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**PhD IN CLINICAL PHYSIOPATHOLOGY**

# *miRNA expression profiling of Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: more light on its behavior.*

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# ABSTRACT

Follicular variant of PTC (FVPTC) is a variant of well differentiated papillary thyroid carcinoma. FVPTCs comprise encapsulated and infiltrative forms with different histological features and clinical behavior, but according cytomorphological features the forms with indolent behavior are not significant different from those with aggressive phenotype. Since the encapsulated forms with no capsular invasion show an indolent clinical behavior similar to benign lesion, recently , an international panel of pathologists and clinicians has reclassified these lesions as a distinct class of thyroid tumors termed as "Non-invasive follicular thyroid neoplasms with papillary-like nuclear features" (NIFTP).

To better classify these tumors by a molecular viewpoint and to evaluate new diagnostic tool to improve pre-surgical differential diagnosis between NIFTPs, Follicular Adenomas (FAs) and Infiltrative FVPTCs, an expression profiling of 798 miRNAs was performed.

miRNA expression levels were detected in 54 thyroid tumors (18 Follicular adenomas, 19 NIFTPs and 17 Infiltrative FVPTCs) using an highly reproducible and sensitive method, nCounter Nanostring assay.

A set of differentially expressed miRNAs was identified. miR-146-5p, miR-221-5p, miR-222-3p, miR-30e-3p and miR-152-3p were able to discriminate benign from malignant lesions with a very high level of significance (p-value<0.001); miR-146-5p,

miR-199a-5p, miR-199b-5p, miR-1915-3p and miR-148b-3p were most probably associated with a infiltrative growth of FVPTCs. miR-152-3p, miR-185-5p, miR-574-3p were found to be strongly down-regulated in NIFTPs compared with FAs whereas a significant overexpression of miR-10a-5p was reported in NIFTPs compared with infiltrative forms of FVPTC.

In conclusion, our data suggest that a panel of these markers could have a high diagnostic potential and could be applied in the preoperative assessment of thyroid nodules, mainly for the lesions that cannot be reliably distinguished on cytology alone and are classified as indeterminate nodules.

# 1.INTRODUCTION

## 1.1 Thyroid Tumors: Classification and General Considerations

Thyroid nodules are a common clinical founding, with an estimated prevalence on the basis of palpation that ranges from 3% to 7% [1-5].

The prevalence of clinically inapparent thyroid nodules is estimated with ultrasound examination (US) at 20% to 76% in the general population, with a prevalence similar to that reported from autopsy data. Thyroid cancer is the primary clinical concern in a patient with thyroid nodules, although only 5%–15% of thyroid nodules are malignant [6-8].

The vast majority of thyroid tumors arise from thyroid follicular epithelial cells, whereas 3–5% of cancers originate from parafollicular or C cells (Figure 1).

The follicular cell-derived cancers are further subdivided into well-differentiated, poorly differentiated carcinoma and anaplastic (undifferentiated) carcinoma.

Well differentiated thyroid tumors are subdivided into follicular thyroid adenoma (FA), follicular thyroid carcinoma (FTC), and papillary thyroid carcinoma (PTC) [9].

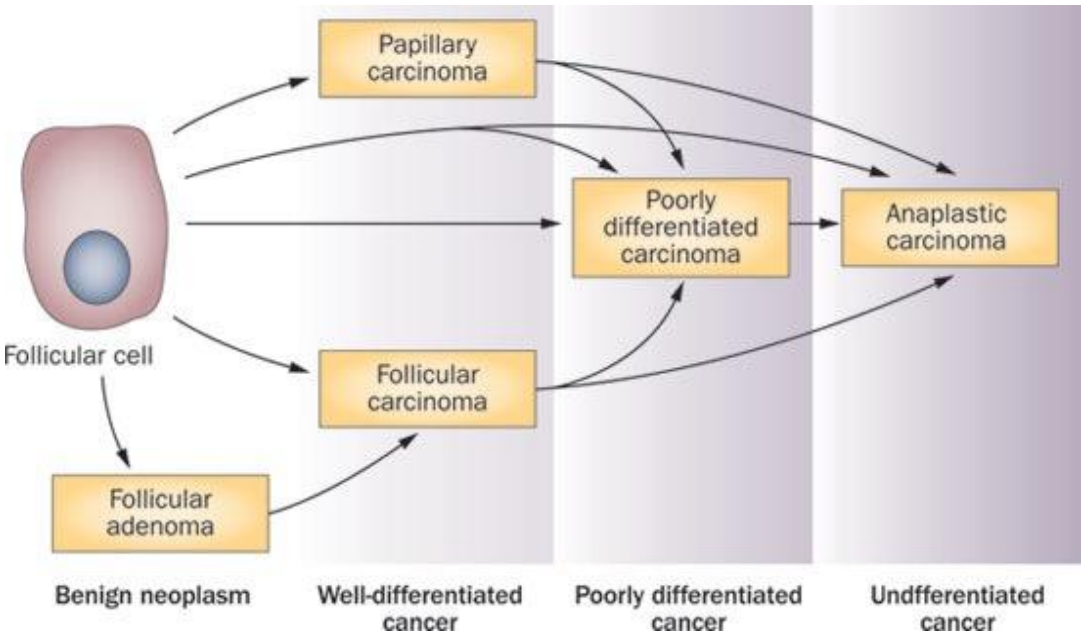


**Fig.1 Scheme of putative progression and dedifferentiation of follicular cell-derived thyroid tumors.**

Approximately 10% to 15% of thyroid cancers are classified as follicular. These tumors are typically encapsulated, solid neoplasms with minimal colloid, microfollicle formation, and variable degrees of nuclear atypia. This histologic pattern is similar in benign and malignant follicular neoplasms; therefore, the distinction between the adenoma and carcinoma is based on identification of vascular and/or capsular invasion by tumor.

Age older than 45, sex, extrathyroid invasion, greater tumor size, completeness of surgery and responsiveness to radioactive iodine at presentation are recognized risk factors for poorer prognosis of FTC [10-13].

The prognosis also depends on the degree of invasiveness of tumors: minimally invasive carcinomas carry a much better prognosis than do widely invasive carcinomas.

More recently, it is acknowledged the prognostic importance of angioinvasion [12, 13].

The behavior of follicular cancer is thought to be somewhat worse than papillary carcinoma. However, this may be secondary to an older age and more advanced stage at diagnosis when compared with papillary cancer [14, 15].

When controlled for age, gender, and stage, the prognosis of follicular and papillary cancer are similar. Mazzaferri and Jhiang report similar 30-year survival and recurrence rates for these tumors types after eliminating those patients found to have distant metastatic disease at diagnosis [15].

Papillary thyroid carcinoma is the most common endocrine malignancy (70% of tumors). The nuclear features of papillary carcinoma (e.g., nuclear clearing, grooves, pseudoinclusions) characterize a carcinoma that belongs to the PTC family. By definition, typical PTC often average 2–3 cm, although lesions may be quite large or commonly subcentimeter in size. PTCs measuring less than 1 cm in size are called papillary microcarcinomas, and have been reported in 10% to 30% of autopsy studies [16-18].

In the past, these tumors were incidentally detected in thyroidectomy specimens, but they are now detected with increasing frequency by high-resolution ultrasound. They are believed to have an excellent prognosis, but some may behave more aggressively than previously appreciated, and management remains controversial [19, 20].

PTC has an excellent prognosis, with a 5-year survival rate of greater than 97% [21].

Poor prognostic factors in papillary carcinoma include older age at diagnosis, male sex, large tumor size, and extrathyroidal growth. Pathologic variables associated with a more guarded prognosis include less differentiated or solid areas, vascular invasion, and aneuploid cell population [10, 22-24].

Lymphatic metastases are the second most common pathologic feature of papillary thyroid cancer. These occur in >35% of adults with papillary cancer and an even higher proportion, 40% to 75%, in patients older than 40 years of age at diagnosis.)

Between 10% and 20% of papillary thyroid cancers will show evidence of local invasion. When this extends beyond perithyroidal fat, adjacent structures may be invaded.

Distant metastases are shown in 2 % to 3 % of papillary thyroid cancers at diagnosis.

Whether certain subtypes of papillary carcinoma are in and of themselves associated with more aggressive clinical behavior are unclear. However, at least four subtypes should be mentioned in this regard: tall cell variant, diffuse sclerosis variant, solid variant, and follicular variant.

## 1.2 Molecular Genetics of Thyroid Cancer

The molecular pathogenesis of Thyroid tumors involves several known mutational events, including point mutations in the *BRAF* and *RAS* gene, as well RET/PTC, PAX8-PPAR and TRK rearrangements.

Point mutations in the RAS genes typically occur in codons 12, 13, and 61, and are found in 40-50% of follicular carcinomas and in 10-20% of papillary thyroid carcinomas [21, 25, 26]. RAS-mutated papillary thyroid carcinomas typically are of the follicular variant [27]. RAS mutations are also seen in 20-40% of follicular adenomas [21, 25, 26].

Point mutation in *BRAF* shows a high specificity for PTC, especially the classic variant, whereas it was never found in follicular thyroid carcinoma or in benign thyroid neoplasms. It is found in approximately 45% of papillary thyroid cancers [21, 28, 29].

In nearly all cases (98-99% of cases) activating point mutations of *BRAF* involve codon 600 and result in the V600E mutation, and in 1-2% of cases other *BRAF* mutations such as the K601E mutation, small in-frame insertions or deletions, or *BRAF* rearrangement can occur [30-32].

