Long Non-Coding RNAs in the Cell Fate Determination of Neoplastic Thymic Epithelial Cells

Alessia Iaiza†, Claudia Tito†, Federica Ganci‡, Andrea Sacconi‡, Enzo Gallo§, Silvia Masciarelli*†, Giulia Fontemaggi‡, Alessandro Fatica‡, Enrico Melis‡, Vincenzo Petrozza‡, Federico Venuta‡, Mirella Marino‡, Giovanni Blandino‡ and Francesco Fazi‡*

1 Department of Anatomical, Histological, Forensic and Orthopedic Sciences, Section of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy, 2 Oncogenomic and Epigenetic Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy, 3 Department of Pathology, IRCCS Regina Elena National Cancer Institute, Rome, Italy, 4 Department of Life Science and Public Health, Histology and Embryology Unit, Catholic University of the Sacred Heart, Rome, Italy, 5 Department of Biology and Biotechnology ‘Charles Darwin’, Sapienza University of Rome, Rome, Italy, 6 Thoracic Surgery, IRCCS Regina Elena National Cancer Institute, Rome, Italy, 7 Pathology Unit, ICOT, Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy, 8 Department of Thoracic Surgery, Sapienza University of Rome, Rome, Italy

Keywords: thymoma, thymic carcinoma, thymic epithelial tumors (TETs), ncRNAs (non coding RNAs), miRNA - microRNA, IncRNA - long noncoding RNA, MALAT1, myasthenia gravis

INTRODUCTION

The thymus is the primary lymphoid organ located at the level of the anterior mediastinum. The thymus plays an essential role in educating and enabling the maturation of prelymphocytes into mature T lymphocytes, which are involved in the adaptive immune response. Thymic epithelial-reticular cells (TECs) play a major role in the maturation of T lymphocytes. However, TECs may
undergo neoplastic transformation, resulting in certain types of tumors, such as thymic epithelial tumors (TETs) (1).

TETs are relatively rare neoplasms in middle-aged or elderly adults that represent 0.2–1.5% of all cancers and comprise thymoma, thymic carcinoma (TC), and thymic neuroendocrine tumors (2). TETs are characterized by wide variability and heterogeneity in their malignant behavior. In 30% of cases, TETs are asymptomatic; however, in 40% of cases, local symptoms, such as chest pain, cough, dyspnea, and hoarseness, are displayed while in the remaining 30% of cases, systemic symptoms, with superior vena cava syndrome (SVC), and in the most aggressive forms, weight loss, ensue. To date, the etiology of TETs has not been established and the risk factors remain unclear (3).

TETs classification has remained controversial subject for many years. After different classification approaches [Bernatz et al. in 1961, Rosai and Levine in 1976, Marino and Muller-Hermelink, in 1985 (4)], Rosai and Sobin published a new classification in the World Health Organization (WHO) series in 1999, dividing thymic tumors into three major subgroups based on the morphology of epithelial cells and the percentage of epithelial and lymphocyte populations: type A, type B, and type C (thymic carcinoma). Type A thymomas are tumors with a component of spindle-oval EC but lack lymphocytes, whereas type B thymomas are characterized by large EC with dendritic or plump (epithelioid) morphology, forming networks where lymphocytes are attracted. Notably, the combination of these two morphologies has been designated as type AB (5). Thymic carcinoma (type C) is a rare malignancy, representing less than 1% of thymic tumors, and is characterized by cytological atypia, more aggressive behavior, and local and distant metastases (liver, lymph nodes, or bones) (6).

Recently, a new WHO classification of thoracic cancers was established, which includes new diagnostic criteria and rare entities, such as hyalinizing clear cell carcinoma (7). Interestingly, thymoma is strongly associated with various paraneoplastic syndromes (PNS), such as myasthenia gravis (MG), red cell aplasia, polymyositis, systemic lupus erythematosus, Cushing syndrome, and syndrome of inappropriate antidiuretic hormone secretion (8).

Thymomas are associated with MG in 30–50% of cases, and thymoma occurs in 10–15% of cases of MG (9). MG (myos = muscle, asthenos = weakness, gravis = severe) is an autoimmune disease that affects the neuromuscular junction (NMJ) of the skeletal muscle, causing muscle weakness of different severity, several complications such as myasthenia crisis, and in some cases, acute respiratory paralysis (10–13). MG can develop at any age, particularly in young women (>30 years) and older men (>60 years) (14).

Thymoma-associated myasthenia gravis (TAMG) is frequently reported in adults and is characterized by alterations in thymus function (14). The association between MG and thymoma is due to the dysregulation of positive and negative selection of T cells in the thymus (15). For example, in cortical thymoma, the lack of medullary epithelial cell function and defects in the autoimmune regulator complex (AIRE), which is responsible for negative selection, leads to the production of autoreactive T cells specific for acetylcholine receptors (AChR) that are exported to the periphery, where autoreactive T cells stimulate and activate B cells to produce antibodies against AChR (14, 16). Although the required treatment for thymoma-associated MG is tumor removal, remission is not inevitable. Moreover, removal of thymoma in non-myasthenic patients does not prevent the subsequent onset of MG. Patients with thymoma have been observed to develop antibodies against AChR and symptoms of myasthenia after the resection of the tumor (17).

MOLECULAR PATHWAYS IN TETs AND MG

Although the etiology of TETs is still poorly understood, advanced next-generation sequencing (NGS) techniques have recently allowed the mapping of gene mutations and epigenetic alterations occurring in thymic tumors.

One of the most frequently mutated genes in TETs is GTF2I, whose mutation is specifically associated with types A and AB (78%), but is less frequent in more aggressive histological types, such as thymic carcinomas (8%) (18). The overexpression of EGFR and HER2 and mutations in KIT, IGR-1, and neurotrophin receptors have been recently demonstrated in some cases, as reviewed by Scorsetti et al. (6). Gain-of-function mutations in HRAS and NRAS and loss-of-function mutations in TP53 are less common, but are considered founder mutations (19).

Thymic carcinoma is characterized by loss of chromosome 16q, mutations in epigenetic regulatory genes (BAPI, ASXL1, SETD2, SMARCA4, TET2, DNMT3A, and WTI), and anti-apoptotic genes (BCL2 copy number gains) (8, 20).

