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Review

MicroRNA Expression in the Progression and Aggressiveness of Papillary Thyroid Carcinoma

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Abstract. *In the last two decades microRNAs have received great attention in research because of their ability to regulate gene expression. Many studies have shown that defects in different microRNA molecules are linked to many diseases; however, their contribution towards thyroid disease has not been fully explored. Herein, we present a short review of the present state of knowledge on microRNAs, such as their origin, their biogenesis and biological function, as well as their differential expression in papillary thyroid carcinoma. Dysregulated microRNA has been closely linked to thyroid dysfunction and oncogenicity leading to this type of thyroid cancer. The effects of Single Nucleotide Polymorphisms in microRNA are also discussed with respect to papillary thyroid carcinoma.*

MicroRNAs (miRNA, miR) are a class of endogenous non-coding RNA molecules. Mature miRNAs are short, single-stranded RNA molecules ranging from 18 to 22 nucleotides in length (1). These molecules play a substantial role in the regulation of gene expression, through the induction of translational repression or silencing effects by complementary binding to target mRNAs (2-4). They may also act as tumor suppressor genes and oncogenes (5). Although miRNAs constitute only 3% of the human genome, it is believed that these molecules altogether regulate more than half of the protein-coding genes. Noteworthy, one single miRNA can alter the expression of hundreds of different transcripts (6). MiRNAs are expressed in a tissue-specific fashion (7) and can be found, apart from tissues, also in blood as components of serum, plasma, mononuclear cells and in other body fluids (*i.e.* urine, semen, saliva, tears, ascitic fluid, amniotic fluid and breast milk) (8, 9). Circulating miRNA molecules are very stable in the blood plasma and serum because they are incorporated in microparticles, such as exosomes and apoptotic bodies (10, 11). Biochemical analyses have revealed that miRNA is resistant to RNase activity as well as to extreme acidic and alkaline pH and temperature (12, 13).

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Biogenesis of microRNAs

Briefly, biogenesis of miRNA is initiated by the generation of non-coding primary miRNA (pri-miRNA) transcripts (14). MiRNA is first transcribed as pri-miRNA by RNA polymerase II in the nucleus and then, split into precursor microRNA molecules (pre-miRNA) (15). Next, pre-miRNA is transported through exportin 5 (XPO5) to the cytoplasm where it is processed by the Dicer RNase III enzyme, to form mature miRNA (16, 17). Mature miRNAs can promote or inhibit mRNA translation and degradation by targeting with precision complementary sequences in 3'UnTranslated Regions (3'UTR) (14, 18). In this way, miRNAs modulate different cellular pathways and can be used as therapeutic means to treat pathological conditions, such as cancer (19).

Discovery of microRNAs

This novel class of small regulatory RNAs were first described in 1993 by Lee *et al.* in *Caenorhabditis elegans* (20). Since their discovery and original description in the 90s, the number of miRNA sequences deposited in the microRNA database (miRBase) has grown exponentially (21). Concomitant with this, the amount of research studies on

miRNA has greatly increased, especially following the identification of miRNA in human blood in 2008 (22). This discovery generated vast interest for the potential use of plasma or serum miRNAs as biomarkers of neoplastic and non-neoplastic disease (23). To-date more than 2,800 miRNA sequences have been recorded in the miRBase, which acts as a public repository for miRNAs (24).

Function of microRNAs

MiRNA is involved in regulating almost all biological processes, including cell proliferation, differentiation, apoptosis, hematopoiesis, stress responses and metabolism (1, 25). Recent findings demonstrate a crucial role for certain miRNA molecules in immune cell differentiation and immune responses (26-28), making them very relevant for the progression of many diseases, including tumors (29). MiRNA molecules have been shown to act both as post-transcriptional regulators of gene expression and as messengers of intercellular signaling (30). Encapsulated within exosomes and other microvesicles, or bound to complexes with proteins, miRNAs are secreted into the extracellular space, where they can be transported to both neighboring and distant cells, infiltrate biological fluids, and travel throughout the whole organism, where they can exert their regulatory role on different processes (31).

MicroRNA as a Promising Biomarker

MiRNA is a promising biomarker in many diseases, as various cell-free circulating miRNA molecules have been found to be dysfunctional in cancers (32, 33), cardiovascular diseases (34), neurological disorders (35) and metabolic diseases (36).

