MECHANISMS IN ENDOCRINOLOGY

MicroRNA in diagnostics and therapy of thyroid cancer

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Abstract

MicroRNAs, short non-coding regulators of the gene expression, are subjects of numerous investigations assessing their potential use in the diagnostics and management of human diseases. In this review, we focus on studies that analyze the utility of microRNAs as novel diagnostic and therapeutic tools in follicular cell-derived thyroid carcinomas. This very interesting and promising field brings new insight into future strategies for personalized medicine.

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Introduction on microRNAs

Due to their unique biological and functional properties, microRNAs (miRNAs, miRs) are emerging as potent diagnostic and therapeutic tools. The first feature of miRNAs is a specificity of their expression profile, which is highly specific for a given cell and disease, and it can serve as a specific fingerprint for both healthy and disordered tissues (1, 2). The second unique feature of miRs is their length, which usually does not exceed 24 nucleotides; this makes them resistant to endonucleolytic cleavage. As such, miRs are relatively stable and can be detected and reliably measured in various biological materials, including archived formalin-fixed and paraffin-embedded samples (3) or serum (4).

MicroRNA function and biogenesis

MiRNAs act via annealing to transcripts of protein-coding genes and inhibiting further steps of their expression. This interaction depends on the Watson-Crick complementarity between the seed region, located between the second and the eighth miRNA nucleotides, and the target sequence located in mRNA (5). Although most miRs recognize sequences within the 3' untranslated regions (3'UTRs) of target mRNAs, still a large number of interactions occurs within the coding sequence of the target gene (6). It is estimated that microRNAs regulate the expression of at least a half of the human protein-coding transcripts (7).

Tissue specificity of microRNA expression adds to the stringency of mechanisms, which maintains the

Invited Author's profile

Krystian Jażdżewski, MD, PhD, is the head of an interdisciplinary group comprising medical doctors, molecular biologists, and geneticists from Genomic Medicine (Medical University of Warsaw) and the Laboratory of Human Cancer Genetics (Centre of New Technologies, University of Warsaw) in Poland. The group analyzes the function of regulatory RNAs and the role of their disturbed expression in the development and progression of cancer. They aim to elucidate noninvasive molecular panels based on microRNA expression that will allow for diagnostics and prognostics of human cancers. Prof. Jażdżewski has published several papers in all leading peer-reviewed journals.

development and differentiation of cells. MiRNAs play an important role in apoptosis (8) and proliferation (9). Importantly, because a single miR regulates several target mRNAs, and a single mRNA is regulated by ~100 miRNAs (10), the combined effects of deregulation of miRNA expression can induce strong biological responses. MiRs are involved in the regulation of many different types of genes, including oncogenes and tumor suppressors, and more than one-half of annotated miRNAs are located in fragile sites associated with cancer (11, 12).

MicroRNAs are encoded all over the genome, mainly in introns of protein-coding genes. MiRs are first expressed as long primary transcripts (pri-miRNAs) that are cleaved to produce ~60 nt long microRNA precursors (pre-miRNAs) (13). Pre-miRNAs fold into specific secondary structures of hairpins and are further cleaved by the Dicer RNase to produce mature miRNA molecules from each strand of the hairpin (5) (Fig. 1).

Recent studies revealed that a single miRNA gene might give rise to isomiRs, numerous mature miRNA molecules of different length (14). This fact is of profound importance for miRNA's function, because the addition or deletion of nucleotides at the isomiR 5' end results in a change in the seed region and, consequently, leads to the regulation of a distinct set of target genes compared to

the isomiR's canonical counterpart. As functional partners of their reference canonical molecules, isomiRs are tissue specific; therefore, the proper interpretation of microRNA-regulated networks requires comprehensive information on the miRNome for each particular tissue type (15, 16, 17).

MicroRNAs of the thyroid gland

This review focuses on microRNAs implicated in the pathogenesis of the follicular cell-derived thyroid carcinomas. A recent next-generation sequencing study revealed that although 2588 human microRNAs are deposited in miRbase 21 (18), only 427 miRs are expressed at a significant level (> 5 reads per million reads (RPMs)) in the thyroid gland (19). The study also showed that the 427 thyroid miRs exist in 1749 length isoforms, and novel isomiRs are often expressed at higher levels compared to their canonical counterparts. If the length variation affects the miRNA 5' end, a novel seed is created leading to an altered regulatory function. This fact is of great importance for the proper understanding of the miR-mediated processes in the thyroid cells; however, traditionally used microarray and TaqMan analyses could not distinguish between most of the length variants. In papillary thyroid carcinoma (PTC), 427 expressed miRs created almost 100 novel regulatory seeds, while the total number reached 513. Even though the expression of a single microRNA can be relatively low, the action of all isoforms carrying the same seed region (so-called seed power) can induce strong biological responses (19).

