ANTICANCER RESEARCH *35*: 2037-2048 (2015)

Review

The Role of MicroRNAs in Thyroid Carcinomas

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Abstract. *Thyroid cancers (TCs) are the most common malignancies of endocrine organs. They originate from cells of different origin within the thyroid gland, which is located at the base of the neck. Several forms of TCs have been classified and great variability is observed in molecular, cellular and clinical features. The most common forms have favorable prognosis but a number of very aggressive TCs, which are characterized by a less differentiated cellular phenotype, have no effective treatment at the moment. While TC causes are not completely understood, many genetic factors involved in their onset have been discovered. In particular, activating mutations of BRAF, RET or RAS genes are known to be specifically associated with TC initiation, progression and outcome. The involvement of microRNAs in thyroid neoplasms has recently changed the paradigm for biomarker discovery in TC, suggesting that these small non-coding RNAs could be used to develop, refine or strengthen strategies for diagnosis and management of TCs. In this review, the importance of microRNA profiling in TC is explored suggesting that these molecules can be included in procedures that can perform better than any known clinical index in the identification of adverse outcomes.*

Thyroid cancer (TC) is a group of uncommon neoplastic diseases affecting the thyroid gland. Among carcinomas of follicular cell origin, three categories of TCs can be defined according to specific histological features: well-differentiated thyroid carcinomas (WDTCs), poorly differentiated thyroid carcinomas (PDTCs) and undifferentiated thyroid carcinomas (UDTCs) (1). A sequential model in which UDTCs represent the final step of WDTC progression through the spectrum of PDTC has been suggested by evidence of pre- or co-existing WDTCs with less differentiated types that also share the original genetic alteration (2).

Medullary thyroid cancer (MTC), unlike the previously mentioned, is a neuroendocrine tumor that arises from parafollicular C cell and constitutes types fewer than 5% of all TC cases (3).

WDTCs are the most common TC forms and include papillary thyroid carcinoma (PTC), which accounts for about 75-80% of worldwide TC occurrences, and the less frequent (10-15%) follicular thyroid carcinoma (FTC). PTC differential diagnosis from FTC is determined by histology. PTC diagnosis relies on the presence of follicular cell differentiation, typically with papillary and follicular structures, as well as characteristics nuclear changes, such as large size, pale staining, 'ground glass' appearance, irregular outlines or inconspicuous nucleoli. FTC is characterized by evidence of follicular differentiation that lacks papillary architecture and typical nuclear features of PTC (4, 5). Even if WDTCs are usually considered an homogeneous group of neoplastic diseases in terms of outcome, follicular cancer is often more aggressive, leading less favorable survival rates. On the other hand, much evidence suggests that tumor histology cannot be considered alone as a determinant of WDTC prognosis and patient- (*e.g.* sex and mainly age) and tumor- (*e.g.* TNM or size) related factors are usually taken into account to define risk categories (6-9).

PDTCs are more aggressive than WDTCs and comprise nonfollicular, nonPTCs. While PDTCs can be easily placed between differentiated and undifferentiated carcinomas in term of prognosis, a rigorous and widely accepted histologic

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Key Words: Thyroid carcinomas; microRNA; diagnostic biomarkers; risk assessment; prognosis prediction, review.

Table I. *Prevalence, clinicopathological features and most frequent genetic defects of thyroid neoplasms. Genetic alterations are in order of prevalence from the most to the less frequent.*

Tumor type	Prevalence	Lymph node metastasis	Distant metastasis	5-Year survival	Genetic alteration	References
PTC	75-80%	<50%	5-7%	~ 100% stage I -II 93% stage III 51% stage IV	*BRAF* mutation *RET* rearrangement *NTRK1* rearrangement *RAS* mutation *TP53* mutation (uncommon) *BRAF* rearrangement (uncommon)	(28, 30, 77, 97, 98, 99, 101, 102)
FTC	10-15%	<5%	20%	~ 100% stage I -II 71% stage III 50% stage IV	*RAS* mutation *PPARγ* rearrangement *TP53* mutation (uncommon)	(44, 101, 102)
PDTC	<7%	30-80%	30-80%	50%	*TP53* mutation *RAS* mutation β-Catenin mutation *RET* rearrangement *BRAF* mutation	(28, 30, 98, 99, 101)
UDTC	2%	40%	20-50%	7%	*TP53* mutation β-Catenin mutation *RAS* mutation *BRAF* mutation	(28, 30, 46, 101)
MTC	<5%	50%	15%	86%	*RET* mutation	(100)

PTC: Papillary thyroid carcinoma, FTC: follicular thyroid carcinoma; PDTC: poorly differentiated thyroid carcinoma; UDTC: undifferentiated thyroid carcinoma; MTC: medullary thyroid carcinoma.

classification is still controversial. According to some authors, they can be classified on the base of two major characteristics: presence of a solid-trabecular-insular (STI) growth pattern and absence of conventional nuclear features that define papillary carcinomas (10, 11). Some authors, on the other hand, propose histological grading as the only determinant irrespective of growth pattern (12). Controversial designations also arise from the different thresholds used by authors in defining classification requirements. Many authors provide evidence of the impact of different cutoff usage for the identification of STI areas in comparing prognoses (13). For this reason, PDTCs can be considered quite rare and heterogeneous tumors and their prevalence can only be roughly estimated at between 1.5% and 6% of malignant TCs (10, 14).

Anaplastic thyroid carcinoma (ATC) is a highly aggressive form of UDTC. ATCs constitute the most uncommon subgroup of TCs and are characterized by rapid growth, metastatic invasion and, consequently, a dramatically unfavorable prognosis. The 5-year relative survival rate for anaplastic carcinoma has been defined at round 7%. All the other forms of TC have a much better prognosis, with 5-year relative survival rates of 50% for PDTC (15, 1), 86% for MTC (16) and from 50% to near 100%, according to stage, for WDTCs (17).

Many diagnostic algorithms that evaluate histological, surgical and molecular features of the thyroid neoplasms can often be used to easily define TC subgroups and patient risk. Nevertheless, in some cases, diagnosed TC tumors show intermediate or confounding profiles that make the classification more difficult. Some of the above- mentioned disease groups present intermediate characteristics and comprise tumors that are heterogeneous for prognosis or sensitivity to treatment. Many efforts have been made to improve the accuracy of disease profiling for a better classification of tumors that may actually behave differently, despite their apparent histological similarities; however, TC management is still an important medical challenge. The identification of unambiguous biological and molecular features that can be used to discriminate indolent from aggressive disease, for example, would be fundamental in defining clinically useful predictor risks. On this basis, it is interesting to note that deregulation of microRNA (miRNA) function was recently identified as an important driver for tumor development and progression in TCs (18, 20). miRNAs are a class of small noncoding RNAs that are able to regulate gene expression through mRNA cleavage or translational repression. The specificity of miRNA–mRNA interaction is directed by nucleotide sequence complementarity between the miRNA seed region and its corresponding binding sequence on the target mRNA. The flexibility of this recognition system allows miRNA to target multiple molecules and mRNAs to be regulated simultaneously by more than one miRNA, therefore

constituting the basis for a complex and diffused regulatory network. These molecules finely regulate many biological processes (including proliferation, apoptosis and differentiation) and deeply influence pathological pathways (20).

