

REVIEW

Long noncoding RNAs: emerging players in thyroid cancer pathogenesis

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Abstract

Thyroid cancer continues to be the most common malignancy of endocrine glands. The incidence of thyroid cancer has risen significantly over the past 4 decades and has emerged as a major health issue. In recent years, significant progress has been achieved in our understanding of the molecular mechanisms of thyroid carcinogenesis, resulting in significant diagnostic, prognostic and therapeutic implications; yet, it has not reached a satisfactory level. Identifying novel molecular therapeutic targets and molecules for diagnosis and prognosis is expected to advance the overall management of this common malignancy. Long noncoding RNAs (lncRNAs) are implicated in the regulation of various key cellular genes involved in cell differentiation, proliferation, cell cycle, apoptosis, migration and invasion mainly through modulation of gene expression. Recent studies have established that lncRNAs are deregulated in thyroid cancer. In this review, we discuss extensively the tumor-suppressive (for example, LINC00271, MEG3, NAMA, PTCSC1/2/3, etc.) and oncogenic (for example, ANRIL, FAL1, H19, PVT1, etc.) roles of various lncRNAs and their possible disease associations implicated in thyroid carcinogenesis. We briefly summarize the strategies and mechanisms of lncRNA-targeting agents. We also describe the potential role of lncRNAs as prospective novel therapeutic targets, and diagnostic and prognostic markers in thyroid cancer.

Key Words

- ▶ thyroid cancer
- ▶ papillary thyroid cancer
- ▶ lncRNAs
- ▶ oncogene
- ▶ tumor suppressor
- ▶ diagnosis
- ▶ prognosis

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Introduction

Thyroid cancer continues to be the most common endocrine malignant tumor. Over the past 40 years, the incidence of thyroid cancer has steadily escalated in many countries of the world (Fagin & Wells 2016, Howlader *et al.* 2017, Siegel *et al.* 2017). Thyroid cancers generally originate either from follicular or parafollicular C cells. The majority of thyroid malignant tumors such as papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC) originate from follicular cell-derived thyroid cells. Differentiated thyroid cancer (DTC) comprises the common thyroid cancer types,

PTC and FTC. Medullary thyroid cancer is derived from chief cells or parafollicular C cells which account for a minor portion of thyroid cancer (DeLellis *et al.* 2004, Fagin & Wells 2016). Among these subtypes (PTC, FTC, PDTC and ATC) of thyroid cancers, PTC is the major form of thyroid cancer accounting for approximately more than 85% (Fagin & Wells 2016). ATC is the most aggressive and deadly thyroid cancer accounting for less than 3% and its overall survival rate is only 3–5 months after initial diagnosis (Fagin & Wells 2016, Howlader *et al.* 2017). Currently, the common treatment methods for DTC are total thyroidectomy followed in some patients

with radioactive iodine remnant ablation and thyroid hormone-suppressive therapy (Cooper 2009, Fagin & Wells 2016). In recent years, after the initial treatment, patients are closely followed by neck ultrasonography, serum thyroglobulin measurement and in some cases by whole body scanning of radioactive iodine and computed tomography, magnetic resonance imaging or positron emission tomography on a long-term basis in order to detect the recurrence at an early stage and implement therapeutic measures when necessary (Cooper 2009, Pacini & Castagna 2012, Fagin & Wells 2016). Although the outcome of DTC is excellent in the majority of cases, it partly shows a poor satisfactory outcome as tumor persistence, and the recurrence rate is ~30% even after many years of remission (Cooper 2009, Pacini & Castagna 2012, Grant 2015).

Though cancer is a multifactorial disease, it has been well ingrained that genetic, epigenetic alterations and environmental factors are the basis for carcinogenesis and these factors play pivotal roles in the pathogenesis and/or progression of the various types of human cancers including thyroid cancer (Vogelstein & Kinzler 2004, Xing 2013, Fagin & Wells 2016). For the past 30 years of study of molecular mechanisms, thyroid cancer facilitated understanding of various genetically deregulated genes of many pathways: among them, two key signaling pathways are at the heart of thyroid cancer pathogenesis. The mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/Akt pathways have been shown to be frequently altered by different mechanisms in thyroid cancer (Xing 2013, Fagin & Wells 2016, Landa *et al.* 2016). PTC frequently harbors activating mutations in the genes such as *RET/PTC*, *BRAF*, *RAS*, etc., of the MAPK pathway (Grieco *et al.* 1990, Xing 2005, Hou *et al.* 2007, Agrawal *et al.* 2014, Murugan *et al.* 2016) except the ERK (Murugan *et al.* 2009) and infrequently harbors mutation in the genes such as *EGFR*, *P53*, *EIF1AX*, *PPM1D*, *CHEK2*, *NF1*, etc., (Masago *et al.* 2009, Murugan *et al.* 2011, Ricarte-Filho *et al.* 2012, Agrawal *et al.* 2014) but not commonly in the genes of PI3K/Akt pathway (Xing 2010, Murugan *et al.* 2015a). FTC and ATC commonly harbor genetic mutations/amplifications/fusions in genes such as *P53*, *RAS*, *ALK*, *PIK3CA*, *PTEN*, *AKT*, *PDK1*, *RASAL1*, *RET*, *NTRK1/3*, *PAX8-PPAR γ* , etc. (Hou *et al.* 2007, Liu *et al.* 2008, 2013a, Murugan & Xing 2011, Landa *et al.* 2016). Recently, PDTC and ATC have been shown to harbor *BRAF* mutations: 33% and 45%, respectively (Landa *et al.* 2016). Thyroid cancers have been shown to be mutated in certain genes of the metabolic and regulatory pathways such as *IDH1* (Hemerly *et al.* 2010, Murugan *et al.* 2010)

and *TERT* (Landa *et al.* 2013, Liu *et al.* 2013b, 2014, Xing *et al.* 2014, Qasem *et al.* 2015), respectively. Genetic deregulation of these genes results in hyperactivation of MAPK and PI3K/Akt signaling pathways that drive the cell for uncontrolled cell proliferation, growth, survival, invasion and metastasis in thyroid cancer.

In thyroid cancer, genetic and epigenetic deregulations in the MAPK, PI3K/Akt and other signaling pathway-associated genes altogether account for 90–95% (Xing 2013, Agrawal *et al.* 2014, Fagin & Wells 2016, Landa *et al.* 2016); the remaining ~5–10% of genetic and epigenetic factors involved in thyroid cancer are unidentified and their molecular basis is not well understood. Recently, long noncoding RNAs (lncRNAs) have been demonstrated to play crucial roles in thyroid tumorigenesis (Lan *et al.* 2015a, Yang *et al.* 2016, Wang *et al.* 2016, Liyanarachchi *et al.* 2016, Xu *et al.* 2016, Li *et al.* 2017a). lncRNAs and their potential role in follicular cell-derived thyroid tumorigenesis are the central focus of this review. To date, this is the first review to address lncRNA involvement in thyroid cancer pathogenesis. This review mainly focuses on the role of lncRNAs as being tumor-suppressive and oncogenic, and further discusses their potential importance as a therapeutic target, and diagnostic and prognostic biomarkers for the ever increasing deadly thyroid cancer.

Noncoding ribonucleic acids (ncRNAs)

In mammals, although >90% of the genome is transcribed, only about 2% of the genome is subsequently translated. The remaining majority of the transcribed genes lack protein-coding capacity and are left un-translated. As these RNA transcripts do not encode for any proteins, they are grouped as ncRNAs. The noncoding genomic regions serve as a template for the transcription of a large number of ncRNAs in addition to their role as a substrate for DNA binding protein (Carninci *et al.* 2005). Based on the size of the ncRNAs, they are categorized into two groups including short ncRNAs and long ncRNAs. MicroRNAs (miRNAs), piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), transfer RNAs (tRNAs) and some ribosomal RNAs (rRNAs) are grouped under short ncRNAs. lncRNAs include long intergenic noncoding RNA (lincRNA), intronic long noncoding RNA (ilncRNA), natural sense or antisense transcript, promoter-associated long noncoding RNA (paRNA), promoter upstream transcript (PROMPT), repetitive element-associated noncoding RNA, pseudogene long noncoding RNA (plncRNA), transcribed ultraconserved region (T-UCR) and enhancer-like noncoding RNA (elncRNA). Among these

ncRNAs, miRNAs are the most extensively studied ncRNAs in human cancers (Huarte 2015, Murugan *et al.* 2015b, Tye *et al.* 2015). Other types of ncRNAs have already been reviewed in thyroid cancer (Li & Wang 2012, Kentwell *et al.* 2014, Zhang *et al.* 2016), while this review addresses only lncRNAs in thyroid cancer.

lncRNAs and their biological functions

lncRNAs, >200 nucleotides in length, do not code for proteins and they are shorter than mRNAs as lncRNAs have fewer exons. These RNA molecules vary in size with the length ranging from 200bp to 100kb. Recently, the ENCODE project revealed that ~80% of the human genome is transcribed into 14,880 lncRNAs from 9277 loci (Derrien *et al.* 2012). The concept of lncRNAs was introduced more than 2 decades ago. However, the

focus on lncRNAs has been neglected generally as the expression of lncRNAs are much lower than those of mRNAs (Carninci *et al.* 2005, Derrien *et al.* 2012), and exhibit evolutionarily poor sequence conservation across species (Taft *et al.* 2007). Currently, lncRNA research has moved to the forefront of human cancer research as recent findings revealed a crucial role of lncRNAs in gene modulation. lncRNAs are mainly involved in the epigenetic regulation of expression of various genes at different levels including chromatin, splicing, transcriptional and post-transcriptional. They epigenetically regulate expressions of many salient genes involved in vital cellular biological processes such as autophagy, cell differentiation, cell cycle regulation, cell proliferation, migration and invasion, apoptosis and mesenchymal stem cell differentiation (Fig. 1) (Huarte 2015, Tye *et al.* 2015).