Recent data have revealed an important role for miRNA molecules performing either as pro-metastatic (oncogenes) or anti-metastatic (tumor suppressors) agents (37-39). Some miRNAs have been reported to be important prognostic markers for evaluating the stage or progression of diseases (40). A feature of a clinically-useful miRNA molecule as a biomarker should be at least that it is expressed in a cell type that is specifically involved in a certain disease process (23). Recent studies suggest that serum miRNA profiles may be useful as biomarkers of certain cancers (41). In fact, the relationship between miRNA dysregulation and cancer was only recognized in 2002 (42) and aberrant expression of miRNA molecules, particularly the ones circulating in serum, has been linked to cancer progression. Blood samples are easy to obtain and minimally invasive, making this source of mRNA attractive for exploring the role of potential circulating biomarkers (10). The identification and use of molecular biomarkers in cancer aims to increase the diagnostic accuracy and assist clinicians in the preoperative management of patients (43). As such, certain miRNA molecules can be useful with better estimating the prognosis of patients, predicting the efficacy of the therapeutic approach, better surveillance following surgery and planning in case of disease recurrence (10).

MicroRNAs in Papillary Thyroid Carcinoma

Thyroid Carcinoma (TC) is the most frequent endocrine malignancy, and affects mostly women (44). Incidences of this cancer are steadily increasing in many regions across the world over the years (45, 46). In 2018 alone, according to the Surveillance, Epidemiology and End Results Program (SEER) from the National Cancer Institute, - nearly 54,000 new cases of thyroid cancer were registered, accounting for 3.1% of all new cases of cancer during this year so far (47).

Thyroid cancers develop from abnormal parafollicular (medullary) and follicular cells (non-medullary) in over 95% of all thyroid cancer (TC) cases. Four distinct clinical types of TC exist: i) papillary thyroid carcinoma (PTC), ii) follicular thyroid carcinoma (FTC), iii) medullary thyroid carcinoma (MTC), and iv) anaplastic thyroid carcinoma (ATC) (48). The most common type of TC is the papillary thyroid carcinoma (PTC) (49) followed by the follicular thyroid carcinoma (FTC). The anaplastic carcinoma is the most poorly differentiated, aggressive and invasive form of all types of TC, occurring in less than 2% of cases (50).

Aberrant Expression of microRNA in Papillary Thyroid Carcinoma

The relationship between miRNA dysregulation and cancer was recognized in 2002 (42), but the first published information of the role of miRNAs in thyroid tumorigenesis appeared in 2005 (51). Several miRNAs involved in PTC tumor initiation, progression and aggressiveness have been found abnormally expressed in serum/plasma or in tissue (up-regulated or down-regulated) (Table I) (52, 53).

Circulating miRNAs in PTC

A large number of studies have demonstrated that different levels of circulating miRNAs are associated with thyroid dysfunction. In 2012, Yu and coworkers demonstrated that levels of serum miRNA precursor Let-7e, as well as miR-151-5p and miR-222 were significantly higher in the PTC group than in healthy controls or those with benign nodules (41). Yu *et al*., were the first to show that the levels of miR-151-5p and miR-222 in serum decreased after thyroidectomy compared to the pre-operative status of patients (41). Furthermore, they confirmed that levels of these miRNA molecules are associated with cancer multifocality and increased tumor size, and advanced TNM stage (41). Not