Molecular Biology and Biomarkers of TCs

The growth of follicular thyroid cells is physiologically regulated by the thyroid-stimulating hormone (TSH or thyrotropin). TSH receptor, upon thyrotropin binding, activates the GSα-adenylyl cyclase-cyclic AMP (cAMP) cascade, triggering cell differentiation and proliferation through transcriptional activation of specific molecular effectors. Even though mutations that constitutively activate TSH receptor or GSα have been identified in 60-70% of benign adenomas (21-23), the hypothesis of a causative role in malignant transformations of thyroid cells has been rejected (24-27). While the constitutive activation of cAMP-elicited transduction cascade alone is not sufficient for neoplastic transformation, many genetic abnormalities have been described in different histological types of TC (Table I).

In WDTCs, the vast majority of cases present genetic alterations affecting the MAPK signaling pathway. In particular, activating mutations of *BRAF*, *RET* or *NTRK1* genes have been identified as causative and mutually exclusive events in PTC onset. The T1799A transversion mutation of *BRAF* that produces the V600E variant of the encoded kinase occurs in about 45% of sporadic PTCs (28). It has been demonstrated that this mutation is associated with a poorer outcome and it can be used as a predictor of clinical features, such as extranodal invasion, lymph node metastasis, advanced staging and disease recurrence (29). While *BRAF* mutations have not been found in FTC, they are present in 13% of PDTC. They have been also observed more frequently in UDTC with a PTC component than in those without such a component (30). The expression of hexogen V600E *Braf* in mouse thyroid cells produces PTC with some of the PDTC specific features (31). While the V600E variant of *BRAF* is the most common alteration in sporadic PTC, other variants (*e.g.* the K601E for the follicular variant papillary carcinomas and the in-frame VK600-1E deletion for the solid variant papillary carcinoma) define a phenotype specific range of genetic alterations targeting *BRAF* exons (32). Much evidences (33-35) suggests that deregulated activation of the MAPK cascade can increase genomic instability of TC cells, thus promoting the acquisition of additional somatic mutations during TC progression.

The receptor-tyrosine kinase encoded by the *RET* gene is an essential molecular actor required for renal organogenesis and enteric neurogenesis. It has been shown that while gain-of-function *RET* mutations are associated with MTC (36), the chromosomal rearrangements that originate a family of chimeric RET oncoproteins are involved in PTC development. At least 10 chimeric PTC- associated *RET* variants have been identified in PTC (37) and all of them result from the fusion of the 3'- terminal sequence of *RET* that encodes for a tyrosine kinase domain with the 5' portion of different genes.

The neurotropic receptor-tyrosine kinase (*NTRK1*) gene encodes for a tyrosine-kinase receptor that is activated by NDF. Its expression is typically neural and usually restricted to sympathetic, trigeminal and dorsal root ganglia or to cholinergic neurons of the basal forebrain and the striatum (38). This receptor, upon NGF binding, transduces the signal activating several pathways, including ERK, PI3K and the PLCγ cascades. *NTRK1* rearrangements observed in TC, like those in *RET-PTC*, involve the tyrosine kinase domain of NTRK1 that is fused to another gene. Only three fusion partners, which have practically identical prevalence in sporadic PTC, have been identified so far: *TFG*, *TPM3* and *TPR*.

RAS mutations are the second most abundant mutations identified in TCs. They comprise mutations affecting a family of three homologous isoforms *HRAS*, *KRAS* and *NRAS*, the latter of which is the most frequently mutated in TC. These membrane-associated guanine nucleotide-binding proteins transduce and amplify extracellular signals to initiate important cellular responses. The type of guanine nucleotide bound to RAS regulates their activation state: while GTP promotes RAS activity, GDP inhibits its function. The GTP-ase function of these proteins promotes their deactivation in a typical feedback manner: when GDP is bound to RAS, the resulting conformation is inactive. The physiological role of *RAS* genes is dual: they are involved in the activation of both MAPK and PI3K-AKT pathways. TC-related *RAS* variants, which predominantly exhibit mutations of codons 12, 13 and 61, preferentially activate the PI3K-AKT axis as suggested by the observed association between the occurrence of these mutations and phospho-AKT increase (39). Oncogenic variants produced by point mutation in codons 12 and 13 are characterized by increased affinity for GTP, while those resulting from mutations of codon 61 show deficiency in the autocatalytic GTP-ase function (40). Among WDTCs, *RAS* mutations have been observed more frequently in tumors with follicular histology (FTC and follicular variant of PTC) than in typical papillary TCs (41, 42). In FTC, *NRAS* codon 61 mutations are also associated with distant metastasis, supporting the hypothesis that aberrantly active proteins participate in providing a more aggressive clinical phenotype. *RAS* mutations have been observed also in PDTCs and UDTCs with variable frequencies (1).

Some authors suggested that *RAS* mutations and rearrangements involving the peroxisome proliferator-activated receptor-γ (*PPARγ*) and paired box 8 (*PAX8*) genes, are, in FTC, mutually exclusive events with distinct molecular mechanisms (43, 44). It has been shown that the *PPARγ* gene, which encodes for a steroid nuclear hormone receptor that forms heterodimers with retinoid X receptor,

can generate a chimeric fusion gene with *PAX8*, a transcription factor normally involved in thyroid follicular cell development. The *PPARγ–PAX8* chimera acts as a negative dominant affecting the transcriptional activity of the wild type *PPARγ* gene. The fusion oncoprotein contributes to malignant transformation in FTC and some authors indicate that it promotes the vasculo-invasive phenotype.

Cadherin-associated protein beta 1, encoded by the gene *CTNNB1* and usually called β-catenin, is a protein that by acts -regulating both cell-cell adhesion and signal transduction through the Wnt pathway. This transduction cascade is essential for embryogenesis and integrates signals from other molecular pathways (retinoic acid, fibroblast growth factor, transforming growth factor beta 1, bone morphogenetic protein) within different cell types and tissues. The cascade starts when the Wnt ligand binds to the extracellular portion of the Frizzled receptor that in turn activates a number of molecular partners releasing β-catenin from its post translational regulation exerted by proteasomal degradation. The subsequent accumulation of cytosolic β-catenin allows the protein to translocate to the nucleus where it promotes the transcription of target genes. Aberrant expression of β-catenin has been reported in many tumors (45). Increased cytoplasmic β-catenin is observed in TC but its increased nuclear localization, which is promoted by specific mutations, appears to be limited to PDTC and UDTC (46). Different authors recently reported a clear functional link between RET oncoprotein activation and the modulation of downstream β-catenin axis (47, 48).