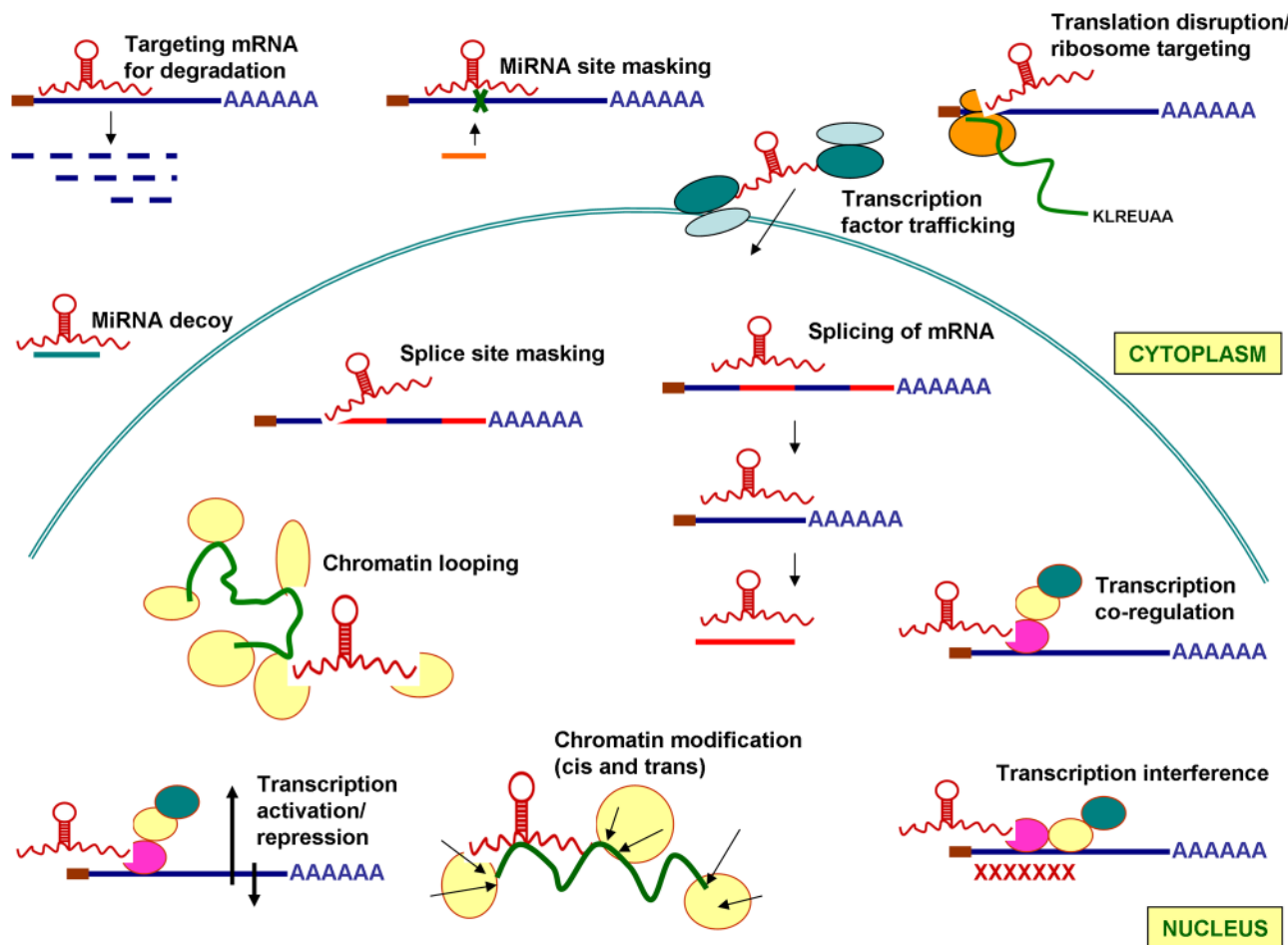


Figure 1

lncRNAs and their functions. This illustration shows various functions of lncRNAs. In the nucleus, lncRNAs are involved in chromatin looping, chromatin modification in both *cis* and *trans*, transcription regulation (activation or repression) and mRNA splicing. lncRNAs target mRNA for degradation or protection, transcription factor trafficking, miRNA site masking and translation disruption/ribosome targeting in the cytoplasm. A full colour version of this figure is available at <https://doi.org/10.1530/ERC-17-0188>.

lncRNAs in human cancers

Concurrent knowledge on lncRNAs clearly shows that they are implicated in many types of human diseases, including cancer (Huarte 2015). Based on cellular function, lncRNAs could also be classified broadly into oncogenic and tumor-suppressive lncRNAs in a way similar to the protein-coding genes. For example, lncRNA HOTAIR has been known to be deregulated in a wide spectrum of human cancers such as oral, nasopharynx, breast, esophagus, lung, liver, pancreas, colon, endometrium and cervix cancers (Huarte 2015). Expression of PCA3 (prostate cancer antigen 3) is restricted to prostate tissue and it is highly overexpressed in tumors when compared to normal prostatic tissue of the same cases (Bussemakers *et al.* 1999). PCGEM1 is highly prostate-specific and its elevated expression has been associated with high-risk populations, such as African-Americans compared to Caucasian-Americans (Petrovics 2004). PRNCR1 (prostate cancer noncoding RNA 1) is upregulated in prostate cancer and has been reported to be highly associated with prostate cancer susceptibility in Europeans and African-Americans (Chung *et al.* 2011). HULC (highly upregulated in liver cancer) has been found to be overexpressed both in hepatocellular carcinoma (Panzitt *et al.* 2007) and in gastric cancer and is also implicated in hepatic colorectal carcinoma metastasis (Matouk *et al.* 2009). Nonetheless, the variant rs7763881 in HULC has been found to be associated with a decreased risk of HCC in persistent carriers of HBV (Liu *et al.* 2012). Recently, CCAT1 has been demonstrated to be upregulated and shown to predict poor prognosis in oral squamous cell carcinomas (Arunkumar *et al.* 2017).

lncRNAs in thyroid cancer

Genome-wide profile of lncRNAs in thyroid cancer

lncRNAs play a crucial role in many key cellular functions and deregulations of lncRNAs have been identified in many complex human diseases including cancers. Recently, a genome-wide analysis (GWAS) of lncRNA expression profile in PTCs identified various up- and downregulated lncRNAs in PTC samples (Lan *et al.* 2015a). This group investigated the lncRNA and mRNA expression profiles in 62 PTC tissues with paired normal thyroid tissue by microarray and validated 10 differentially expressed lncRNAs using qRT-PCR. Gene functions were investigated using gene ontology and KEGG pathway analyses and two independent algorithms were used to predict possible

target genes of these lncRNAs. In this microarray-based study, Lan and coworkers found thousands of significantly differentially expressed lncRNAs and mRNAs compared to nonthyroid cancer tissue (Lan *et al.* 2015a). Furthermore, 1805 deregulated lncRNAs have been found to have *cis* or *trans* target genes. In the *cis* target genes, 463 of them were found to be differentially expressed and it has been suggested that they might be regulated by lncRNAs in the process of PTC carcinogenesis. Among the top 20 upregulated lncRNAs, a minimum of 86-fold to a maximum of 204-fold change has been observed. Furthermore, they observed a minimum of 47-fold to a maximum of 148-fold changes among the top 20 downregulated lncRNAs. Of these lncRNAs, 10 lncRNAs have been identified as statistically significant and are differentially expressed as up/downregulated with the similar trend (Lan *et al.* 2015a). A human lncRNA-based microarray study found abnormal expression of 675 lncRNAs in 3 pairs of PTC compared with paired normal thyroid tissues (Yang *et al.* 2016). Moreover, analysis of 12 samples with matched normal tissue by RNA sequencing and qRT-PCR identified 188 differentially expressed lncRNAs. Among them, NONHSAT076747 and NONHSAT122730 were associated with lymph node metastasis (LNM), while the expression of NONHSAG051968 was correlated negatively with tumor size (Wang *et al.* 2016). However, except for a few, the majority of lncRNAs identified in these GWAS are largely uncharacterized. In order to understand the functional role of these deregulated lncRNAs in thyroid cancer, an individual functional characterization of these lncRNAs is warranted at least for the top 10–20 up/downregulated lncRNAs in PTCs.

Recently, the genome-wide RNA sequencing of 12 PTC tumors with adjacent normal tissue disclosed 218 differentially expressed lncRNAs in PTC. Among the deregulated lncRNAs, two (XLOC_051122 and XLOC_006074) were found to be significantly overexpressed in patients with wild-type *BRAF* and LNM (Liyanarachchi *et al.* 2016). Moreover, overexpression of these 2 lncRNAs was observed to correlate significantly with LNM and *BRAF* mutation status. If confirmed in further studies, these 2 lncRNAs could serve as a prognostic biomarker in PTC as they were associated with adverse prognostic risk factors.

In addition, Xu and coworkers performed array hybridization of 22 PTC tumor samples with matched normal tissues using SBC 4X180K human microarrays. Compared to the normal tissues, a total of 777 differentially expressed lncRNAs were identified in PTCs. Among them, 325 upregulated and 452 downregulated

lncRNAs have been identified. Microarray expression data were further validated by the RT-PCR in 46 PTC tumor samples with paired normal tissues and they found consistency with the microarray data. It has also been shown that ENST00000537266 and ENST00000426615 were found to be upregulated in thyroid cancer (Xu *et al.* 2016). Recently, a microarray-based study detected 1593 differentially expressed lncRNAs in PTC (Li *et al.* 2017a). The later two study results are discussed in detail in this review under the subtitle: Oncogenic lncRNAs and their role in thyroid cancer.

Tumor-suppressive lncRNAs and their role in thyroid cancer

NAMA (noncoding RNA associated with MAP kinase pathway and growth arrest)

NAMA, the first lncRNA in thyroid cancer, was delineated by Albert de la Chapelle and his group a decade ago (Yoon *et al.* 2007). It showed several unique characteristic features distinguishing it from typical protein-coding counterpart genes. NAMA was assumed not to be a protein-coding gene but a noncoding RNA (ncRNA) as it lacked significant ORFs and most exons showed very low sequence conservation between human and mouse. The NAMA has been downregulated in PTCs with the BRAF (V600E) mutation that led the researchers to investigate whether NAMA could be modulated by the MAPK pathway. It has been demonstrated that NAMA was triggered upon treatment with BRAF or MEK inhibitors and it was also induced by serum depletion in NPA87 cells, but not in K1 cells with mutant BRAF that confers constitutive activation and helps the cells to continuously grow even in the absence of upstream signals. Moreover, NAMA has also been induced by inhibiting MEK in the HCT116 cell line suggesting that regulation of NAMA expression by the MAPK pathway was not exclusively specific to thyroid cells. These experimental observations suggested clearly that the NAMA could be a downstream target of MAPK pathway.

Treatment with the MEK inhibitor U0126 inhibited K1 and NPA87 cells at the G1 phase. Although >90% of K1 cell arrest at the G1 phase resulted in clear induction of NAMA, under serum-free conditions, no significant shift of the K1 cell population into G1 or S was seen when compared with controls, while the NAMA expression was unchanged. On the other hand, under serum-free conditions, U0126-treated NPA87 cells showed >20% increased cell cycle arrest at the G1 phase with NAMA induction. This association was further evidenced

by sustained cell cycle arrest after DNA damage that accompanied NAMA upregulation, which suggests that NAMA is a direct target of the MAP kinase pathway, and hence NAMA induction results in cell growth arrest and apoptosis. Alternatively, it has been speculated that induction of NAMA could be secondary to cell cycle arrest and apoptosis. To rule this out, spliced forms of NAMA was overexpressed and unspliced NAMA was knocked down by siRNA. Nonetheless, it failed to detect any phenotypic changes, while NAMA was transfected and overexpressed in K1 cells. As only unspliced forms of NAMA are seen in thyroid cells, it has been hypothesized that the unspliced NAMA is likely to be the active form, perhaps in a tissue-specific manner (Yoon *et al.* 2007). Furthermore, siRNA-mediated knockdown of NAMA expression in K1 cells resulted in differential effects. Of two siRNAs, siN-210 induced apoptosis with a longer-lasting effect while siN-630 induced cell cycle arrest with minimal strength. These observations suggested that the NAMA is likely to mediate a protective response. They also concluded that NAMA may also behave as a multifaceted molecule and/or has dual effects depending on the molecular interaction with various stimuli (Yoon *et al.* 2007).