RET-PTC and TRK rearrangements are specific for papillary thyroid cancer. The former occurs in 10–20% of PTC, while the latter occurs with a significantly lower prevalence (<5%) than *RET/PTC* rearrangements [21, 33, 34].

By contrast, PAX8-PPAR$\gamma$ is an alteration found in follicular thyroid carcinoma, where it occurs with a frequency of 30–35% [21, 35]. In most studies, this rearrangement has also been found in some (2–13%) follicular adenomas and in a small proportion (1–5%) of the follicular variant of papillary carcinomas [21, 35, 36].

Additional genes mutated in thyroid cancer include *TP53* and *CTNNB1* (beta-catenin). TP53 is a tumor suppressor that plays important roles in cell cycle regulation and DNA repair and *CTNNB1* is involved in Wnt signaling. These genes tend to be mutated in more aggressive and advanced thyroid tumors [33, 37, 38].

More recently, novel markers have been identified, including *ETV6/NTRK3*, *STRN/ALK*, and *TERT* [33].

**1.3 Other molecular events: microRNAs**

Recent studies have revealed the emerging role of microRNAs (miRNAs), a class of non-coding RNAs of 19 to 24 nucleotides that regulate gene expression through post-transcriptional, RNA interference, gene silencing pathways.

Consequently deregulated miRNAs have been linked to a variety of different cancers, including thyroid tumors.

In the latest version of miRBase (v.21) there are currently annotated at more than 1880 human microRNA loci [39].

MicroRNAs are sequentially processed from longer precursor molecules that are encoded by the miRNA genes [40-42] (Figure 2). The encoding DNA sequence is much longer than the mature miRNA. Two ribonuclease enzymes, Drosha and Dicer, subsequently process the primary transcripts (or pri-miRNA) to generate mature miRNAs. The primary transcripts contain one or more stem-loop structures of about 70 bases. The ribonuclease Drosha excises the stem-loop structure to form the precursor

miRNA (or pre-miRNA) [43]. After export into the cytoplasm by RAN-GTP and Exportin-5 [44], the pre-miRNA is cleaved by the ribonuclease Dicer 1 in association with a double-stranded RNA-binding protein, TRBP (TAR (HIV) RNA binding protein 2) to generate a short RNA duplex (21-23 nucleotide long) [45]. These small mature miRNA fragments are then incorporated into RNP (Ribonulcear particle) where the two form the RISC complex (RNA-induced silencing complex). When the Dicer-like endonuclease cleaves the pre-miRNA, only one of the two complementary RNA strands is integrated with the RISC complex [42] and becomes the mature single-stranded miRNA. While the complementary strand, termed miRNA*, is usually rapidly degraded. The responsibility of selecting the guiding strand to incorporate into the RISC belongs to the Argonaute protein, which is the RNase in the RISC complex. MicroRNAs recognize their targets based on sequence complementarity [46].

The mature miRNA is partially complementary to one or more messenger RNAs.

In humans, the complementary sites are usually within the 3′-untranslated region of the target messenger RNA. To become effective, the mature miRNA forms a complex with proteins, termed the RNA-induced silencing complex. The miRNA incorporated into the silencing complex can bind to the target messenger RNA by base pairing. This base pairing subsequently causes inhibition of protein translation and/or degradation of the messenger RNA [47] [48].

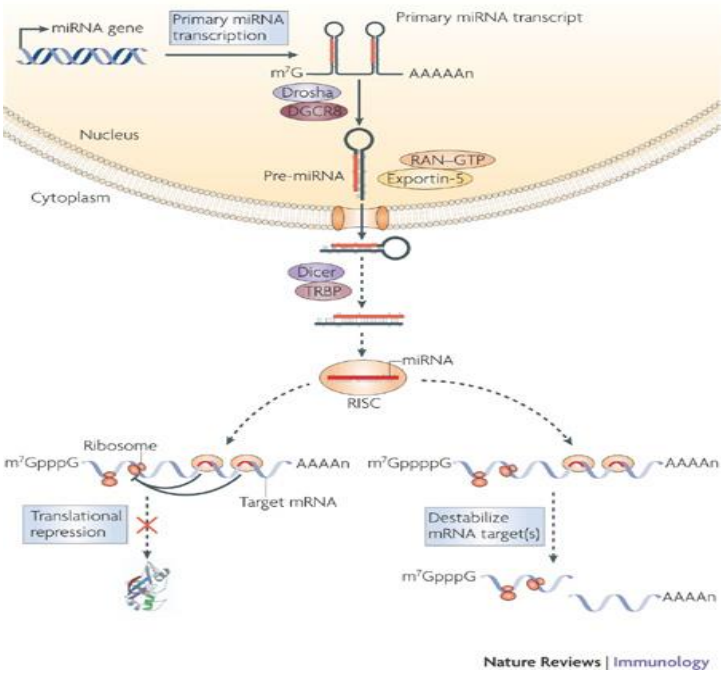


**Fig.2 Canonical biogenesis of miRNA**

Thus, microRNA are regulators of gene expression, and have been found to regulate a large number of genes that play roles in fundamental processes – such as development, differentiation, cell proliferation, apoptosis, and stress response some estimates reporting miRNA regulation of up to 60% of the human genome [49, 50]. Given that miRNA transcripts affect nearly every aspect of cellular function, it is not surprising that they play a critical role in the etiology of a wide variety of disease manifestations [15]. Indeed, miRNAs have been implicated in many types of cancers, as well as specific cardiac and neurologic diseases [51-55].

The first direct evidence for an involvement of miRNAs in cancer was reported by Dr. Croce's group [56].

Specific subsets of miRNAs in certain tumors have increased or reduced expression. Some of microRNAs act through reducing the expression of tumor suppressing genes, cell differentiation regulatory genes and apoptosis. Others act through targeting proto-oncogenic mRNAs and silencing such mRNAs. Thus, altered expression of some miRNAs may cause cells to become cancerous, affect cell growth through interfering with the regulation of cell cycle [57-63]. miRNA profiles can distinguish not only between normal and cancerous tissue and identify tissues of origin, but they can also discriminate different subtypes of a particular cancer, as well as between tumors with distinct biological properties [64] [65-67].

In this regard, thyroid cancer represents an attractive model to study because it include several histopathological tumor types originating from the same cell and tumors with distinct levels of differentiation and different degrees of malignancy.

Over the last years, several independent studies have analyzed miRNA expression in numerous and different types of thyroid tumors, evidencing a miRNAs deregulation in cancer tissues compared to their normal counterparts [68-73].

In details, the majority of known human miRNAs were expressed in normal thyroid tissue, by contrast 32% of miRNAs resulted being upregulated and 38% to be down-regulated with more than a 2-fold change in thyroid neoplasm as compared to normal tissues [74]. As reported by Nikiforova et al the thyroid tumors originating from follicular cell and C-cell-derived Medullary Carcinomas (MCs) showed completely different miRNA expression profiles [68, 74].

Moreover, it was reported a significant variability of miRNA expression profile between different kinds of thyroid cancers, even if they originate from the same type of thyroid cells. Follicular adenoma, Follicular Carcinoma and Papillary Thyroid Carcinoma are characterized by a separate clusters.

Most studies have reported comparisons of miRNA profiles between papillary thyroid carcinomas and follicular tumors (adenomas and carcinomas), multinodular goiter, and normal thyroid tissues [69-71, 75]. As a result, a number of miRNAs that are significantly upregulated in papillary thyroid carcinomas have been identified [76, 77]; by contrast, several miRNAs have been found to be downregulated in malignant lesions compared with normal thyroid tissue [69, 70, 78-80]. Other reports have focused on miRNAs profiling of papillary thyroid carcinomas associated with their clinical behavior. In detail, upregulation of miR-31, miR-146, miR-155, miR-221, and miR-222 and downregulation of miR-1, miR-34b, miR-130b, and miR-138 were observed in the aggressive form of papillary thyroid carcinoma compared with non-aggressive tumors [81].

Although most works so far have analyzed miRNA expression profiles in PTC, over the last few years some studies have started to attain deeper knowledge of miRNA deregulation in Follicular Adenoma and FTC, or anaplastic thyroid carcinoma [68, 79, 82] .

The most highly unregulated miRNA in FTC were miR-187, miR-225, miR-155, miR-221, and miR-222.

In addition, it were identified some miRNA significantly unregulated in FTC as compared to FA [68, 74, 75].

### 1.4 Follicular variant of papillary thyroid cancer: the borderline category of thyroid tumor classification

Among all PTC variants, follicular variant of papillary thyroid carcinoma (FV-PTC) is the most common subtype of PTC, constituting between 9% and 22.5% of all PTC cases [83-85]. FV-PTC was first described by Crile and Hazard in 1953[86].

FVPTC are usually an encapsulated tumor (EFVPTC) and less commonly a non-encapsulated infiltrative neoplasm (IFVPTC) [87, 88].

This variant predominantly has a follicular architecture, which is lined by cells with nuclear features of papillary carcinoma. Thus, FVPTC shares with FA and FTC the presence of follicles. Its prognosis definitely falls between that of classical PTC (cPTC) and FTC [89]. FVPTC has lower mortality and less frequent distant metastases than cPTC. FVPTC has fewer lymph node metastases and less frequent infiltrative disease and extrathyroidal extension that cPTC, but more than FTC.

In addition molecular profile of the follicular variant was shown to be close to the follicular adenoma/carcinoma, with a high prevalence of RAS and a very low BRAF mutation rate [21].