The overall picture of molecular events involved in TC initiation and progression has to be completed adding the fundamental details represented by the regulatory layer controlled by miRNAs.

In the recent years, these small endogenous regulators have been clearly identified as key molecular components in cancer and tumor miRNA profiles have been shown to be capable of defining efficiently relevant pathologic subtypes, patient response and survival.

miRNA as Biomarkers

More than 2,000 miRNAs have been identified in humans (49). It has been shown that about 50% of human miRNA genes are located in cancer-associated genomic regions or in fragile sites (50), supporting their contribution as key drivers of neoplastic transformation that follows genetic injuries. Mutations in miRNA target sites that lead to an incorrect mRNA recognition may induce severe phenotypic consequences and may promote cancer initiation. (51, 52). Chromatin remodeling may also affect miRNA expression through the methylation of promoter sequences. (53).

The first evidence of the role of miRNAs in cancer was reported for chronic lymphocytic leukemia (54), where the cluster containing miR-15 and miR-16 is frequently deleted or down-regulated in cancer cells. During the past decade, many studies reported that miRNAs are often differentially modulated in cancer cells, defining severe miRNA expression patterns that were specifically associated with histological variants and tumor prognosis (55-58). Moreover, it has been accepted that miRNAs can be directly controlled by oncogenic transcription factors (59).

A common feature of tumor-related miRNA profiles is the frequent down-regulation of miRNAs in cancer cells, while their over expression is a much less frequently observed phenomenon. In physiological conditions, indeed, miRNA expression increases, as during cell differentiation. Lower expression levels in cancer cells may be coherent with the acquisition of a less differentiated state, a common feature in malignant transformation. The transcriptional inhibition of miRNA genes in cancer cells can be exerted through repression by oncogenic transcription factors (60), or through the alteration of the miRNA biogenesis pathway (61, 62).

Some authors highlighted the importance of miRNA biogenesis regulation in TC (63). miRNAs are transcribed mainly by RNA polymerase II or more rarely by RNA polymerase III as double-stranded precursors called primary miRNA (pri-miRNA). These molecules, which are up to several thousands nucleotides long, are characterized by distinctive secondary stem-loop structure. Inside the nucleus, pri-miRNAs are enzymatically cleaved into smaller (70- to 80-nucleotide-long) molecules, defined pre-miRNAs, that present hairpin structure due to reverse complementary regions in their sequence. A multicomponent complex called a microprocessor, whose core components are Drosha and DGCR8 enzymes, carries out this cleavage. (64). DGCR8 (also known as PASHA in invertebrates) stabilizes the pri-miRNA, while Drosha, an RNAse type III protein, cuts it into the pre-miRNA. The pre-miRNAs are then transported to the cytoplasm by their specific transporter, the exportin-5, which utilizes nuclear Ran-GTP (65, 66, 20). It is interesting to note that knockdown of the *EXP5* gene, which encodes for exportin-5, produces a reduction of cytoplasmic miRNA molecules without an increase of nuclear ones, suggesting that the transporter may also be involved in pre-miRNA stabilization and protection from nucleases (67).

According to the well-known and accepted processing model, after nuclear export, the pre-miRNA is further processed by the enzyme Dicer, which recognizes the terminal loop and after cleavage releases a small RNA duplex (68). The two strands of these molecules are defined as the guide strand and the passenger strand according to the role they will have on the effector complex for the silencing, which is called RNA-induced silencing complex (RISC; also referred to as the miRISC). After pre-miRNA processing, the RISC-loading complex (RLC), which comprises the Dicer and other molecules,

Table II. *miRNA modulation in thyroid neoplasms. Green boxes indicate down-regulation, red boxes indicate up-regulation and yellow boxes indicate conflicting evidence. Define abbreviated terms.*

miRNA	PTC	PDTC	FTC	ATC	MTC	References
let-7						(92, 85, 80, 55)
miR-100						(92)
miR-107						(92)
miR-122a						(79)
miR-125b						(92, 85, 80)
miR-127						(93, 79)
miR-128b/b						(92)
miR-129						(79)
miR-130b						(92)
miR-135b						(92)
miR-137						(79)
miR-138						(91, 87)
miR-139						(92)
miR-141						(92, 96)
miR-142-3p						(92)
miR-145						(92)
miR-146						(19, 92)
miR-146b						(79, 80)
miR-148						(92)
miR-149						(92)
miR-154						(92)
miR-155						(79)
miR-15a						(92)
miR-17-92 cluster						(86)
miR-181a						(92)
miR-181b						(80)
miR-183						(79, 96)
miR-185						(92)
miR-187						(79)
miR-191						(93)
miR-192						(77)
miR-197						(77)
miR-200a						(92, 96)
miR-200b						(92, 96)
miR-200c						(96)
miR-20a						(19)
miR-205						(79)
miR-21						(80)
miR-211						(92)
miR-213						(92)
miR-216						(92)
miR-218						(92)
miR-219						(87)
miR-221						(19, 80, 94, 79)
miR-222						(19, 80, 94, 79, 85)
miR-224						(79)
miR-26a						(80, 85)
miR-299						(92)
miR-302b						(92)
miR-302c						(92)
miR-30a-5p						(80, 85)
miR-30d						(80)
miR-31						(79)
miR-323						(92)
miR-328						(77)
miR-34a						(92)
miR-34c						(92)
miR-345						(87)
miR-346						(77)
miR-370						(92, 95)
miR-375						(95)
miR-9*						(95)
miR-96						(92)
miR-99a						(92)

Table II. *Continued*

PTC: Papillary thyroid carcinoma, PDTC: poorly differentiated thyroid carcinoma; FTC: follicular thyroid carcinoma; UDTC: undifferentiated thyroid carcinoma; MTC: medullary thyroid carcinoma.

catalyses the duplex loading into one of the four Argonauts (AGO) known in humans.

The loading mechanism and the dispensability of proteins that constitute the RLC are still unclear in mammals and contrasting evidence on RISC assembly has been provided (69-71). While in flies and in *C. elegans* the loading process is specific for the miRNA duplex, which is targeted to specific AGO proteins according to its sequence, in humans, a similar system seems not to exist (72). After miRNA duplex loading, the passenger strand is quickly removed and its degradation may be exerted by the AGO2 protein through cleavage, even if cleavage does not seem to be the most frequent mechanism in miRNA processing. It has been shown that AGO proteins are stabilized only when they are bound to miRNA: empty AGO proteins are quickly removed by proteasome-mediated degradation and autophagy.

As soon as the mature, single-stranded miRNA is in place inside the RISC complex, it drives the specificity of target mRNA recognition and subsequent negative regulation. RISC-mRNA interaction is, in fact, elicited through nucleotide pairing between the 'seed' sequence on the miRNA and the corresponding complementary 'seed-match' sequence on its target mRNA.