A recent study investigated the role of NAMA in thyroid carcinogenesis by analyzing the expression of NAMA in a total of 40 pairs of PTC tissue and paired normal tissue by qRT-PCR. Expression of NAMA was significantly downregulated in PTC compared with normal tissue. Furthermore, as thyroid cell proliferation is mediated by the interaction of TSH and its receptor (TSHR), the effect of NAMA suppression on TSHR induction was evaluated using a PTC cell line (IHH-4). Nonetheless, silencing NAMA did not modulate TSHR level in IHH-4 cells suggesting that NAMA is likely to play a role in PTC not via TSHR (Zheng *et al.* 2016). These results suggest that NAMA is likely to function as tumor-suppressive lncRNA by playing a protective role in thyroid cancer. However, current knowledge on the understanding of the roles of NAMA in thyroid cancer is very limited. More work will be necessary, including the identification of the molecular targets of NAMA as the majority of lncRNAs are thought to play their key regulatory functions by interacting with DNA, RNA and proteins.

PTCSC3 (papillary thyroid carcinoma susceptibility candidate 3)

GWAS addressed the genetic predisposition to PTC and found two independent SNPs (rs944289 and rs965513) in the chromosomal regions containing nonannotated genes

and a significant association with PTC (Gudmundsson *et al.* 2009, 2012, Takahashi *et al.* 2010). At 3.2-kb downstream of the SNP rs944289 in 14q.13.3, Albert de la Chapelle and his group identified a lincRNA gene and named it *PTCSC3*. Its expression was evaluated in 46 PTC cases using qRT-PCR (Jendrzejewski *et al.* 2012). *PTCSC3* expression was strongly downregulated in thyroid tumor tissue and the expression was associated with the risk allele (T) genotype (TT) ($n=21$) compared with the genotype (CT) ($n=19$). On the other hand, the genotype (TT) was associated with upregulation of *PTCSC3* in normal thyroid tissue. The SNP rs944289 was identified in the binding site for C/EBP α and β . Risk allele has been shown to destroy the binding site *in silico*. Furthermore, in reporter assays, both C/EBP α and β activated the *PTCSC3* promoter and the risk allele (T) reduced the activation compared with the nonrisk allele (C). Transient transfection of a *PTCSC3*-expression plasmid in PTC cell lines which lack *PTCSC3* expression (TPC-1 and BCPAP) resulted in decreased cell growth and downregulation of expression of genes involved in DNA replication, recombination, motility, morphology and apoptosis (Jendrzejewski *et al.* 2012). This study included only two cell lines and they usually accumulate genetic changes over time as a consequence; this was the major limitation of this study, although the results were distinct and conclusive. Furthermore, a future study is warranted with multiple cell lines and original tumor samples. Another study showed that the SNP rs944289 was found to be associated with an increased risk of both benign and malignant thyroid tumors in Japanese patients (Rogounovitch *et al.* 2015). Although the expression of *PTCSC3* is highly thyroid-tissue-specific, no somatic mutations or new SNPs were found in the *PTCSC3* gene. The study suggested that the SNP rs944289 is likely to predispose to PTC via *PTCSC3*, a novel, tumor-suppressive lincRNA. Recently, five variants including the above-studied SNPs (rs944289 and rs965513) were assessed for the utility in the management of PTCs. It was found that larger tumor size and extrathyroidal expansion were associated with risk allele of the variant rs965513. Association with LNM and tumor multifocality was found with the rs2439302 suggesting an important function in clinical course, a role beyond predisposition to PTC (Jendrzejewski *et al.* 2016).

The same group investigated the possible molecular target of *PTCSC3* as they have previously reported that the lincRNA located close to the variant rs944289 predisposes to PTC (Jendrzejewski *et al.* 2012) and alters expression of various cancer-associated key genes such as *S100A4*, *RHOB*, *MOAP1*, *AKT*, etc. (Jendrzejewski

et al. 2015). The PTC cell lines (BCPAP and TPC-1) were stably transfected with a mammalian expression vector containing the *PTCSC3* or empty vector. Analysis of clones of both TPC-1 and BCPAP cell lines which stably express *PTCSC3* by qPCR found that the transcript of *S100A4* is the most significantly downregulated gene under stable expression of *PTCSC3*. Furthermore, analysis of *S100A4* and *PTCSC3* expression in 74 PTC tumor tissues with paired normal thyroid tissues revealed that *S100A4* was significantly upregulated, while *PTCSC3* was downregulated in tumor tissues compared to normal tissues. The expression level of *S100A4* has been associated moderately with *PTCSC3* expression in normal thyroid tissue. Stable overexpression of *PTCSC3* suppressed the migration and invasion potential of BCPAP cells through transcriptionally downregulating the VEGF and MMP-9. Moreover, *PTCSC3* stable overexpression also downregulated the expression of *S100A4* in BCPAP cells (Jendrzejewski *et al.* 2015). Furthermore, *PTCSC3* was shown to counteract miR-574-5p and inhibit significant cell growth, arrest cell cycle and enhance apoptosis in thyroid cancer cells (Fan *et al.* 2013). In support of this idea, recently, *PTCSC3* was identified to be significantly downregulated in PTCs (Zheng *et al.* 2016). These experiments clearly demonstrated that *PTCSC3* has tumor-suppressive properties and downregulates *S100A4*, which results in inhibition of PTC cell migration and invasion.

PTCSC2 (papillary thyroid cancer susceptibility candidate 2)

Another SNP identified by GWAS was rs965513 and the risk allele (A) of this SNP has been demonstrated to predispose strongly to PTC. It was localized at 9q22 (a 60-kb length from the *FOXO1* gene). Albert de la Chapelle and his group found yet another novel lincRNA gene and named it *PTCSC2* (He *et al.* 2015). Spliced *PTCSC2* isoform is expressed specifically in normal thyroid, while the *PTCSC2* unspliced form is invariably highly expressed in thyroid, in both adult and fetal brain. Expression analysis of unspliced *PTCSC2* and 4 spliced isoforms in 6 thyroid cancer cell lines showed that the expression of *PTCSC2* was undetectable in BCPAP, C646 and FTC133, and found to be significantly downregulated in KTC1, SW1736 and TPC1 cells compared with normal thyroid tissue. On the other hand, expression analysis in 65 PTC cases showed a low expression of both unspliced and spliced isoforms compared with matched normal tissue. Nevertheless, they found no significant association of rs965513 with expression levels of *PTCSC2* in both unspliced and spliced

transcripts from PTC tumors. Besides, in normal thyroid tissue, a low expression of unspliced *PTCSC2*, *FOXE1* and *TSHR* has been strongly correlated with the risk allele (AA) of rs965513. The *PTCSC2* unspliced form has also been correlated significantly with age and CLT. To understand the cellular role of *PTCSC2*, BCPAP cells were transiently transfected with a *PTCSC2-c* expression construct that strongly modulated the expression of cell cycle and cancer-associated genes (coding and noncoding) (He *et al.* 2015). Recently, the same group experimentally demonstrated that myosin-9 could bind to *PTCSC2* and regulate *FOXE1*: this could be the mechanism underlying predisposition to PTC, induced by rs965513 (Wang *et al.* 2017). These data suggest that the lincRNA gene *PTCSC2* is likely to function as a tumor-suppressor gene and genetically predisposes to PTC.

MEG3 (maternally expressed 3)

lncRNA-MEG3 was significantly downregulated in PTC tissues with LNM compared to that of primary thyroid cancer. Furthermore, it has been found that the low expression level of MEG3 was strongly correlated with LNM (Wang *et al.* 2015). Moreover, forced expression of MEG3 in TPC-1 and HTH83 thyroid cancer cell lines could significantly decrease cell migration and invasion. Besides, dual luciferase assay results revealed that Rac1 was downregulated by MEG3 at the post-transcriptional level, through targeting 3'UTR. In PTC tissues, MEG3 expression level has been found to be negatively correlated with expression of Rac1 (Wang *et al.* 2015). These results collectively suggest that MEG3 could act as a novel tumor suppressor involved in the regulation of thyroid cancer cell migration and invasion by molecular targeting of Rac1.

NONHSAT037832

NONHSAT037832, a novel lncRNA, has recently been investigated for its expression in some cell lines and 87 PTCs with matched adjacent normal thyroid tissues by qRT-PCR. NONHSAT037832 has been found to be downregulated in PTC tissues compared with normal tissues (median fold change 4.2). It has also been found to be significantly decreased in two PTC cell lines K1 and IHH4 compared with Nthy-ori 3-1 (a normal thyroid cell line) but there was no difference between BCPAP and normal cells. Moreover, NONHSAT037832 downregulation was significantly associated with LNM and tumor size but not with other clinical features (Lan *et al.* 2015b). These results suggest that NONHSAT037832 is likely to be a tumor-suppressive lncRNA. Although NONHSAT037832

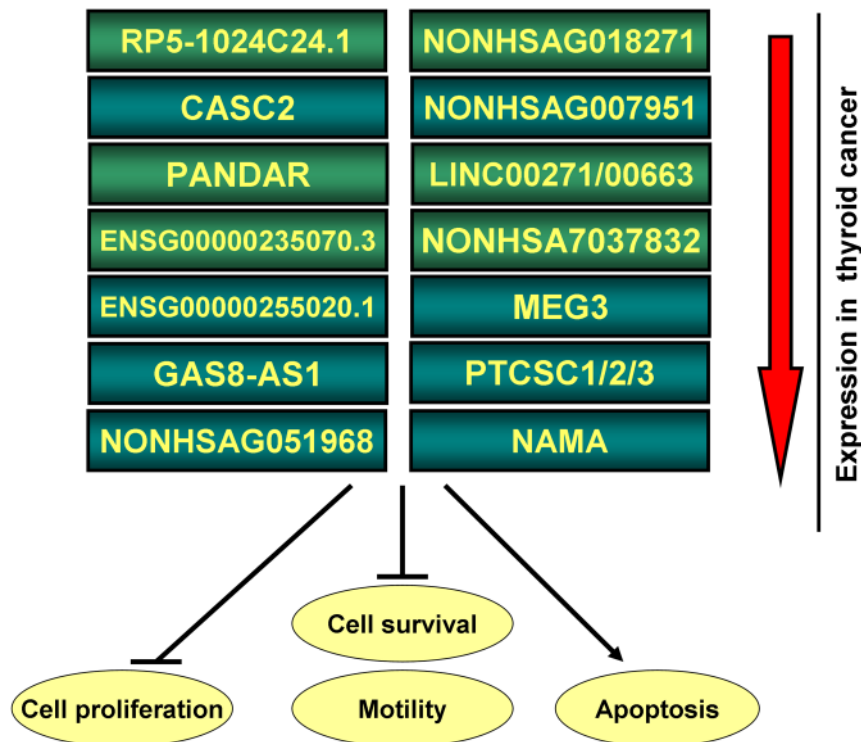
has been suggested to play a role in tumorigenesis via regulation of PGF expression, the mechanism is not clearly known and warrants further investigation.