The role of microRNAs in thyroid tumors has been studied for a long time, revealing specific expression patterns of miRs. Most profiling studies in thyroid cancers were performed using oligo DNA microarrays (see Table 1). The studies showed that different cancer types exhibit deregulation of different microRNAs, proving the existence of cancer-specific signatures of microRNA expression (20, 21, 22, 23, 24). These observations were recently confirmed in the next-generation sequencing of thyroid tumors (19, 25). The studies showed that PTC is accompanied by a significant upregulation of the miR-146, -181, -221/222, and -224 families; follicular thyroid carcinoma (FTC) exhibits upregulation of the miR-181 and -200 families and downregulation of the miR-199 family, and anaplastic thyroid carcinoma (ATC) is characterized by elevated levels of miR-17 and -221/222 and downregulation of the let-7 and miR-30 and -29 families. A list of the top deregulated miRs identified in different studies is presented in Table 1.

Figure 1

MicroRNA biogenesis. MicroRNA gene is expressed in a form of a long (hundreds to thousands of nucleotides) primary transcript (pri-miRNA), which is cleaved to produce ~60 nt long pre-miRNA. In cytoplasm, pre-miRNA is further cleaved by the RNase complex to give a miRNA duplex comprising two mature miRNA molecules, each arising from one arm of the hairpin. One of the mature miRs is loaded into RNA-Induced Silencing Complex (RISC), the other miR is degraded.

Table 1 Top deregulated miRNAs in differentiated thyroid carcinomas.

Upregulated miRNAs	Downregulated	Method	References
PTC			
miR-146, miR-221, miR-222, miR-21, miR181a/c, miR155 miR-221, miR-222, miR-181b		miRNA microarrays, northern blot miRNA microarrays, northern blot, real-time PCR TaqMan MicroRNA Assays Human Panel	(21) (22) (36)
miR-31, miR-122a, miR-146b, miR-155, miR-187, miR-205, miR-221, miR-222, miR-224		Real-time PCR TaqMan TaqMan Human Microarray Arrays v3.0	(37) (46)
miR-146b, miR-155, miR-187, miR-221, miR-222, miR-224 miR-146b-3p, miR-146-5p, miR-221, miR-222, miR-222-5p, miR-181a	miR-1179, miR-138, miR-144-5p, miR-199b-5p, miR-204, miR-219-5p, miR-451 miR-7, miR144, miR-199a-1, miR-486-3p	Next-generation sequencing Quantitative RT-PCR	(19) (72) (51) (49)
miR-146b, miR-221, miR-551b-3p miR-155 miR-146b, miR-221, miR-222 miR-145 miR-182		Real-time RT-PCR Quantitative RT-PCR Quantitative RT-PCR Quantitative RT-PCR	(73) (39) (44) (45)
miR-25-3p, miR-451a, miR-140-3p, let-7i miR-146b, miR-221, miR-222	miR-7, miR-204 miR-21 (patients with tumor recurrence), miR-9 (in recurrent PTC, compared to non-recurred)	Next-generation sequencing Quantitative RT-PCR	(25) (53)
FTC			
miR-192, miR-197, miR-328, miR-346 miR-146b, miR-155, miR-187, miR-222, miR-221, miR-224 miR-122 (compared to FA)		MiRNA microarrays TaqMan MicroRNA assays human panel Quantitative RT-PCR MiRNA microarrays	(20) (36) (78) (76)
let-7a miR-10b, miR-92a, miR-221, miR-222 miR-183-3p, miR-183-5p, miR-107, miR-146b-3p, miR-221-3p	miR-486-5p, miR-486-3p, miR-9-5p, miR-9-3p, miR-205-5p, miR-199-5p, miR-34b-3p, miR-1 miR-7-5p miR-7-2-3p miR-1179 miR-144-5p miR-130b-5p, miR-328-3p miR-30a-3p miR-10a-5p miR-296-5p miR-138-5p miR-199	TaqMan MicroRNA array panels MiRNA microarrays	(54) (75)
miR-137, miR-767-5p, miR-663b, miR-30d-3p, miR-874-3p, miR-125a-3p, miR-1287-5p, miR-30c-1-3p, miR-125b-2-3p, miR-375 miR-181, miR-200		MiRNA microarrays	(41)
ATC			
miR-137, miR-155, miR-187, miR-205, miR-214, miR-221, miR-222, miR-224, miR-302		Next-generation sequencing	(25)
miR-17, miR-221, miR-222	miR-26a, miR-30a-5p, miR-30d, miR-125 let-7, miR-29, miR-30	TaqMan MicroRNA assays human panel MiRNA microarrays, northern blot Next-generation sequencing	(36) (24) (25)