Table I. *Expression of dysregulated miRNAs in papillary thyroid carcinoma.*

MicroRNA dysregulation	Sample type	Up-regulated/down-regulated	References
MiR-31	Serum	Up-regulated	Yoruker *et al*. (40)
	Plasma		Samsonov *et al*. (65)
	Tissue		Yip *et al*. (64)
MiR-151-5p	Serum	Up-regulated	Yoruker *et al*. (40)
			Yu *et al*. (41)
Let-7	Serum	Up-regulated	Yoruker *et al*. (40)
Let-7a	Tissue, thyroid cancer cell lines	Down-regulated	Zhou *et al*. (62)
Let-7b	Tissue, thyroid cancer cell lines, serum	Up-regulated	Li *et al*. (63)
			Perdas *et al*. (57)
Let-7e	Serum	Up-regulated	Yu *et al*. (41)
MiR-21	Serum	Down-regulated	Yoruker *et al*. (40)
MiR-146a	Tissue	Up-regulated	Qiu *et al*. (76)
MiR-146b	Plasma	Up-regulated	Lee *et al*. (20)
	Serum		Yip *et al*. (64)
	Tissue		Wei *et al*. (84)
			Acibucu *et al*. (75)
			Qiu *et al*. (76)
			He *et al*. (51)
			Pallante *et al*. (73)
MiR-221	Serum	Down-regulated	Yoruker *et al*. (40)
	Tissue		Acibucu *et al*. (75)
MiR-221-3p	Tissue		Chou *et al*. (74)
			He *et al*. (51)
MiR-222	Serum	Up-regulated	Yorukerе*t al*. (40)
	Plasma		Yu *et al*. (41)
	Tissue		Chou *et al*. (74)
MiR-222-3p	Tissue		Acibucu *et al*. (75)
			Lee *et al*. (20)
			Yip *et al*. (64)
			Chou *et al*. (62)
			He *et al*. (51)
			Pallante *et al*. (73)
MiR-126	Tissue	Down-regulated	Xiong *et al*. (66)
			Kitano *et al*. (67)
			Wen *et al*. (68)
			Qian *et al*. (60)
MiR-7	Tissue	Down-regulated	Xiong *et al*. (66)
			Kitano *et al*. (67)
			Mancikova *et al*. 70)
MiR-148-a	Tissue	Down-regulated	Han *et al*. (77)
MiR-TG	Tissue	Down-regulated	Kolanowska *et al*. (71)

surprisingly, the levels of miR-151-5p and miR-222 appear to decrease following tumor excision (54). In the following year, Lee *et al*. came to similar conclusions. More specifically, they analyzed the levels of circulating miRNA before and after thyroidectomy in patients with or without recurrent PTC, and found that miR-222 and miR-146b present in plasma were reduced after total thyroidectomy in both groups (54). The researchers examined plasma instead of serum and the quantitative determination was made more than 2 weeks (from 2 to 6 weeks) after surgery (54).

Another group, Yoruker *et al*. analyzed a set of seven miRNA molecules in the serum of patients with PTC, multinodular goiter (enlarged thyroid) and in healthy controls (40). They noticed that Let-7 as well as miR-222, miR-31 and miR-151-5p levels were substantially higher in patients with PTC when compared to healthy controls and those with benign tumors, while miR-21 levels were lower. Furthermore, after thyroidectomy, miR-222 levels decreased dramatically in the PTC group, regardless of tumor size (40).

The Let-7 family is a very interesting group of miRNA precursors. Generally, the Let-7 family (Let-7a, Let-7b, Let-7c, Let-7d, Let-7e, Let-7f, Let-7g and Let-7i) exhibit an important tumor suppressor activity in various cancers, including TC (55-61). The Let-7 family is considered as a

tumor suppressor, since it inhibits the expression of several oncogenes, such as *RAS, MYC*, and *HMGA2* in the thyroid tissue itself as well as in blood (serum or plasma) (41, 62, 63). Zhou *et al*. also demonstrated that Let-7a is significantly down-regulated in tissues of PTC patients as well as in TC cell lines (62). In addition, overexpression of Let-7a suppressed PTC cell proliferation, migration and invasion to distal sites. AKT2 was found to be a direct target of Let-7a and its expression levels inversely correlated to Let-7a expression in PTC tissues (62). Li *et al*. investigated the expression of Let-7b miRNA and high-mobility group A2 (HMGA2) mRNA in different PTC tissues and cell lines (PTC cell lines and normal human thyroid epithelial cell line) (63). They demonstrated that the expression of Let-7b was down-regulated, but the expression of HMGA2 was up-regulated in thyroid tissues and cells compared to normal tissues and cells. Overexpression of Let-7b or knockdown of HMGA2 successfully suppressed the proliferation, migration, and invasion of PTC cells, while these effects were reversed by HMGA2 reintroduction. Moreover, Let-7b induced down-regulation of HMGA2, and HMGA2 could affect the biological function of Let-7b in PTC cells, suggesting that Let-7b might function as a tumor suppressor in PTC (63).

