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Review Article

Functional roles of non-coding RNAs regulated by thyroid hormones in liver cancer



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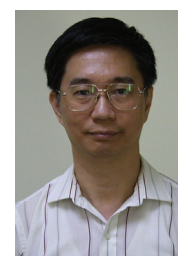
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ABSTRACT

Recent reports have shown the important role of the non-coding part of human genome RNA (ncRNA) in cancer formation and progression. Among several kinds of ncRNAs, microRNAs (miRNA) play a pivotal role in cancer biology. Accumulating researches have been focused on the importance of non-coding genes in various diseases. In addition to miRNAs, long non-coding RNAs (lncRNAs) have also been extensively documented. Recently, the study of human liver cancer has gradually shifted to these non-coding RNAs that were originally considered “junk”. Notably, dysregulated ncRNAs may influence on cell proliferation, angiogenesis, anti-apoptosis, and metastasis. Thyroid hormones play critical roles in human development and abnormalities in thyroid hormone levels are associated with various diseases, such as liver cancer. Thyroid hormone receptors (TR) act as ligand-activated nuclear transcription factors to affect multiple functions through the gene-level regulation in the cells and several studies have revealed that thyroid hormone associated with ncRNAs expression. TR actions are complex and tissue- and time-specific, aberrant expression of the various TR isoforms have different effects and are associated with different types of tumor or stages of development. In this review, we discuss various aspects of the research on the thyroid hormones modulated ncRNAs to affect the functions of human liver cells.

Thyroid hormones (TH) play critical roles in human growth and development and abnormalities in TH levels are associated with various human diseases, such as cancer. Thyroid

hormones in the human body affect multiple functions predominantly through gene-level regulation and act as ligand-activated nuclear transcription factors that interact with

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their cognate hormone response elements (TRE) in the promoters of different genes to induce positive or negative regulation. In recent years, accumulating research has focused on the importance of non-coding genes in various diseases. In addition to the originally identified microRNAs (miRNA), the physiological relevance of frequently fragmented long non-coding RNAs (lncRNA) has been extensively documented. MiRNAs exert their specific effects via direct interactions with three prime untranslated regions (3'-UTR) of various target genes, leading to decreased mRNA expression. The molecular mechanisms of lncRNAs in cells are relatively more complex, with different modes of action in the nucleus and cytoplasm. More and more studies, including therapeutic strategies and biomarkers, have been focused on the non-coding RNAs. For example, compared with coding genes, miRNA has the characteristics to affect different target genes' expression and potentially alter several carcinogenic or tumor suppressor pathways at the same time. Besides, the design and implementation of inhibitors or drugs formulated for nucleic acids are easier than those for proteins. Thus, non-coding RNAs have been considered as the most potential targets in cancer treatment. Further, several studies have revealed that the thyroid hormone acts as a transcription factor in association with non-coding genes. The application of thyroid hormones in cancer has long been recognized. Therefore, to understand the relationship between thyroid hormone and non-coding RNAs is an urgent need for clinical application. In the current review, we specifically focus on the miRNAs and lncRNAs affected by thyroid hormones in liver cancer, along with their roles and mechanisms of action in tumor development.

Thyroid hormone in human liver cancer

Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma (HCC) is a common primary malignant tumor of the liver and one of the leading causes of cancer-related death worldwide. The incidence of HCC increases progressively with advancing age in all populations and global cases have continued to rise over the last decade [1]. The burden of liver cancer varies considerably by geographic region and sex due to the distribution of underlying diseases, genetic factors and variable exposure to environmental and behavioral risk factors. Globally, hepatitis B virus (HBV) is the leading cause of liver cancer death, accounting for 33% mortality, followed by alcohol and hepatitis C virus (HCV) infection [2]. While prognosis of patients with HCC is generally poor, the 5-year survival rate is >70% in patients diagnosed at an early stage. However, early diagnosis of HCC is complicated by the coexistence of inflammation and cirrhosis, highlighting the urgent requirement for novel biomarkers of early-stage HCC. Currently, diagnosis of HCC without pathological correlation is achieved by analysis of serum α -fetoprotein (AFP) levels combined with imaging techniques. Advances in genomics and proteomics platforms and biomarker assay techniques over the last decade have resulted in the identification of numerous novel biomarkers

and improved diagnosis of HCC. These biomarkers are not only useful for early diagnosis of HCC but also provide insights into the mechanisms driving oncogenesis that could facilitate the development of effective treatment strategies [3]. For instance, Sorafenib is an oral multikinase (receptor tyrosine kinase, RTK) inhibitor that suppresses tumor cell proliferation and angiogenesis and promotes apoptosis. The drug was approved by FDA as a unique target for advanced hepatocellular carcinoma (HCC) in 2007 [4,5] as one of the therapeutic options based on in-depth understanding of the carcinogenic mechanisms underlying liver cancer in recent years. Regrettably, sorafenib prolongs life expectancy by only about 3 months. One of the possible factors is that HCC is a complex disease with multiple signaling pathways involved in its pathogenesis. As a result, the targeted treatment of liver cancer is not effective. Combining the use of a variety of drugs with different mechanisms of action is a new clinical treatment strategy. For example, Sorafenib is combined with a MEK kinase inhibitor. The effect has also been validated to be helpful to patients [6]. The development of different technologies to identify genes with important roles in liver cancer remains a significant focus of scientific and clinical research. To date, several biotechnological methods, including next-generation sequencing and multiple "omics" data analyses including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and metagenomics, have been developed and applied for HCC diagnostic biomarker screening [7].