Previously studies found also a different distribution of the RAS and BRAF mutations according to presence of tumor capsule. EFVPTC definitely have a molecular profile

very close to follicular adenomas/carcinomas (high rate of RAS and absence of BRAF mutations), IFVPTC has an opposite molecular profile closer to classical papillary thyroid carcinoma than to follicular adenoma/carcinoma (BRAF>RAS mutations) [90].

When FVPTC is non encapsulated and infiltrates the surrounding thyroid parenchyma or diffusely involves the thyroid, the diagnosis of carcinoma usually presents no problem.

Otherwise, for the encapsulated tumor without invasion of surrounding thyroid tissue, the diagnosis of malignancy relies solely on the presence of the nuclear features of PTC, which often can be borderline.

Therefore, the diagnosis of noninvasive, encapsulated FVPTC (EFVPTC) versus follicular adenoma is prone to considerable interobserver variability.

A recent multi-institutional study examined a large cohort of well-annotated EFVPTC and established that none of 109 patients with noninvasive EFVPTC followed for 10-26 years developed recurrence or other disease manifestations. Based on this information, the international multidisciplinary group of authors recommended to reclassify these tumors as a distinct class of thyroid tumors with very low risk of adverse outcome, namely "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) [91].

NIFTP is defined by a set of reproducible diagnostic criteria that include:

| Diagnostic criteria |
| --- |
| □ Encapsulation or clear demarcation[1] |
| □ Follicular growth pattern[2] with: |
| <1% papillae; no psammoma bodies |
| <30% solid/trabecular/insular growth pattern |
| □ Nuclear Score 2-3 |
| □ No vascular or capsular invasion[3] |
| □ No tumor necrosis |
| □ No high mitotic activity[4] |

[1]High Thick, thin, or partial capsule or well circumscribed with a clear demarcation from adjacent thyroid tissue.
[2-]Including microfollicular, normofollicular, or macrofoelicular architecture with abundant colloid.
[3]Requires adequate microscopic examination of the tumor capsule interface.
[4]High mitotic activity defined as at least 3 mitoses per 10 high-power fields (400×).

This reclassification will have a profound impact on patient management, as the lesions classified as NIFTP will not require completion thyroidectomy or postsurgical radioiodine therapy.

Although histological features of NIFTP have been well characterized, how reliably these features can be identified in preoperative cytology samples remains unknown. Thus, yet, the presurgical differential diagnosis of NIFTPs, and invasive or infiltrative carcinoma is extremely difficult.

Molecular characterization could be definitely useful for classifying these lesions.

From a molecular point of view, the multi-institutional re-examination of these tumors confirmed previously observations demonstrating a high prevalence of RAS mutations PPARg and THADA gene fusions, which have been associated with follicular-pattern thyroid tumors, including follicular adenoma, follicular thyroid carcinoma, and EFVPTC [90, 92].

Therefore these known mutations are not sufficient to definitely classify as NIFTP on FNA.

Consequently, additional methods and other molecular markers should be examined to improve the preoperative diagnosis.

# 2. AIM

The present study aimed to delineate the molecular profile of the follicular variant of papillary thyroid carcinomas in noninvasive encapsulated and infiltrative forms to better classify the new pathological class of tumors, NIFTPs, into biologically and clinically relevant entity.

In detail, it was performed high throughput miRNA expression profiling to determine and identify a cluster-specific expression and it was evaluated the mutational status to explore the correlation between miRNA expression patterns and specific genetic mutation.

# 3. MATERIALS AND METHODS

## 3.1 Patients and Study Design

The study included 18 follicular adenomas and 36 follicular variant of papillary thyroid carcinomas obtained from patients who underwent surgery at the Department of Surgical, Medical, Molecular Pathology and Critical Area of the University of Pisa, Italy, from 2013 to 2015. In the latter group, 19 were diagnosed with NIFTPs and 17 with infiltrative follicular variant of papillary thyroid carcinomas. Tissue samples

were fixed in 10% buffered formalin and embedded in paraffin for routine histopathological examination. Histological sections (2–4μm thick) were cut and stained with hematoxylin and eosin (Automatic Stainer Varistain Gemini Sheldon). Hematoxylin–eosin-stained sections of patients from the archives of the Section of Pathology of the University of Pisa were independently re-evaluated by two pathologists (CU and FB). A diagnostic concordance rate of 98% was achieved between the two investigators. Rare, discordant cases were eliminated. Follicular adenomas and infiltrative follicular variant of papillary thyroid carcinomas were classified according to the WHO 2004 histo- pathological criteria and noninvasive encapsulated follicular variant of papillary thyroid carcinomas were reconsidered according to Nikiforov et al. [91]. The capsules of all encapsulated cases were re-evaluated (cases without total capsule evaluation were excluded). Noninvasive encapsulated and infiltrative type of follicular variant of papillary thyroid carcinoma are shown in Figure 3.

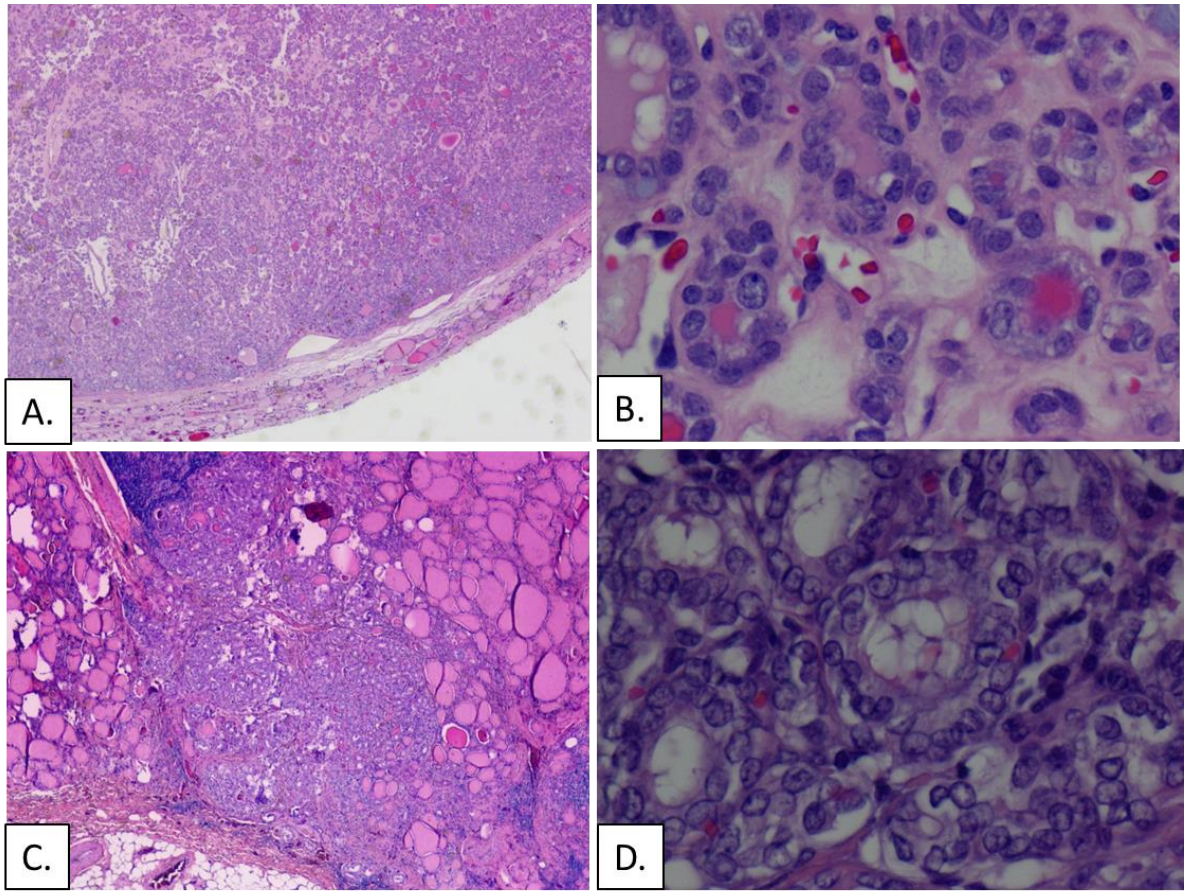


**Fig.3 Follicular variant of Papillary Thyroid Carcinoma: A., B. encapsulated type; C., D. infiltrative type**

Institutional approval from the local ethics com- mittee was obtained for this study. Surviving patients gave written informed consent for the use of their tissue specimens in present and future investigations.

### 3.2 miRNA Extraction

Total RNA, including miRNA, was isolated from formalin-fixed and paraffin-embedded tissue using a miRNeasy Mini Kit (Qiagen, Hilden, Germany)

The miRNeasy Mini Kit combines phenol/guanidine-based lysis of samples and silica- membrane–based purification of total RNA. QIAzol Lysis Reagent, included in the kit, is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of tissues, to inhibit RNases, and also to remove most of the cellular DNA and proteins from the lysate by organic extraction.

Tissue samples are homogenized in QIAzol Lysis Reagent. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase.

The upper, aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions for all RNA molecules from 18 nucleotides (nt) upwards. The sample is then applied to the RNeasy Mini spin column, where the total RNA binds to the membrane and phenol and other contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water.

The RNA concentration was assessed using a NanoDrop  spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

The concentration of RNA was determined by measuring the absorbance at 260 nm ($A_{260}$).

### 3.3 NanoString nCounter Assay

The nCounter v3 miRNA Expression Assays used in this study were designed and synthesized by Nano- string Technologies (Nanostring, Seattle, WA, USA).