LINC00663 (long intergenic noncoding RNA 663)

LINC00663 is normally well expressed in tissues of the thyroid and uterus but not in skeletal muscle tissues (Bozgeyik *et al.* 2016). Expression analysis of human cancer cell lines and normal tissues using qRT-PCR demonstrated that LINC00663 is differentially expressed in cancer cell lines. LINC00663 expression has been discovered to be highly decreased in the BCPAP thyroid cancer cell line. Further analyses exhibited the presence of a novel exonic region between exons 2 and 3 and that led to the identification of five potential splice variants showing a high level of variation among them. This group also studied the secondary structures of these variants with minimum free energy and demonstrated that they have potential putative miRNA binding sites in these variants (Bozgeyik *et al.* 2016). Nevertheless, the role of LINC00663 in human carcinogenesis is yet not well understood; further works are essential and indispensable to find the role of LINC00663 in human cancers including thyroid cancer. Moreover, judging from the expression analysis, LINC00663 is likely to act as a tumor suppressor rather than an oncogene as this lncRNA is demonstrated to be downregulated in the majority of human cancer cell lines (DU-145, PC3, HGC-27, CRL-1469, A549 and MCF7) including BCPAP, a thyroid cancer cell line.

LINC00271

Annotation of 2773 lncRNAs from the TCGA dataset found 220 differentially expressed lncRNAs, of which CTBP1-AS2, HAR1A, HAS2-AS1, FAM41C and LINC00271 have been observed to be closely associated with recurrence. In multivariate-mediated analyses, LINC00271 has been found to be an independent risk factor for ETE, LNM and tumor stage III/IV and recurrence after adjusting the BRAF^{V600E} clinicopathological factors. In addition, expression analysis of 50 PTC patients with paired normal tissues revealed that LINC00271 has been downregulated significantly in PTCs. Moreover, gene set enrichment analysis (GSEA) predicted that genes involved in the p53 and JAK/STAT signaling pathways, and genes implicated in cell adhesion and cell cycle were remarkably enriched in PTCs with a lower expression of LINC00271 (Ma *et al.* 2016). This study concluded that LINC00271 is likely to play the role of a tumor-suppressor gene and it could also serve as a potential predictor of poor prognosis in PTC

**Figure 2**

Tumor-suppressive effects of various lncRNAs in thyroid cancer. Thyroid cancer-specific lncRNAs shown above regulate expression by repressing/tightly controlling transcription of various genes involved in proliferation, cell cycle regulation, cell motility, etc. In thyroid cancer, context-dependent downregulation of these lncRNAs results in transcriptional activation of various genes of key cellular signaling pathways. A full colour version of this figure is available at <https://doi.org/10.1530/ERC-17-0188>.

patients. The lncRNAs discussed above and listed in Table 1 are more likely to function as a tumor suppressor in thyroid cancer (Fig. 2 and Table 1).

Oncogenic lncRNAs and their role in thyroid cancer

BANCR (BRAF-activated long noncoding RNA)

BANCR has been implicated in cell proliferation and autophagy in PTC (Wang *et al.* 2014a). A qRT-PCR-mediated study on a total of 40 pairs of PTC and its paired normal tissues identified that BANCR expression was significantly upregulated in PTCs compared to adjacent normal tissue. Thyroid cell proliferation and its subsequent function are implicated by the interaction of TSHR. Transient transfection of siRNAs specifically targeting BANCR showed significant downregulation of TSHR in IHH-4 cells (Zheng *et al.* 2016). Furthermore, siRNA-BANCR-transfected cells showed much slower proliferation than the cells transfected with the scrambled siRNA control. Consistent with the proliferation assay, fewer colonies were formed in cells lacking BANCR compared to the control siRNA-transfected IHH-4 cells in the colony formation assay. Interestingly, expressions of cyclin D1 both at the mRNA and the protein level were observed to be significantly decreased in siRNA-BANCR-transfected cells compared to the control-siRNA-transfected IHH-4 cells. In contrast, mRNAs of cyclin A1, E1 and B1 were

unaltered after silencing BANCR, although cyclin A1 and E1 proteins were slightly but not significantly decreased in BANCR-silenced cells. BANCR was found to be highly enriched in the EZH2 group compared to the IgG control group and expression of TSHR was significantly lower in BANCR-knockdown cells compared to the control cells suggesting that BANCR may enhance TSHR expression through binding to EZH2 (Zheng *et al.* 2016). Taken together, this series of experimental results suggest that BANCR is a typical oncogenic lncRNA and an important molecule for a therapeutic target in PTC (Fig. 3). In contrast, a recent study demonstrated that BANCR functions as a tumor suppressor in PTC (Liao *et al.* 2017). Although it has been speculated that BANCR could function as both an oncogene and a tumor-suppressor gene (Liao *et al.* 2017), it is not very clear whether it could be bifunctional in PTC itself and warrants further studies to clarify these findings.

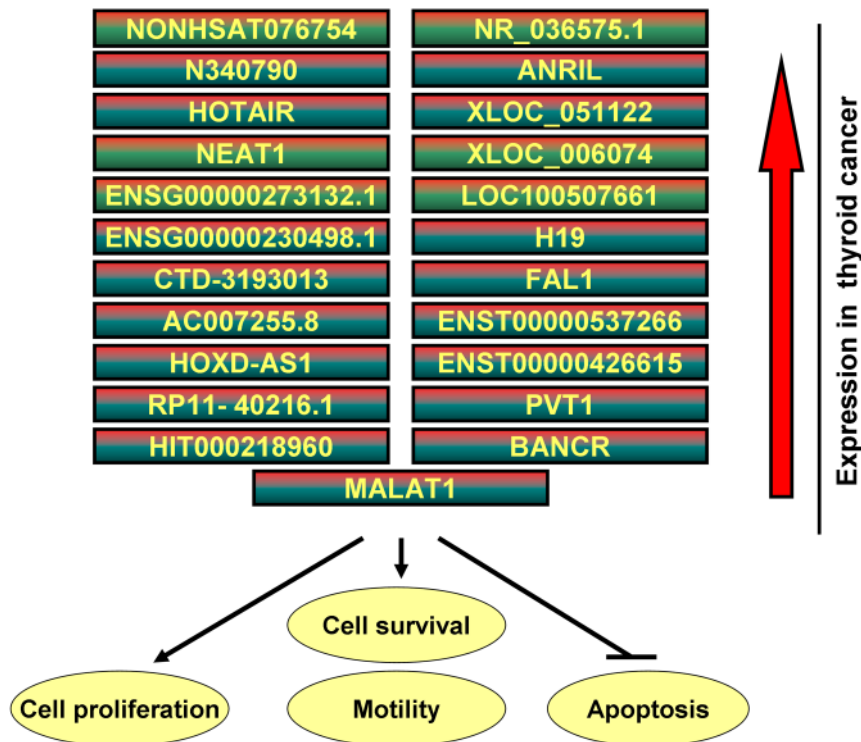
PVT1 (plasmacytoma variant translocation 1)

An extensive expression analysis of PVT1 in 84 thyroid cancer tissues showed that the relative mRNA expression was significantly upregulated in cancer cells compared to adjacent normal cells. Consistent with these results, the PVT1 expression was identified to be significantly higher in various thyroid cancer cell lines such as IHH-4 (PTC), FTC-133 (FTC) and 8505c (ATC) compared to the normal

Table 1 Summary of tumor-suppressive lncRNAs in thyroid cancer.

S. No.	lncRNAs	C. location	Analyzed samples		Variant	Expression	Functions	References
			Cell lines	Tumor tissues				
1.	RP5-1024C24.1	Chr. 11p14.1	Yes	45	PTC	Low	Associates negatively with late clinical stages	Du <i>et al.</i> (2017)
2.	CASC2	Chr. 10q26.11	Yes	18 (GWAS) 86	PTC	Low	Expression correlates with multifocality and TNM	Xiong <i>et al.</i> (2017)
3.	PANDAR	Chr. 6q21.2	Yes	84 (TCGA) 64	PTC	Low	Inhibits proliferation, cell cycle and promote apoptosis	Li <i>et al.</i> (2017f)
4.	ENSG00000235070.3	Chr. 2q36.3	Yes	59	PTC	Low	Correlates directly to BRAF (V600E)	Goedert <i>et al.</i> (2017)
5.	ENSG00000255020.1	Chr. 8q23.1	Yes	59	PTC	Low	Correlates directly to BRAF (V600E)	Goedert <i>et al.</i> (2017)
6.	GAS8-AS1	Chr. 16q24.3	Yes	402	PTC	Low	Suppresses cell proliferation in thyroid cancer	Pan <i>et al.</i> (2016)
7.		Chr. 16q24.3	Yes	97	PTC	Low	Expression negatively correlates with LNM	Zhang <i>et al.</i> (2017b)
8.	NONHSAG051968	Chr. 9p21.2	Yes	36	MNG	Low		
9.	NONHSAG018271	Chr. 16p13.3	Yes	12	PTC	Low	Correlates negatively with tumor size	Wang <i>et al.</i> (2016)
10.	NONHSAG007951	Chr. 11q12.1	Yes	12	PTC	Low	Suppresses tumor cell growth	Wang <i>et al.</i> (2016)
11.	LINC00271	Chr. 6q23.3	Yes	50	PTC	Low	Suppresses tumor cell growth Involves in extrathyroidal invasion, LNM, advanced tumor stage and recurrence in TC	Wang <i>et al.</i> (2016) Wang <i>et al.</i> (2016) Wang <i>et al.</i> (2016) Ma <i>et al.</i> (2016)
12.	LINC00663	Chr. 19p13.11	Yes	471 (TCGA) 185 (FUSCC)	PTC	Low		
13.	NONHSAT037832	Chr. 14	Yes	No	PTC	Low	N/A	Bozgeyik <i>et al.</i> (2016)
14.	MEG3	Chr. 14q32	Yes	87	PTC	Low	Plays role in LNM and determines tumor size	Lan <i>et al.</i> (2015b)
15.	PTCSC2	Chr. 9q22	Yes	16	PTC	Low	Inhibits invasion and associates with LNM	Wang <i>et al.</i> (2015)
16.	PTCSC3	Chr. 14q13.3	Yes	65	PTC	Low	Predisposes genetically to thyroid cancer	He <i>et al.</i> (2015)
17.		Chr. 14q13.3	Yes	46	PTC	Low	Suppresses cell growth and invasion	Jendrzewski <i>et al.</i> (2012)
18.	PTCSC1	Chr. 8q24	Yes	73	PTC	Low		Jendrzewski <i>et al.</i> (2016)
19.	NAMA	Chr. 9	Yes	26	PTC	Low	A candidate susceptibility gene for PTC	He <i>et al.</i> (2009)
20.		Chr. 9	Yes	40	PTC	Low	Targets MAPK signaling pathway	Yoon <i>et al.</i> (2007)
			Yes	21	PTC	Low		Zheng <i>et al.</i> (2016)