Thyroid hormone and receptor

The thyroid hormone system starts from the hypothalamus, where thyrotropin-releasing hormone (TRH) is synthesized and released from the paraventricular nucleus (PVN). TRH binding to its receptor on the thyrotroph of the anterior pituitary gland stimulates proliferation, synthesis and secretion of thyroid stimulating hormone (TSH). TSH subsequently interacts with the TSH receptor (TSHR) on individual thyroid follicular cells of the thyroid gland to stimulate synthesis and release of the two major thyroid hormone forms, tetraiodothyronine (T_4) and 3,3',5-tri-iodo-L-thyronine (T_3). T_3 is the active form of thyroid hormone and T_4 the prohormone activated by deiodinases at the cellular and circulatory levels [8]. The actions of T_3 are mediated by nuclear thyroid hormone receptors (TR) located on human chromosomes 17 and 3, which primarily serve as T_3 -inducible transcription factors. Two major isoforms of TRs exist, specifically, THRA ($TR\alpha$) and THRB ($TR\beta$). Different TRs are composed of similar domains, including an amino-terminal A/B domain to recruit regulatory proteins, a central DNA-binding domain (DBD) or C region displaying high affinity for DNA sequences of TREs, a linker D region necessary for nuclear translocation of the receptor, and a carboxy-terminal ligand-binding domain (LBD) that interacts with thyroid hormones [9]. Expression of TR isoforms is tissue-dependent. $TR\alpha$ is predominantly expressed in bone and heart and $TR\beta$ in liver and kidney. Within cells, TR forms a heterodimer with the retinoid X receptor (RXR) that interacts with thyroid hormone response elements (TRE) in regulatory regions of target genes [10].

Role of thyroid hormone and receptor in human liver cancer

Thyroid hormone plays a critical regulatory role in cellular homeostasis and its imbalance in the body is significantly associated with multiple chronic diseases, including obesity, diabetes, cardiovascular, and liver disorders [9]. Under physiological conditions, thyroid hormones control tumor cell development, differentiation, metabolism, and growth pathways. Evidence of TR involvement in human carcinogenesis is based on the discovery of TR mutations in hepatocellular carcinoma, renal clear cell carcinoma, breast cancer, pituitary tumor, and thyroid cancer. Loss of normal expression of *THRB* located on chromosome 3p due to truncation or deletion has been additionally reported in several malignancies including lung, melanoma, breast, head-and-neck, renal cell, uterine cervical, ovarian, and testicular tumors [11]. In liver, the effects of thyroid hormones appear to be particularly important. The liver has regenerative capacity but is subject to molecular pathologies that may lead to cancer, such as fibrosis, cirrhosis, and non-alcoholic fatty liver disease. In addition, cancer cells undergo reprogramming of their metabolism that results in drastic changes, such as aerobic glycolysis in lieu of oxidative phosphorylation [12]. Hypothyroidism is associated with poorer overall and recurrence-free survival of HCC patients receiving liver transplantation [13]. T_3 or its analogs, in particular, agonists of TR β , may serve as useful tools in HCC therapy. The T_3 /TR β axis has been shown to cause a shift in the global expression profile of the most aggressive preneoplastic nodules towards that of normal liver [14].

Thyroid hormone regulates non-coding RNAs in human liver cancer

Non-coding RNAs

Traditionally, the central dogma of molecular biology explaining the destiny of genes is stated as “DNA makes RNA and RNA makes protein”, which subsequently performs the physiological function of its encoded gene. Over the years, the focus of research has gradually shifted to other non-coding RNAs that were originally considered “junk” [15]. These nucleic acids that do not encode proteins not only store and transmit genetic information but also perform vital intracellular functions as regulators of gene expression. Non-coding RNAs are defined by inability to code for peptide chains long enough to constitute proteins (amino acids encoded within the open reading frame (ORF) are <100) and classified based on length of the nucleic acid chain [16]. Typically, microRNAs (miRNA), long non-coding RNAs (lncRNA) and circular RNAs (circRNA) play crucial roles in regulating vital biological processes, especially in malignant diseases [17]. Among these molecules, miRNAs and lncRNAs have been a considerable focus of tumor-related research in recent years. MiRNAs are non-coding RNAs about 21–23 nucleotides in size. Compared with other non-coding RNAs with longer fragment sizes, miRNAs perform simple functions and were discovered at an early stage of research. These RNA molecules undergo a series of biogenesis steps that convert primary miRNA transcripts into mature miRNAs, which are subsequently loaded into the

RNA-induced silencing complex (RISC) targeting the 3'-translated regions (3' UTR) of target genes, eventually leading to translational repression or target mRNA degradation [18]. In contrast, long non-coding RNAs (>200 nucleotides transcribed by RNA polymerase II) contain introns and present a 7-methylguanosine cap at the 5' end and a poly(A) chain at the 3' end, similar to mRNAs.

Compared with miRNA, the mechanisms of action of lncRNAs are complex. LncRNAs can interact with protein, DNA or RNA (RNA-protein, RNA-DNA or RNA-RNA) to perform their functions and are distributed in both nucleus and cytoplasm. In the nucleus, lncRNAs affect downstream gene transcription via multiple roles (enhancer, guide, and decoy or chromatin architect). In the cytoplasm, lncRNAs serve as miRNA/protein sponges or micropeptide templates to affect mRNA translation and stability. Indicating lncRNAs participate many process (epigenetic modification, transcriptional regulation and post-transcriptional regulation) in the cells [19,20].