The miRNA panel consisted of unique oligo- nucleotide tags onto 798 human miR-NAs (from miRBase v21) and five housekeeping mRNAs for reference (ACTB, B2M, GAPDH, RPL19, and RPLP0). Moreover, each assay included 25 control probes that recognize synthetic mRNA or miRNA targets to monitor the efficiency and specificity in each reaction step (nCounter Human v3 miRNA Expression Assay Transcript List at end of text).

Sample preparation involves a multiplexed annealing of specific tags to their target miRNAs, a ligation reaction, and an enzymatic purification step to remove unligated material. Sequence specificity between a miRNA and its synthetic sequence tag is ensured by careful, stepwise control of hybridization and ligation temperatures. Con-trol RNA included in the nCounter Human v2 miRNA Sample Preparation Kit allows for the monitoring of ligation efficiency and specificity throughout each step of the reaction.

After miRNA sample preparation, the expression data were obtained directly the nCounter Analysis System. The nCounter Analysis System delivers direct, multi-plexed measurement of miRNA gene expression, providing digital readouts of the relative abundance of hundreds of transcripts simultaneously.

The system is based on target-specific probe pairs that are hybridized to the sample in solution. In detail, the miRNAs were hybridized using 150ng of total RNA in addition

to the probe pairs consisting of a Reporter Probe and a Capture Probe. The Reporter Probe carries the fluorescent signal on its 5′ end; the Capture Probe, which carries biotin on their 3′ end allows the complex to be immobilized for data collection. Moreover, the protocol does not include any amplification steps that might introduce bias into the results. Hybridization was performed for 16 h at 65 °C in a SensoQuest (SensoQuest, Göttingen, Germany) thermal cycler. After hybridization of the Human v2 miRNA CodeSet with the tagged miRNA preparation, samples are transferred to the nCounter Prep Station where excess probes are removed and probe/target complexes are aligned and immobilized in the nCounter Cartridge. Cartridges are then placed in the nCounter Digital Analyzer for data collection. Each miRNA of interest is identified by the "color code" generated by six ordered fluorescent spots present on the Reporter Probe. The Reporter Probes on the surface of the cartridge are then counted and tabulated for each miRNA species.

### 3.4 Data normalization

Raw data were collected and exported into an Excel worksheet using NanoString nSolver version 1.1 software. The data was normalized using internal positive spike controls to account for variability in the hybridization process and negative spike controls to estimate the background. Sample input amounts were normalized to the geo-

metric mean of five housekeeping mRNA controls (ACTB, B2M, GAPDH, RPL19 and RPL10) included in the assay, and finally to total miRNA count.

miRNA input levels were normalized using the geometric mean of the top 100 miRNAs with the lower variability coefficients according to the manufacturer's protocol.

**3.5 DNA purification**

Serial 10 µm-thick sections were taken from paraffin blocks for DNA extraction from the primary tumor. The presence of tumor tissue was confirmed in the first and last section for each section series. DNA was purified using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany).

The QIAamp DNA purification procedure comprises four steps (lysis of sample, binding to QIAamp silica-gel membrane and two washing steps) and is carried out using QIAamp Spin Columns in a standard microcentrifuge.

The lysate buffering conditions are adjusted to allow optimal binding of the DNA to the QIAamp membrane before the sample is loaded onto the QIAamp Spin Column. DNA is adsorbed onto the QIAamp silica-gel membrane during a brief centrifugation. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the QIAamp membrane.

DNA concentration was assessed spectrophotometrically using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA; A260/A230 ratio was over 1.7, and the A260/A280 ratio was greater than 1.8).

### 3.6 BRAF and Ras gene family mutational analysis

Mutational testing was performed for BRAF, H-N and K-Ras according to the standard procedures using High Resolution Melt (HRM) analysis followed by Sanger sequencing (18,19). Briefly, DNA (~80 ng) was amplified in a final volume of 25 μl containing 12.5 ul Master Mix (Qiagen GmbH, Hilden, Germany) 0·8 μmol/l of each primer and 1 μl of EvaGreen 20X. PCR and HRMA were performed on a Rotorgene 6000™ realtime analyser (Qiagen GmbH, Hilden, Germany). Postamplification fluorescence melting curve analysis was performed by gradual heating of samples at a rate of 1 C/sec from 45C to 95C. A high-resolution melt was immediately performed from 75°C to 85°C rising at 0.1°C/s. The resulting data were analyzed using Rotorgene Series software V1.7. All HRMA altered and a number of non altered PCR products were sequenced as required. Postamplification fluorescence melting curve analysis was performed by gradual heating of samples at a rate of 1 C/sec from 45C to 95C. A high-resolution melt was immediately performed from 75°C to 85°C rising at 0.1°C/s. The resulting data were analyzed using Rotorgene Series software V1.7.

All HRMA altered and a number of non altered PCR products were sequenced as required.

### 3.7 Statistical Analysis

The Shapiro–Wilk test was performed to verify the normality of the distributions. The Mann–Whitney test, t-test, Kruskal–Wallis test and ANOVA test were performed; the Dunn's test and the Bonferroni's test were used for the multiple comparisons. Power analyses were conducted to estimate the sample sizes of the groups. The 1-β values of the significant variables were 40.8, assuring a low risk of type II error and appropriate sample sizes. The statistical analysis was performed using IBM SPSS software package, version 17.0.1.

# 4. RESULTS

The expression profiling of 798 miRNAs was evaluated using a nCounter Nanostring platform on 54 thyroid tumors, including 18 (33%) follicular adenomas, 19 (35%) NIFTPs and 17 (32%) infiltrative follicular variant of papillary thyroid carcinomas. Six-hundred-thirty miRNAs with an average count of less than 20 (mean ± 2 s.d. of negative controls) were excluded to subtract the background noise. The remaining 168 miRNAs were suitable for analyses. Among these, we identified 42 miRNAs with significantly different expression between follicular adenomas and follicular variant of papillary thyroid carcinomas (NIFTPs and infiltrative follicular variant of papillary thyroid carcinomas). In addition, multiple comparison analysis between histological groups was performed to identify miRNAs that could be associated with a specific subtype or were likely involved in the infiltrative growth of follicular variant of papillary thyroid carcinomas.

## 4.1 miRNAs Differentially Expressed between Follicular Variant of Papillary Thyroid Carcinomas and Follicular Adenomas

As reported in Table 1, 42 miRNAs had significant deregulation between malignant lesions, follicular variant of papillary thyroid carcinomas (NIFTPs and infiltrative follicular variant of papillary thyroid carcinomas), and benign counterparts (follicular adenomas). Nineteen were upregulated in follicular variant of papillary thyroid carcinomas, whereas 23 were downregulated. In detail, miR-146b-5p, miR -221-5p, and miR-222-3p were highly upregulated (P-value <0.001), whereas miR-30e-3p and

miR-152- 3p were downregulated with the same statistical significance. In addition, we found another 37 miRNAs that were significantly deregulated between these two groups; in 11, the P-value was <0.01, whereas in the other 26, the P-value was <0.05.

***Table1. Deregulated miRNA in FVPTCs vs FAs***

| **Up-regulated** | **Down-regulated** |
|---|---|
| **p value<0.001** | |
| miR-146b-5p | miR-30e-3p |
| miR-221-5p | miR-152-3p |
| miR-222-3p | |
| **p value<0.01** | |
| miR-135a-5p | miR-22-3p |
| miR-181c-5p | miR-148a-3p |
| miR-221-3p | miR-185-5p |
| miR-551b-3p | miR-195-5p |
| miR-3151-5p | miR-204-5p |
| **p value<0.05** | |
| let7c-5p | miR-574-3p |
| miR-31-5p | let-7f-5p |
| miR-100-5p | miR-7-5p |
| miR-125b-5p | miR-19b-3p |
| miR-130a-3p | miR-30a-3p |
| miR-223-3p | miR-30c-5p |
| miR-302d-3p | miR-30e-5p |
| miR-424-5p | miR-148b-3p |
| miR-548ar-5p | miR-194-5p |
| miR-1915-3p | miR-200a-3p |
| miR-6721-5p | miR-200b-3p |
| | miR-345-5p |
| | miR-362-3p |
| | miR-423-3p |
| | miR-497-5p |

Hierarchical clustering according to differentially expressed miRNAs was performed using nSolver Analysis software with Pearson correlation. This approach separated the groups into two different clusters, 14 out of 18 (78%) follicular adenomas and 26 out of 36 (72%) follicular variant of papillary thyroid carcinomas (Figure 4).