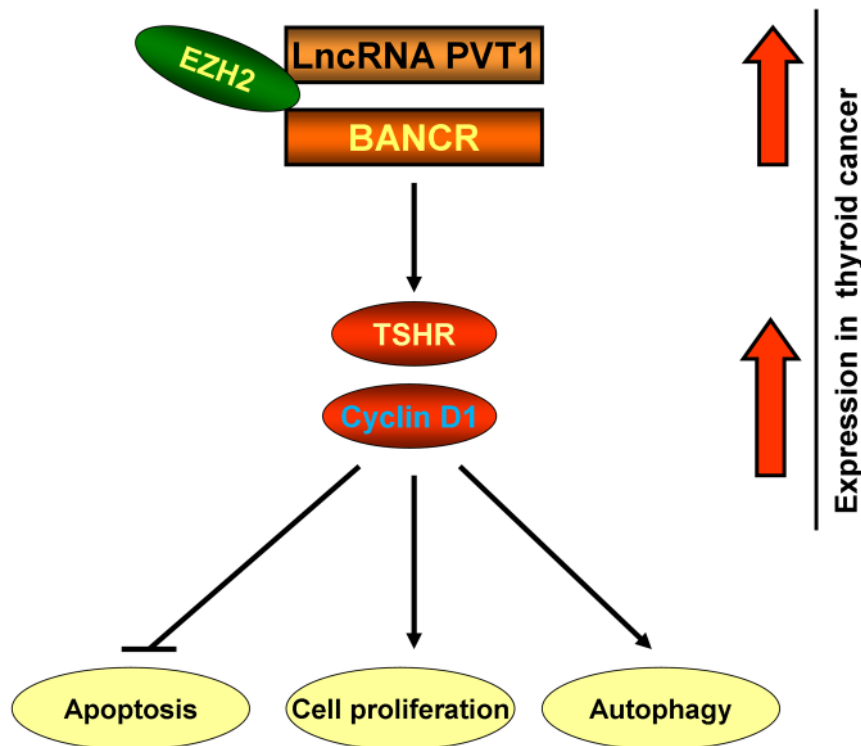
Chr, chromosome; C, location, chromosomal location; FUSCC, Fudan University Shanghai Cancer Center; GWAS, Genome-Wide Association Study; LNM, lymph node metastasis; MNG, Multi-Nodular Goiter; N/A, data not available; PTC, papillary thyroid cancer; S. No., study number; TC, thyroid cancer; TCGA, The Cancer Genome Atlas; TNM, tumor node metastasis.

**Figure 3**

Various oncogenic lncRNAs implicated in thyroid cancer. Deregulation of various lncRNAs is implicated in thyroid cancer. lncRNAs regulate expression by controlling transcription of various genes involved in the key cellular processes such as proliferation, cell cycle, survival, apoptosis, cell motility, etc. In thyroid cancer, context-dependent upregulation of these lncRNAs results in transcriptional activation of various genes of important cell signaling pathways that result in deregulated cellular processes paving the way to carcinogenesis. A full colour version of this figure is available at <https://doi.org/10.1530/ERC-17-0188>.

counterpart Nthy-ori 3-1 cell line (Zhou *et al.* 2015). Moreover, siRNA-mediated inhibition of PVT1 in IHH-4 cells (transfected with an siRNA-PVT1 vector) showed significantly slower growth of IHH-4 cells from 48 to 96 h when compared to vector-alone-transfected cells. On the other hand, FTC-133 and 8505C cells transfected with siRNA-PVT1 showed slower growth from 72 to 96 h. Similar to these proliferation assay results, the number of colonies formed by all three cell lines in the colony formation assay was significantly decreased in cells lacking PVT1. Furthermore, flow cytometry-mediated exploration of cellular DNA content, cell cycle profiling and cell proliferation revealed that the percentage of cells at the G1 stage was significantly enhanced when the PVT1 was silenced in all three of the thyroid cancer cell lines (IHH-4, FTC-133 and 8505C) as compared with control cell lines which suggests that suppression of PVT1 could result in cell cycle arrest at G1. This was clearly reflected in the expression of various proteins involved in cell cycle kinetics, as cyclin D1 was significantly decreased when cells were transfected with siRNA-PVT1 compared with controls cells. On the contrary, it has been shown that mRNA expressions of cyclin A1, B1 and E1 were not significantly changed, albeit proteins of A1, B1 and E1 were slightly but not significantly reduced in PVT1-silenced cells. In addition, it has been shown that

siRNA-mediated PVT1 suppression resulted in a decreased expression of TSHR (mRNA and protein) in IHH-4 cells. A similar tendency has also been observed in two other thyroid cancer cell lines (FTC-133 and 8505C) suggesting a positive correlation of PVT1 expression with TSHR (Zhou *et al.* 2015). As observed with BANCR (Zheng *et al.* 2016), PVT1 was also accumulated by EZH2 compared to that in control cells in all three thyroid cancer cell lines (IHH-4, FTC-133 and 8505C) implying that lncRNA is likely to bind to EZH2. Further experiments confirmed that silencing PVT1 could significantly decrease TSHR expression by targeting its promoter in three different cell lines. These results collectively suggest that PVT1 suppression decreases TSHR expression via binding to EZH2 in thyroid cancer cell lines (Fig. 4). Apart from thyroid cancer, PVT1 has also been implicated in the tumorigenesis of various human cancers (Takahashi *et al.* 2014, Wang *et al.* 2014b). It has been shown that PVT1 overexpression is correlated with colon cancer prognosis via the regulation of cell apoptosis (Takahashi *et al.* 2014) and overexpression of lncRNA has been shown to be involved in promoting stem cell proliferation in hepatocellular carcinoma (Wang *et al.* 2014b). Moreover, the results of these studies suggest that PVT1 is likely to function as an oncogene in various types of human cancers including thyroid cancer.

**Figure 4**

Oncogenic effect of PVT1 and BANCR lncRNAs in thyroid cancer. lncRNAs PVT1 and BANCR activate the transcription of various genes implicated in regular cell growth signaling pathways of normal thyroid cells. Deregulation of lncRNA machinery results and upregulation of lncRNAs PVT1 and BANCR that hyperactivate the transcription of various key cellular growth controlling genes in thyroid cancer. A full colour version of this figure is available at <https://doi.org/10.1530/ERC-17-0188>.

ENST00000537266 and ENST00000426615

As a result of array hybridization, 10 candidate lncRNAs were identified including ENST00000537266 and ENST00000426615. They were found to be expressed more than tenfold differentially in PTC tissues compared to normal tissues (Xu *et al.* 2016). A subsequent RT-PCR analysis found that ENS-266 and ENS-615 were found to be consistently upregulated compared with adjacent normal tissues in 46 cases. Transfection of siRNAs against ENS-266 and ENS-615 in TPC-1 cells revealed that cell proliferation was significantly reduced in Si-266/Si-615-transfected TPC-1 cells at various time points (24, 36, 48 and 72h). In Edu assay, Hoechst-stained nuclei in all three (Si-NC, Si-266 and Si-615) transfected TPC-1 cells were dense and contracted. Despite the percentage of Edu-positive cells, the number of colonies was significantly decreased in Si-266/Si-615-transfected TPC-1 cells compared with Si-NC cells.

Interestingly, a further functional analysis showed no significant difference in cell apoptosis between Si-266/Si-615-transfected groups and the control group in TPC-1 cells. However, in cell cycle analysis, the cell proportion was significantly enhanced in the G1 phase but lowered in S and G2 phases in TPC-1 cells transfected with Si-266. On the other hand, they also observed an enhanced amount of cells at the G1 phase and also showed a reduction in the S phase and no change in the G2 phase in TPC-1

cells transfected with Si-615. A significant decrease in cell migration was observed in both trans-well and scratch assay in Si-615-transfected TPC-1 cells, while there was no significant change observed in Si-266-transfected TPC-1 cells suggesting that ENS-615 has a major role in cell motility but not ENS-266. Moreover, structural prediction of both ENS-266 and ENS-615 revealed that these lncRNAs are likely to interact with *BRAF*, *FOXE1*, *SIN1*, *TERT*, *NRG1*, *GLI* and *MBIP* (Xu *et al.* 2016). These results strongly suggest that both lncRNAs are likely to play an oncogenic role in thyroid carcinogenesis.

FAL1 (focally amplified long noncoding on chromosome 1)

An extensive expression level analysis of FAL1 and *p21* in 100 PTC samples and matched normal thyroid tissues revealed that the expression of FAL1 was significantly upregulated in PTCs than in normal tissues (Jeong *et al.* 2016). Unlike in other human cancers, *p21* expression was increased when compared to the matched normal tissues, suggesting that *p21* is not negatively modulated by FAL1 in PTC cells and this result was consistent with Western blot analysis of protein samples. In addition, PTC cell lines also showed increased *p21* levels. Furthermore, immunohistochemical staining data from the Human Protein Atlas revealed that *p21* was

easily detectable only in PTC tissues, not in follicular neoplasms, suggesting that p21 is active in PTC tumor tissues. A comparison of 30 PTC patients with the highest FAL1 expression against 30 cases with the lowest FAL1 expression showed that patients with high FAL1 expression could increase the risk of multifocality, suggesting that FAL1 expression is likely to affect tumor behavior and generate aggressive features of PTC. Furthermore, GSEA from open-source microarray data revealed a unique gene set for the cell cycle, including *E2F1/2*, *VEGFA* and *cyclin D1*, which was accumulated in the FAL1-upregulated samples, and this result has also been consistently reflected upon FAL1 overexpression in TPC1 and BCPAP cell lines (Jeong *et al.* 2016).

H19 (imprinted maternally expressed transcript)

lncRNA H19 was shown to be overexpressed and implicated as an oncogene in many human cancers (Liu *et al.* 2016). Recently, H19 has been demonstrated to be highly expressed in thyroid cancer cell lines and tumor samples when compared with normal thyroid cell lines and tissues (Liu *et al.* 2016). Furthermore, overexpression of H19 in TPC-1 and NIM thyroid cancer cell lines showed increased proliferation, migration and invasion. On the other hand, knockdown of H19 in the same cell lines decreased the cell viability, migration and invasion, and triggered growth arrest both *in vitro* and *in vivo*. This oncogenic lncRNA has been identified as a target of miR-17-5p. Moreover, H19 has been shown to antagonize miR-17-5p upon overexpression of its target *YES1* and suppressed miR-17-5p-induced cell cycle progression (Liu *et al.* 2016). These results suggest that the oncogenic H19 is likely to also function as a competitive endogenous RNA.

LOC100507661

A recent study analyzed 2394 tumor SNP arrays from 12 types of cancer (Hu *et al.* 2014). Upon verification of 56 potential cancer-promoting genes from the aforementioned study, Kim *et al.* (2016) finally selected 3 candidate lncRNAs for further functional study. Among those 3 lncRNAs, LOC100507661 has been shown to be strongly overexpressed in thyroid cancer tissues compared to paired normal tissue. LOC100507661 has been demonstrated to be highly expressed and easily detectable in PTC (TPC1 and BCPAP) and ATC (C643 and 8505C) cell lines but not in FTC (FTC133) cell lines. Furthermore, stable overexpression of LOC100507661 resulted in

enhanced cell proliferation, migration and invasion of thyroid cancer cells. In addition, it has been found that LOC100507661 expression was always high (60/64) and the high expression was more frequently associated with LNM in PTC samples (Kim *et al.* 2016). These results suggest that LOC100507661 is likely to function as an oncogenic lncRNA and could be a predictive prognostic biomarker in thyroid cancer.

XLOC_051122 and XLOC_006074

A genome-wide expression screening of 12 PTC tumors with paired normal tissue identified 218 differentially expressed lncRNAs (Liyanarachchi *et al.* 2016). Among them, a significant overexpression of 2 lncRNAs (XLOC_051122 and XLOC_006074) was found, particularly in PTC patients with no BRAF mutation. It has also been shown that higher levels of these 2 lncRNAs were detected in PTC cases with LNM suggesting oncogenic and strong prognostic roles of these novel lncRNAs in PTC. Though a distinct prognostic role of novel lncRNAs has been identified, the molecular mechanisms and their role in thyroid cancer are largely unknown and future works are warranted to supplement these findings.