Non-coding RNAs in liver cancer

Non-coding RNAs have been reported to participate in cancer hallmarks, including uncontrolled cell growth, invasion and metastasis, angiogenesis, resistance to cell death, and evasion of immune destruction [21,22]. MicroRNAs participate in cell proliferation, apoptosis, and transformation, as they can regulate gene expression and intracellular signal transduction for various physiological processes [23]. The tumor-suppressive effects that binding specificities, target genes, and regulated pathways of the miRNA-449 family (miR-449a, miR-449b, and miR-449c) are slightly different in HCC. They inhibited cell proliferation and migration, induced apoptosis, and reduced tumor growth to different extents by directly target to SRY-Box Transcription Factor 4 (SOX4), which codes for a transcription factor involved in epithelial–mesenchymal transition (EMT) and HCC metastasis, and thereby inhibited TGF- β mediated cell migration [24]. MiRNA-196a has been reported to be overexpressed in many types of cancers, including HCC. A mouse model demonstrated that down-regulation of miR-196a inhibited human liver cancer cell migration and invasion in vivo through direct targeting Forkhead box protein O1 (FOXO1) and could benefit the clinical therapy of HCC in the future [25]. MiR-122 and miR-22 are known to be related to the occurrence and progression of HBV related HCC. MiR-122 and miR-22 were downregulated in HBV related HCC patients and were related to tumor size, lymph node metastasis, TNM stage, pathological type, differentiation grade, liver cirrhosis, AFP and HBV DNA in clinical [23].

LncRNAs are a kind of non-coding RNAs [26]. Dysregulated lncRNAs are gradually considered to be closely related to the growth, metastasis, and deterioration of liver cancer [20]. LncRNA Highly Up-regulated in Liver Cancer (HULC) is highly upregulated in HCC and negatively associated with the expression of Phosphatase and Tensin Homolog (PTEN) and miR15a. Further, HULC is related to highly specific upregulation characterized in HCC tissues and associated with intrahepatic metastases, TNM stage, and HCC recurrence [20,27]. In addition to the miR-449 mentioned above, HOXD cluster antisense RNA 1 (HOXD-AS1) also interacts

with SOX4. HOXD-AS1 is significantly upregulated in HCC tissues and associated with poor prognosis and high tumor node metastasis stage of HCC patients. HOXD-AS1 competitively binds to miR-130a-3p that prevented SOX4 from miRNA mediated degradation, thus activates the expression of the Enhancer of zeste homolog 2 (EZH2), Matrix Metalloproteinase-2 (MMP-2), and facilitated HCC metastasis [28]. lncRNAs play emerging EMT regulators to regulate different types (1–3) of EMT, such as Tripartite Motif Containing (TRIM) 71 interacting long non-coding RNA (Trincr)1 that can promote embryonic stem cells self-renewal and suppress ERK target genes through inhibiting TRIM71. Besides, lncRNAs have been found to target EMT-related genes and signaling pathways to increase epithelial plasticity [29]. Moreover, some evidence indicates that lncRNAs are aberrantly expressed in diverse cancer stem cell (CSC)s and regulate CSC properties at different molecular levels. As an example, Differentiation Antagonizing Non-protein Coding RNA (DANCR) can interact with CTNNB1 mRNA and significantly promotes stemness features in HCC [30].

Non-coding RNAs as biomarker/therapeutic targets for liver cancer

Serum Alpha-fetoprotein (AFP) is the most used biomarkers for HCC. But, AFP lacks the specificity for use as the standard diagnostic tool for HCC in clinical. Also other biomarkers, such as Desgamma Carboxyprothrombin (DCP) and fucosylated AFP, have been investigated for their clinical usefulness as well but showed equally low accuracy as AFP. Thus, non-coding RNA based assays could be considered as an alternative diagnostic tool for HCC, since most protein-based assays lack the desired accuracy [31]. Many reports suggest that non-coding RNAs alone or in combinations act as candidate biomarkers for HCC diagnosis. The lncRNA Urothelial Carcinoma Associated-1 (UCA1) is an example of lncRNA based HCC diagnostic biomarker. And the simultaneous detection of JUN mRNA was more accurate than the single detection of UCA1 (the sensitivity and specificity were 97.1% and 80%) [32]. Similarly, several miRNAs show their value in clinical diagnosis. For example, miR-26a was identified as a promising biomarker for the diagnosis of early HCC. Even further, the expression levels of miR-221 and miR-101-1 could be used as non-invasive biomarkers for the diagnosis of early HCC from HCV patients [33]. In the choice of biomarker, in addition to the specificity and accuracy of the test, the method of obtaining the specimen is also a very important consideration. Therefore, non-invasive sample acquisition has become the most common consideration. Among them, in the serum of patients, detecting non-coding RNAs in exosomes secreted by cells is a way to obtain higher quality targets to be tested, and it has attracted attention in clinical development [34]. Like circulating exosomal miRNA-21 and lncRNA Activated By TGF- β (ATB) were related to the TNM stage and other prognostic factors, including the T stage and portal vein thrombosis that can novel prognostic markers and therapeutic targets for HCC [35]. These values underline the utility of RNA based detection methods for early HCC diagnostic.

The application of non-coding RNAs in the HCC therapeutic strategy has great potential. Based on the action

characteristics of lncRNA, its function similar to the miRNA plays the role of sponge. Therefore, lncRNAs with different tumor suppressor roles can be designed to inhibit oncogenic miRNAs with the abnormal performance for treatment [36]. Further, lncRNA targeting approaches hold some advantages than protein targeting methods in terms of base pairing principle is much more straightforward than designing a specific protein-binding inhibitor, such as antisense oligonucleotides (ASOs) and RNA interference (RNAi) [37]. For example, linc00210 is highly expressed along with liver tumorigenesis and linc00210 drives the self-renewal and propagation of liver tumor-initiating cells (TICs) through activating Wnt/ β -catenin signaling. Also, ASO-mediated linc00210 silence has been shown to repress the self-renewal and invasion of HCC cells [38]. Besides, knockdown of oncogenic lncRNA cancer susceptibility 9 (CASC9) by RNAi also significantly reduced the tumor formation in an HCC mouse model [39]. Nucleic acid inhibitors can inhibit endogenous miRNAs or piRNAs that exhibit tumor-promoting functions in cancer. Thus, miR-122 inhibitor has been tested in phase 2a clinical trials for treating patients with HCV infection [40].