A limited complementarity (2-9 nt) between these regions is sufficient for a functional interaction that usually lead to a variable degree of mRNA degradation and reduced protein expression. The partial complementarity between seed and seed-match sequences permits miRNAs to interact with several targets. Recent studies showed that non-canonical pathways of miRNA biogenesis exist, as well as Drosha- and DGCR8- independent pathway, terminal uridylyl transferase-dependent (TUTase) pathway and Dicer-independent pathway. One of the first non-canonical pathways was identified and demonstrated with experimental procedures that showed the ability of cells to generate pre-miRNA-like

molecules that bind directly AGOs, including AGO2 that mediates the direct maturation of miRNA (73). An example of Drosha and DGCR8-independent non-canonical pathway is represented by the processing of microRNAs located inside intronic regions (miRtrons) when the introns excised by the spliceosome are directly cleaved and loaded by Dicer. Additionally, recent studies identified miRtrons, like miR-1225 and miR-1228, that are splicing-independent and, accordingly, have been defined simtron (splicing-independent mirtron-like miRNAs) (74). It is important to underline that the functionality of only a small number of these non-canonical miRNAs processing pathways has been demonstrated and that further studies are needed to better understand all the processes involved in miRNA biogenesis. However, it seems clear at this point that the miRNA biogenesis pathway will continue to be a rich source of exciting new discoveries (75).

The identification of miRNAs that may potentially be used as diagnostic and prognostic markers for TC diagnosis and treatment will surely provide valuable means to improve TC management. A considerable degree of observer-related variability exists in morphological diagnosis of TC, especially for certain tumor types like follicular pattern neoplasm. Patient outcome prediction is also biased by this subjective evaluation and enhancements are desirable to improve prognosis determination and therapeutic decision making.

One of the main advantages in the use of miRNAs in practical molecular diagnosis is that these molecules can be easily identified and profiled through minimally invasive processes. The active population of mature miRNAs is very resistant to post-sampling procedures minimizing the risk of pre-analytical artifacts that may be related to RNA degradation. Moreover, it has been shown that the concentration of specific circulating miRNAs in blood is tightly linked to molecular events occurring in those body regions affected by the disease, thus providing an indirect, while easy to use, way of measuring molecular events of diagnostic importance. It was reported recently that a miRNA-based signature can be used to discriminate benign from malignant thyroid nodules using fine-needle aspiration (FNA) samples. The identification of TC-related miRNAs could also serve to support the discovery of potential therapeutic targets and the development of more efficient therapies.

Many studies have been performed to clarify the role of aberrant miRNA functions in TCs and most authors analyzed miRNA expression comparing different forms of TCs in order to identify unique expression patterns (Table II).

In a Chinese study (58), 51 thyroid tumor and adjacent normal tissues were profiled for the identification of miRNA signatures that discriminate between PTCs and nodular goiters (NG). The authors showed that three miRNAs were significantly associated with PTC: miR-30a-3p, miR-146b and miR199b-5p and, while the first has been found to be down-regulated, the others were up-regulated in tumor tissues. It is obvious that the importance of these profiles in discriminating tissues is limited to comparisons of the same type. Some authors (18), for instance, reported a detailed signature of miRNAs differentially modulated between PTCs and normal tissues that do not include the three miRNAs listed above. They also highlighted the importance of miR-221 using experimental models to evaluate the effects of its over expression. The same authors also investigated the expression of miRNAs in ATC and observed that miR-30d, miR-125b, miR 26a, and miR-30a-5p are drastically down-regulated in tumor tissues. Cantara and co-workers screened miRNA expression in sera from PTC, NG and healthy subjects (76). The analysis revealed eight down-regulated (579, -95, -29b, 5-01-3p, -548d-5p) and three up-regulated (190, -362-3p, -518a-5p) miRNAs in PTC when compared to NG and healthy subjects. After a confirmatory analysis on a wider cohort, miRNAs 579, 95, 29b and 190 maintained their significative differences among sample types. The analysis of 45 primary thyroid samples, comprising FTC samples, follicular adenomas and normal control thyroids (77), resulted in the identification of two miRNAs significantly over expressed in FTC: miR-197 and miR-346. The *in vitro* over expression of both molecules induced proliferation, while their inhibition reduced cellular growth, suggesting an oncogenic role for these miRNAs. In a subsequent study (78), some authors used a more focused technology to characterize the expression of a smaller set of miRNAs in a group of surgically removed thyroid neoplastic and normal samples and in 62 FNA samples. They identified a number of molecules that were specifically modulated in PTC, FTC and ATC. Interestingly, it has been shown that while some miRNAs are usually over expressed in TC (*e.g.* miR-121 and miR-122), the level of their up-regulation is typical of the pathological phenotype considered. A number of studies also focused on miRNA profiling in PDTCs (78-80). It has been shown, for example, that miR-150 and miR-23b can be used to classify conventional and oncocytic PDTC when compared to WDTC (79). Moreover, miR-187, -221, -129, -222, -146b, -339 and -183 (78) were significantly over expressed in PDTCs when compared to normal tissues or hyperplastic nodules.

As mentioned above (1), ATC is likely to arise from PTC and FTC progression. This process requires multiple changes in global gene expression and some of the involved molecular pathways have been already characterized (81-83). According to their role as master gene regulators, the analysis of miRNA expression profiles in ATC has often been investigated in order to elucidate the processes leading to this transition. Even though a number of studies have been performed and reported in the literature, ATC is a very rare disease. For this reason, the data produced so far often lack a strong numeric basis to statistically support the conclusions drawn by

Table III. *miRNA expression profiles associated with specific pathological features. Green boxes indicate down-regulation, while red boxes indicate up-regulation.*

miRNA	Aggressive phenotype	*BRAF* mutation	Extrathyroidal extension	LN metastasis	Multifocal cancer	Relapse-free survival	Tumor-specific survival	Size ≥ 2 cm	TNM stage I/II *vs* III/IV
hsa-let-7e					🟥🟥				
hsa-miR-130b	🟩🟩								
hsa-miR-146b	🟥🟥	🟥🟥							
hsa-miR-151-5p								🟥🟥	
hsa-miR-181	🟥🟥		🟥🟥	🟥🟥		🟥🟥		🟥🟥	
hsa-miR-199b-5p									
hsa-miR-203		🟥🟥							
hsa-miR-21*		🟥🟥		🟥🟥					
hsa-miR-221		🟥🟥		🟥🟥				🟥🟥	🟥🟥
hsa-miR-222	🟥🟥	🟥🟥		🟥🟥					🟥🟥
hsa-miR-23b						🟩🟩			
hsa-miR-34b	🟩🟩								
hsa-miR-99b						🟥🟥			
hsa-miR151-5p								🟥🟥	
hsa-miR-150							🟩🟩		

authors. In 2007, Visone and co-workers investigated the genome-wide miRNA expression in 10 human ATCs and compared it with 10 normal thyroid profiles. While they proposed a miRNA signature associated with ATC, they also showed that according to functional studies *in vitro*, miR125b and miR-26a down-modulation may be critical for thyroid carcinogenesis (84). Another study (78) indicate that miR-302c, -205 and -137 are over expressed in ATC samples when compared to hyperplastic nodules. Different miRNA signatures have been proposed to discriminate ATC from other TCs. It has been shown (85, 86) that the down-regulation of miR-138 may be sufficient to distinguish ATC from PTC. It was also recently suggested, also, that the up-regulation of miR-200 and mR-30 family members can efficiently discriminate ATC from PTC and FTC.