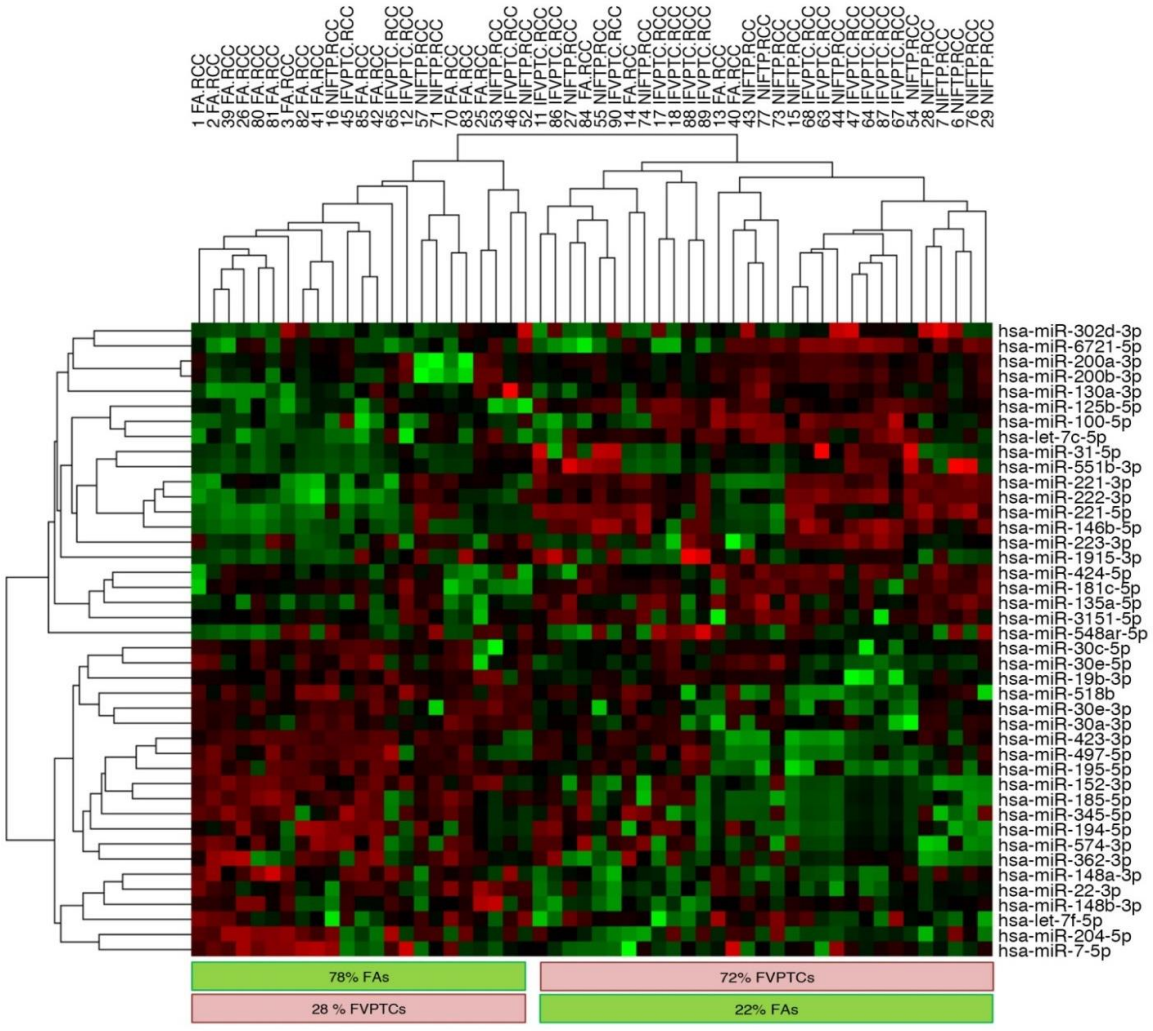


**Fig.4 Hierarchical clustering of follicular adenomas and follicular variant of papillary thyroid carcinomas using statistically significant deregulated miRNAs.** The columns represent the samples and the rows represent the miRNAs. Only those miRNAs with a different expression statistically significant (P-valueo0.05) between malignant (NIFTPs and IFVPTCs) and benign lesions (FAs) were used for hierarchical clustering. Red and green indicate a high and a low level of expression, respectively.

## 4.2 Evaluation of Differential miRNA Expression between NIFTPs, Follicular Adenomas and Infiltrative Follicular Variant of Papillary Thyroid Carcinomas

### *4.2.1 NIFTPs versus Follicular Adenomas*

Seven miRNAs exhibited overexpression in NIFTPs compared with follicular adenomas with a very high significant difference in only one miRNA, miR-222-3p. Moreover, 6 miRNAs were upregulated, but they had P-values <0.0167. By contrast, 13 miRNAs were downregulated, but only miR-152-3p, miR-185-5p, and miR-574-3p had P-values <0.001 (Table 2).

***Table2. Deregulated miRNAs in NIFTPs vs FAs***

| **Up-regulated** | **Down-regulated** |
|---|---|
| **p value<0.001*** | |
| miR-222-3p | miR-152-3p |
| | miR-185-5p |
| | miR-574-3p |
| **p value<0.0167*** | |
| miR-135a-5p | miR-7-5p |
| miR-181c-5p | miR-30e-3p |
| miR-221-5p | miR-194-5p |
| miR-551b-3p | miR-200b-3p |
| miR-3151-5p | miR-204-5p |
| miR-6721-5p | miR-296-5p |
| | miR-362-5p |
| | miR-423-3p |

*p-values correct for multiple comparisons (Bonferroni)

*4.2.2 NIFTPs versus infiltrative follicular variant of papillary thyroid carcinomas*

Eight miRNAs showed upregulation and 5 downregulation in infiltrative follicular variant of papillary thyroid carcinomas compared to NIFTPs (Table 3). Among these miRNAs, miR-10a-5p, miR-199b-5p, miR-1285-5p and miR-320e were highly significant (P-value <0.001).

***Table3. Deregulated miRNAs in IFVPTCs vs NIFTPs***

| **Up-regulated** | **Down-regulated** |
|---|---|
| **p value<0.001*** | |
| miR-320-5 | |
| miR-10a-5p | |
| miR-199b-5p | |
| miR-1285-5p | |
| **p value<0.0167*** | |
| miR-135a-3p | miR-32-5p |
| miR-1915-3p | miR-95-3p |
| miR-199a-5p | miR-148b-3p |
| miR-4516 | miR519d-3p |
| | miR-598-3p |

***p-values correct for multiple comparisons (Bonferroni)**

*4.2.3 Infiltrative follicular variant of papillary thyroid carcinomas versus follicular adenomas*

In addition to those reported above, we also analyzed and compared miRNA expression in infiltrative follicular variant of papillary thyroid carcinomas and follicular adenomas. Twenty miRNAs had a different pattern of expression; 11 were upregulated and 9 were

downregulated in infiltrative follicular variant of papillary thyroid carcinomas. Further details are provided in Table 4.

***Table4. Deregulated miRNAs in IFVPTCs vs FAs***

| **Up-regulated** | **Down-regulated** |
|---|---|
| **p value<0.001*** | |
| miR-146-5p | |
| miR-199b-5p | |
| miR-222-3p | |
| miR-1915-3p | |
| **p value<0.0167*** | |
| miR-31-5p | Let-7f-5p |
| miR-199a-5p | miR-22-3p |
| miR-221-3p | miR-30e-3p |
| miR-221-5p | miR-30e-5p |
| miR-551b-3p | miR-95-3p |
| miR-1285-5p | miR-143-3p |
| miR-4516 | miR-144-3p |
| | miR-148a-3p |
| | miR-148b-3p |

*p-values correct for multiple comparisons (Bonferroni)

### 4.3 Differentiating between NIFTPs Versus Follicular Adenomas and Infiltrative Follicular Variant of Papillary Thyroid Carcinomas

The aim of the following evaluation was to identify miRNAs that are specifically deregulated in different lesions. In particular, we focused on miRNAs that are differentially expressed in NIFTPs versus follicular adenomas and in NIFTPs versus infiltrative follicular

variant of papillary thyroid carcinomas. In detail, we found that 16 miRNAs were able to distinguish between NIFTPs and follicular adenomas; we excluded miR-222-3p, miR-221-5p, miR-551b-3p, and miR-30e-3p because their expression levels were also significantly different between follicular adenomas and infiltrative follicular variant of papillary thyroid carcinomas (Table 5).

***Table5. Specific miRNAs expression in NIFTPs vs FAs***

| **Up-regulated** | **Down-regulated** |
|---|---|
| **p value<0.001*** | |
| | miR-152-3p |
| | miR-185-5p |
| | miR-574-3p |
| **p value<0.0167*** | |
| miR-135a-3p | miR-7-5p |
| miR-181c-5p | miR-194-5p |
| miR-3151-5p | miR-195-5p |
| miR-6721-5p | miR-200b-3p |
| | miR-204-5p |
| | miR-296-5p |
| | miR-362-5p |
| | miR-423-3p |
| | miR-497-5p |

*p-values correct for multiple comparisons (Bonferroni)

Moreover, six specific miRNAs (miR-10a-5p, miR-132-3p, miR-320e, miR-32-5p, miR-519d-3p, and miR-598-3p) were capable of dividing infiltrative follicular variant of papillary thyroid carcinomas from NIFTPs, as reported in Table 6; seven miRNAs have been excluded from this analysis because they were deregulated in infiltrative follicular variant of papillary thyroid carcinomas versus follicular adenomas.

***Table 6. Specific miRNA expression in IFVPTCs vs NIFTPs***

| **Up-regulated** | **Down-regulated** |
|---|---|
| **p value<0.001*** | |
| miR-10a-5p | |
| miR-320e | |
| **p value<0.0167*** | |
| miR-132-3p | miR-32-5p |
| | miR519d-3p |
| | miR-598-3p |

*p-values correct for multiple comparisons (Bonferroni)

The hierarchical clustering analysis showed that 16 out of 18 (84%) and 17 out 18 (89%) of NIFTPs were, respectively, separated from follicular adenomas and infiltrative follicular variant of papillary thyroid carcinomas (Figure 3a and b).