Such tumor specificity of microRNA expressions suggests their possible use as cancer biomarkers. Indeed, numerous studies aimed at the elaboration of microRNA-based prognostic and diagnostic tools for thyroid cancers. The results of this quest are described in more detail below.

MicroRNA-mediated thyroid cancer risk

The familial risk for PTC is associated with a single nucleotide polymorphism (SNP) within the miR-146a-3p (rs2910164 G>C), which alters both expression levels and gene targeting by the miR (26, 27). A case-control study performed in 608 patients from Polish, American, and Finnish populations revealed that the G/C heterozygosity is a risk factor predisposing to PTC (OR=1.62; 95% CI 1.3–2.0; $P=0.000007$), with the CC state being protective (OR=0.42; 95% CI 0.24–0.73, $P=0.0027$). The same study revealed that tumor DNA of up to 6% of patients undergoes somatic mutations within rs2910164, which additionally confirmed the role of this variant in the pathogenesis of PTC (26). The rs2910164-mediated predisposition was further analyzed in 781 thyroid cancer patients recruited in the United Kingdom (28), and the study revealed no association between the polymorphism and thyroid cancer risk. Similar results were obtained in 753 Chinese patients (29) and in 307 Italian patients (30). However, meta-analyses revealed a strong association of the SNP with the cancer risk in the Caucasian population (30). Interestingly, another study performed in FTC and follicular adenomas (FA) showed that the CC genotype within the rs2910164 was completely absent in FTC tumors. The authors proposed that the SNP could be correlated with the transition from normal to neoplastic thyroid tissue (23). It is worth mentioning that rs2910164 has been later shown to predispose to hepatocellular carcinoma in males (31), prostate cancer (32), bladder cancer (33), and colorectal cancer (34). Rs2910164 is also proposed to contribute to the pathogenesis of breast and ovarian cancer, as the target genes of miR-146a include *BRCA1* and *BRCA2*. Breast cancer patients who had at least one miR-146a variant allele were diagnosed at an earlier age than the patients with no variant alleles (35).

MicroRNAs in thyroid cancer diagnostics: analysis of microRNAs in fine-needle aspiration (FNA) and tissue specimens

Thyroid nodules are the most common thyroid disease and an estimated 50% of the general population has nodules detectable by ultrasonography. However, only 5%

of all thyroid nodules harbor malignancy; therefore, preoperative differentiation of benign and malignant lesions is crucial, although not always possible with the use of traditional FNA biopsy and cytological examination. Several studies attempted to elaborate specific microRNA diagnostic panels that would enable higher specificity and sensitivity in differentiation between benign and malignant thyroid nodules.

Nikiforova *et al.* performed microRNA profiling of various thyroid tumors and proposed a set of miR-146b, -155, -187, -197, -221, -222, and -224 as potentially distinguishing between the analyzed sample types. The chosen miRs were quantified in 62 FNA samples and the authors claimed that, when at least one miRNA was overexpressed over twofold, the sensitivity of cancer detection was 100%, specificity 94%, and accuracy 95%. When three or more miRNAs were upregulated, the sensitivity of cancer detection was 88%, with a specificity of 100% and accuracy of 98% (36). The clinical utility of the same microRNA panel was further evaluated in a set of 141 FNA specimens, revealing that the decision model based on the expression of miR-146b, -155, and -221 had a validity of 97.7% (59% in nodules with undetermined cytology) and reliability of 78.4% (37). In a study by Kitano *et al.*, the authors chose a diagnostic set of four different microRNAs: miR-7, -126, and -374a and let-7g. The analysis performed in 154 FNA specimens revealed that among the chosen miRs only miR-7 actually distinguished between malignant and benign tissue. The proposed predictor model had an overall accuracy of 37% with the sensitivity of 100% and specificity of 20%, while positive predictive values and negative predictive values reached 25 and 100% respectively (38). Another study analyzed the expression of miR-146b-5p and miR-21 in various thyroid nodules using *in situ* hybridization in formalin-fixed paraffin-embedded specimens (39). The study showed high overexpression of miR-146b-5p in PTC (89%) and follicular variant of PTC (fvPTC, 41%), whereas the miR was not detected in most FTCs, ATCs, poorly differentiated thyroid carcinomas (PDTCs), or FAs. MiR-21 was overexpressed in 83% of ATCs, 79% of PTCs, 34% of fvPTCs, and 19% of PDTCs, but not in FAs or FTCs. The utility of quantification of the miR-221/222 cluster together with analysis of *BRAF* V600E mutation and galectin-3 protein expression was assessed in 120 FNA samples with indeterminate cytology. Compared with a postoperative histopathological diagnosis, the proposed markers showed the sensitivity of 73.5%, specificity of 89.8%, and accuracy of 75.7% (40).