MicroRNA for Risk Assessment and TC Prognosis

Many efforts have been made for the identification of molecular biomarkers that can be easily used for diagnosis, prognosis and risk assessment. As previously reported, miRNAs have been widely studied and sometimes unequivocally associated with specific types of TCs. Scientific works that aim to reveal associations between miRNA-altered expression and clinically relevant features in PTC are not so numerous but the proposed evidence deserves special attention for their practical relevance. Table III summarizes the involvement of miRNAs in these phenomena according to literature published so far.

An observational study was conducted to identify possible associations between deregulated miRNA expression and clinicopathological features, including *BRAF* mutational status, in a Chinese cohort of 52 patients with PTC (87). It was demonstrated that four miRNAs (221, 222, 146b and 181) had significantly higher expression levels in patients with *BRAF* mutations. The transcript levels of miRNA- 221 and miRNA-181 were also higher in patients with tumor diameter ≥2 cm, while over expression of miRNA-221 and miRNA-222 was observed in patients with advanced TNM stage and lymph node metastasis. Another Asian study (88) complemented these findings with the identification of a significantly relevant association between miR-21* and miR-203 expression and some of the above mentioned features (*BRAF* mutations, TNM stage and occurrence of lymph node metastases). Increased expression of miR-199b-5p in PTC was also shown to be associated with extrathyroidal extension and lymph node metastasis (58).

The opportunity to use circulating miRNAs as biomarkers for minimally invasive molecular tests has been also explored (89) and these small non-coding molecules demonstrated to be predictive of features, like tumor size, multifocality, lymph node metastasis and TNM staging. These molecules, in fact, are very stable in sera and miRNA profiles obtained after blood sampling are often recognizable and reproducible. The mechanisms underlying miRNA release from tissues into the bloodstream, however, is still unclear. Moreover, sera-isolated miRNAs are actually released by many cells belonging to different body regions not all of which are involved in the pathological process. For these reasons, blood sampling-based techniques must take into account the risks for false- positive (*e.g.* increased miRNA production by disease -unrelated regions) and false- negative (*i.e.* when the event to be monitored is the decrease of tumor-specific miRNA level) occurrences.

The impact of miRNA deregulations in prognosis has been investigated in different TC types, like PTC, follicular variant of papillary thyroid carcinoma (FVPTC) and PDTC (90-91, 79). In FVPTC, for example, increased transcription levels of miR-181 and miR-99b produced a decrease in a 10-year relapse-free survival from ~75% to ~18% for the former and from 60% to near 0% for the latter miRNA. On the contrary, a decrease in miR-23b expression in PDTC is reflected in a reduced relapse-free survival. While these studies underline that miRNA profiles can actually perform better than any known clinical indexes (*e.g.* tumor necrosis, increased mitotic index or convoluted nuclei occurrence) in the identification of adverse outcome, further studies will have to prove their potential diagnostic and predictive clinical value.

Conclusion

TC exhibits a wide range of clinical behaviors comprising indolent to highly aggressive forms. While the identification of genetic injuries, like *BRAF, RAS, RET* and *NTRK1* mutations and rearrangements have greatly aided our understanding of thyroid cell transformation, in many cases a clear distinction between risk categories in specific TC subtypes is far from being achieved. Many studies offered insights into the stepwise neoplastic progression, providing molecular signatures that can be ideally used for clinical management of TC. miRNA profiling adds information of critical importance to the molecular picture represented by gene regulation and thus provides completeness and strength to those signatures. According to the literature cited herein, it is possible to conclude that while miRNA signatures are mature enough for TC- type classification, further evidence is needed to confirm that these molecules can be used as valuable biomarkers for outcome prediction in thyroid neoplastic diseases.

Acknowledgements

Valeria Pecce and Francesca Rosignolo are recipients of the Sapienza Ph.D. program in Biotechnology in Clinical Medicine.

References

1 Kondo T, Ezzat S and Asa SL: Pathogenetic mechanisms in thyroid follicular-cell neoplasia. Nat Rev Cancer *6*: 292-306, 2006.

2 Hunt JL, Tometsko M, LiVolsi VA, Swalsky P, Finkelstein SD and Barnes EL: Molecular evidence of anaplastic transformation in coexisting well-differentiated and anaplastic carcinomas of the thyroid. Am J Surg Pathol *27*: 1559-1564, 2003.

3 Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel D, Schlumberger M and Wells SA Jr: Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid *19*: 565-612, 2009.

4 LiVolsi VA: Papillary neoplasms of the thyroid. Pathologic and prognostic features. Am J Clin Pathol *97*: 426-34, 1992.

5 Rosai J, Carcangui ML and DeLellis RA: Tumors of the Thyroid gland. Atlas of tumor pathology. Armed Forces Institute of Pathology, Washington DC, vol 3rd Series, Fascicle 5, 1992.

6 Schneider DF, Chen H and Sippel RS: Impact of lymph node ratio on survival in papillary thyroid cancer. Ann Surg Oncol *20*: 1906-1911, 2013.

7 Edge S, Byrd DR, Compton CC, Fritz AG, Greene FL and Trotti A: AJCC American Joint Committee on Cancer Staging Manual 2010 7th ed.

8 Beal SH, Chen SL, Schneider PD and Martinez SR: An evaluation of lymph node yield and lymph node ratio in well-differentiated thyroid carcinoma. Am Surg *76*: 28-32, 2010.

9 Lang BH, Wong KP, Wan KYand Lo CY: Significance of metastatic lymph node ratio on stimulated thyroglobulin levels in papillary thyroid carcinoma after prophylactic unilateral central neck dissection. Ann Surg Oncol *19:* 1257-1263, 2012.

10 Volante M, Rapa I and Papotti M: Poorly differentiated thyroid carcinoma: diagnostic features and controversial issues. Endocr Pathol *19*: 150-155, 2008.

11 Sakamoto A, Kasai N and Sugano H: Poorly differentiated carcinoma of the thyroid. A clinicopathologic entity for a high-risk group of papillary and follicular carcinomas. Cancer *52*: 1849-1855, 1983.

12 Akslen LA and LiVolsi VA: Poorly differentiated thyroid carcinoma–it is important. Am J Surg Pathol 24: 310-313, 2000.

13 Rufini V, Salvatori M, Fadda G, Pinnarelli L, Castaldi P, Maussier ML and Galli G: Thyroid carcinomas with a variable insular component: prognostic significance of histopathologic patterns. Cancer *110*: 1209-1217, 2007.