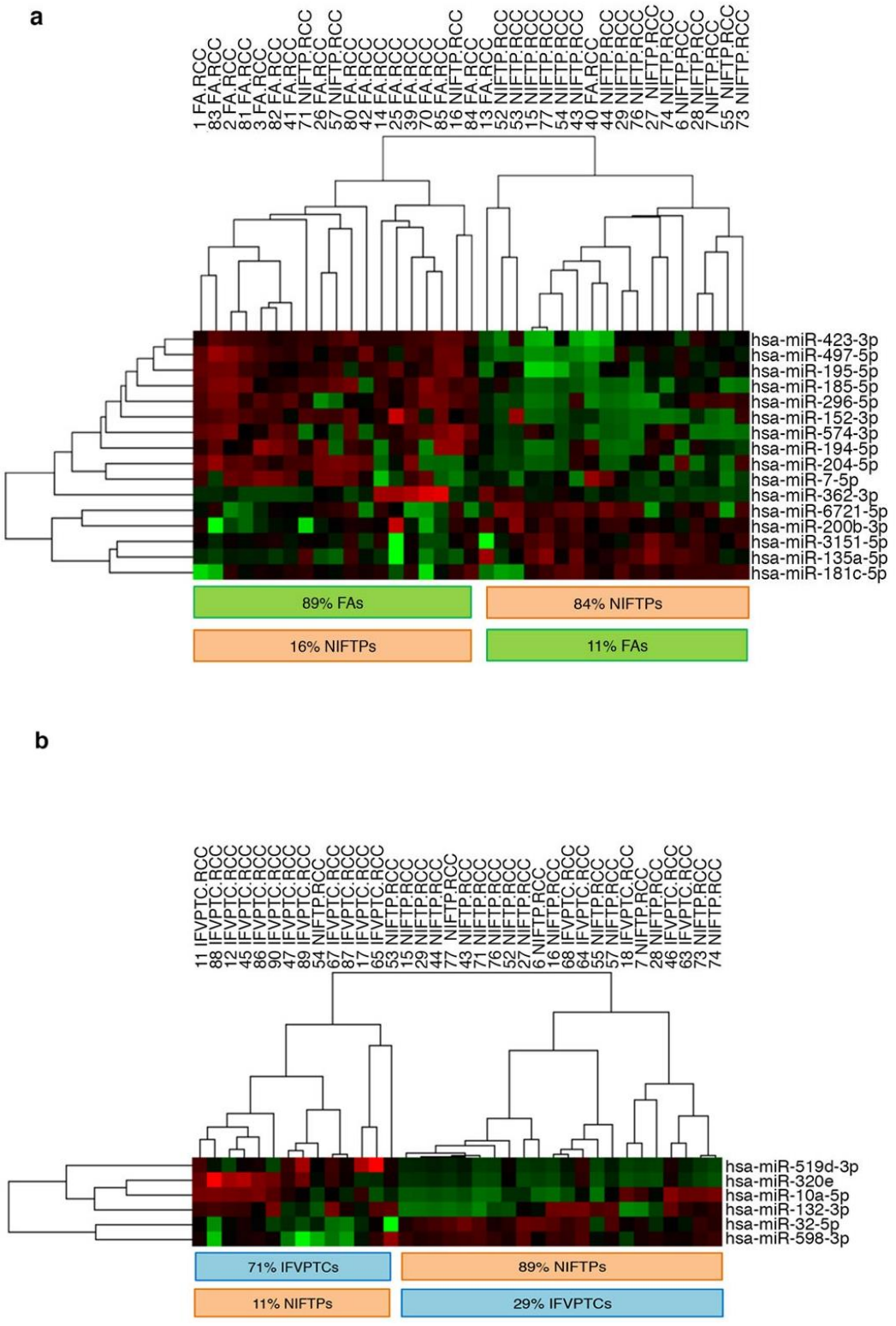


**Fig.5 Hierarchical clustering performed using miRNAs expressed with a significant difference between NIFTPs and FAs (a), NIFTPs and IFVPTCs**

### 4.4 miRNAs involved in the Infiltrative Growth of Follicular Variant of Papillary Thyroid Carcinomas

In comparing infiltrative follicular variant of papillary thyroid carcinomas with all encapsulated tumors (follicular adenomas and NIFTPs), we observed significant differential expression in 33 miRNAs; 14 were upregulated and 19 downregulated (Table 7). miR-222-3p and miR-30e-3p were excluded from this list because they were simultaneously significantly deregulated between follicular adenomas and NIFTPs.

***Table 7. Deregulated miRNAs in IFVPTCs vs Encapsulated Tumors***

| Up-regulated | Down-regulated |
|---|---|
| **p value<0.001** | |
| miR-146b-5p | miR148b-3p |
| miR-199a-5p | |
| miR-199b-5p | |
| miR-1285-5p | |
| miR-1915-3p | |
| miR-4516 | |
| **p value<0.01** | |
| miR-222-3p | miR-22-3p |
| miR-320e | miR-95-3p |
| miR-10a-5p | miR-143-3p |
| | miR-144-3p |
| **p value<0.05** | |
| miR-10b-5p | let-7f-5p |
| miR-21-5p | miR-25-3p |
| miR-221-3p | miR-30e-3p |
| miR-1290 | miR-30e-5p |
| miR-1972 | miR-32-5p |
| | miR-98-5p |
| | miR-126-3p |
| | miR-141-3p |
| | miR-148a-3p |
| | miR-181a-3p |
| | miR-301a-3p |
| | miR-519d-3p |
| | miR-598-3p |
| | miR-1180-3p |

As reported in Table 8, considering all results of infiltrative follicular variant of papilary thyroid carcinomas versus encapsulated tumors (infiltrative follicular variant of papillary thyroid carcinomas versus follicular adenomas and infiltrative follicular variant of papillary thyroid carcinomas versus NIFTPs), 33 miRNAs (15 upregulated and 18 downregulated) are likely involved in the infiltrative growth of follicular variant of papillary thyroid carcinomas. miR-146b-5p, miR-199a-5p, miR-199b-5p, miR-1285- 5p, miR-1915-3p, and miR-4516 were significantly upregulated (P-value<0.001).

***Table 8. miRNAs involved in infiltrative growth of FVPTCs***

| **Up-regulated miRNAs** | **IFVPTCs vs FAs+NIFTPs** p-value | **IFVPTCS vs FAs** p-value* | **IFVPTCs vs NIFTPs** p-value* | **Down-regulated miRNAs** | **IFVPTCs vs FAs+NIFTPs** p-value | **IFVPTCs vs FAs** p-value* | **IFVPTC vs NIFTPs** p-value* |
|---|---|---|---|---|---|---|---|
| miR-199b-5p | <0.001 | <0.001 | <0.001 | miR148b-3p, | <0.001 | <0.0167 | <0.0167 |
| miR-1915-3p | <0.001 | <0.001 | <0.0167 | miR-95-3p | <0.01 | <0.0167 | <0.0167 |
| miR-199a-5p | <0.001 | <0.0167 | <0.0167 | miR-25-3p | <0.05 | | |
| miR-4516 | <0.01 | <0.0167 | <0.0167 | miR-98-5p, | <0.05 | | |
| miR-1285-5p | <0.05 | <0.0167 | <0.001 | miR-126-3p | <0.05 | | |
| miR-10b-5p | <0.05 | | | miR-141-3p | <0.05 | | |
| miR-1290 | <0.05 | | | miR-181a-3p, | <0.05 | | |
| miR-21-5p | <0.05 | | | miR-301a-3p, | <0.05 | | |
| miR-1972 | <0.05 | | | miR-1180-3p | <0.05 | | |
| miR-146b-5p | <0.001 | <0.001 | | miR-22-3p, | <0.01 | <0.0167 | |
| miR-221-3p | <0.05 | <0.0167 | | miR143-3p | <0.01 | <0.0167 | |
| miR-31-5p | | <0.0167 | | miR144-3p | <0.01 | <0.0167 | |
| miR-10a-5p | <0.05 | | <0.001 | let-7f-5p | <0.05 | <0.0167 | |
| miR-320e | <0.05 | | <0.0167 | miR-30e-5p | <0.05 | <0.0167 | |
| miR132-3p | | | <0.0167 | miR-148a-3p | <0.05 | <0.0167 | |
| | | | | miR-32-5p | <0.05 | | <0.0167 |
| | | | | miR-519d-3p | <0.05 | | <0.0167 |
| | | | | miR-598-3p | <0.05 | | <0.0167 |

*p-values correct for multiple comparisons (Bonferroni)

Among the downregulated miRNAs, only miR-148b-3p was significantly deregulated in infiltrative follicular variant of papillary thyroid carcinomas (P-valueo0.001). Moreover, three upregulated (miR-146b-5p, miR-221-3p, and miR-31-5p) and six downregulated miRNAs (miR-22-3p, miR-143-3p, miR-144- 3p, let-7f-5p, miR-30e-5p, and miR-148a-3p) were differentially expressed when infiltrative follicular variant of papillary thyroid carcinomas were compared with follicular adenomas (Table 8).

When hierarchical clustering analysis was performed using miRNAs involved in infiltrative growth, 36 out of 37 (97%) encapsulated tumors were grouped in a different cluster with respect to infiltrative follicular variant of papillary thyroid carcinomas (Figure 4).