Notably, gene expression profiling in TGCA study revealed four molecular subtypes, represented respectively by type B (subtype 1), TC (subtype 2), AB (subtype 3), and a mix of types A and AB (subtype 4). TGCA study also revealed four distinct molecular clusters using PARADIGM analysis. In particular, the upregulation of TP53 and downregulation of oncogenes, such as MYC/Max, MYB, and FOXM, characterize the A-like cluster, while the downregulation of TP53 and upregulation of MYC/Max, MYB, FOXM1, and E2F1 in AB-, B-, and C-like clusters are consistent with the high aggressiveness of B3 and TC tumors. Furthermore, this study highlighted that types A, AB, B, and TC are not a continuum of diseases, but are instead distinct biological entities (5, 19). Recently, metaanalytic thymomas were reported to harbor the YAP1-MAML2 translocation, whereas 6% of pretreated types B2 and B3 and a combined TC and B3 thymoma (but not in thymoma and “pure” TCs) may be associated with KMT2A-MAML2 translocation (7).

Alterations in inflammatory and thymus function, which occur in thymic neoplasms, and mutations in the AIRE gene locus promote the development of autoimmune diseases, such as MG (16). EOMG is associated with HLA-DRA3, HLA-B8, and other autoimmune risk genes, whereas LOMG is weakly associated with HLA-DRA2, HLA-B7, and mLRA-DR-B1*15:0 (14). Aneuploidy and intratumoral overexpression of genes
that have a similar sequence to autoimmune targets (CHRNA1, RYR3, and NEFM) are common in patients (19).

In addition to mutations in protein-coding genes, alterations in ncRNA molecules have been reported to significantly impact the initiation, progression, and response of TETs and MG to therapy, as described in the next section.

IDENTIFICATION OF ncRNAs IN TETs AND MG

Despite being considered “junk” for a long time, ncRNAs have emerged as functionally relevant in nearly all physiological and pathological cellular processes (21, 22). These new discoveries have been aided by powerful high-throughput approaches, such as next-generation sequencing (NGS), transcriptome studies, molecular network analyses, and artificial intelligence-guided prediction of ncRNA function (23).

ncRNAs play a role in many biological, physiological, and developmental processes, including several diseases and tumors. ncRNAs are produced by transcription from different genomic regions and post-transcriptional maturation and modification. ncRNAs can be divided into two classes according to their length (24): small non-coding RNAs (ncRNAs) and IncRNAs (usually >200 nt).

In recent years, ncRNAs have been found to play an important role in gene regulation at different levels. Several studies have demonstrated the involvement of ncRNAs in transcriptional regulation, RNA maturation, chromatin remodeling, post-transcriptional RNA regulation, and modification (25). Dysregulation of ncRNAs is involved in many human diseases and in tumor initiation and progression (26). Regulatory ncRNAs can be divided into two classes: circular and linear.

Circular RNAs (circRNAs) are a class of covalently closed RNA molecules and are thus more stable than linear RNAs. The expression patterns of circRNAs are cell type-, tissue-, and developmental stage-specific (27, 28). Depending on their localization, circRNAs can exert different functions, including acting as microRNA (miRNA) sponges, modulating the activity of RNA-binding proteins (RBPs), or acting as protein scaffolds, which can be translated into polypeptides owing to the presence of internal ribosome entry sites (IRES) or m6A modifications (27, 29). Many circRNAs are differentially expressed in several cancer types compared with their untransformed counterparts and are related to tumor growth, metastasis, and therapy resistance (30, 31). Interestingly, several studies have demonstrated that circRNAs are differentially expressed in thymoma and MG (32, 33), and have highlighted their important role as biomarkers for the diagnosis of this disease (34).

Another major group of regulatory ncRNAs is represented by linear ncRNAs, comprising small ncRNA (18-200 nt) and IncRNAs (>200 nt) (35).

Among small ncRNAs, miRNAs play a predominant role in post-transcriptional regulation, binding to specific mRNA targets and causing their degradation or translation inhibition. miRNAs are single-stranded RNAs with an average length of 22 nucleotides that are derived from hairpin-structured precursors (36). Several miRNAs are altered in different human diseases, including cancer. The roles of miRNAs in thymus differentiation, development, and involution have been extensively described by Cron et al. (37). Moreover, their relevance in the treatment, diagnosis, and prognosis of TETs and MG is emerging (38).

Several research groups have identified miRNAs that are differentially expressed between thymic tumors and normal samples, as well as between thymic carcinoma and thymoma and histotype classes (39–41). In particular, Bellissimo et al. found that miR-145-5p was epigenetically downregulated in thymic carcinoma cells (42), confirming its well-known tumor suppressor role (43). Similar to circRNAs, miRNAs are excellent biomarkers for cancer diagnosis found in the serum of patients with TETs and MG (44–49).

LncRNAs IN TETs

Although miRNAs represent one of the most studied biomarkers involved in thymic tumorigenesis among all ncRNA classes, the study of LncRNAs is becoming more relevant. Based on their localization, LncRNAs can bind to genes, transcripts, miRNAs, and proteins that regulate different cellular processes, such as gene expression, transcription, and post-transcriptional regulation, through different mechanisms of action. Further, LncRNAs can act as guides of chromatin modification complexes, including DNA methyltransferase and histone-modifying enzymes, on specific genomic loci that induce activation or inhibition of target genes in cis or trans (50); function as decoys for transcription factors and other effectors, impairing their regulatory activity (50); serve as modular scaffolds, and bind and drive two or more physically distant proteins into specific genomic regions to regulate gene expression (51); act as sponges of miRNAs, sequestering them and preventing their ability to promote degradation or repression of target genes (52, 53); and influence splicing (54) and the stability of miRNAs, regulating their post-transcriptional expression (55). Therefore, according to their mechanisms of action, LncRNAs are involved in the regulation of various biological processes, including cellular survival, proliferation, differentiation, apoptosis, invasion, and metastasis. Consequently, aberrant LncRNA expression is tightly correlated with cancer development (56).