MiR-31 is another miRNA molecule up-regulated in PTC and with significant role in the aggressiveness of this type of cancer (64). Samsonov *et al*. have demonstrated that high levels of MiR-31 were contained within exosomes in PTC patients prior to surgery, which were significantly reduced following the removal of the tumor (65). Similar results have been obtained for MiR-151, that has been found to be highly expressed in the blood (serum and plasma) of PTC patients compared to patients with benign thyroid tumors (40, 41).

Recently, Zhang *et al*. found that the expression levels of 3 miRNA molecules (miR-222, miR-221 and miR-146b) are much higher in patients with PTC compared to the healthy control group and to individuals with Benign Thyroid Nodules (BTN). High expression of these miRNAs was associated with certain poorer prognostic outcome as well as with PTC with recurrence (PTC-RC) (13).

MiR-126 is one of the miRNA molecules that when down-regulated it appears to be associated with aggressive behavior of TC (66-69), while its overexpression inhibits the proliferation of TC cells and significantly reduces tumor growth and metastasis *in vivo* (66).

By developing a diagnostic technique using fine-needle aspiration thyroid biopsies, Kitano *et al*. identified four miRNA molecules differentially expressed between benign and malignant tumors, with low levels of miR-7 being the most precise diagnostic marker for thyroid carcinomas (67). Furthermore, Mancikova *et al*. have reported that papillary thyroid carcinomas with BRAF mutation, which is one of the most frequent ones in PTC, displayed an extreme down-regulation of miR-7 and miR-204. The down-regulation of miR-7 was also correlated to tumor aggressiveness (70). Similarly, a Polish group discovered that the down-regulation of a novel, functional microRNA encoded within the thyroglobulin gene (miR-TG) acting *via* the MAP kinase signaling, may also be a potential biomarker for PTC (71).

Tissue miRNAs in PTC

Specific patterns of several miRNA molecules detected in thyroid tumors of PTC patients correlate with clinicopathological features depending on, tumor size, status of multifocal lesion, capsular and vascular invasion, TNM stage and in some cases on the aggressiveness of the tumor (72). Studies by He and Pallante and their colleagues have shown a high expression of miR-146b-5p, miR-221-3p and miR-222-3p in papillary thyroid carcinomas (51, 73). One of the first studies to investigate the role of miRNAs in TC aggressiveness was published by Chou *et al*. (74). They demostrated that the expression of miR-146b, miR-221 and miR-222 were connected with extrathyroidal invasion and were higher in the high-risk PTC group compared to the low-risk group. Furthermore, they demonstrated that expression of miR-146b in PTCs with BRAF mutation were much more higher compared to those without this mutation (74). Overexpression of the same miRNAs was found in papillary thyroid carcinoma patients with capsule and vascular invasion or lymph node metastasis, along with distant metastases (64). A study from Acibucu *et al*. revealed that the expression of miR-146b-5p, miR-221, miR-222 and p27Kip1, a member of the universal cyclin-dependent kinase inhibitor (CDKI) family, was higher in patients with distant metastases and lower levels of p27Kip1 and was associated with the aforementioned clinicopathological parameters in papillary TC (75). Similar results were obtained by Qiu *et al*. for miR-146a and miR 146b, who showed that these molecules appear to have an impact on the cancer cell proliferation and migration, as well as on regulation of IRAK1 (proteins which take part in one of the intracellular signaling control- TLRs/IL-1 signaling pathway) expression in cancer cells (76). In another study, Han *et al*. demonstrated that miR-148a negatively regulates PTC cell proliferation, migration, invasiveness, and tumor growth by down-regulating the expression of the target gene, cyclin-dependent kinase 8 (*CDK8*). Overexpression of miR-148a significantly represses expression of CDK8 by directly targeting the 3'-UTR of the *CDK8* gene in PTC tissues and in cell lines, while overexpression of *CDK8* reverses the inhibitory effects of miR-148a on PTC cell growth, migration and invasiveness (77). A meta-analysis of published studies comparing miRNA expression data in invasive thyroid carcinoma compared to non-invasive tumors or normal tissues by a Chinese group showed 29 dysregulated miRNAs associated with TC in 6 studies (78).