Thyroid hormone regulates microRNA expression in liver cancer

Recent studies have highlighted that thyroid hormone as a transcription factor is closely associated with various non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). In liver cancer, thyroid hormone affects tumor progression through effects on multiple non-coding genes. Our group has extensively investigated miRNAs and lncRNAs with potential clinical value in diagnosis and treatment of liver cancer. As a result, several miRNAs and lncRNAs clearly affected by thyroid hormones and receptors have been identified [9,41].

MiR-214 is reported to act as either a tumor suppressor or oncogene in various malignancies [42]. The miRNA has been characterized as a tumor suppressor in HCC. Interactions of miR-214 with different target genes exert a range of effects on different cells in HCC. Hepatoma-derived growth factor (HDGF) is a heparin binding protein purified from conditioned medium of HuH-7 hepatoma cells and expressed in a range of tissues, such as liver and lung. Overexpression of HDGF has been reported in multiple cancer types and the HDGF level is correlated with poor prognosis in breast, colorectal, pancreatic, non-small cell lung and gallbladder cancers as well as HCC [43]. Dysregulated miR-214 contributes to the unusual hypervascularity of HCC via direct activation of target genes in the HDGF paracrine pathway of angiogenesis [44,45]. MiR-214 additionally suppresses cell proliferation, migration and metabolism by targeting Flotillin 1 (FLOT1), pyruvate dehydrogenase kinase 2 (PDK2), and plant homeodomain finger protein 6 (PHF6) in hepatocellular carcinoma [42,46]. Previous studies suggest that T₃ induces miR-214 expression and suppresses cell proliferation through interactions with the target proto-oncogene, the serine/threonine-protein kinase PIM-1, thus contributing to inhibition of HCC tumor formation [47].

An miRNA cluster is a set of two or more miRNAs transcribed from physically adjacent miRNA genes. In common cognition processes, miRNA clusters are located in similar

chromosomes under the same circumstances and therefore often subject to the same regulation mechanisms or play a consistent role [48]. For instance, the miR-106b-25 cluster regulates atherosclerosis by influencing clearance of VLDL and LDL from the plasma and dysregulation of the miR-183 cluster causes defects in differentiation of photoreceptors and other retinal neurons through effects on target gene expression patterns [49,50]. The miR-199a/miR-214 cluster has additionally been identified as two miRNAs with the similar effects in a number of studies. Both miR-199a and miR-214 are significantly downregulated during differentiation and induce expression of a series of genes correlated with differentiation (COX-2, NF- κ B p50/p65, and CREB1) [51]. In addition, miR199/miR214 play roles in the differentiation of mammalian skeletal precursor cells into osteoblasts or chondrocytes, function in the development of muscle and heart, and regulate the development and progression of various cancer types [52]. The transcription factor, Twist-1, drives the expression of a 7.9 kb non-coding RNA transcript (from the *Dynammin-3* gene intron) encoding the miR-199a/miR-214 cluster [53].

MiR-199 family members are downregulated in HCC tumors and cell lines in association with poor overall survival. MiR-199a overexpression in HCC cell lines has been shown to inhibit cell proliferation, migration and invasion by targeting Rho-associated coiled-coil kinase 1 (ROCK1), Regulators of G-protein signaling (RGS) and X-box binding protein 1 (XBP1) [54–56]. Interestingly, the miR-199a/miR-214 cluster is subject to the same regulation processes, which seems not so complete. In terms of the effects of thyroid hormones on these miRNAs, the results were unexpected. Earlier studies by our group revealed an interesting regulatory mechanism whereby thyroid hormones exert opposite effects on miR-199a and miR-214. Notably, miR-199a is downregulated by T_3 /TR while miR-214 is upregulated. The differential regulatory effects on miR-199a and miR-214 are attributed to different TREs in the region between the two miRNAs [47]. Analogous to this finding, miR-17-92 clusters [play distinct roles in lymphomas and solid tumors [57].

MiR-130b acts as an oncogene in HCC, showing significantly higher expression in tumor tissues compared to matched normal tumor-adjacent tissues. Clinical analyses disclosed that high expression of miR-130b is significantly correlated with venous infiltration, high Edmondson-Steiner grade and advanced tumor-node-metastasis (TNM) stage [58]. The serum miR-130b level may present an ideal marker for monitoring the recurrence and prognosis of primary hepatocellular carcinoma (PHC) after radiofrequency ablation (RFA) treatment [59]. The miR-130b gene inhibits protein expression of Notch-Dll1 to promote proliferation, invasion and metastasis of liver cancer cells [60]. Negative regulation of miR-130b by T_3 /TR has been reported with the identification of a novel pathway crosslink? T_3 /TR, miR-130b and its target gene, interferon regulatory factor 1 (IRF1), which affects the EMT-related genes, p-mTOR, p-STAT3, and the p-AKT cascade, in turn, regulating motility and invasion of hepatoma cells [61].

In addition to miR-130b, miR-21 is a potential tumor biomarker of liver cancer, and combined detection of miR-130b and miR-21 in serum is suggested to aid in diagnosis of HCC [62]. Another study confirmed an oncogenic role of miR-21 and its utility as a potential diagnostic biomarker for

early diagnosis of HCC [63]. MiR-21 expression in exosomes is positively correlated with that in cells and negatively correlated with expression of its target genes, such as PTEN, PTENp1 and TETs, to affect HCC cell growth [64]. Serum visfatin is associated with histology and metastasis of HCC. Serum levels of both visfatin and miR-21 were shown to be significantly higher in HCC patients. Visfatin promoted miR-21 expression and migration of HepG2 cells, suggesting that visfatin-induced HCC cell migration is attributable to upregulation of miR-21 [65]. In addition to visfatin, thyroid hormone affects the performance of miR-21 in HCC. MiR-21 is activated by T_3 through its binding to a native T_3 response element in the primary promoter region. Stimulation of miR-21 by T_3 and subsequent suppression of T-cell lymphoma invasion and metastasis 1 (TIAM1) promotes hepatoma cell migration and invasion [66].