Deregulation of LncRNAs in TETs

Similar to many types of human tumors, such as breast cancer (57), lung cancer (58), colorectal carcinoma (59), ovarian cancer (60) and prostate cancer (61), dysregulated LncRNAs in TETs may contribute to tumor onset and progression. Different studies have identified a large spectrum of LncRNAs in thymic epithelial tumors using high-throughput sequencing technologies. In this context, most of the identified LncRNAs act as miRNA sponges, playing oncogenic or tumor suppressor roles. An example of this type of regulation is represented by LncRNA LOXL1-AS1, miR-525-5p, and the HSPA9 gene network. Data from TCGA study showed that high expression of LOXL1-AS1 and downregulation of miR-525-5p correlated with poor prognosis in TET (62).
Similar to other cancer types (63, 64) in thymic tumors, miR-525-5p acts as a tumor suppressor, inhibiting cell growth and invasion, and inducing apoptosis by repressing the target gene, HSPA9. HSPA9 is upregulated in thymoma and thymic carcinoma and correlated with poor patient survival. The positive association between LOXL1-AS1 and HSPA9, which is consistent with the downregulation of miR-525-5p, was confirmed by in vitro experiments. The silencing of LOXL1-AS1 promotes thymic tumor progression by acting as a sponge of miR-525-5p and increasing the expression of HSPA9 (62).

Similar to LOXL1-AS1, another network is represented by the interaction between lncRNA LINC00174, miR-145-5p, and miR-145-5p predicted target genes involved in thymic tumorigenesis (65). In this study, the upregulation of lncRNA LINC00174 in frozen tissue samples of thymoma compared to its normal counterparts was identified. LINC00174 is negatively associated with miR-145-5p, a well-known tumor suppressor of miRNA downregulation in TETs (39, 42), and positively correlated with miR-145-5p predicted targets (Figure 1). The inhibition or overexpression of miR-145-5p modulates LINC00174 expression and its associated genes (65). Notably, the poor prognosis of TET patients, characterized by high expression of LINC00174 and its associated genes and low expression of miR-145-5p, suggests an oncogenic role of LINC00174 in TETs. According to these data, LINC00174 silencing impairs cell growth and proliferation, cell migration, and lipid metabolism. Similar to LINC00174, MALAT1 can act as a sponge for miR-145-5p in TET. MALAT1 is a well-known oncogenic IncRNA that regulates different biological processes, such as cell proliferation, apoptosis, angiogenesis, invasion, and metastasis, and contributes to cancer development (66–68). Using a luciferase assay, the interaction between miR-145-5p and MALAT1 was demonstrated in the thymic cancer cell line, IU-TAB1. In this thymic tumor context, the downregulation of MALAT1 increased miR-145-5p expression and led to a reduction in cell proliferation and an increase in the apoptosis rate compared to that observed in the control. Additionally, the combination of MALAT1 silencing and miR-145-5p overexpression induces a synergistic effect, suggesting that MALAT1 may regulate the thymic cancer phenotype by inhibiting miR-145-5p (69).

Recently, the expression of MALAT1 has been a focus in our studies where the relationship between lncRNA MALAT1 and METTL3, a methyltransferase enzyme that catalyzes the N6-methyladenosine (m6A) modification, were described in the thymic carcinoma cell line, TC1889. Of note, the expression of lncRNAs can be regulated by m6A modifications (70), and the downregulation of METTL3 leads to increased localization of MALAT1 in nuclear speckles and decreased m6A modification of MALAT1 lncRNA (71), which probably impinges on its functional activity. In the past, we observed a similar delocalization of MALAT1 in nuclear speckles with consequent altered splicing (72) due to the presence of mutant p53 protein in breast cancer cells. Another interesting lncRNA-miRNA-target network in the...
control of thymoma progression was reported by Yang et al. (73).
The lncRNA RP11-424C20.2 regulates the expression of the UHRF1 gene (ubiquitin-like containing PHD ring finger 1) by sponging miR-378a-3p; the RP11-424C20.2/UHRF1 axis is strongly associated with a better outcome in thymoma patients, which is related to the different types of infiltrating immune cells, such as B cells, macrophages, CD8+ and CD4+ cells, neutrophils, and dendritic cells. The role of UHRF1 is well established; it is an epigenetic modifier that regulates immune infiltration and the tumor immune microenvironment through its interaction with DNA histone deacetylase genes (73, 74). Therefore, RP11-424C20.2 expression can influence the prognosis of patients with thymoma by regulating the expression of UHRF1 via miR-378a-3p sponging (73).

Owing to this recent evidence, the identification of altered lncRNAs in TETs and the characterization of their role in the promotion of tumorigenesis could provide new potential therapeutic targets relevant for the treatment of TETs.

**LncRNAs as a Predictive Factor of Patient Prognosis in TETs**

Studies on the profiles of lncRNAs expressed in thymoma tissue samples have revealed that altered expression of specific lncRNAs may correlate with overall or disease-free survival. For example, Su et al. (75) identified a panel of lncRNAs that predicts the recurrence of thymic epithelial tumors. They analyzed a cohort of 114 TET patients from TCGA study and identified four lncRNAs, ADAMTS9-AS1, HSD52, LINC00968, and LINC01697, which are significantly related to recurrence-free survival (RFS). These lncRNAs can be used to divide TET patients into high-risk and low-risk groups, respectively, with shorter and longer RFS. Based on ROC analysis, these lncRNAs represent a better prognostic model for the RFS of patients than the WHO classification and Masaoka stage. Although these lncRNAs constitute a good factor of discrimination between different TETs subtypes and their associated stages, the trial had some limitations: few samples from TCGA, absence of evidence of their predictive power in other types of cancer, and biological characterization of their role in TETs (75).