HIT000218960

lncRNA microarray analysis of 6 pairs of PTCs with their paired normal tissue and further validation in 55 pairs of PTCs detected that HIT000218960 was significantly overexpressed in PTC. It was significantly correlated with TNM stage, LNM and multifocality and was also associated positively with the *HMGA2* (high-mobility group AT-hook 2) oncogene. Moreover, downregulation of *HMGA2* was observed in HIT000218960-knockdown PTC cell lines (TPC1 and BCPAP) and further functional analysis showed decreased cell growth, migration and invasion *in vitro* (Li *et al.* 2017a). These results suggest that HIT000218960 is likely to function as an oncogenic lncRNA by modulating *HMGA2* expression.

ANRIL (antisense noncoding RNA in the INK4 locus)

This study detected expressions of ANRIL by qRT-PCR in 105 thyroid cancer patients compared with adjacent normal tissues. It has been shown that ANRIL was highly expressed in thyroid cancer, and siRNA-mediated ANRIL silencing in TPC-1 and SW579 cells inhibited proliferation, invasion and metastasis via the TGF- β /Smad pathway (Zhao *et al.* 2016). These results collectively suggest that ANRIL is a typical oncogene.

NR_036575.1

Expressions of this novel lncRNA were evaluated in 83 paired thyroid cancer and normal tissues and the results revealed that NR_036575.1 was significantly upregulated in thyroid cancer tissues. Furthermore, the expression of NR_036575.1 was associated with tumor size and extrathyroidal invasion. Establishment of two receiver-operating characteristic (ROC) curves predicted that NR_036575.1 could be a potential biomarker for identifying PTC (Sun *et al.* 2016). Moreover, NR_036575.1 knockdown experiments showed significant inhibition of TPC-1 cells suggesting that NR_036575.1 is likely to act as an oncogene in thyroid cancer.

MALAT1 (metastasis-associated lung adenocarcinoma transcript 1)

Higher level expressions of MALAT1 and *IQGAP1* were detected in a thyroid cancer cell line and tissues compared with normal control. A series of experiments in this study demonstrated that MALAT1 could upregulate the expression of *IQGAP1*. *MALAT1* knockdown resulted in decreased proliferation and invasion of FTC-133 cells. In turn, *IQGAP1* knockdown reversed *MALAT1*-overexpression-induced increased cell proliferation and invasion of thyroid cancer cells. MALAT1-induced tumor growth has been verified in nude mice (Huang *et al.* 2016). These results suggest that MALAT1 functions as an oncogene via regulation of *IQGAP1* expression. Recently, expression analysis of MALAT1 on 195 benign and thyroid cancer cases by tissue microarrays for RNA *in situ* hybridization and RT-PCR revealed that MALAT1 is well expressed in both normal and tumor tissues of a thyroid. However, it has been found that MALAT1 is upregulated during the progression of normal tissue to cancer tissue. In contrast, compared to normal tissue, MALAT1 was shown to be significantly downregulated in PDTCs and ATCs. Furthermore, TGF- β -mediated induction of epithelial to mesenchymal transition (EMT) in TPC-1 cells resulted in upregulation of MALAT1 expression, suggesting a potential role of MALAT1 in EMT-mediated thyroid cancer progression (Zhang *et al.* 2017a).

HOTAIR (HOX transcript antisense RNA)

Although HOTAIR has been known to be widely deregulated in human cancers, its role is largely unclear as only a few studies were conducted in thyroid cancer. Initially, a study investigated the involvement of 3 haplotype-tagging SNPs of HOTAIR in PTC and found that HOTAIR SNP (rs920778) was a PTC-susceptibility

variant and further demonstrated that HOTAIR acts as an oncogene (Zhu *et al.* 2016). A study based on TCGA and Gene Expression Omnibus data analysis showed that HOTAIR has been significantly overexpressed, associated with poor survival of thyroid cancer patients and likely to play a role in thyroid carcinogenesis via the Wnt signaling pathway (Li *et al.* 2017b). These results suggest that HOTAIR is an onco-lncRNA and its overexpression could serve as a biomarker associated with poor outcome in thyroid cancer.

NONHSAT076754

Expression analysis of NONHSAT076754 in 72 PTC samples (37 metastatic and 35 nonmetastatic) detected upregulation of NONHSAT076754 in 37 metastatic compared with nonmetastatic PTC cases and overexpression has been associated with LNM in PTC. Although NONHSAT076754 has been detected originally as an upregulated lncRNA, the experimental results of an overexpression study did not support its role in cell proliferation. Nevertheless, it strongly promoted migration and invasion of TPC1 cells and this activity was significantly reversed upon NONHSAT076754 knockdown in K1 cells, suggesting that it could serve as a potential auxiliary biomarker which may aid ultrasonography in predicting cervical LNM of PTCs (Xia *et al.* 2017).

NEAT1 (nuclear-enriched abundant transcript 1)

NEAT1 has been identified to be overexpressed, while miRNA-214 had been downregulated in thyroid cancer patients. Knockdown of NEAT1 resulted in decreased cell survival and motility with β -catenin (a direct target of miRNA-214) downregulation. Moreover, in thyroid cancer cells, upregulation of NEAT1 strongly promoted tumor progression and tumor size increase *in vitro* and *in vivo*, respectively. On the other hand, these cells showed a lower expression of miRNA-214 (Li *et al.* 2017c). These results suggest that overexpression of NEAT1 could result in the onset of thyroid cancer and that NEAT1 may act as an oncogenic lncRNA as it drives progression of thyroid cancer via modulation of miRNA-214 expression. The lncRNAs studied above and other lncRNAs listed in Table 2 are more likely to function as oncogenes in thyroid cancer (Fig. 3 and Table 2).

Role of lncRNAs as therapeutic targets, and diagnostic and prognostic biomarkers

lncRNAs have been found to be differentially expressed in human cancer and their overexpression or knockdown

Table 2 Summary of oncogenic lncRNAs in thyroid cancer.

S. No.	lncRNAs	C. location	Analyzed samples		Variant	Expression	Functions	References
			Cell lines	Tumor tissues				
1.	NONHSAT076754	Chr. 2	Yes	72	PTC	High	Correlates to LNM in PTC	Xia <i>et al.</i> (2017)
2.	n340790	N/A	Yes	85	PTC	High	Accelerates TC cell growth, motility and inhibit apoptosis	Li <i>et al.</i> (2017d)
3.	HOTAIR	Chr. 12q13.13	Yes	35	PTC	High	Triggers cell growth and invasion	Zhang <i>et al.</i> (2017c)
4.	NEAT1	Chr. 11q13.1	Yes	496 (TCGA)	PTC	High	Associates with poor survival of TC patients	Li <i>et al.</i> (2017b)
5.	ENSG00000273132.1	Chr. 6q25.1	Yes	No	PTC	High	Promotes tumor progression and tumor size	Li <i>et al.</i> (2017c)
6.	ENSG00000273132.1	Chr. 6q25.1	Yes	59	PTC	High	Overexpression correlates directly to BRAF (V600E)	Goedert <i>et al.</i> (2017)
7.	ENSG00000230498.1	Chr. 1q31.1	Yes	59	PTC	High	Overexpression correlates directly to BRAF (V600E)	Goedert <i>et al.</i> (2017)
8.	CTD-3193013	N/A	Yes	45	PTC	High	A node of co-regulation with other lncRNAs and tumor size	Du <i>et al.</i> (2017)
9.	AC007255.8	Chr. 7p14.3	Yes	45	PTC	High	Correlates to clinical stage (patient age)	Du <i>et al.</i> (2017)
10.	HOXD-AS1	Chr. 2q31.2	Yes	45	PTC	High	Correlates to clinical stage	Du <i>et al.</i> (2017)
11.	RP11-40216.1	N/A	Yes	45	PTC	High	Expression proportional to LNM	Du <i>et al.</i> (2017)
12.	HIT000218960	Chr. 12	Yes	18 (GWAS)	PTC	High	Correlates to TNM stage, LNM, and multifocality	Li <i>et al.</i> (2017a)
13.	MALAT1	Chr. 11q13.1	Yes	195	PTC	High	Regulates proliferation, migration and EMT via TGF- β	Zhang <i>et al.</i> (2017a)
14.	NR_036575.1	Chr. 19p13.11	Yes	83	PTC	High	Promotes proliferation and migration of thyroid cancer	Sun <i>et al.</i> (2016)
15.	ANRIL	Chr. 9p21.3	Yes	105	TC	High	Proliferation, invasion and metastasis via TGF- β /Smad	Zhao <i>et al.</i> (2016)
16.	XLOC_051122	N/A	Yes	12	PTC	High	Oncogenic with metastatic potentials	Liyanarachchi <i>et al.</i> (2016)
17.	XLOC_006074	N/A	Yes	12	PTC	High	Oncogenic with metastatic potentials and prognostic role	Liyanarachchi <i>et al.</i> (2016)
18.	LOC100507661	Chr. 3q26.2	Yes	64	PTC	High	Enhances proliferation, migration and invasion	Kim <i>et al.</i> (2016)
19.	H19	Chr. 11p15.5	Yes	30	TC	High	Increases proliferation, migration and invasion	Liu <i>et al.</i> (2016)
20.	FAL1	Chr. 1q21.2	Yes	100	PTC	High	Associates positively with risk of multifocality	Jeong <i>et al.</i> (2016)
21.	ENST00000537266	Chr. 19	Yes	46	PTC	High	Promotes proliferation and inhibit apoptosis	Xu <i>et al.</i> (2016)
22.	ENST00000426615	Chr. 2	Yes	46	PTC	High	Regulates proliferation, migration, apoptosis and cell cycle	Xu <i>et al.</i> (2016)
23.	PVT1	Chr. 8q24.21	Yes	84	TC	High	Increases thyroid cancer cell proliferation	Zhou <i>et al.</i> (2015)
24.	BANC1	Chr. 9q21.12	Yes	6	PTC	High	Enhances TC cell proliferation and inhibits apoptosis	Wang <i>et al.</i> (2014a)
25.			Yes	40	PTC	High		Zheng <i>et al.</i> (2016)

Chr, chromosome; C, location, chromosomal location; GWAS, Genome-Wide Association Study; LNM, lymph node metastasis; N/A, data not available; PTC, papillary thyroid cancer; S. No., study number; TC, thyroid cancer; TCGA, The Cancer Genome Atlas; TNM, tumor node metastasis.

resulted in altered phenotypical responses owing to malignant transformation, such as changes in proliferation, cell survival, migration and invasion or apoptosis. This unique feature of lncRNAs opens a new arena for application of lncRNAs as potential therapeutic targets and biomarkers for cancer diagnosis and prognosis.