Reports on the role of miR-17 in HCC are controversial and the issue of whether this miRNA plays a carcinogenic or tumor suppressor role is yet to be established. Several studies have shown that miR-17 is highly expressed in HCC, especially in patients with higher risk of metastasis, supporting its utility as a novel prognostic marker [67,68]. The p38 MAPK pathway plays a crucial role in miR-17-induced phosphorylation of HSP27 and enhances HCC cell migration [69]. MiR-17 affects cell growth through direct targeting of SMAD family member 3 (SMAD3) in HCC cells. Downregulation of SMAD3 was shown to be consistently associated with high miR-17 levels in HCC tissues of patients, compared with normal liver tissues [70]. However, a few studies put forward the opposite view that miR-17 has the ability to inhibit tumor spread in liver cancer patients with a higher risk of metastasis. In one of these studies, HCC patients with lower miR-17 and higher ERBB3 levels had significantly shorter overall survival (OS) and progression-free survival (PFS) rates after surgical resection [71]. In another study, miR-17 suppressed postoperative metastasis of hepatocellular carcinoma through blocking the HGF/ERBB3-NF- κ B positive feedback loop [71]. Global gene expression profiling data from another investigation indicate that miR-17 targets and inhibits MYC to affect cell cycle progression, supporting the potential of anti-miR-17 as a therapeutic strategy for MYC-driven HCC [72]. T_3 /TR negatively regulates miR-17 expression in HCC. MiR-17 expression was significantly negatively associated with TR α 1 and matrix metalloproteinase-3 (MMP-3) in HCC, suggesting that T_3 /TR, miR-17 and MMP3 activities are interlinked in the regulation of metastasis [73].

MiR-206 plays an important regulatory role in cell growth in multiple cancer types. Reduced expression of miR-206 has been reported in HCC [74,75]. MiR-206 directly targets the 3' UTRs of cyclin-dependent kinase 9 (CDK9), cMET and protein tyrosine phosphatase 1B (PTP1B) genes for silencing, in turn, inhibiting HCC proliferation and invasion and simultaneously promoting apoptosis [74–76]. Liver cancer stem cells (CSC) are involved in tumor progression, drug resistance and recurrence of HCC. MiR-206 was reduced in patients with chemoresistant and recurrent HCC and shown to suppress HCC cell dedifferentiation and liver CSC expansion by targeting the epidermal growth factor receptor (EGFR) signaling pathway [77]. The action of thyroid hormone on lipid metabolism in liver is associated with a number of genes involved in lipogenesis and

lipid metabolism. Serum miR-206 expression is reduced in patients with hyperthyroidism. In addition, miR-206 is involved in T₃-mediated regulation of lipid metabolism in HCC cells, indicating a role in thyroid hormone-induced disorders of lipid metabolism in liver [78].

Hepatic lipid droplets (LD) are associated with metabolic syndrome, type 2 diabetes, hepatitis C, and both alcoholic and non-alcoholic fatty liver disease. The novel miRNA, miR-181d, has been identified as an inhibitor that decreases LD levels by about 60%. Data from biochemical assays showed that miRNA-181d reduces cellular triglyceride and cholesterol ester levels that may be relevant in hepatic diseases arising from obesity and alcohol abuse [79]. Thyroid hormone regulates the transcription of numerous metabolic genes in the liver through interactions with its nuclear receptors. MiRNA-181d is regulated by thyroid hormone in hepatic cells and functions in negative regulation of key metabolic genes through effects on two novel thyroid hormone-regulated target genes, caudal type homeobox 2 (CDX2) and sterol O-acyltransferase 2 (SOAT2 or ACAT2) [80].

Thyroid hormone regulates long non-coding RNA expression in liver cancer

In addition to miRNAs, several studies have revealed increasingly important roles of lncRNAs that also belong to the non-coding RNA family. The mechanism of action of lncRNAs is relatively complex, compared to that of miRNAs. Emerging evidence suggests a crucial role of lncRNAs in tumor progression of HCC. Various HCC-related lncRNAs have been shown to display aberrant expression patterns and participate in cancerous phenotypes, such as proliferation, evasion of apoptosis, promotion of vessel formation, and metastasis capability [37]. The most typical lncRNA in HCC associated with the thyroid hormone is brain cytoplasmic RNA 1 (BCYRN1/BC200), a 200-nucleotide ncRNA originally identified as a neuron-specific transcript that is abnormally overexpressed in several tumor types, such as colorectal, breast, lung and liver cancer [81–83]. A number of recent studies have focused on the mechanisms by which abnormal expression of BC200 RNA contributes to cancer development. BC200 expression is reported to be significantly higher in HCC tissues and effective as an independent prognostic marker. Moreover, BC200 affects cell proliferation and migration through influencing c-Myc, Bax and Bcl-xL expression [84] and induces cell growth and tumor sphere formation via regulation of cell cycle-related genes and stemness markers (CD133, CD44, Nanog and Sox2). Simultaneously, BC200 protects cyclin E2 mRNA from degradation via direct interactions and promotes CDK2–cyclin E2 complex formation [85]. Using the Human Disease-Related lncRNA Profiler to identify the lncRNAs regulated by thyroid hormone in HepG2 cells overexpressing thyroid hormone receptor $\alpha 1$ (HepG2-TR $\alpha 1$), BC200 was identified as an lncRNA downregulated by the thyroid hormone. The results support the existence of a novel pathway involving thyroid hormone-mediated regulation of BC200, cyclin E2 and CDK2, which, in turn, modulates proliferation and tumor sphere formation of hepatoma cells, highlighting the potential of therapeutic strategies targeting BC200 and associated molecules for treatment of HCC [85].