Furthermore, the altered expression of ADAMTS9-AS1 (one of the four RFS-related lncRNAs) with five other lncRNAs, namely AFAP1-AS1, LINC00324, VLDLR-AS1, LINC00968, and NEAT1, was detected in another study by RNA-seq and profiling expression analysis in 25 thymoma patients and 25 healthy individuals (76). These lncRNAs are involved in the development of different types of cancers and regulate several biological processes and molecular pathways. For example, ADAMTS9-AS1 induces cell migration and proliferation in colorectal carcinoma, affecting β-catenin expression (77); LINC00968 reduces drug resistance and invasion of tumor cells in breast cancer (78); AFAP1-AS1 increases epithelial-mesenchymal transition by impairing RhoC, ROCK1, p38MAPK, and Twist1 signaling pathways in osteosarcoma (79); LINC00324 inhibits the NOTCH pathway, regulating apoptosis and cell proliferation in papillary thyroid cancer (80); VLDLR-AS1 modulates the expression of genes involved in fat loss in cancer cachexia, acting as a sponge of hsa-miR-1224-3p (81) and finally, NEAT1, by sponging miR-193b-3p, activates cyclin D, promoting cell proliferation in cervical cancer (82). Notably, the differential expression of these lncRNAs in TETs affects the disease-free survival of patients. In particular, the high expression of ADAMTS9-AS1 and low expression of LINC00324 are correlated with the worst prognosis of patients. Similar to other types of cancer, the expression of these lncRNAs was found to be correlated with the deregulation of miRNA clusters and target genes involved in the regulation of tumorogenic signaling pathways, including PI3K/Akt, FoxO, HIF-1, and Notch, supporting their oncogenic role in the tumorigenesis process (76). Moreover, Gong et al. found that AFAP1-AS1, LINC00324, and VLDLR-AS1 were associated with the RFS of patients with TETs (83).

According to these data, bioinformatic analysis performed for different types of TETs (A, B, AB, and TC) revealed that different competitive endogenous RNA (ceRNA) networks were significantly associated with the overall survival of individuals. The two most important lncRNAs in this ceRNA network were LINC00665 and NR2F1-AS1. The association between their expression and patient prognosis aligns with their biological function (84). LINC00665 binds to mRNAs MYO10 and WASF3 through the miRNAs, hsa-miR-140 and hsa-miR-3199. LINC00665 is upregulated in lung cancer, regulates cellular proliferation and invasive ability in lung adenocarcinoma, and is a predictive factor of this tumor (85). NR2F1-AS1 can indirectly interact with FBN1, GALNT16, HAND2, and MCAM through miR-140, miR-139, and miR-141. NR2F1-AS1 leads to the impairment of osteosarcoma, acting as a sponge of miR-483-3p and increasing FOXA1 gene expression (86).

Based on these recent studies, profiling analysis of lncRNA expression can be used as a potential and innovative strategy for the detection and follow-up of thymic epithelial tumors (Table 1).

**LncRNAs IN MG**

The alteration of lncRNA expression could play a prominent role in distinguishing thymomatous and non-thymomatous MG and clarifying the molecular mechanisms underlying its pathogenesis. In this context, by using lncRNA and mRNAs microarray analyses, Luo et al. (87) identified an aberrant expression of different lncRNAs between MG patients with thymoma and healthy controls, and MG patients without thymoma and normal individuals. In the first case, lncRNAs upregulated in MG patients with thymoma were associated with different regulatory pathways that contribute to thymic cancer progression and immune cell proliferation, such as cell response to interferon-γ, positive regulation of cytokine production, chemokine receptor binding, and regulation of smooth muscle cell proliferation. In particular, the most upregulated lncRNA in MG patients with thymoma is Oebiotech_11933, an lncRNA related to the MAPK, chemokine, and Toll-like receptor signaling pathways (87–89). In the second case, although altered lncRNAs in MG patients without thymoma revealed...
their association with the same cellular pathways in MG patients with thymoma (i.e., positive regulation of cytokine production and chemokine receptor binding), they showed a lower association with cell response to interferon-γ. These data highlight that the discrimination between MG patients with or without thymoma may depend on the presence of altered lncRNAs involved in the regulation of IFN-γ expression (90). Additionally, these lncRNAs have been observed to function by regulating the transcription of genes in cis or trans (87). Consistent with this study, Ke et al. found another lncRNA, XLOC_003810, which is highly expressed in MG-associated thymoma patients, and revealed an increase in activated CD4+ T cells compared to that in control samples. In vitro experiments using thymic mononuclear cells demonstrated that the overexpression of XLOC_003810 leads to an increase in CD4+ T cells and production of the inflammatory cytokines IFN-γ, TNF-α, and IL-1β. In contrast, the downregulation of XLOC_003810 caused the opposite results. Consequently, as the activation of CD4+ T cells and inflammatory cytokines plays an important role in the development of thymoma-associated MG, XLOC_003810 lncRNA could contribute to the pathogenesis of these cellular pathways (91). The study by Niu et al. supports the role of XLOC_003810 in MG with thymoma. XLOC_003810 affects the balance between T helper 17 (Th17) and T regulatory cells (Tregs) (92). T helper cells are active in the adaptive immune response against antigens and pathogens, and Tregs have suppressive potential, preventing autoimmune diseases (93). The overexpression of XLOC_003810 leads to higher levels of Th17 cells than Tregs, which, on the contrary, increases upon silencing of this lncRNA. This association is also evident in MG-T patients and is characterized by an increase in CD4+ T cells and Th17 cells and a decrease in Treg cells (92). As the number of Tregs increases in MG-T patients upon immunosuppressive treatment (94) and the number of Th17 cells correlates with the severity of the disease (95), the alteration of XLOC_003810 expression could enhance the imbalance in the Th17/Treg ratio, favoring the pathogenetic mechanism. Moreover, the discrimination between patients with MG with or without thymoma is also determined by the different hypomethylation and hypermethylation levels associated with the aberrant expression of lncRNAs. The presence of DNA methylation sites has been observed in three immune-related lncRNAs, namely AC004943.1, FOXG1-AS1, and WT1-AS, in (MG-T) patients. DNA methylation is an epigenetic modification catalyzed by DNA methyltransferase enzymes that promote the silencing of gene expression. In this context, tissue samples of thymoma patients with MG are characterized by lower methylation levels of these lncRNAs than those without MG. Consequently, MG-T patients showed a higher expression of these immune-related lncRNAs that correlate with their involvement in pathogenesis, regulating different biological processes, such as transmission at the neuromuscular junctions, cell cycle, actin and Ras GTPase binding, and herpes simplex virus 1 infection associated with MG development (96). Although the lncRNA, MALAT1, is a known oncogene that promotes thymic cancer development, as described previously, it plays a protective role in MG (Figure 2). Compared to healthy individuals, lower expression of MALAT1 has been observed in MG patients, together with higher expression of miR-338-3p, an oncosgenic miRNA that directly targets MSL2, a gene involved in chromatin organization and DNA damage response. A previous study revealed the interaction between MALAT1, miR-338-3p, and MSL2 by luciferase assay, demonstrating that the silencing of MALAT1 leads to an increase in miR-338-3p expression, reducing MSL2 protein levels (97). The downregulation of MALAT1 in MG patients suggests its involvement in the inhibition of T lymphocytes, suggesting that it could be a specific target for MG treatment. Finally, different lncRNAs are