lncRNAs: therapeutic targets and mechanisms of lncRNA-targeting agents

lncRNAs are therapeutic targets in thyroid cancer

High-resolution microarray, genome sequencing and RNA sequencing revealed a massive amount of novel coding and noncoding transcripts which resulted in the identification of abundant ncRNAs, including lncRNAs (Carninci *et al.* 2005, Trapnell *et al.* 2010, Agrawal *et al.* 2014). lncRNAs and their association with cancer have been well summarized (Gutschner & Diederichs 2012). As a result of state-of-the-art technologies in genomics, epigenomics and transcriptomics, tremendous data has been accumulated and were analyzed to find lncRNAs associated with human cancer (Prensner *et al.* 2011, Sinicropi *et al.* 2012). The DNA/RNA sequencing data granted the possibility of characterizing the structure and function of various disease-related SNPs of lncRNAs. The importance of lncRNA-based cancer therapy and the roles of several lncRNAs in cancers have been characterized and strategies targeting them resulted in inhibitory effects on malignant cells *in vitro* and *in vivo* (Zhao *et al.* 2010, Chu *et al.* 2011, Jin *et al.* 2011, Wang *et al.* 2011). Extensive studies were carried out to interrogate the molecular functions of lncRNAs, which resulted in the development of targeting approaches specific to individual lncRNAs (Lee 2012). Advanced RNA sequencing methods also accelerated the progress of developing lncRNA-based cancer management. These findings support the notion that lncRNAs are prospective novel therapeutic targets in cancers. Besides, recent improvements in the development of biological drugs have broadened the types of therapeutic targets and paved way for the development of various novel methodologies to target RNA molecules (Castanotto & Rossi 2009, Davies *et al.* 2010). All these methodologies showed a hopeful outcome in cancer inhibition as manifested by RNA-targeted therapeutics in various phases of clinical trials. Taken together, the lncRNAs summarized and discussed above (BANCR, PVT1, ENST00000537266, ENST00000426615, FAL1, H19, LOC100507661, XLOC_051122, XLOC_006074, HIT000218960, ANRIL, NR_036575, MALAT1, HOTAIR,

NONHSAT076754 and NEAT1) and other lncRNAs in Table 2 with oncogenic features could be a potential therapeutic target in thyroid cancer including human cancers and warrant further research on the development of lncRNA-based cancer therapy. Therapeutic agents that potentially target lncRNAs mostly function via reducing the intracellular transcript level of lncRNAs or attenuating their activities and molecular functions in cancer cells. The onco-lncRNAs possess pro-cancerous effects since their expression level or activity is often elevated in cancer cells become druggable targets, although we should not overlook the important tumor-suppressor roles of other lncRNAs in cancer. Therapeutic options which directly target onco-lncRNAs are more likely to have a prominent anticancer effect. At this end, siRNAs and ASOs (antisense oligonucleotides) could easily be used to regulate lncRNA expression and/or function.

Prospective strategies and mechanisms of lncRNA-targeting agents

lncRNAs are identified to be critically involved in cancer and modulations of their functions have been shown to have promising anticancer effects. As illustrated in Fig. 5, recently, in order to regulate the level of lncRNAs in cancer cells, nucleic acid-based strategies are more frequently employed in targeting lncRNAs either by modifying their structures or mature sequences (Gupta *et al.* 2010). RNA interference technique have been one of the most relevant methods to target lncRNAs in cancer cells. siRNAs are short, double-stranded RNAs (19–30 nt) which target lncRNA molecules by complementary base pairing. Initially, siRNA duplex unwinds and becomes a linear single-stranded molecule and subsequently assembles into an active RISC (RNA-induced silencing complex). Complementary base pairing to target RNA chops at a single phosphodiester bond found in the middle of the complementary nucleotide sequence to the siRNA (Gupta *et al.* 2010). Moreover, both siRNAs and shRNAs have been shown to exhibit greater RNA selectivity and knockdown efficiency. The simplicity of siRNA and shRNA synthesis, and versatile design for specific targeting facilitate them as promising therapeutic agents. The stability of these nucleic acid drugs could be greatly improved by various chemical alterations (Geary *et al.* 2001). ASOs, single-stranded DNAs/RNAs (8–50 nt), are designed specifically for targeting lncRNAs. Generally, ASOs precisely bind to the transcripts of lncRNAs by pairing of complementary nucleotides; the resulting complexes (hybrids) become prey for the intracellular RNase H1 that chops the

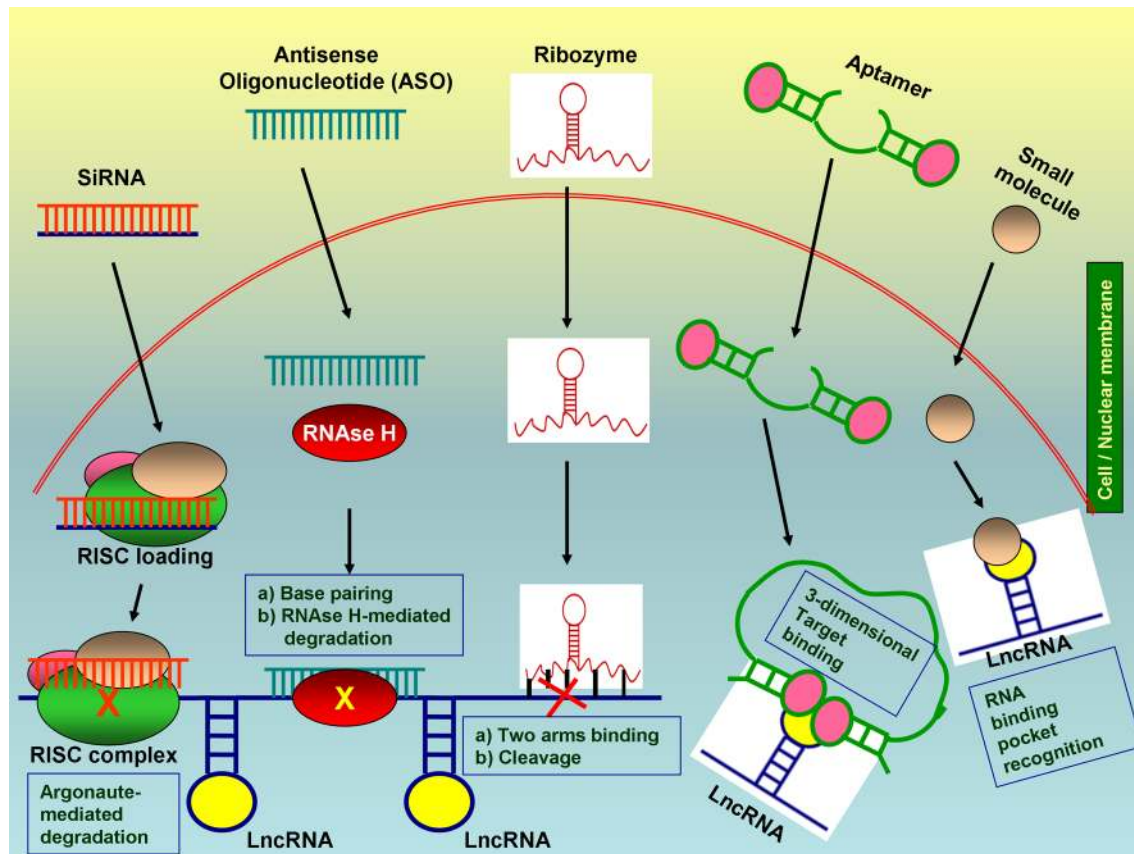


Figure 5

Schematic representation of various lncRNA-targeting agents and their mechanisms. Deregulated lncRNAs in cancer cells are targeted using various molecules such as siRNA, an antisense oligonucleotide, ribozyme, aptamer, small molecules, etc. siRNAs: guide strands initially loaded to AGO2 (argonaute 2) that results in formation of RISC (an active RNA-induced silencing complex). RISC binds to the target RNA by complementary base pairing and cleaves the target RNA. ASOs: RNase-H identifies the DNA-RNA duplex and cleaves the RNA target. Ribozyme: catalyzes the cleavage of the flanked RNA region downstream to a NUH site via destabilizing the phosphodiester backbone of target RNA. Aptamers: bind to their target lncRNAs specifically fitting to the 3-D structures of lncRNA and suppress the interactions of lncRNAs. Small molecules: specifically bind to the RNA binding pockets of lncRNAs, and compete with protein factors/intracellular small ligands for the binding of lncRNAs. It causes conformational distortion within lncRNA which results in suppression of unique lncRNA. These target-specific molecular agents have an efficient binding capacity and destroy the targeted lncRNAs that exclusively depend on the cellular context. A full colour version of this figure is available at <https://doi.org/10.1530/ERC-17-0188>.

transcripts of the lncRNAs and they are shown to effectively target lncRNAs in human cancer cells (Gupta *et al.* 2010, Tripathi *et al.* 2010). Hammerhead ribozyme is a short, single-stranded catalytic RNA molecule with self-cleavage activity. Under neutral cellular physiological conditions, two of the three flanking helices of the catalytic core make tertiary interactions. Active inhibition of the target lncRNA by the ribozyme is exclusively limited by its base pairing capability. Making an active catalytic motif requires the binding of 2 stems of the ribozyme to the target lncRNAs. After binding, ribozyme catalyzes the cleavage of the flanked RNA region downstream to an NUH site via the destabilization of the phosphodiester backbone of target RNA. Ribozymes have been implicated in targeting lncRNAs in cancer (Pavco *et al.* 2000). An aptamer represents short DNA or RNA oligonucleotides or

peptides which have a stable 3-dimensional (3-D) structure *in vivo*. They bind to their target lncRNAs specifically fitting to the 3-D structures of lncRNA. Aptamers suppress the interactions of lncRNAs and their critical components and certain distinct characteristics of lncRNAs show advantages over siRNAs (Watrin *et al.* 2009). On the other hand, small molecules are synthesized to specifically bind to the RNA binding pockets of lncRNAs and compete with protein factors or intracellular small ligands for the binding of lncRNAs. It causes conformational distortion within lncRNA which results in suppression of unique lncRNA (Pushchnikov *et al.* 2009). Moreover, in cancer treatment, inductions of post-transcriptional processing pathways and miRNAs have also been shown to be valid therapeutic options to suppress lncRNAs (Mimura *et al.* 2012, Jalali *et al.* 2013). Developing various innovative

approaches facilitates the use of combination therapy to augment lncRNA targeting in cancer therapy (Fig. 5).

lncRNAs: diagnostic markers in thyroid cancer

lncRNAs are potent biomarkers as they do not code for proteins and most of their functions correlate with expression. The expression pattern of lncRNAs is tissue-specific and is greater than the coding mRNAs that enable them to be highly specific diagnostic biomarkers (Derrien *et al.* 2012). Detection of cancer at the early stages significantly increases the chances of survival. Detection needs robust biomarkers for both invasive and noninvasive methods (sampling of extracellular fluids). For example, circulating nucleic acids (CNAs) are fragments of DNA and RNA molecules found in blood serum and other extracellular fluids. Altered levels of CNAs are associated with tumor and malignant progression, serving as potential tumor biomarkers that could easily be screened by PCR assays (Schwarzenbach *et al.* 2011). Further ROC curve-mediated analysis of the diagnostic value of NONHSAT076754 revealed that the lncRNA could aid ultrasonography in predicting PTC with LNM (Xia *et al.* 2017). MALAT1 could serve as a biomarker for thyroid cancer classification and diagnosis (Zhang *et al.* 2017a). Therefore, these lncRNAs could be used as novel minimally invasive diagnostic and prognostic biomarkers for the assessment of these metastatic PTCs.