Our group used microarray analysis for profiling of other important lncRNAs in HepG2-TR $\alpha 1$ cells treated with/without T₃ and HCC specimens. These oncogenic lncRNAs were identified through random sampling verification and down-regulated by thyroid hormones [Table 1] [86].

Taurine up-regulated gene 1 (TUG1) is an lncRNA shown to play an oncogenic role in various cancers. TUG1 is highly expressed in HCC tissues and contributes to proliferation, metastasis and apoptosis via activation of distal-less homeobox 2 (DLX2) in HCC [94]. In view of the finding that TUG1 can be easily detected in patient samples and has clinical significance, this lncRNA may serve as a novel potential biomarker in HCC, as determined with a valid non-invasive technique [95]. The majority of research to date suggests that TUG1 predominantly acts as a miRNA sponge in HCC, exerting effects on miR-216b-5p, miR-142-3p, miR-144 and miR-132. Interactions of TUG1 with multiple miRNAs lead to its participation in various tumor processes in HCC [94,96–98]. Cancer cells can alter their glucose metabolism and activate aerobic glycolysis [99]. TUG1 is reported to induce cell migration, invasion, and glycolysis through suppression of miR-455-3p. MiR-455-3p directly interacts with the 3'UTR of adenosine monophosphate-activated protein kinase subunit beta 2 (AMPK $\beta 2$). The TUG1/miR-455-3p/AMPK $\beta 2$ axis regulates cell growth, metastasis, and glycolysis through modulation of hexokinase 2 (HK2) in HCC [100]. Hypothyroidism in patients is associated with high risk of HCC and levels of the glycoprotein, alpha-fetoprotein (AFP), are increased in the majority of patients with HCC. Despite the controversy regarding the utility of AFP, it still presents a useful diagnostic biomarker. TUG1 is regulated by thyroid hormone to affect AFP. Specifically, thyroid hormone suppresses TUG1 expression, leading

Table 1 lncRNAs negatively associated with thyroid hormones in the HepG2 liver cancer cell line.

lncRNAs Negatively Affected by Thyroid Hormones			
lncRNA	In HCC (Fold)	T ₃ /48 h (Fold)	In HCC Ref.
FAM215A	7.23	0.42	[87]
ANKRD20A8P	2.20	0.43	
HCG18	2.23	0.43	
PVT1	7.47	0.44	[88]
SNHG1	4.69	0.45	[89]
SNHG3	3.2	0.46	[90]
UNK	3.49	0.46	
TUG1	3.25	0.47	[86]
SNHG7	3.30	0.47	[91]
DDX11-AS1	10.20	0.47	[92]
DUSP5P	9.46	0.48	
AGSK1	2.79	0.49	
MFI2-AS1	5.07	0.49	[93]

Microarray analysis of long non-coding RNA (lncRNA) expression in two pairs of HCC specimens. Data are presented as average tumor/adjacent normal (T/N ratio) of the two specimen pairs. The specified lncRNAs were highly expressed (>2-fold) in HCC specimens. HepG2 cell lines overexpressing TR $\alpha 1$ were treated with thyroid hormone (10 nM) and subjected to microarray analysis of lncRNA expression after 48 h. Notably, the above lncRNAs were negatively affected (<0.5-fold) upon thyroid hormone stimulation. The references pertain to studies on the roles of these lncRNAs in liver cancer.

to downregulation of AFP. AFP is positively correlated with TUG1 levels and unfavorable prognosis in patients with non-hepatitis B/non-hepatitis C (NBNC) HCC [86].

While correlations of thyroid hormone with other lncRNAs have only been observed in our preliminary research, their oncogenic roles in HCC are clear-cut. Family with sequence similarity 215 member A (FAM215A) is a tissue-specific lncRNA with different roles in ovarian cancer and HCC. High expression of FAM215A in low-grade ovarian cancer and early-stage disease has been reported, compared to high-grade ovarian cancer and late-stage disease [101]. In HCC, overexpression of FAM215A is positively correlated with tumor size, vascular invasion, and pathology stage [87]. Overexpression of FAM215A accelerates proliferation and metastasis of HCC cells. FAM215A additionally increases doxorubicin (DOX) resistance in HCC in association with enhanced expression of lysosome-associated membrane protein 2 (LAMP2). Furthermore, FAM215A interacts with and stabilizes LAMP2 to increase tumor progression along with decreasing doxorubicin sensitivity [87].

Long non-coding small nucleolar RNA host genes (SNHG) are abnormally expressed in multiple cancers, including urologic neoplasms, respiratory tumors, and digestive cancers, and crucial for tumor progression [102]. SNHGs, including SNHG1, SNHG3, SNHG6, SNHG16, and SNHG20, are reported to play variable roles in HCC via multiple regulatory mechanisms, with both promotory and inhibitory effects on tumorigenesis [90,103]. SNHG1 is an oncogenic lncRNA highly expressed in different tumor types, including colorectal, liver, lung, prostate and gastric cancer. Expression of SNHG1 is significantly positively associated with advanced tumor stage, tumor size, TNM stage, and decreased overall survival in liver cancer. Furthermore, aberrant expression of SNHG1 contributes to cell proliferation, metastasis, migration and invasion of HCC cancer cells [104]. Mechanistically, SNHG1 is proposed to act as an miRNA sponge (miR-195-5p and miR-21) and enhance tumor cell proliferation and invasion ability through targeting and inhibition of p53 by binding DNA methyltransferase 1 (DNMT1) [105–107]. Another member of the SNHG family, SNHG3, is highly expressed in HCC tissues and positively correlated with tumor size, portal vein tumor thrombus (PVTT), and relapse. Additionally, high expression of SNHG3 is correlated with OS, recurrence-free survival (RFS), and disease-free survival (DFS) [108]. Simultaneously, SNHG3 is suggested to promote tumor invasion and sorafenib resistance by affecting MET via the miR-128/CD151/Akt/PI3K [109] and miR-326/Sma and Mad Related Family 3 (SMAD3)/zinc finger E-box binding homeobox 1 (ZEB1) signaling pathways [90].