TABLE 1 | LncRNAs deregulated in TETs.

| lncRNAs     | Expression | Biological function                                                                 | Prognostic clinic value                          | References     |
|-------------|------------|-------------------------------------------------------------------------------------|-------------------------------------------------|----------------|
| LOXL1-AS1   | Upregulated in thymoma and thymic carcinoma | LOXL1-AS1 acts as a sponge for miR-525-5p, increasing HSPA9 expression. | High levels of LOXL1-AS1 and HSPA9 are associated with poor prognosis | Wang et al. (62) |
| LINC00174   | Upregulated in thymoma and thymic carcinoma | LINC00174 acts as a sponge for miR-145-5p. | High levels of LINC00174 and low level of miR-145-5p are associated with poor prognosis | Tito et al. (63) |
| MALAT1      | Upregulated in thymic carcinoma | MALAT1 acts as a sponge for miR-145-5p. MALAT1 localization is m7A-dependent and is involved in c-MYC induction. | High levels of MALAT1 are associated with poor prognosis | Tan et al. (69) |
| RP11-424C20.2| Upregulated in thymoma | RP11-424C20.2 acts as a sponge for miR-378a-3p, increasing UHRF1 expression. | High levels of RP11-424C20.2 and UHRF1 are associated with better prognosis | Yang et al. (73) |
| AFAP1-AS1   | Upregulated in thymoma | They are involved in the regulation of cell proliferation. | High levels of AFAP1-AS1 and low levels of LINC00324 and VLDLR-AS1 are associated with poor disease-free survival | Ji et al. (76) |
| LINC00665   | Upregulated in thymoma | LINC00665 acts as a sponge for miR-140 and miR-3199, increasing MYO10 and WASF3. | High levels of LINC00665 are associated with poor overall survival | Chen et al. (84) |
| NR2F1-AS1   | Upregulated in thymoma | NR2F1-AS1 acts as a sponge for miR-140, miR-139 and miR-141, increasing FB1, GALNT16, HAND2, and MCAM expression. | High levels of NR2F1-AS1 are associated with poor overall survival | Chen et al. (84) |
involved in the regulation of hydrolase, phosphorylase, and dephosphorylase enzyme activities, which affect the activation of T cells during selection in the thymus, promoting MG development (98).

Based on the evidence, a large spectrum of lncRNAs can regulate different signaling pathways that contribute to the development of associated MG-thymoma. As a result, they could be used as biomarkers to distinguish between two types of MG, namely MG with or without thymoma. Moreover, the data suggest the possible use of these pathogenesis-related molecules as therapeutic targets (Table 2).

**CONCLUDING REMARKS**

Recent advances in next-generation sequencing technologies have enabled the study of the role of ncRNAs in the development and progression of cancer. Particularly, the aberrant expression of the most studied groups of ncRNAs, such as miRNAs, circRNAs, and lncRNAs, is associated with tumorigenesis, highlighting the role of ncRNAs as oncogenes or tumor suppressors. In this review, we sought to provide an overview of lncRNA regulation in the initiation and progression of TET and MG. Many lncRNAs identified in these diseases play an oncogenic role, acting as...
sponges of tumor suppressor miRNAs and consequently regulating many cellular pathways that contribute to the cancer phenotype. The identification of aberrant expression of lncRNAs and studies on their inhibition or overexpression allow us to understand their contribution to the thymic cancer phenotype and suggest specific targeted therapies. Various lncRNAs differentially expressed in tumor vs. normal tissues in patients with TET are potential powerful biomarkers for the detection and follow-up of diseases.

The role of the lncRNA, MALAT1, which has several opposing functions, is particularly intriguing. In thymoma and thymic carcinoma, MALAT1 regulates cell proliferation by acting as an miR-145-5p sponge and contributing to c-MYC induction, following its change in subnuclear localization due to METTL3 methylation. In contrast, MALAT1 has a protective role in MG, acting as an miRNA sponge and inhibiting T lymphocyte activation. Although several lncRNAs have been identified to date, the function and expression of many lncRNAs in TETs and MG pathogenesis and progression remain unclear. Therefore, further studies on these ncRNAs are necessary. Moreover, the development of novel lncRNA-directed therapeutic strategies could represent a promising and powerful approach for the management of TET and MG.