Cytological diagnostics of follicular lesions is considered a grey area in thyroid pathology. Distinguishing between FTC and follicular adenoma in FNA material is one of the most challenging tasks, thus another study specifically addressed the search for a malignancy marker in follicular tumors. The identified two-miR-classifier combining miR-486-5p and miR-7-2-3p had the accuracy of 62%, sensitivity of 82.1%, and specificity of 48.8% (41).

MicroRNAs in thyroid cancer diagnostics: analysis of circulating microRNAs

Specific microRNAs deregulated in thyroid tumors are secreted to the bloodstream, which makes them promising noninvasive biomarkers for cancer patients. In a large-scale analysis of serum microRNA profiles in 245 subjects, Yu *et al.* revealed that the levels of let-7e and miR-151-5p and miR-222 were significantly elevated in PTC patients in comparison either to patients with benign nodules or to healthy subjects (42). Depending on the cutoff values, the test achieved 87.8% sensitivity and 88.4% specificity. Another recent study revealed that levels of miR-181a-5p were significantly elevated in the plasma of thyroid cancer patients compared with both control subjects and with patients with other types of cancer, including breast, lung and colon cancer or melanoma (43). In yet another study, the authors analyzed the possible use of circulating miR-146b and miR-155, -221, and -222. The mean levels of miR-146b and miR-155 were higher in the PTC group than in the benign group. MiR-146b distinguished between the groups with 61.4% sensitivity and 57.9% specificity, while miR-155 with 74.3% sensitivity and 63.2% specificity. The levels of miR-146b, -155, and -222 increased proportionally to tumor size (44). Alternatively, plasma levels of miR-25-3p and miR-451a were used to distinguish PTC patients from subjects with benign nodules with the sensitivity of 92.8 and 88.9% and specificity of 68.8 and 66.7%, respectively (45). It is worth mentioning that some studies found miR-451 downregulated, not upregulated, in thyroid cancer (46, 47). Most importantly, miR-451 and miR-16 are known to be related to hemolysis and thus not easy to be used as a circulating marker (48). Another study showed a diagnostic utility of miR-145 in serum and FNA (49), even though the expression of this miR is lower in cancer tissue.

MicroRNAs in cancer prognostics

MicroRNA expression might also serve as a prognostic factor for the clinical outcome of thyroid cancer patients. Although PTC is associated with relatively low mortality

rates, nodal and distant recurrence is observed in ~20% of patients, significantly worsening the disease outcome. Elucidation of sensitive biomarkers allowing for identification of such patients would significantly facilitate the treatment and monitoring of their health status. In PTC, miR-146b promotes cell migration and invasion and is associated with an increased risk of recurrence (50). Recently, it has been shown that PTC cases with overexpression of miR-146b-5p and miR-21 had significantly poorer disease-free survival rates. Because miR-146b-5p was significantly overexpressed in PTC, including fvPTC, but not in other analyzed tissues, the authors suggested its possible use as both a diagnostic and a prognostic marker for PTC (39). Similar conclusions were drawn from a study analyzing miR-146b and miR221/222 cluster. Higher expression levels of those miRs were detected in cancers presenting with capsular and vascular invasion or lymph node metastasis (51). OncomiRs miR-221 and miR-222 were reported to play a role in PTC aggressiveness (52) and, in the Cancer Genome Atlas (TCGA) data, were associated with less-differentiated tumors (47). In TCGA data, a more aggressive phenotype of thyroid cancer was also correlated with overexpression of miR-21 and miR-146b, as well as the loss of miR-204 (47). Later, the expression levels of miR-21 and miR-9 were also associated with the metastatic potential of tumor cells and proved to be good predictors of nodal metastasis (53).