DDX11-AS1 expression is markedly higher in HCC tissues and cell lines and associated with poor predicted overall survival [110]. DDX11-AS1 promotes cell proliferation in gastric cancer through inhibiting miR-30-5p and miR-145-5p [111]. Proliferation, cell cycle progression, migration, and invasion of HCC cells are reduced upon DDX11-AS1 silencing. RNA immunoprecipitation (RIP) and chromatin immunoprecipitation (ChIP) findings suggest that DDX11-AS1 reduces expression of large tumor suppressor kinase 2 (LATS2) by interacting with Enhancer of zeste homolog 2 (EZH2) and DNA (cytosine-5)-methyltransferase 1 (DNMT1) in HCC cells [92].

Discussion

The majority of the human genome is not translatable into protein but can be transcribed into RNA. While non-coding RNAs do not encode proteins, aberrations in these molecules contribute to several disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), Huntington's disease (HD) and various cancer types [112]. These findings are mainly attributable to the development of high-throughput RNA sequencing technology over the years that has facilitated the discovery of thousands of non-coding RNA genes. A simple sub-classification system is generally used, with ncRNAs divided into short (sncRNAs) and long ncRNAs (lncRNAs) based on a size cut-off of >200 bases. The sncRNA group comprises microRNAs (miRNAs) with small fragment sizes of 21–23 bases [113]. Non-coding RNAs have been shown to be involved in liver cancer progression. For instance, miR-296-5p attenuates the epithelial–mesenchymal transition (EMT) program through Neuregulin (1NRG1)/erb-b2 receptor tyrosine kinase 2 (ERBB2)/ERBB3 signaling. Moreover, miR-296-5p exerts an inhibitory effect on the stemness potency of HCC cells via direct targeting of the Brahma-related gene-1 (BRG1)/Sal-like protein 4 (SALL4) axis [114]. MiR-486-3p is downregulated in sorafenib-resistant HCC cell lines and tumor tissue relative to adjacent normal tissue in HCC patients and could induce apoptosis by targeting fibroblast growth factor receptor 4 (FGFR4) and EGFR [115]. Golgin A2 pseudogene 10 (GOLGA2P10) is frequently upregulated in HCC tissues as well as hepatoma cells subjected to endoplasmic reticulum (ER) stress inducers. Higher GOLGA2P10 levels are correlated with shorter recurrence-free survival of HCC patients. GOLGA2P10 enhances Bcl-xL protein levels and BAD phosphorylation, and confers resistance to ER stress-induced apoptosis in tumor cells [116]. Expression of cancer susceptibility candidate 15 (CASC15) is elevated in HCC tissues and positively correlated with tumor size, TNM stage and metastasis. CASC15 activates the Wnt/ β -catenin pathway via enhancing expression of SRY-Box Transcription Factor 4 (SOX4), thus promoting tumor progression [117]. While several non-coding RNAs with different sizes, functions and significance have been identified over the years, this review has mainly focused on the miRNAs and lncRNAs involved in liver cancer and their correlations with the thyroid hormone pathway.

A number of molecular mechanisms have been proposed to explain the involvement of the thyroid hormone and effects on non-coding RNAs in liver cancer [Fig. 1], and thyroid hormone-associated non-coding RNAs including their roles and expression patterns in liver cancer [Table 2] are listed.

Thyroid hormones, a transcription factor affect the expression of various non-coding RNAs (miRNAs and lncRNAs). This leads to several regulatory pathways in liver cells.

Previously, from ours or other reports indicate that the thyroid hormone is involved in various mechanisms underlying the growth and development of liver cells, inevitably resulting in different roles. An earlier study reported hypothyroidism over ten years in female patients with