REFERENCES

1. Thapa P, Farber DL. The Role of the Thymus in the Immune Response. Thorac Surg Clin (2019) 29(2):123. doi: 10.1016/j.thorsurg.2018.12.001
2. Benjamin DJ, Klaphake A, Lara PN, Cress RD, Riess JW. A Population-Based Study of Incidence and Survival of 1588 Thymic Malignancies: Results From the California Cancer Registry. Clin Lung Cancer (2019) 20(6):477–83. doi: 10.1016/j.cllc.2019.06.005
3. Venuta F, Rendina EA, Anile M, de Giacomo T, Vitulo D, Coloni GF. Thymoma and Thymic Carcinoma. Gen Thorac Cardiovasc Surg (2012) 60(1):1–12. doi: 10.1007/s11748-011-0814-0
4. Kashima J, Okuma Y. New Histological Classification and Staging of Thymic Malignancies: ITMIG Consensus Statements and the 8th TNM Staging System. J Thorac Dis (2017) 9(10):3565–74. doi: 10.21037/jtd.2017.09.126
5. Marino M, Marx A, Anemona L, Lauriola L, Ströbel P, Müller-Hermelink HK, Juan Rosai as Master of Our Comprehensive Understanding of Thymus and Thymoma. Pathologica (2021) 113(5):360–70. doi: 10.32071/1591-951X-539
6. Scorsetti M, Leo F, Trama A, D’Angellilo R, Serpico D, Macerelli M, et al. Thymoma and Thymic Carcinomas. Crit Rev Oncol Hematol (2016) 99:332–50. doi: 10.1016/j.critrevonc.2016.01.012
7. Marx A, Chan JKC, Chalabreysse L, Daccis S, Detterbeck F, French CA, et al. The 2021 WHO Classification of Tumors of the Thymus and Mediastinum: What Is New in Thymic Epithelial, Germ Cell, and Mesenchymal Tumors? J Thorac Oncol (2022) 17(2):200–13. doi: 10.1016/j.jtho.2021.10.010
8. Conforti F, Pala L, Giaccone G, De Pas T. Thymic Epithelial Tumors: From Biology to Treatment. Cancer Treat Rev (2020) 86:102014. doi: 10.1016/j.ctrv.2020.102014
9. Zhang Z, Cui Y, Jia R, Xue L, Liang H. Myasthenia Gravis in Patients With Thymoma Affects Survival Rate Following Extended Thymectomy. Oncol Lett (2016) 11(6):4177–82. doi: 10.3892/ol.2016.4528
10. Beloore Suresh A, Asuncion RMD. Myasthenia Gravis. StatPearls Publishing, Treasure Island (FL) (2022).
11. Konœczny I, Herbst R. Myasthenia Gravis: Pathogenic Effects of Autoantibodies on Neuromuscular Architecture. Cells (2019) 8(7):671. doi: 10.3390/cells8070671
12. Wang S, Breskovska I, Gandhy S, Punga AR, Guptill JT, Kaminjhi H. Advances in Autoimmune Myasthenia Gravis Management. Expert Rev Neurother (2018) 18(7):573–88. doi: 10.1080/14737175.2018.1491310
13. Lazaridis K, Tzartos SJ. Autoantibody Specificities in Myasthenia Gravis: Implications for Improved Diagnostics and Therapeutics. Front Immunol (2020) 11:212. doi: 10.3389/fimmu.2020.00212
14. Gilhus NE, Verschuuren JJ. Myasthenia Gravis: Subgroup Classification and Therapeutic Strategies. Lancet Neurol (2015) 14(10):1023–36. doi: 10.1016/S1474-4422(15)00145-3
15. Romi F, Hong Y, Gilhus NE. Pathophysiology and Immunological Profile of Myasthenia Gravis and its Subgroups. Curr Opin Immunol (2017) 49:9–13. doi: 10.1016/j.coi.2017.07.006
16. Marx A, Yamada Y, Simon-Keller K, Schalk W, Wilcock P, et al. Thymus and Autoimmunity. Semin Immunopathol (2021) 43(1):45–64. doi: 10.1007/s00281-021-00842-3
17. Fuji Y. The Thymus, Thymoma and Myasthenia Gravis. Surg Today (2013) 43(5):461–6. doi: 10.1007/s00595-012-0318-2
18. Petroni I, Meltzer PS, Kim J-K, Lucchi M, Park K-S, Fontanini G, et al. A Specific Missense Mutation in GTF2I Occurs at High Frequency in Thymic Epithelial Tumors. Nat Genet (2014) 46(8):844–9. doi: 10.1038/ng.3016
19. Radovich M, Pickering CR, Felau I, Hsu G, Zhang H, Ho E, et al. The Integrated Genomic Landscape of Thymic Epithelial Tumors. Cancer Cell (2018) 33(2):244–258.e10. doi: 10.1016/j.ccell.2018.01.003
20. Wang Y, Thomas A, Lau C, Rajan A, Zhu Y, Källjan JK, et al. Mutations of Epigenetic Regulatory Genes Are Common in Thymic Cancers. Sci Rep (2015) 4(1):7336. doi: 10.1038/srep07336
21. Anastasiadou E, Jacob LS, Slack FJ. Non-Coding RNA Networks in Cancer. Nat Rev Cancer (2017) 17(1):5–18. doi: 10.1038/nrc.2017.99
22. Palazzo AE, Lee ES. Non-Coding RNA: What Is Functional and What Is Junk? Front Genet (2015) 6:2. doi: 10.3389/fgene.2015.00002
23. Wolfien M, Brauer DL, Bagnacani A, Wolkenhauer O. Workload Development for the Functional Characterization of ncRNAs. In: Methods in Molecular Biology. Clifton, NJ: Methods Mol Biol (2019). p. 111–32.
24. Zhang P, Wu W, Chen Q, Chen M. Non-Coding RNAs and Their Integrated Networks. J Integr Bioinform (2019) 16(3). doi: 10.1515/jib-2019-0027
25. Grillone K, Rullo C, Scionti F, Rocca R, Tradigo G, Guzzi PH, et al. Non-Coding RNAs in Cancer: Platforms and Strategies for Investigating the Genomic "Dark Matter". J Exp Clin Cancer Res (2020) 39(1):117. doi: 10.1186/s13046-020-01622-x
26. López-Jiménez E, Andrés-León E. The Implications of ncRNAs in the Development of Human Diseases. Non-Coding RNA (2021) 7(1):17. doi: 10.3909/nccrna7001017

AUTHOR CONTRIBUTIONS

AI and CT designed and wrote the manuscript. SM, GF, MM, and FF reviewed and edited the manuscript. FG, AS, EG, AF, EM, VP, FV, and GB conceptualized the study and provided feedback regarding the content of the manuscript. All authors approved the final version of the manuscript.

FUNDING

AIRC IG 2018 - ID. 21406 project, ‘Progetti Ateneo’ Sapienza University of Rome and PRIN 2017-Prot. 2017TATYMP_003 to FF; AIRC IG 2018 - ID. 21434 Project to GF; and ‘Progetti Ateneo,’ Sapienza University of Rome to VP.