Findings illustrate that miRNAs have been deregulated in TC and moreover they have been demonstrated to function as tumor suppressors and as oncogenes, as well. These molecules are key regulators of thyroid cell function, and influence thyroid hormone production and cell proliferation. As mentioned previously, the expression of miRNAs is strictly tissue-specific and, during pathological conditions, alterations in miRNA expression can cause loss-of-function and tumorigenesis in thyroid cells (32, 79). In summary, overexpression of certain miRNAs can result in inhibition of some tumor suppressor genes and down-regulation of certain miRNAs can lead to increased expression of oncogenes, leading to tumor growth and progression in PTC (52).

MicroRNA Single Nucleotide Polymorphism in Papillary Thyroid Carcinoma

The role of miRNA can be greatly affected by single nucleotide polymorphisms (SNPs), since these variations can change miRNA affinity to particular transcripts, while they may also produce novel miRNA-specific binding sites or destroy them (80). Polymorphisms in microRNA may therefore have a distinct impact on gene and protein expression that can influence the risk of developing or the progression of some diseases, such as PTC (81), since an SNP located within the miRNA mature sequence or within the seed region of the mature miRNA sequence (1) may modify its normal function. Such is the case of a common (>1% in at least one population) SNP in pre-miR-146a that can inhibit mature miRNA expression and boost the risk of developing PTC (51, 82).

The first evidence proving that the SNP played an important role in PTC progression was demonstrated in 2005 by He *et al*. The group sequenced the regions harboring two miRNA-binding sites in the KIT gene from PTC patients and found that the G>A SNP is located within the KIT 3' UTR, complementary to the seed region of miR-221/222, while the G>C SNP in exon 18 is located within the site complementary to the seed region of miR-146a/146b. These two SNPs have been linked to dysregulated expression of the KIT protein and may have a causative role in the development of PTC (51). Jażdżewski *et al*. also found that a common SNP within the pre-miR-146a sequence weakens its maturation and leads to reduced mRNA target recognition. This SNP-related reduction of miR-146a expression has been implicated in PTC (82), with high risk of PTC confirmed in both North American and European populations (82, 83). In addition, this SNP in a heterozygous state (GC) differs from either GC or CC homozygotes by producing 3 mature miRNAs. These modulate genes mainly involved in the regulation of apoptosis, causing an exaggerated DNA-damage response, and thus more severe consequences in heterozygotes than in homozygotes (84). A new somatic mutation (c.5438A>G; E1813G) within *the DICER1* gene was discovered by Penha *et al*., who showed an up-regulation ofDICER1 expression in human TC cells of PTC (TPC-1, BCPAP, FRO and 8505c) and anaplastic thyroid carcinoma samples. XPO5, as mentioned previously, is a miRNA-related nuclear export protein, and its dysfunction may cause the dysregulation of miRNAs and contribute to carcinogenesis (85). Wen *et al*. showed that the expression of *XPO5* in TC tissues was significantly lower than in normal tissues and XPO5 expression was dependent on the XPO5 SNP rs11077. Furthermore, G allele of rs11077 was strongly associated with low expression of *XPO5* in TC patients, thus providing a potential marker for TC diagnosis (86).

Finally, a novel network application, called Rank miRNA, helps analyze the impact of genomic variations on miRNA-target interactions. This can also be used to predict the impact of SNPs on miRNA-mRNA binding capability and identify target genes of (possibly new) miRNA molecules based on their sequences (80).

Taken together, all these studies indicate that SNPs located within the binding sites of miRNA molecules may affect the expression of their target genes and as such, influence the development or progression of PTC.

Conclusion

This review provides a brief overview of the regulation and different roles of miRNAs in PTC. MiRNAs might be especially useful tools in diagnosis, prognosis and prediction of recurrence of PTC since many are deregulated in PTC. As it was presented in this review, the molecular epidemiology of PTC is also associated with polymorphisms of miRNA genes, which can affect the expression of miRNAs and subsequently affect the expression of their target genes.

More studies on specific miRNA expression in the thyroid are required for the identification of new sequences and delineation of their role in the development and progression of PTC. Serum miRNA profiling may be one of the best methods to further expand our knowledge on this subject.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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