14 Hiltzik D, Carlson DL, Tuttle RM, Chuai S, Ishill N, Shaha A, Shah JP, Singh B and Ghossein RA: Poorly differentiated thyroid carcinomas defined on the basis of mitosis and necrosis: a clinicopathologic study of 58 patients. Cancer *106*: 1286-1295, 2006.

15 Hundahl SA, Fleming ID, Fremgen AM and Menck HR: A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995. Cancer *83*: 2638-2648, 1998.

16 Sippel RS, Kunnimalaiyaan M and Chen H: Current management of medullary thyroid cancer. Oncologist *13*: 539-547, 2008.

17 Edge SB and Compton CC: The American Joint Committee on Cancer: the 7th edition of the AJCC Cancer Staging Manual and the future of TNM. Ann Surg Oncol *17*: 1471-1474, 2010.

18 Pallante P, Visone R, Ferracin M, Ferraro A, Berlingieri MT, Troncone G, Chiappetta G, Liu CG, Santoro M, Negrini M, Croce CM and Fusco A: MicroRNA deregulation in human thyroid papillary carcinomas. Endocr Relat Cancer *13*: 497-508, 2006.

19 He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM and de la Chapelle A: The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci *102*: 19075-19080, 2005.

20 Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell *116*: 281-297, 2004.

21 Van Sande J, Parma J, Tonacchera M, Swillens S, Dumont J and Vassart G: Somatic and germline mutations of the TSH receptor gene in thyroid diseases. J Clin Endocrinol Metab *80*: 2577-2585, 1995.

22 Tonacchera M, Vitti P, Agretti P, Ceccarini G, Perri A, Cavaliere R, Mazzi B, Naccarato AG, Viacava P, Miccoli P, Pinchera A and Chiovato L: Functioning and nonfunctioning thyroid adenomas involve different molecular pathogenetic mechanisms. J Clin Endocrinol Metab *84*: 4155-4158, 1999.

23 Kimura T, Van Keymeulen A, Golstein J, Fusco A, Dumont JE and Roger PP: Regulation of thyroid cell proliferation by TSH and other factors: a critical evaluation of *in vitro* models. Endocr Rev *22*: 631-656, 2001.

24 Cetani F, Tonacchera M, Pinchera A, Barsacchi R, Basolo F, Miccoli P and Pacini F: Genetic analysis of the TSH receptor gene in differentiated human thyroid carcinomas. J Endocrinol Invest *22*: 273-278, 1999.

25 Matsuo K, Friedman E, Gejman PV and Fagin JA: The thyrotropin receptor (TSH-R) is not an oncogene for thyroid tumors: structural studies of the TSH-R and the alpha-subunit of Gs in human thyroid neoplasms. J Clin Endocrinol Metab *76*: 1446-1451, 1993.

26 Spambalg D, Sharifi N, Elisei R, Gross JL, Medeiros-Neto G and Fagin JA: Structural studies of the thyrotropin receptor and Gs alpha in human thyroid cancers: low prevalence of mutations predicts infrequent involvement in malignant transformation. J Clin Endocrinol Metab *81*: 3898-3901, 1996.

27 Goretzki PE, Lyons J, Stacy-Phipps S, Rosenau W, Demeure M, Clark OH, McCormick F, Röher HD and Bourne HR: Mutational activation of *RAS* and *GSP* oncogenes in differentiated thyroid cancer and their biological implications. World J Surg *16*: 576-581, 1992.

28 Xing M: *BRAF* mutation in thyroid cancer. Endocr Relat Cancer *12*: 245-262, 2005.

29 Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G, Tolaney S, Holt EH, Hui P, Umbricht CB, Basaria S, Ewertz M, Tufaro AP, Califano JA, Ringel MD, Zeiger MA, Sidransky D and Ladenson PW: *BRAF* mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab *90*: 6373-6379, 2005.

30 Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F, Zhu Z, Giannini R, Salvatore G, Fusco A, Santoro M, Fagin JA and Nikiforov YE: *BRAF* mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab *88*: 5399-5404, 2003.

31 Knauf JA, Ma X, Smith EP, Zhang L, Mitsutake N, Liao XH, Refetoff S, Nikiforov YE and Fagin JA: Targeted expression of *BRAF*V600E in thyroid cells of transgenic mice results in papillary thyroid cancers that undergo dedifferentiation. Cancer Res *65*: 4238-4245, 2005

32 Barollo S, Pezzani R, Cristiani A, Redaelli M, Zambonin L, Rubin B, Bertazza L, Zane M, Mucignat-Caretta C, Bulfone A, Pennelli G, Casal Ide E, Pelizzo MR, Mantero F, Moro S and Mian C: Prevalence, tumorigenic role, and biochemical implications of rare *BRAF* alterations. Thyroid *24*: 809-819, 2014.

33 Saavedra HI, Knauf JA, Shirokawa JM, Wang J, Ouyang B, Elisei R, Stambrook PJ and Fagin JA: The *RAS* oncogene induces genomic instability in thyroid PCCL3 cells *via* the MAPK pathway. Oncogene *19*: 3948-3954, 2000.

34 Mitsutake N, Knauf JA, Mitsutake S, Mesa C Jr, Zhang L and Fagin JA: Conditional *BRAF*V600E expression induces DNA synthesis, apoptosis, dedifferentiation, and chromosomal instability in thyroid PCCL3 cells. Cancer Res *65*: 2465-2473, 2005.

35 Knauf JA, Ouyang B, Knudsen ES, Fukasawa K, Babcock G and Fagin JA: Oncogenic RAS induces accelerated transition through G_2/M and promotes defects in the G_2 DNA damage and mitotic spindle checkpoints. J Biol Chem *281*: 3800-3809, 2006.

36 Marx SJ: Molecular genetics of multiple endocrine neoplasia types 1 and 2. Nat Rev Cancer *5*: 367-375, 2005.

37 Nikiforov YE: RET/PTC rearrangement in thyroid tumors. Endocr Pathol *13*: 3-16, 2002.

38 Barbacid M: The Trk family of neurotrophin receptors. J Neurobiol *25*: 1386-1403, 1994.

39 Xing M: Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 13: 184-199, 2013.

40 Howell GM, Hodak SP and Yip L: *RAS* mutations in thyroid cancer. Oncologist *18*: 926-932, 2013.

41 Zhu Z, Gandhi M, Nikiforova MN, Fischer AH and Nikiforov YE: Molecular profile and clinical-pathologic features of the follicular variant of papillary thyroid carcinoma. An unusually high prevalence of ras mutations. Am J Clin Pathol *120*: 71-77, 2003.

42 An JH, Song KH, Kim SK, Park KS, Yoo YB, Yang JH, Hwang TS and Kim DL: *RAS* Mutations in Indeterminate Thyroid Nodules are Predictive of the Follicular Variant of Papillary Thyroid Carcinoma. Clin Endocrinol (Oxf): 1-7, 2014.