ACKNOWLEDGMENTS

The authors thank the Biobank of the IRCCS Regina Elena National Cancer Institute (BBIRE), Rome, Italy, for providing study samples and for preserving the data.
27. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The Biogenesis, Biology and Characterization of Circular RNAs. Nat Rev Genet (2019) 20(11):675–91. doi: 10.1038/s41576-019-0158-7

28. Qu S, Liu Z, Yang X, Zhou J, Yu H, Zhang R, et al. The Emerging Functions and Roles of Circular RNAs in Cancer. Cancer Lett (2018) 414:301–9. doi: 10.1016/j.canlet.2017.11.022

29. Shi Y, Jia X, Xu J. The New Function of circRNA: Translation. Clin Transl Oncol (2020) 22(12):2162–9. doi: 10.1007/s12094-020-02371-1

30. Patop IL, Kadener S. circRNAs in Cancer. Curr Opin Genet Dev (2018) 44:18–7. doi: 10.1016/j.gde.2017.11.007

31. Guarrerojo J, Bezzi M, Jeong IC, Paffenholz SV, Berry K, Naldini MM, et al. Oncogenic Role of Fusion-circRNAs Derived From Cancer-Associated Chromosomal Translocations. Cell (2016) 165(2):289–302. doi: 10.1016/j.cell.2016.03.020

32. Wu Q, Luo X, Li H, Zhang L, Su F, Hou S, et al. Identification of Differentially Expressed Circular RNAs Associated With Thymoma. Thorac cancer (2021) 12(9):1312–9. doi: 10.1111/1759-7714.13873

33. Lai X, Bi Z, Yang X, Hu R, Wang L, Jin M, et al. Upregulation of Circ-FBL Promotes Myogenic Proliferation in Myasthenia Gravis by Regulation of miR-133/Pax7. Cell Biol Int (2021) 45(11):208676. doi: 10.1016/j.cbi.2021.108676

34. Lv J, Ren L, Han S, Zhang J, Zhao X, Zhang Y, et al. Peripheral Blood Hsa-Mi01886-3p and miR-124-3p as New Non-Coding RNA Biomarkers of Esophageal Squamous Cell Carcinoma. Cancer Lett (2021) 525:192–97. doi: 10.1016/j.canlet.2021.04.008

35. Rimoano G, Venkata D, Acquino M, Small Non-Coding RNA and Cancer. Carcinogenesis (2017) 38(5):485. doi: 10.1093/carcin/bgs026

36. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol (2018) 141(4):1202. doi: 10.1016/j.jaci.2017.08.034

37. Cron MA, Guillochon E, Kusner L, Le Panse R. Role of miRNAs in Normal and Myasthenia Gravis Thymus. Front Immunol (2020) 11:1074. doi: 10.3389/fimmu.2020.01074

38. Blandino G, Fazi F, Donzelli S, Kedmi M, Sas-Chen A, Muti P, et al. Tumor Epigenetics: Cancer Cell Biology and the Role of lncRNA in Chromosomal Translocations. Cell (2016) 165(2):289–302. doi: 10.1016/j.cell.2016.03.020

39. Wu Q, Luo X, Li H, Zhang L, Su F, Hou S, et al. Identification of Differentially Expressed Circular RNAs Associated With Thymoma. Thorac cancer (2021) 12(9):1312–9. doi: 10.1111/1759-7714.13873

40. Enkner F, Pichlhöfer B, Zaharie AT, Krunic M, Holper TM, Janik S, et al. Long Non-Coding RNA WDR5 and KAT2A Complexes to Specify the Histone Modification Pattern. Cancer Discovery (2016) 6(7):784–801. doi: 10.1158/2159-2890.CD-15-0921

41. Radovich M, Ahmad SM, Muntz PT, Malik AA, Dar MA, Urvat W, et al. Long Non-Coding RNA HOTAIR Inactivates Mediator Subunit 17 and Promotes Metastasis in Breast Cancer. Cancer Med (2017) 5(8):2162–6. doi: 10.1002/cam4.1353

42. Bortone F, Scandiffio L, Marcuzzo S, Bonanno S, Frangiamore R, Motta T, et al. miR-146a in Myasthenia Gravis Thymus Bridges Innate Immunity With Autoimmunity and Is Linked to Therapeutic Effects of Corticosteroids. Front Immunol (2020) 11. doi: 10.3389/fimmu.2020.00142

43. Chen M, Liu L. MiR-525-5p Repressed Metastasis and Anoikis Resistance in Esophageal Squamous Cell Carcinoma. J Exp Clin Cancer Res (2015) 34(1):7. doi: 10.1186/s13046-014-01293-4

44. Wang J, Lu A, Chen L. LncRNAs in Ovarian Cancer. Cell Mol Life Sci (2018) 75(3):482–55. doi: 10.1007/s00018-016-2253-1

45. Radovich M, Solzak JP, Hancock BA, Conces ML, Atale R, Porter RF, et al. Analysis of Serum miRNA Profiles From Thymic Epithelial Tumors. Autoimmunity and Is Linked to Therapeutic Effects of Corticosteroids. Cell Lung Cancer: Functions and Distinctions From Other Malignancies. Transl Cancer Res (2019) 8(7):2636–53. doi: 10.21037/tcr.2019.10.22

46. Yoshida K, Kondo M, Nishida K, Ohtani Y, Yonekura N, et al. Oncogenic Role of circRNA in Myasthenia Gravis. Mol Neurobiol (2019) 56(1):457–63. doi: 10.1007/s12035-018-0305-y

47. Zhang H, Lu Y, Wu J, Feng J. LINCR00460 Hypomethylates Promotes Metastasis in Colorctal Carcinoma. Front Genet (2019) 10. doi: 10.3389/fgene.2019.00880

48. Wang J, Lu A, Chen L. LncRNAs in Ovarian Cancer. Cervical Cancer (2019) 4(9):17–27. doi: 10.1080/15532444.2018.12.013

49. Wens S, Wei Y, Zen C, Xiong W, Niu Y, Zhao Y. Long Non-Coding RNA NEAT1 Promotes Bone Metastasis of Prostate Cancer Through N6-Methyladenosine. Mol Cancer (2020) 19(1):171. doi: 10.1186/s12943-020-01293-4

50. Wang J, Huang H, Zhang X, Ma H. LOXL1–AS1 Promotes Thymoma and Thymic Carcinoma Progression by Regulating miR–525–5p–HSP9A. Oncol Lett (2021) 21(5):117. doi: 10.3892/ol.2021.10686