Several lncRNAs have been described to be highly specific for a particular cancer type. For example, in thyroid cancer, the level of tumor-suppressive lncRNAs is invariably much lower in malignant tumor tissues when compared to the paired nonneoplastic tissues. The differential expressions of those lncRNAs have also been found to be tissue-specific. Hence, thyroid cancer-associated tumor-suppressive lncRNAs listed in Table 1 could be readily used as diagnostic biomarkers for thyroid cancer (Fig. 2 and Table 1). In addition to the tumor suppressors, oncogenic lncRNAs have also been reported to be tissue-specific and frequently overexpressed in malignant thyroid tumors compared with the paired normal tissues, suggesting that the thyroid cancer-associated oncogenic lncRNAs listed in Table 2 could also be used as a potential diagnostic marker in thyroid cancer (Fig. 3 and Table 2).

In human cancer, several lncRNAs have been characterized as potential biomarkers from human body fluids. Detection of PCA3 in the urine has been shown to be a more specific diagnostic marker in prostate cancer

than the other commonly used prostate-specific antigen (Lee *et al.* 2011). UCA1 (urothelial carcinoma associated 1) transcript detected in urine has been demonstrated to be a sensitive biomarker for bladder carcinoma (Wang *et al.* 2006). HULC is posed as a promising diagnostic biomarker for hepatocellular carcinoma since it has been detected with high frequency in the plasma of HCC patients (Xie *et al.* 2013). In oral cancers, a set of lncRNAs in the saliva were identified as potential markers (Tang *et al.* 2013). Detection of AA174084 in gastric juice was shown to be an indicator of gastric cancer (Shao *et al.* 2014). Plasma-mediated detection of the MALAT1 fragment has been shown to serve as a biomarker for prostate cancer (Ren *et al.* 2013).

lncRNAs: prognostic markers in thyroid cancer

lncRNAs have been shown to be aberrantly expressed in cancer and correlate with tumorigenesis, tumor progression and metastasis in various human cancers. lncRNAs could be involved either in oncogenic or tumor-suppressive pathways and their expression may directly correlate with the good/bad prognosis that makes them promising prognostic biomarkers. In thyroid cancer, the expression level of NONHSAT037832 was drastically decreased in PTCs compared with paired normal tissues. A similar pattern was also observed in PTC cell lines (K1 and IHH-4) when compared with the normal Nthy-ori 3–1 cell line. Moreover, downregulated NONHSAT037832 has been significantly associated with LNM and tumor size (larger tumor) suggesting that NONHSAT037832 and rs2439302 of PTCSC3 could serve as a potential prognostic marker of PTC, as it clearly showed a high value to differentiate between PTCs and noncancerous tissues (Lan *et al.* 2015b). The rs2439302 of PTCSC3 has also been associated with LNM and tumor multifocality reflecting a pivotal role in clinical course (Jendrzewski *et al.* 2016). Besides, LOC100507661 has been frequently detected as a highly expressed lncRNA in PTC and ATC cases, and significantly associated with LNM and the classical *BRAF* mutation (Kim *et al.* 2016). NR_036575.1 and FAL1 have been positively associated with extra thyroidal extension (ETE) and tumor size, and the risk of multifocality, respectively (Jeong *et al.* 2016, Sun *et al.* 2016). XLOC_051122 and XLOC_006074 have also been demonstrated to correlate with LNM especially in patients carrying wild-type *BRAF* and shown to be overexpressed in these cases and associated with risk factors of adverse prognosis (Liyararachchi *et al.* 2016). Similarly, NONHSAT076747 and NONHSAT122730

Table 3 lncRNAs associated with clinicopathological features of thyroid cancer patients.

No.	Clinicopathological features	lncRNAs	Chr. location	References
1.	BRAF (V600E) mutation	LOC100507661 BANCR	Chr. 3q26.2 Chr. 9q21.12	Kim <i>et al.</i> (2016) Wang <i>et al.</i> (2014a), Zheng <i>et al.</i> (2016)
2.	Differentiation	LOC100507661	Chr. 3q26.2	Kim <i>et al.</i> (2016)
3.	Extrathyroidal extension	NR_036575.1	Chr. 19p13.11	Sun <i>et al.</i> (2016)
4.	Metastasis	HIT000218960 LINC00271 LOC100507661 MEG3 NONHSAT037832 NONHSAT076754	Chr. 12 Chr. 6q23.3 Chr. 3q26.2 Chr. 14q32 Chr. 14 Chr. 2 Chr. 1q21.2 Chr. 1p34.1 Chr. 16q24.3 Chr. 9q21.12 Chr. 6q23.3	Li <i>et al.</i> (2017a) Ma <i>et al.</i> (2016) Kim <i>et al.</i> (2016) Wang <i>et al.</i> (2015) Lan <i>et al.</i> (2015b) Xia <i>et al.</i> (2017) Jeong <i>et al.</i> (2016) Xiang <i>et al.</i> (2017) Pan <i>et al.</i> (2016) Wang <i>et al.</i> (2014a), Zheng <i>et al.</i> (2016)
5.	Multifocality	FAL1	Chr. 2	Ma <i>et al.</i> (2016)
6.	Prognosis	SLC6A9-5:2 GAS8-AS1 BANCR LINC00271 ENST00000462717 ENST00000415582 NR_028494	Chr. 1q21.2 Chr. 1p34.1 Chr. 16q24.3 Chr. 9q21.12 Chr. 6q23.3 Chr. 3p14.1 Chr. 1q32.1 N/A	Ma <i>et al.</i> (2016) Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016)
7.	Recurrence	TCONS_00024700 LINC00271	Chr. 16q21 Chr. 6q23.3	Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016)
8.	Resistance ^(131I)	ENST00000462717 ENST00000415582 NR_028494	Chr. 3p14.1 Chr. 1q32.1 N/A	Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016) Qiu <i>et al.</i> (2016)
9.	TNM stage	TCONS_00024700 HIT000218960 LINC00271 NONHSAT076754	Chr. 1p34.1 Chr. 16q21 Chr. 12 Chr. 6q23.3	Xiang <i>et al.</i> (2017) Qiu <i>et al.</i> (2016) Li <i>et al.</i> (2017a) Ma <i>et al.</i> (2016)
10.	Tumor size	NONHSAT037832 NONHSATG051968 NR_036575.1	Chr. 2 Chr. 14 Chr. 9p21.2 Chr. 19p13.11	Xia <i>et al.</i> (2017) Lan <i>et al.</i> (2015b) Wang <i>et al.</i> (2016) Sun <i>et al.</i> (2016)

Chr, chromosome; C, location, chromosomal location; N/A, data not available; S, No., study number; TNM, tumor node metastasis.

expression have also been associated with *LMN* and hence could serve as potent prognostic biomarkers in thyroid cancer (Wang *et al.* 2016). A study explored plasma lncRNAs using lncRNA microarray chip as a novel biomarker panel for the diagnosis of non-¹³¹I-avid lung metastases of PTCs. In the non-¹³¹I-avid lung metastases of PTC cases, two lncRNAs (ENST00000462717 and ENST00000415582) were discovered to be overexpressed, while two (TCONS_00024700 and NR_028494) were downregulated. Low (ENST00000462717 and ENST00000415582) and high lncRNA levels in plasma (TCONS_00024700 and NR_028494) have been associated with better prognosis of these PTC cases. ROC-mediated analysis of these 4 lncRNAs revealed that they have high diagnostic specificity and sensitivity for predicting non-¹³¹I-avid lung metastases of PTC (Qiu *et al.* 2016). Furthermore, PTCSC1 and PTCSC2 were associated with thyroid cancer risk (He *et al.* 2009, Wang *et al.* 2017). HOTAIR overexpression has been associated with poor outcome (Li *et al.* 2017b). LINC00271 and HIT000218960 have been associated with ETE, LNM, TNM stage III & IV and recurrence (Ma *et al.* 2016, Li *et al.* 2017a). A recent study found that expression of SLC6A9-5:2 have been significantly decreased in ¹³¹I-resistant thyroid cancer cell lines and ¹³¹I-insensitive patients and were positively correlated with the expression of *PARP-1*. Artificial knockdown of SLC6A9 or inhibiting *PARP-1* in the sensitive PTC cell lines resulted in a resistant phenotype under ¹³¹I exposure. In turn, SLC6A9-5:2 overexpression restored the sensitivity. Furthermore, the SLC6A9 low expression has been associated with poor prognosis of PTCs, suggesting that this lncRNA could be a prognostic biomarker (Xiang *et al.* 2017). Therefore, the lncRNAs discussed above including those listed in Table 3 could readily serve as a valuable prognostic marker in PTC cases as they were significantly associated with various clinical and pathological features (Table 3).

Conclusion and future perspectives

Understanding the mechanism of lncRNAs and their role is important not only to normal thyroid tissues but also in thyroid cancer and its disease stages. Various high-throughput next-generation genomic technologies facilitated the rapid screening of tissue-specific differentially expressed lncRNA transcripts. From the above-discussed studies, it is evident that lncRNAs are emerging as a new class of ncRNAs with a significant and

crucial role in thyroid cell proliferation, carcinogenesis and metastasis. These differentially expressed lncRNAs play a pivotal role either as a tumor suppressor or as an oncogene. The oncogenic lncRNAs could be molecular therapeutic targets. The tumor-suppressive and oncogenic lncRNAs may serve as potential diagnostic biomarkers, while the disease-associated lncRNAs listed in Table 3 could serve as prognostic biomarkers. Many differentially expressed lncRNAs are understudied and they need further *in vitro* and *in vivo* functional studies to investigate their functions and contribution to thyroid cancer pathogenesis. The function of lncRNAs could be initially predicted by various *in silico* analysis tools. Based on these predictions, siRNA, shRNA, small molecule and ASO-mediated inhibition of target lncRNA and overexpression of a mammalian expression vector with the cDNA of a target lncRNA *in vitro* are valid methodologies for loss-of-function and gain-of-function studies, respectively. Phenotypic assays such as proliferation, cell death or apoptosis, cell cycle, migration, invasion and metastasis and immunoblotting for detecting signaling network can be performed upon implementing transient/stable *in vitro* methodologies. Xenograft tumor on nude mice and knockout mice are functional *in vivo* animal models and these experimental methods could determine the functions of hundreds of uncharacterized differentially expressed lncRNAs in thyroid cancer. Moreover, translation of lncRNAs from bench to bedside warrants a thorough and detailed study of their structure, and their regulation in normal and malignant cells, large case-control studies and possible side effects and rectifying methods upon gene therapy. Although many groups have studied lncRNAs in thyroid cancer, the majority of them have focused only on PTC. On the other hand, to date, other follicular cell-derived thyroid cancer subtypes have not been studied apart from LOC100507661 in two ATC cell lines (C643 and 8505C) (Kim *et al.* 2016). There are many areas which are less studied: for example, subtypes of PTCs (tall cell-PTC and follicular variant PTC), FTC and ATC. Further studies in these areas may not only advance the understanding of molecular pathogenesis of these subtypes but may also improve the therapeutic, diagnostic and prognostic options of these subtypes including ATC, the most deadly thyroid cancer subtype.

Declaration of interest

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

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