43 Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM and Fletcher JA: PAX8–PPARγ1 fusion oncogene in human thyroid carcinoma (corrected). Science *289*: 1357-1360, 2000.

44 Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW 2nd, Tallini G, Kroll TG and Nikiforov YE: RAS point mutations and *PAX8–PPAR* gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab 88: 2318-2326, 2003.

45 Morin PJ: beta-catenin signaling and cancer Bioessays *21*: 1021-1030, 1999.

46 Garcia-Rostan G, Camp RL, Herrero A, Carcangiu ML, Rimm DL and Tallini G: Beta-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and *CTNNB1* exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. Am J Pathol *158*: 987-996, 2001.

47 Castellone MD, De Falco V, Rao DM, Bellelli R, Muthu M, Basolo F, Fusco A, Gutkind JS and Santoro M: The beta-catenin axis integrates multiple signals downstream from RET/papillary thyroid carcinoma leading to cell proliferation. Cancer Res *69*: 1867-1876, 2009.

48 Tartari CJ, Donadoni C, Manieri E, Mologni L, Mina PD, Villa A and Gambacorti-Passerini C: Dissection of the RET/β-catenin interaction in the TPC1 thyroid cancer cell line. Am J Cancer Res *1*: 716-725, 2011.

49 Kozomara A and Griffiths-Jones S: miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res *42*: D68-73, 2014.

50 Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M and Croce CM: Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA *101*: 2999-3004, 2004.

51 Mayr C, Hemann MT and Bartel DP: Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science *315*: 1576-1579, 2007.

52 Veronese A, Visone R, Consiglio J, Acunzo M, Lupini L, Kim T, Ferracin M, Lovat F, Miotto E, Balatti V, D'Abundo L, Gramantieri L, Bolondi L, Pekarsky Y, Perrotti D, Negrini M and Croce CM: Mutated beta-catenin evades a microRNA-dependent regulatory loop. Proc Natl Acad Sci USA *108*: 4840-4845, 2011.

53 Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA and Jones PA: Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell *9*: 435-443, 2006.

54 Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F and Croce CM: Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA *99*: 15524-15529, 2002.

55 Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM and Harris CC: Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell *9*: 189-198, 2006.

56 Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M and Croce CM: A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med *353*: 1793-1801, 2005.

57 Fassina A, Cappellesso R, Simonato F, Siri M, Ventura L, Tosato F, Busund LT, Pelizzo MR and Fassan M: A 4-MicroRNA signature can discriminate primary lymphomas from anaplastic carcinomas in thyroid cytology smears. Cancer Cytopathol *122*: 274-281, 2014.

58 Peng Y, Li C, Luo DC, Ding JW, Zhang W and Pan G: Expression profile and clinical significance of microRNAs in papillary thyroid carcinoma. Molecules 19: 11586-11599, 2014.

59 O'Donnell KA, Wentzel EA, Zeller KI, Dang CV and Mendell JT: c-Myc-regulated microRNAs modulate E2F1 expression. Nature *435*: 839-843, 2005.

60 Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, Dang CV, Thomas-Tikhonenko A and Mendell JT: Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet *40*: 43-50, 2008.

61 Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T and Hammond SM: Extensive post-transcriptional regulation of microRNAs and its implications for cancer. Genes Dev *20*: 2202-2207, 2006.

62 Kumar MS, Lu J, Mercer KL, Golub TR and Jacks T: Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet *39*: 673-677, 2007.

63 Puppin C, Durante C, Sponziello M, Verrienti A, Pecce V, Lavarone E, Baldan F, Campese AF, Boichard A, Lacroix L, Russo D, Filetti S and Damante G: Overexpression of genes involved in miRNA biogenesis in medullary thyroid carcinomas with *RET* mutation. Endocrine *47*: 528-536, 2014.

64 Quick-Cleveland J, Jacob JP, Weitz SH, Shoffner G, Senturia R and Guo F: The DGCR8 RNA-binding heme domain recognizes primary microRNAs by clamping the hairpin. Cell Rep *7*: 1994-2005, 2014.

65 Lund E, Güttinger S, Calado A, Dahlberg JE and Kutay U: Nuclear export of microRNA precursors. Science *303*: 95-98, 2004.

66 Bohnsack MT, Czaplinski K and Gorlich D: Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA *10*: 185-191, 2004

67 Yi R, Qin Y, Macara IG and Cullen BR: Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev *17*: 3011-3016, 2003.

68 Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ and Plasterk RH: Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. Genes Dev *15*: 2654-2659, 2001.

69 Kawamata T and Tomari Y: Making RISC. Trends Biochem Sci *35*: 368-376, 2010.

70 Betancur JG, and Tomari Y: Dicer is dispensable for asymmetric RISC loading in mammals. RNA *18*: 24-30, 2012.

71 Ye X, Huang N, Liu Y, Paroo Z, Huerta C, Li P, Chen S, Liu Q and Zhang H: Structure of C3PO and mechanism of human RISC activation. Nat Struct Mol Biol *18*: 650-657, 2011.

72 Azuma-Mukai A, Oguri H, Mituyama T, Qian ZR, Asai K, Siomi H and Siomi MC: Characterization of endogenous human Argonauts and their miRNA partners in RNA silencing. Proc Natl Acad Sci USA *105*: 7964-7969, 2008.

73 Yang JS and Lai EC: Dicer-independent, Ago2-mediated microRNA biogenesis in vertebrates. Cell Cycle 9: 4455-4460, 2010.

74 Havens MA, Reich AA, Duelli DM and Hastings ML: Biogenesis of mammalian microRNAs by a non-canonical processing pathway. Nucleic Acids Res *40*: 4626-4640, 2012.

75 Ha M and Kim VN: Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol *15*: 509-524, 2014.

76 Cantara S, Pilli T, Sebastiani G, Cevenini G, Busonero G, Cardinale S, Dotta F and Pacini F: Circulating miRNA95 and miRNA190 are sensitive markers for the differential diagnosis of thyroid nodules in a Caucasian population. J Clin Endocrinol Metab *99*: 4190-4198, 2014.

77 Weber F, Teresi RE, Broelsch CE, Frilling A and Eng C: A limited set of human MicroRNA is deregulated in follicular thyroid carcinoma. J Clin Endocrinol Metab *91*: 3584-3591, 2006.

78 Nikiforova MN, Tseng GC, Steward D, Diorio D and Nikiforov YE: MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab *93*: 1600-1608, 2008.

79 Dettmer MS, Perren A, Moch H, Komminoth P, Nikiforov YE and Nikiforova MN: MicroRNA profile of poorly differentiated thyroid carcinomas: new diagnostic and prognostic insights. J Mol Endocrinol *52*: 181-189, 2014.

80 Schwertheim S, Sheu SY, Worm K, Grabellus F and Schmid KW: Analysis of deregulated miRNAs is helpful to distinguish poorly differentiated thyroid carcinoma from papillary thyroid carcinoma. Horm Metab Res *41*: 475-481, 2009.