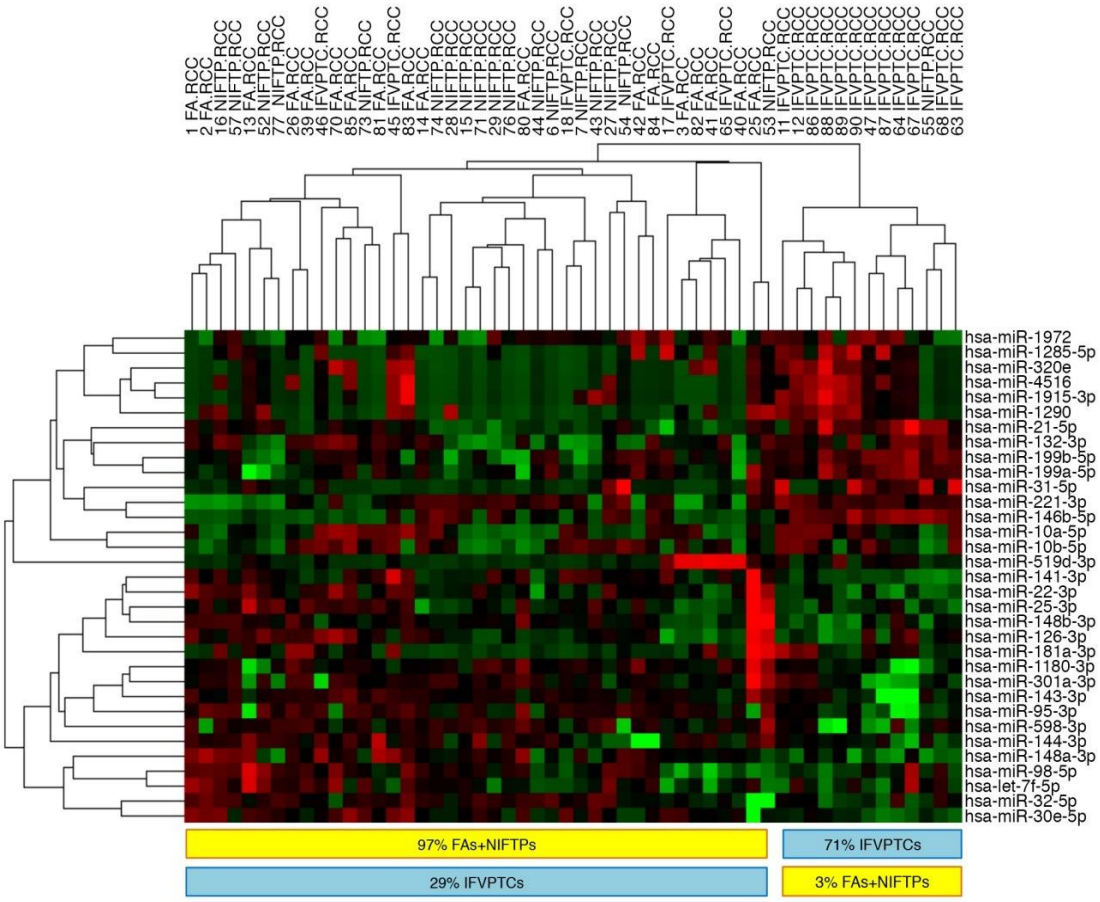


**Fig.4 Hierarchical clustering of encapsulated lesions (NIFTPs and FAs) and IFVPTCs using significant miRNAs involved in infiltrative growth..**

## 4.5 Genotyping Analysis

Mutational status of the BRAF and RAS (NRAS, HRAS, and KRAS) genes in follicular adenomas, NIFTPs and infiltrative follicular variant of papillary thyroid carcinomas was tested. In detail, 17 out of 18 (94.4%) follicular adenomas were wild type

for BRAF (exon 15) and NRAS, HRAS and KRAS (exons 2 and 3) genes, whereas 1 (5.6%) had BRAF K601E mutation; 9 out of 19 (47.4%) NIFTPs were mutation negative, 8 out of 19 (42.1%) were mutated for RAS genes (5 NRAS Q61R and 3 HRAS Q61R), and 2 out of 19 (10.5%) had a BRAF K601E mutation; finally, as regards infiltrative follicular variant of papillary thyroid carcinomas, we found that 7 out of 17 (41.2%) were wild type for BRAF (exon 15) and NRAS, HRAS, and KRAS (exons 2 and 3) genes, 5 out of 17 (29.4%) were mutated for RAS genes (4 NRAS Q61R and 1 HRAS Q61R), 4 out of 17 (23.5%) had a BRAF V600E mutation and 1 was not evaluable.

# 5. DISCUSSION

Follicular variant of papillary thyroid carcinomas can have very different clinical behavior and out- comes. Differentiating between follicular variant of papillary thyroid carcinomas (in particular encapsulated forms) and follicular adenoma is sometimes a challenge when considering only the morphological, cytological, or clinical features.

It is extensively reported that completely encapsulated follicular variant of papillary thyroid carci- noma is a very low-risk form [88, 93, 94].

One recent noticeable change in thyroid pathology has formally proposed reclassifying noninvasive encapsulated follicular variant of papillary thyroid carcinomas as 'NIFTPs'[91].

For presurgical fine-needle aspiration, the cytological distinction between NIFTP versus invasive and infiltra- tive follicular variant of papillary thyroid carcinoma has been very difficult, although it was recently observed that there is a good correlation between the cytological and histological nuclear features [95]. Therefore, to reduce the number of preoperative indeterminate lesions as much as possible, analysis of mutations and rearrange- ments of specific genes involved in thyroid tumori- genesis have been reported by several studies [96, 97].

Nikiforov's group has extensively reported that improved management for patients who bear thyroid nodules with an indeterminate cytology is now possible using a multi-gene panel of molecular markers with high sensitivity and specificity [96] . Nevertheless, the molecular test results should be interpreted in the context of cytological, clinical, and ultrasonographic examinations [98].

In combination, these reports indicate that the addition of extensive molecular characterization to strictly morphological criteria significantly increases the diagnostic accuracy of follicular lesions in 480% (CI: 88–97%) of the cases. Therefore, ~ 20% of patients undergo unnecessary surgery [96].

Furthermore, mutational analysis is not useful for discriminating between NIFTP versus other follicular-patterned thyroid tumors (follicular carci- nomas and follicular adenomas) because of their similar mutational status [91].

Several studies have evaluated the miRNA profiles of thyroid tumors, highlighting that the miRNA signature could be useful (a) for diagnosis because several miRNAs are deregulated in papillary thyroid carcinoma compare to normal thyroid tissue and (b) for prognosis because miRNA profiles differ in papil- lary thyroid carcinomas that have different clinical behavior [69, 70, 78-80]. Although most prior observations have focused on miRNA expression in papillary thyroid carcinomas, some reports have revealed several miRNA expression differences between follicular carcinoma and follicular adenoma [75, 99].

Interestingly, in situ hybridization analysis has revealed that miR-146b-5p is mainly expressed in papillary thyroid carcinoma, including follicular variant of papillary thyroid carcinoma and is not expressed in most follicular, poorly differentiated, and anaplastic thyroid carcinoma samples [100].

A study by Dettmer et al. showed that miR-146b is consistently overexpressed in both the classical and follicular variants of papillary thyroid carcinoma [101]; moreover,

others have reported a correlation between miR-146b deregulation and tumor aggressiveness [76, 81].

miR-146b-5p positively regulates the migration and invasion of normal thyroid and tumor follicular cells through a mechanism involving the actin cytoskeleton [102]. Consequently, miR-146 could be involved in thyroid tumorigenesis considering its important role as a regulator of NF-kb [103, 104], although this mechanism is not completely understood. In addition, it has recently been found that the miRNA-146 family could interfere with the RARb gene, which seems to be involved in promoting tumor growth and the inefficiency of retinoic acid and radioactive iodine treatment [78].

In our study, we found that miR-146b-5p was upregulated in malignant lesions compared to benign tumors. Furthermore, miR-146b-5p seems to be specifically useful in differentiating between the infiltrative form of follicular variant of papillary thyroid carcinomas and follicular adenomas and could be a marker of papillary thyroid carcinoma and infiltrative lesions in presurgical fine-needle aspiration.

Other miRNAs, namely miR-221 and miR-222, seem to have important roles. The deregulation of these miRNAs has been reported in several human cancers (colorectal carcinoma, non-small cell lung carcinoma, breast cancer, pancreatic carcinoma, glioma, hematological malignancies, and thyroid cancer, and so on) [70].

Other different studies have consistently reported the upregulation of miR-221 and miR-222 in thyroid cancer, suggesting that this phenomenon is crucial to thyroid carcinogenesis. The finding that these two miRNAs modulate growth receptors, tran-

scription factors and tumor suppressor genes as well as target genes involved in cell growth and apoptosis may explain, at least in part, their role in tumorigenesis [70].

We confirmed these data, validating that miR-221- 5p and miR-222-3p are upregulated in follicular variant of papillary thyroid carcinomas compared with follicular adenomas. Their upregulation has also been observed in NIFTP compared with folli- cular adenomas as well as in infiltrative follicular variant of papillary thyroid carcinomas compared to follicular adenomas. These data strongly suggest that miR-221-5p and miR-222-3p may be markers of malignancy.