In another study, miR-181a-2-3p and miR-99b-3p predicted a relapse-free survival of fvPTC patients, providing a potentially important diagnostic and predictive value (46). Similar markers were proposed for minimally invasive FTCs. MiR-10b, -92a and -221/222 cluster were significantly elevated in a group of metastatic cancers, and the expression of miR-10b was proposed to be a prognostic factor for the evaluation of the metastatic potential of minimally invasive FTC with an OR=19.8 (54). In a study by Xiong *et al.*, the expression levels of miR-126-3p were negatively correlated with tumor size and worse clinical outcome of both FTC and PTC patients. *In vitro* and *in vivo* analyses showed that overexpression of miR-126-3p led to the inhibition of cell proliferation, colony formation, vascular endothelial growth factor secretion, and a decrease of the metastatic potential (55). Thus, it seems that microRNAs could be potentially useful for the noninvasive monitoring of thyroid status and thyroid cancer recurrence.

MicroRNAs in cancer therapies

The role of microRNAs in the pathogenesis of cancer is becoming more and more striking. A novel study revealed

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that microRNAs and microRNA processing complexes are directly involved in the cellular adhesion mediated by E-cadherin and p120 catenin (56). Unlike genetic changes, microRNA alterations can be modulated, thus the significant deregulation of microRNA levels in cancer seems to be a promising therapeutic target. The biological impact of upregulated microRNAs can be abolished with specific inhibitors or anti-miRs, which are synthetic molecules that prevent binding of a miR to its targets. Lowered levels of a microRNA can be reversed by its directed upregulation, accomplished through the delivery of synthetic mature microRNA mimics, as in the synthetic RNA (siRNA) technology (57, 58), or the delivery of primary microRNAs (shmiR) or microRNA precursors (shRNA) expressed from plasmids (59, 60, 61).

Studies on mouse models confirmed the potential utility of microRNA targeted therapies; however, numerous questions still have to be answered before most of these results can be further directed to clinical trials. The first milestone in the studies on the use of microRNAs as therapeutic agents was a preclinical study in which miR-122 inhibitors were tested in chimpanzees as a therapy for chronic hepatitis C. i.v. delivery of the microRNA inhibitor resulted in a significant decrease of hepatitis C virus viremia, and the tested molecule proved to be of low toxicity (62). Another study, which reached the first clinical phase, was conducted in patients with unresectable primary liver cancer. The patients were treated with a MRX34 molecule, mimicking miR-34, a potent regulator of 24 oncogenes overexpressed in liver cancer (63). The closing date of the study is scheduled for December 2015. The quest to identify microRNA-based strategies continues. According to ClinicalTrials.gov, a service maintained by the U.S. National Institutes of Health, 272 microRNA studies are currently registered, and patients are being recruited or the recruitment will begin soon for another 145 studies. However, the registered clinical trials are mainly focused on the diagnostic use of miRs, specifically on the elaboration of diagnostic panels based on circulating microRNAs.

The potential of microRNAs in thyroid cancer treatment

Although so far there have been no reports on the clinical use of microRNAs in the thyroid cancer treatment, numerous functional and preclinical studies indicate strong potential for this field. The vast number of studies focuses on PTC as the most common thyroid carcinoma. Functional analyses led to the identification of microRNAs

with a tumor-suppressive role in this cancer. Overexpression of miR-101 (64) and miR-145 (49), both downregulated in PTC, led to decreased cell proliferation, migration, and invasion. Similar effects were observed for miR-199a-3p, which induced non-apoptotic cell death in a PTC-derived cell line (65). Similarly, miR-291-5p was downregulated in PTC tumors, and the forced expression of the miR suppressed PTC cell proliferation and migration, as well as promoted apoptosis. This effect of miR-291-5p on PTC occurrence and behavior was possibly mediated by estrogen receptor α (ER α), its direct target (66). Among the top upregulated microRNAs in PTC, miR-146b-5p plays a role in the cell proliferation and invasion and was shown to be transiently upregulated during epithelial-mesenchymal transition (EMT) (67).

Other studies indicate the potential of microRNAs not only in the regulation of tumor growth but also in adjuvant therapies in thyroid cancer. Many patients with advanced thyroid cancer do not benefit from the radioiodine therapy due to the reduced expression and function of the Na⁺/I⁻ symporter (NIS). It was shown that lowered levels of NIS in thyroid tumors are at least in part caused by the overexpression of miR-339 and miR-146b, and inhibition of these microRNAs results in the increased uptake of radioactive iodine by the thyroid cancer cells (68, 69, 70).