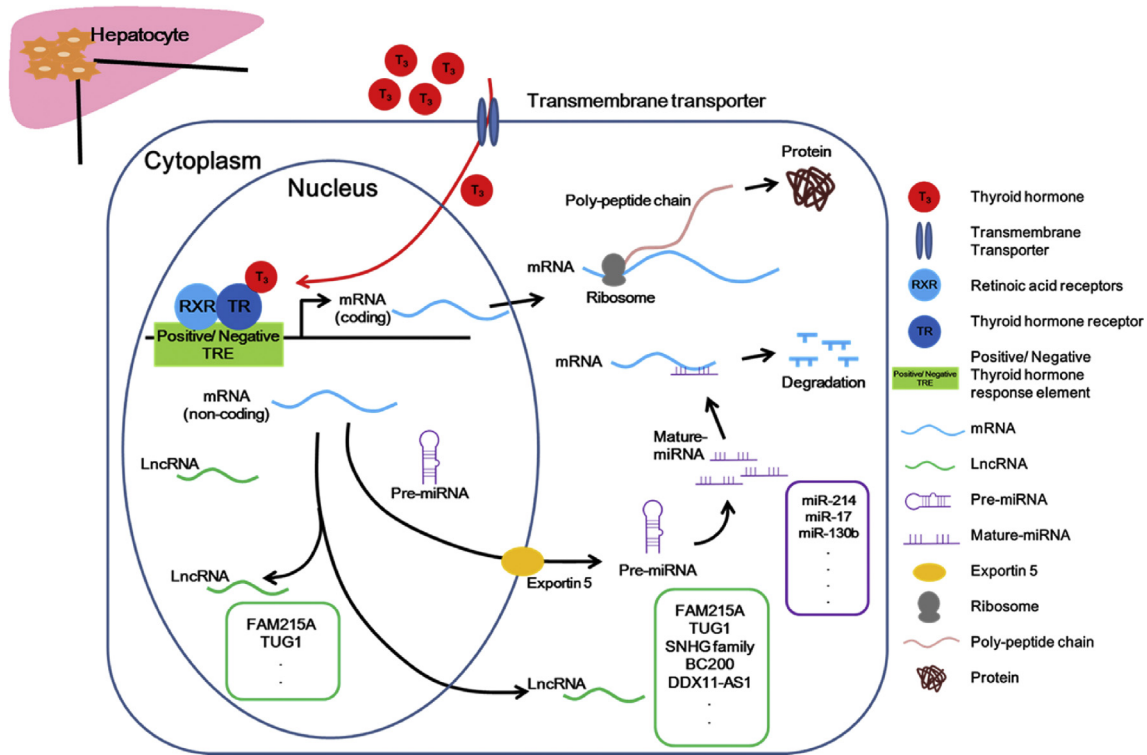


Fig. 1 Thyroid hormones regulate various non-coding RNAs in liver.

hepatocellular carcinoma, indicating a significant association of hypothyroidism with high risk of HCC in females independent of known risk factors [118]. In clinical samples of liver cancer patients, thyroid-stimulating hormone receptor (TSHR) is overexpressed, indicative of a hypothyroid state [119]. At the same time that in terms of therapeutic strategies, thyroid

hormones and/or thymomimetics may be useful for treatment of patients with HCC [14]. Thus, thyroid hormone acts as a transcription factor and its role as a tumor suppressor has been confirmed at the genomic level. We also found that thyroid hormone suppresses HCC development through protecting hepatocytes from HBx-induced damage in transgenic

Table 2 Differential effects of thyroid hormone via regulation of multiple non-coding RNAs in liver cancer.

Gene	Role	Target	Function	T ₃ /TR	Ref.
Thyroid hormone as a tumor suppressor					
miR-214	Suppressor	HDGF	Angiogenesis	Up	[44]
		FLOT1	Proliferation		[46]
		PDK2	Metastasis		[42]
		PHF6	Metabolism		
miR-17	Dual role	PIM-1	Proliferation	Down	[47]
		MAPK	Metastasis		[69]
		SMAD3			[70]
		MYC			[72]
miR-130b	Oncogene	MMP3	Proliferation	Down	[73]
		Notch-Dll1	Metastasis		[60]
		AP-1			
BC200	Oncogene	IRF1	Proliferation	Down	[61]
		c-Myc	Sphere formation		[84]
		Bax			
		Bcl-xL			
		CD133			
		CD44			
		Nanog			
		Sox2			
		Cyclin E2			

(continued on next page)

Table 2 – (continued)

Gene	Role	Target	Function	T ₃ /TR	Ref.
Thyroid hormone as a tumor suppressor					
TUG1	Oncogene	miR-216b-5p miR-142-3p miR-144 miR-132 AFP	Proliferation Metastasis Apoptosis	Down	[94] [96] [97] [98] [86]
FAM215A	Oncogene	LAMP2	Proliferation Metastasis Drug resistance	Down	[87]
SNHG family	Oncogene	miR-195-5p miR-21 miR-128 miR-326 SMAD3	Proliferation Metastasis Drug resistance	Down	[105] [106] [109] [90]
DDX11-AS1	Oncogene	LATS2 EZH2	Proliferation Metastasis	Down	[92]
Thyroid hormone as an oncogene					
Gene	Role	Target	Function	T ₃ /TR	Ref.
miR-199	Suppressor	ROCK1 RGS XBP1	Proliferation Metastasis	Down	[56] [55] [54]
miR-21	Oncogene	PTEN PTENp1 TETs TIAM1	Proliferation Metastasis	Up	[64] [66]
miR-206	Suppressor	CDK9 cMET PTP1B EGFR	Proliferation Metastasis Apoptosis Drug resistance Metabolism	Down	[74] [75] [76]
Potential roles of thyroid hormone-related non-coding RNAs.					

mice via activation of PTEN-induced kinase 1 (PINK1) [120]. And in a murine model of diethylnitrosamine (DEN)-induced HCC, thyroid hormone suppresses the carcinogenic process via activation of death-associated protein kinase 2 (DAPK2), a serine/threonine protein kinase, which enhances phosphorylation of sequestosome 1 (SQSTM1) to promote selective autophagic clearance of protein aggregates [121]. The oncogene, Stathmin (STMN1), is downregulated by thyroid hormone in liver. Thyroid hormone-mediated suppression of STMN1 additionally supports its role as an inhibitor of HCC tumor growth and suggests that lack of normal THR function leads to elevated STMN1 expression and promotes malignancy [122]. As discussed, thyroid hormones play a suppressive role by repressing non-coding RNAs such as miR-214, miR-130b, TUG1 and BC200 to protect liver cells from damage and disease.

Taken together, based on the pathological and clinical analyses and data from different animal models, we propose that thyroid hormone acts as a tumor suppressor to repress several non-coding RNAs as well as coding-genes. In view of the finding that thyroid hormone and its receptor inhibit liver cancer progression, thyroid hormone receptor- β agonists, such as GC-1, MB07344 and KB2115, have been developed as treatment strategies that induce a good response in terms of hepatic metabolism and damage in various animal models. Results from a recent phase II trial consistently showed preventive effects on NAFLD accompanied by improved metabolism (decreased serum levels of LDL cholesterol and TG

[123]. Thus, therapeutic strategies involving modulation of thyroid hormone-regulated non-coding RNAs are feasible for treatment or diagnosis of liver diseases.

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Conflict of interest

The authors declare no competing financial interests.

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