12). The growth of HepG2...
A

PCNA

CK-19

γ-GT

BDL

BDL+T3

B

β-actin

42 kDa

PCNA

36 kDa

BDL

BDL+T3

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cell line overexpressing TRα1 or TRβ1 was inhibited following treatment with T3 (55). The effect of thyroid hormone on cholangiocyte growth is unknown. At physiological doses, T3 triggers cell proliferation in several tissues through a direct (genomic) pathway, in which the activated thyroid hormone receptor induces stimulation of transcription and translation of target genes. Besides the “nuclear” mode of action of T3, a variety of rapid effects exerted by T3 at the cell plasma membrane and cytosol has been described (6, 21). Thus several actions of thyroid hormone do not require intranuclear ligand activation, and rapid effects by activating extranuclear proteins are observed in some cell lines (20, 45). Indeed, thyroid hormone acts on the plasma membrane by membrane-associated THRs regulating ion channels, ion pump activities, certain cytoplasmic proteins, at the ribosome and Golgi apparatus, and upon the cytoskeleton (6, 21). Both TRα and TRβ can localize in the cytoplasm (28), where they mediate T3 rapid effects through extranuclear pathways (28) involving mitogen-activated protein kinase, Src, and IP3-kinase (32).

The values of serum fT3 detected in 1-wk BDL rats were similar to fT3 levels measured in normal rodents (14). This indicates that BDL does not alter serum fT3 levels. By using a high dose of T3 for the in vivo experiments, we sought to obtain high levels of circulating hormone that could mimic a hyperthyroid status of the animal (14). The association between thyroid and liver diseases of an autoimmune nature is clinically frequent (18). In fact, there are clinical and laboratory associations between thyroid and liver disease. For example, patients with chronic liver disease may have thyroiditis, hyperthyroidism, or hypothyroidism (5). This study, in particular, explores the effect of the hyperthyroidism condition on cholangiocyte proliferation, a pathological hallmark of the first stages of cholangiopathies, which is followed by vanishing of biliary ductules at the end stages (30).

Thus, in this work, we tested the effect of high doses of thyroid hormone in cholangiocytes from BDL rats that are actively proliferating differently from normal cholangiocytes that are mitotically dormant (35). To perform our experiments, we used both the in vivo and the in vitro model of BDL rat cholangiocytes (1). With great interest we noted that stimulation with high doses of thyroid hormone was accompanied with a decrease of BDL cholangiocyte proliferation. To confirm the concept that high levels of T3 possess an antiproliferative effect on BDL cholangiocytes, high concentrations of T3 have been associated with inhibitory properties in liver cells as nodule regression and reduction of hepatocellular carcinoma (31). Furthermore, previous studies showed that increased concentrations of T3 (10⁻⁵-10⁻⁶ M) inhibited the growth of bone tissue in vitro (50).

An increase in the aspartate aminotransferase and alanine aminotransferase was reported in 27 and 37% of patients with
tyrotoxicosis, respectively (51). This could be explained by relative hypoxia in the perivenular regions, secondary to an increase in hepatic oxygen demand without an appropriate increase in hepatic blood flow (39). Different degrees of intrahepatic cholestasis and lobular inflammatory infiltrate were shown in liver parenchyma in the course of hyperthyroidism. Histologically, in some cases a mild lobular inflammatory infiltrate of polymorphic neutrophils, eosinophils, and lymphocytes associated with nuclear changes and Kupffer cell hyperplasia is evident in liver parenchyma (39). Moreover, a small number of patients present a progressive liver injury, consisting of centrilobular necrosis and perivenular fibrosis, affecting the areas in which hypoxia may be most prevalent (39). Clinically, this type of injury is usually that of self-limiting hepatitis; however, some cases of fulminant hepatic failure have been described (7). The increase of alkaline phosphates is observed in 64% of patients with thyrotoxicosis (10). Even if this can originate from bone and/or liver, the concomitant increase of γ-GT and bilirubin is an index of cholestasis (10).

The histological features observed in the course of cholestasis are similar to the nonspecific changes seen in hepatic injury, in addition to centrilobular intrahepatic cholestasis (49). The early reports of patients developing a spectrum of pathological changes from focal necrosis with fatty change to cirrhosis can be attributed to untreated hyperthyroidism (11). The hepatic abnormalities associated with hyperthyroidism are generally reversible, following the early recognition and treatment of the disease (11).

In this study, findings such as focal areas of necrosis in the liver parenchyma, hepatic lobular damage, and phlogistic infiltrate were absent in the liver of the two animal groups. Taken together, our data show that chronic administration of T3 to BDL rats reduces cholangiocyte proliferation (1, 2), and this inhibitory effect is not accompanied by an increase of portal inflammation and cholangiocyte apoptosis, events that may contribute to the modulation of tissue growth. Hypothyroidism may directly affect the structure or function of the liver as well. In fact, in some case reports, hypothyroidism has been associated with cholestatic jaundice attributed to reduced bilirubin and bile excretion (52). Reduced bilirubin excretion, hypercholesterolaemia, and hypotonia of the gall bladder increase the incidence of gallstones in hypothyroidism (22). In PBC or chronic autoimmune hepatitis-affected patients, there is an increased prevalence of autoimmune thyroid diseases (9). In fact, autoimmune hypothyroidism occurs in 10–25% of PBC patients (9). Moreover, primary sclerosing cholangitis is associated with an increased incidence of Hashimoto’s thyroiditis, Grave’s disease, and Riedel’s thyroiditis (47).

Recent studies characterized particular thyroid hormone analogs (17), named thyronamines (e.g., T1AM, T0AM), which are decarboxylated and de-iodinated metabolites of the thyroid hormone (17). These analogs link poorly to nuclear THRs but, on the contrary, bind to specific G protein-coupled receptor, such as TARs, far different from the classical THRs, thus activating nongenomic cell signaling pathways (17). Some studies observed that thyronamines have opposite physiological effects than those observed for T3, and it is hypothesized that they may provide a counterregulation to the transcriptional effects of thyroid hormone by nuclear THR (17). However, a further evaluation is required to explore whether thyronamines participate in a certain manner in T3-induced decrease of BDL cholangiocyte proliferation.

In this study, we did not use any specific THR subtype inhibitor. Our primary focus was to explore the nongenomic effects of high doses of T3 in modulating biliary cell growth. Further studies are necessary to establish which receptor subtypes are involved in such a mechanism.

The study shows that 1) T3 inhibits BDL cholangiocyte growth and 2) T3 inhibition of cholangiocyte growth is associated with increased IP3 and Ca2+ levels, decreased p-Src Tyr139, and increased p-Src Tyr530 phosphorylation (indexes of decreased Src activity) (42) and reduced ERK1/2 phosphorylation. U-73122 and BAPTA/AM blocked the inhibitory effect of T3 on cholangiocyte proliferation. These findings indicate that high doses of T3 inhibit BDL cholangiocyte growth.
through a nongenomic mechanism by activation of IP$_3$/Ca$^{2+}$ and subsequent downregulation of Src/ERK1/2.

The study indicates that changes in the expression and cell localization of THRs could be implicated in the development of pathological conditions and that modulation of the intracellular signal triggered by thyroid hormone may be useful to regulate the excessive proliferation of the biliary epithelium in the course of cholestatic liver diseases.

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