Data obtained in the *in vitro* experiments provided the basis for a number of preclinical studies related to the modulation of microRNAs in PTC, currently performed mainly on mouse models. S.c. transplantation of the human PTC cell line stably expressing miR-204-5p to BALB/c nude mice revealed that the overexpression of miR resulted in a lower tumor size compared with tumors induced by the injection of control cells (71). Because miR-204-5p is downregulated in cancer and was proposed a tumor suppressor, this result supports its crucial role in the tumor transformation and growth, possibly mediated via targeting the insulin-like growth factor-binding protein 5 (IGFBP-5). Another interesting study analyzed miR-155, an oncomiR frequently upregulated in PTC. Its overexpression in a mouse model induced the growth of larger and more intensively proliferating tumors (72). A number of studies evaluated the possible use of microRNA inhibitors in modulating cellular miRNA levels. The inhibition of miR-182 in TPC-1 cells injected to BALB/c nude mice resulted in the slower tumor growth compared with mice injected with control cells. These results indicate a potential role of miR-182 as an oncogene in PTC and a putative therapeutic target in this cancer (73). In yet another study, Frezzetti *et al.* proved that the

injection of constitutive Ras-transformed FRTL-5 cells with inhibitors of miR-21 resulted in the decrease of tumor growth (74).

As opposed to PTC, functional studies on the role of miRs in FTC and PDTCs are scarce. The study by Xiong *et al.* showed that similar miRs may play a role in the pathogenesis of PTC and FTC, as miR-126-3p, implicated in the pathogenesis of PTC, was significantly lower in FTC compared with FA specimens (55). Other functional studies suggested the impact of miR-183 and miR-146b, upregulated in FTC, on cancer development. Their overexpression in cell lines induced migration, and overexpression of miR-183 significantly repressed apoptosis (75). In another study, let-7a was found to be downregulated in FTC compared with FA, and functional assays revealed that enforced let-7a expression in the FTC-derived cell line induced epithelial-like phenotype, increased cell adhesion, and decreased cell migration. As a proof of these effects, silencing let-7a in the normal rat thyroid cell line PCCl3 had the opposite outcome (76). Similar results were observed for miR-142-3p that also seems to play a tumor-suppressive role in the thyroid gland. Its downregulation in tumor was associated with the aberrant action of Trithorax group proteins, major regulators of the homeobox gene expression (77). Another study revealed an important role of miR-122 significantly upregulated in FTC compared with FA. This upregulation was associated with the presence of the PAX8/PPAR γ fusion protein, which, paradoxically, led to a minimally invasive behavior of tumor. Functional studies revealed that overexpression of miR-122 in a mouse xenograft model resulted in a significant reduction of tumor progression (78).

In ATC, the role of highly downregulated miR-200 and miR-30 has been studied for some time already. Expression of these microRNAs in mesenchymal ATC-derived cells reduced their invasive potential and induced EMT by regulating the expression of SMAD2 and TGFBR1, upregulated in most ATCs (79). Further studies supported the role of miR-200 deregulation in EMT of thyroid cancer cells (80). The authors showed that re-expression of miR-200 restores the epithelial phenotype, abrogating the epidermal growth factor treatment, and they proposed the upregulation of the miR-200 family as a novel therapeutic strategy in highly invasive thyroid cancers. Another study on the possible use of miRs in therapies of ATC focused on miR-30a, which turned out to be a direct negative regulator of lysis oxidase (LOX). Restored expression of miR-30a in a mouse model resulted in smaller metastases but did not affect the tumor growth

(81). The role of the miR-30 family in the pathogenesis of ATC was additionally supported in a study by Hebrant *et al.* (82), which also identified miR-29a as the second regulator of the LOX gene. Novel studies identified other microRNAs, miR-21 (83) and miR-4295 (84), as potential oncomiRs in ATC. In ATC-derived cell lines, the inhibition of both miRs induced cell differentiation and apoptosis. In the case of miR-4395, this effect was possibly mediated through a direct interaction with its target gene, CDKN1A (84). As already mentioned in the section on FTC studies, miR-122 and miR-375 are upregulated by the PAX8/PPAR γ fusion protein, and these phenomena are associated with decreased angiogenesis and AKT pathway inactivation. Thus, it was proposed that PAX8/PPAR γ -induced expression of miR-122 and miR-375 can serve as a novel therapeutic strategy for ATC (85).

All above studies underline the high potential of miRs as novel therapeutic and diagnostic tools in thyroid carcinomas.

Declaration of interest

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

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