51. Chen M, Liu L. MIR-525-5p Repressed Metastasis and Anoikis Resistance in Cervical Cancer via Blocking UBEC2/ZEB1/2 Signal Axis. Dig Dis Sci (2020) 65(8):2442–51. doi: 10.1007/s10620-019-05916-9

52. Zhao J-Y, Wang Z-J. ADPGK–AS1 Promotes the Progression of colorectal cancer via Sponging miR-525 to Upregulate FUT1. Eur Rev Med Pharmacol Sci (2020) 24(5):2380–90. doi: 10.26355/eurrev_202003_20505

53. Tito C, Ganci F, Sacconi A, Masciarelli S, Fontemaggi G, Pulito C, et al. LINCR00174 Is A Novel Prognostic Factor in Thymic Epithelial Tumors Involved in Cell Migration and Lipid Metabolism. Cell Death Dis (2020) 11(11):959. doi: 10.1038/s41419-020-03171-9

54. Li Z-X, Zhu Q-N, Zhang H-B, Hu Y, Wang G, Zhu Y-S. MALAT1: A Potential Biomarker in Cancer. Cancer Manag Res (2018) 10:6757–68. doi: 10.2147/CMR.S164966

55. Hu L, Wu Y, Tan D, Meng H, Wang K, Bai Y, et al. Up-Regulation of Long Noncoding RNA MALAT1 Contributes to Proliferation and Metastasis in Esophageal Squamous Cell Carcinoma. J Exp Clin Cancer Res (2015) 34(1):7. doi: 10.1186/s13046-015-0123-z

56. Han Y, Wu Z, Wu T, Huang Y, Cheng Z, Li X, et al. Tumor-Suppressive Function of Long Noncoding RNA MALAT1 in Glioma Cells by Downregulation of MMP2 and Inactivation of ERK/MAPK Signaling. Cell Death Dis (2016) 7(3):e2123–3. doi: 10.1038/cddis.2016.12866

57. Tan S, Chen Ji, S1–MALAT1 Attenuates Thymic Cancer Cell Proliferation and Promotes Apoptosis via the Mir–145–5p/HMG2A Pathway. Oncol Lett (2021) 22(2):585.
70. Fazi F, Fatica A. Interplay Between N6-Methyladenosine (M6a) and Non-Coding RNAs in Cell Development and Cancer. Front Cell Dev Biol (2019) 7:103. 10.3389/fcell.2019.01033

71. Iaiza A, Tito C, Iannello Z, Ganci F, Laquintana V, Gallo E, et al. METTL3-Dependent MALAT1 Delocalization Causes C-Myc Induction in Thymic Epithelial Tumors. Clin Epigenetics (2021) 13(1):173. doi: 10.1186/s13148-021-01159-6

72. Pruszko M, Milano E, Forcato M, Donzelli S, Di Agostino S, Ganci F, et al. The Epigenetic Regulator Uhrf1 Facilitates the Proliferation and Maturation of Colonic Regulatory T Cells. Nat Immunol (2014) 15(6):571–9. doi: 10.1038/nm.3286

73. Su Y, Chen Y, Tian Z, Lu C, Chen L, Ma X. IncRNAs Classifier to Accurately Predict the Recurrence of Thymic Epithelial Tumors. Thorac Cancer (2020) 11 (7):1773–83. doi: 10.1111/1759-7714.13439

74. Ji G, Ren R, Fang X. Identification and Characterization of Non-Coding RNAs in Thymoma. Med Sci Monit (2021) 27:e929727. doi: 10.12659/MSM.929727

75. Li N, Li J, Mi Q, Xie Y, Li P, Wang L, et al. Long Non-Coding RNA ADAMTS9-AS1 Suppresses Colorectal Cancer by Inhibiting the Wnt/β-Catenin Signalling Pathway and Is a Potential Diagnostic Biomarker. J Cell Mol Med (2020) 24(19):11318–29. doi: 10.1111/jcmm.15713

76. Ji G, Ren R, Fang X. Identification and Characterization of Non-Coding RNAs for Predicting Prognosis Among Patients With Thymoma. Lab Clin Med (Baltimore) (2021) 119(2):1679–88. doi: 10.1002/jcl.26328

77. Shi D, Wu F, Mu S, Hu B, Zhong B, Gao F, et al. LncRNA AFAP1-AS1 Promotes Tumorigenesis and Epithelial-Mesenchymal Transition of Osteosarcoma Through RhoC/ROCK1/p38MAPK/Twist1 Signaling Pathway. J Exp Clin Cancer Res (2019) 38(1):94. doi: 10.1186/s13046-019-1100-8

78. Han D, Wang J, Cheng G. LncRNA NEAT1 Enhances the Radio-Resistance of Cervical Cancer via miR-193b-3p/CCND1 Axis. Oncotarget (2018) 9(2):2395–409. doi: 10.18632/oncotarget.23416

79. Gong J, Jin S, Pan X, Wang G, Ye L, Tao H, et al. Identification of Long Non-Coding RNAs for Predicting Prognosis Among Patients With Thymoma. Clin Lab (2018) 64(7-8):1193–8. doi: 10.7754/ClinLab.2018.180136

80. Chen K, Bai L, Li L, Wu L, Li G. Bioinformatics Analysis of the Key Potential IncRNA Involved in Fat Loss of Cancer Cachexia. J Cell Biochem (2018) 119(2):1679–88. doi: 10.1002/jcb.26328

81. Gong Z, Diao Y, Xu X, Li L, Jiang Z, Shao C, et al. Long Non-Coding RNA Linc00665 Promotes Lung Adenocarcinoma Progression and Functions as ceRNA to Regulate AKR1B10-ERK Signaling by Sponging miR-98. Cell Death Dis (2019) 10(2):e419. doi: 10.1038/s41419-019-1361-3

82. Li S, Zheng K, Fei Y, Wang W, Zhang X. Long Noncoding RNA NR2F1-AS1 Enhances the Malignant Properties of Osteosarcoma by Increasing Forkhead Box A1 Expression via Sponging of miR-483-3p. Aging (Albany NY) (2019) 11(23):11609–23. doi: 10.18632/aging.102563