81 Cerutti J, Trapasso F, Battaglia C, Zhang L, Martelli ML, Visconti R, Berlingieri MT, Fagin JA, Santoro M and Fusco A: Block of c-MYC expression by antisense oligonucleotides inhibits proliferation of human thyroid carcinoma cell lines. Clin Cancer Res *2*: 119-126, 1996.

82 Califano D, Monaco C, Santelli G, Giuliano A, Veronese ML, Berlingieri MT, de Franciscis V, Berger N, Trapasso F, Santoro M, Viglietto G and Fusco A: Thymosin beta-10 gene over-expression correlated with the highly malignant neoplastic phenotype of transformed thyroid cells *in vivo* and *in vitro*. Cancer Res *58*: 823-828, 1998.

83 De Nigris F, Visconti R, Cerutti J, Califano D, Mineo A, Santoro M, Santelli G and Fusco A: Overexpression of the *HIP* gene coding for a heparin/heparan sulphate-binding protein in human thyroid carcinomas. Cancer Res *58*: 4745-4751, 1998.

84 Visone R, Pallante P, Vecchione A, Cirombella R, Ferracin M, Ferraro A, Volinia S, Coluzzi S, Leone V, Borbone E, Liu CG, Petrocca F, Troncone G, Calin GA, Scarpa A, Colato C, Tallini G, Santoro M, Croce CM and Fusco A: Specific microRNAs are downregulated in human thyroid anaplastic carcinomas. Oncogene *26*: 7590-7595, 2007.

85 Takakura S, Mitsutake N, Nakashima M, Namba H, Saenko VA, Rogounovitch TI, Nakazawa Y, Hayashi T, Ohtsuru A and Yamashita S: Oncogenic role of miR-17-92 cluster in anaplastic thyroid cancer cells. Cancer Sci *99*: 1147-1154, 2008.

86 Mitomo S, Maesawa C, Ogasawara S, Iwaya T, Shibazaki M, Yashima-Abo A, Kotani K, Oikawa H, Sakurai E, Izutsu N, Kato K, Komatsu H, Ikeda K, Wakabayashi G and Masuda T: Down-regulation of miR-138 is associated with over expression of human telomerase reverse transcriptase protein in human anaplastic thyroid carcinoma cell lines. Cancer Sci *99*: 280-286, 2008.

87 Sun Y, Yu S, Liu Y, Wang F, Liu Y and Xiao H: Expression of miRNAs in Papillary Thyroid Carcinomas Is Associated with *BRAF* Mutation and Clinicopathological Features in Chinese Patients. Int J Endocrinol 2013:128735. doi: 10.1155/2013/128735. Epub 2013.

88 Huang Y, Liao D, Pan L, Ye R, Li X, Wang S, Ye C and Chen L: Expressions of miRNAs in papillary thyroid carcinoma and their associations with the *BRAF*V600E mutation. Eur J Endocrinol *168*: 675-681, 2013.

89 Yu S, Liu Y, Wang J, Guo Z, Zhang Q, Yu F, Zhang Y, Huang K, Li Y, Song E, Zheng XL and Xiao H: Circulating microRNA profiles as potential biomarkers for diagnosis of papillary thyroid carcinoma. J Clin Endocrinol Metab *97*: 2084-2092, 2012.

90 Yip L, Kelly L, Shuai Y, Armstrong MJ, Nikiforov YE, Carty SE, Nikiforova MN: MicroRNA signature distinguishes the degree of aggressiveness of papillary thyroid carcinoma. Ann Surg Oncol *18*: 2035-2041, 2011.

91 Dettmer M, Perren A, Moch H, Komminoth P, Nikiforov YE, Nikiforova MN: Comprehensive microRNA expression profiling identifies novel markers in follicular variant of papillary thyroid carcinoma, Thyroid *23*: 1383-1389, 2013.

92 Cahill S, Smyth P, Finn SP, Denning K, Flavin R, O'Regan EM, Li J, Potratz A, Guenther SM, Henfrey R, O'Leary JJ and Sheils O: Effect of RET/PTC 1 rearrangement on transcription and post-transcriptional regulation in a papillary thyroidcarcinoma model. Mol Cancer *5*: 1-12, 2006.

93 Colamaio M, Borbone E, Russo L, Bianco M, Federico A, Califano D, Chiappetta G, Pallante P, Troncone G, Battista S and Fusco A: miR-191 down-regulation plays a role in thyroid follicular tumors through CDK6 targeting. J Clin Endocrinol Metab *96*: E1915-E1924, 2011.

94 Tetzlaff MT, Liu A, Xu X, Master SR, Baldwin DA, Tobias JW, Livolsi VA and Baloch ZW: Differential expression of miRNAs in papillary thyroid carcinoma compared to multinodular goiter using formalin- fixed paraffin- embedded tissues. Endocr Pathol *18*: 163-173, 2007.

95 Abraham D, Jackson N, Gundara JS, Zhao J, Gill AJ, Delbridge L, Robinson BG and Sidhu SB: MicroRNA profiling of sporadic and hereditary medullary thyroid cancer identifies predictors of nodal metastasis, prognosis, and potential therapeutic targets. Clin Cancer Res *17*: 4772-81, 2011.

96 Braun J, Hoang-Vu C, Dralle H and Hüttelmaier S: Down-regulation of microRNAs directs the EMT and invasive potential of anaplastic thyroid carcinomas. Oncogene *29*: 4237-4244, 2010.

97 Tallini G and Asa SL: *RET* oncogene activation in papillary thyroid carcinoma. Adv Anat Pathol *8*: 345-354, 2001.

98 Tallini G, Santoro M, Helie M, Carlomagno F, Salvatore G, Chiappetta G, Carcangiu ML, Fusco A: *RET*/*PTC* oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes. Clin Cancer Res *4*: 287-294, 1998.

99 Lam AK, Montone KT, Nolan KA and Livolsi VA: *RET* oncogene activation in papillary thyroid carcinoma: prevalence and implication on the histological parameters. Hum Pathol *29*: 565-568, 1998.

100 Marsh DJ, Mulligan LM and Eng C: *RET* proto-oncogene mutations in multiple endocrine neoplasia type 2 and medullary thyroid carcinoma. Horm Res *47*: 168-178, 1997.

101 Lemoine NR, Mayall ES, Wyllie FS, Williams ED, Goyns M, Stringer B and Wynford-Thomas D: High frequency of RAS oncogene activation in all stages of human thyroid tumorigenesis. Oncogene *4*: 159-164, 1989.

102 Shi YF, Zou MJ, Schmidt H, Juhasz F, Stensky V, Robb D and Farid NR: High rates of RAS codon 61 mutation in thyroid tumors in an iodide-deficient area. Cancer Res *51*: 2690-2693, 1991.

Received December 15, 2014
Revised January 23, 2015
Accepted January 27, 2015