It has been reported that a group of miRNAs is downregulated in malignant lesions compared to normal tissue and benign counterparts [79, 80], suggest- ing that both overexpression and downregulation of miRNAs have important roles in tumorigenesis. In human tumors, miR-30e has been reported to be downregulated and associated with advanced carci- noma, such as anaplastic thyroid carcinoma and advanced breast cancer [105, 106]. Many functional mecha- nisms have been proposed, including miR-30e's role in the mesenchymal-epithelial transition[79] or, more recently, in suppressing proliferation of hepatoma cells via targeting prolyl-4-hydroxylase subunit alpha-1 (P4HA1) mRNA [107]. In addition, in our study, miR-30e-3p was significantly downregulated in follicular variant of papillary thyroid carcinomas compared with follicular adenomas, which strongly correlated with the malignancy of the lesion. Another study demonstrated significant downregu- lation of miR-152, together with other miRNAs (miR-34a, miR-424, and miR-20b) and genes (CCNE2,

COL4A1, TRAF6, and HSP90B1), in papillary thyr- oid carcinoma and found evidence for its association with aggressive clinical features [108]. Likewise, we observed significant downregulation of miR-152-3p in follicular variant of papillary thyroid carcinomas compared with follicular adenomas.

The main aim of our study was to identify a group of molecular markers that are capable of discriminat- ing between encapsulated lesions (NIFTP and follicular adenomas) versus infiltrative forms of follicular variant of papillary thyroid carcinoma, and NIFTP versus either follicular adenomas or infiltrative follicular variant of papillary thyroid carcinomas both in histology and cytology. This is a critical point because, according to a new definition of NIFTP, patients carrying this lesion type could be treated in a conservative manner.

For this reason, we analyzed the miRNA expression profile of follicular adenomas together with NIFTP versus the infiltrative form of follicular variant of papillary thyroid carcinomas. We identified several miRNAs, including miR-146-5p, miR-199a, miR199b, miR-1285-5p, miR-1915-3p, miR-4516, and miR-148b- 3p, that are highly deregulated (some upregulated and others downregulated) and could discriminate between encapsulated lesions (follicular adenomas and NIFTP) and infiltrative follicular variant of papillary thyroid carcinomas.

Recently, Aragon Han et al [109] reported that the expression levels of several miRNAs, including miR-199b, were significantly associated with aggressive features of thyroid cancer, such as a large tumor size, extrathyroidal extension, multifocality, lymphovascular invasion, lymph node metastases, and distant metastasis. Conversely,

no data have been thus far reported in thyroid tumors for miR-148b, miR-1285-5p and miR-4516, although deregulation of these miRNAs is associated with other cancer types [110-114].

Taken together, our data suggest that evaluating miR-146-5p, miR-199a, miR199b, miR-1285-5p, miR- 1915-3p, miR-4516, and miR-148b could be a useful diagnostic tool for differentiating between encapsu- lated tumors and other more aggressive entities.

In addition to these five miRNAs, we also observed several additional miRNAs (Table 7) that were differentially expressed in the two groups (encapsulated lesions versus infiltrative follicular variant of papillary thyroid carcinomas) albeit with a lower level of significance.

Furthermore, our results suggest that specific miRNA expression patterns could allow us to discri- minate between NIFTP and both follicular adenomas and infiltrative follicular variant of papillary thyroid carcinomas. In detail, miR-152-3p, miR-185-5p, and miR-574-3p are strongly downregulated in NIFTP compared to follicular adenomas. The importance of miR-185-5p and miR-574-3p has also been demon- strated in other human tumors, such as non-small cells lung cancer and breast cancer [115-117].

In the comparison between infiltrative follicular variant of papillary thyroid carcinomas and NIFTP, among six deregulated miRNAs, miRNA-10a-5p and miR-320e were highly upregulated, demonstrating their involvement in a more aggressive form of follicular variant of papillary thyroid carcinomas. Nevertheless, high expression of miR-10a has been already associated with lymph node metastasis in non-small cells

lung cancer through direct targeting of the PTEN/AKT/ERK signaling pathway [118], whereas miR-320e has been reported as a novel prognostic biomarker associated with adverse clinical outcomes in colorectal cancer patients treated with adjuvant chemotherapy [119].

In conclusion, our data provide evidence for the diagnostic potential of a specific miRNA signature. In details, we have shown that there is differential expression of miR-146-5p, miR-221-5p, miR-222-3p, miR-30e-3p, and miR-152-3p in follicular adenomas versus NIFTPPs and infiltrative follicular variant of papillary thyroid carcinomas, which could aid in discriminating between the lesion types. Moreover, among these miRNAs, miR-146-5p as well as miR- 199a-5p, miR-199b-5p, miR-1285-5p, miR-1915-3p, miR-4516, and miR-148b-3p are associated with the infiltrative growth of follicular variant of papillary thyroid carcinomas. Three downregulated miRNAs, miR-152-3p, miR-185-5p, and miR-574-3p, might facil- itate differential diagnosis between NIFTP and follicular adenomas, whereas the differential expression of miR-10a-5p and miR-320e likely helps discriminate between this new entity, NIFTP, and infiltrative follicular variant of papillary thyroid carcinoma.

Besides, from a practical point of view, the results obtained suggest that a panel of these miRNAs could act as a diagnostic marker in pre-surgical fine-needle aspiration diagnosis improving the preoperative assessment of nodules and the management of patients.

Nonetheless our findings require further confirmation in future studies with a large series of well-characterized FNA samples to determine diagnostic accuracy of miRNA signature as a routine diagnostic test for the pre-surgical evaluation of thyroid nodules.

## 6. nCounter Human v3 miRNA Expression Assay Transcript List

| Official Symbol | Accession | Target Sequence |
|---|---|---|
| hsa-let-7a-5p | MIMAT0000062 | UGAGGUAGUAGGUUGUAUAGUU |
| hsa-let-7b-5p | MIMAT0000063 | UGAGGUAGUAGGUUGUGUGGUU |
| hsa-let-7c-5p | MIMAT0000064 | UGAGGUAGUAGGUUGUAUGGUU |
| hsa-let-7d-5p | MIMAT0000065 | AGAGGUAGUAGGUUGCAUAGUU |
| hsa-let-7e-5p | MIMAT0000066 | UGAGGUAGGAGGUUGUAUAGUU |
| hsa-let-7f-5p | MIMAT0000067 | UGAGGUAGUAGAUUGUAUAGUU |
| hsa-let-7g-5p | MIMAT0000414 | UGAGGUAGUAGUUUGUACAGUU |
| hsa-let-7i-5p | MIMAT0000415 | UGAGGUAGUAGUUUGUGCUGUU |
| hsa-miR-100-5p | MIMAT0000098 | AACCCGUAGAUCCGAACUUGUG |
| hsa-miR-101-3p | MIMAT0000099 | UACAGUACUGUGAUAACUGAA |
| hsa-miR-103a-3p | MIMAT0000101 | AGCAGCAUUGUACAGGGCUAUGA |
| hsa-miR-105-5p | MIMAT0000102 | UCAAAUGCUCAGACUCCUGUGGU |
| hsa-miR-106a-5p | MIMAT0000103 | AAAAGUGCUUACAGUGCAGGUAG |
| hsa-miR-106b-5p | MIMAT0000680 | UAAAGUGCUGACAGUGCAGAU |
| hsa-miR-107 | MIMAT0000104 | AGCAGCAUUGUACAGGGCUAUCA |
| hsa-miR-10a-5p | MIMAT0000253 | UACCCUGUAGAUCCGAAUUUGUG |
| hsa-miR-10b-5p | MIMAT0000254 | UACCCUGUAGAACCGAAUUUGUG |
| hsa-miR-1178-3p | MIMAT0005823 | UUGCUCACUGUUCUUCCCUAG |
| hsa-miR-1180-3p | MIMAT0005825 | UUUCCGGCUCGCGUGGGUGUGU |
| hsa-miR-1183 | MIMAT0005828 | CACUGUAGGUGAUGGUGAGAGUGGGCA |
| hsa-miR-1185-1-3p | MIMAT0022838 | AUAUACAGGGGGAGACUCUUAU |
| hsa-miR-1185-2-3p | MIMAT0022713 | AUAUACAGGGGGAGACUCUCAU |
| hsa-miR-1185-5p | MIMAT0005798 | AGAGGAUACCCUUUGUAUGUU |

| | | |
|---|---|---|
| hsa-miR-1193 | MIMAT0015049 | GGGAUGGUAGACCGGUGACGUGC |
| hsa-miR-1197 | MIMAT0005955 | UAGGACACAUGGUCUACUUCU |
| hsa-miR-1200 | MIMAT0005863 | CUCCUGAGCCAUUCUGAGCCUC |
| hsa-miR-1202 | MIMAT0005865 | GUGCCAGCUGCAGUGGGGGAG |
| hsa-miR-1203 | MIMAT0005866 | CCCGGAGCCAGGAUGCAGCUC |
| hsa-miR-1204 | MIMAT0005868 | UCGUGGCCUGGUCUCCAUUAU |
| hsa-miR-1205 | MIMAT0005869 | UCUGCAGGGUUUGCUUUGAG |
| hsa-miR-1206 | MIMAT0005870 | UGUUCAUGUAGAUGUUUAAGC |
| hsa-miR-1224-3p | MIMAT0005459 | CCCCACCUCCUCUCUCCUCAG |
| hsa-miR-1224-5p | MIMAT0005458 | GUGAGGACUCGGGAGGUGG |
| hsa-miR-122-5p | MIMAT0000421 | UGGAGUGUGACAAUGGUGUUUG |
| hsa-miR-1226-3p | MIMAT0005577 | UCACCAGCCCUGUGUUCCCUAG |
| hsa-miR-1228-3p | MIMAT0005583 | UCACACCUGCCUCGCCCCCC |
| hsa-miR-1233-3p | MIMAT0005588 | UGAGCCCUGUCCUCCCGCAG |
| hsa-miR-1234-3p | MIMAT0005589 | UCGGCCUGACCACCCACCCCAC |
| hsa-miR-1236-3p | MIMAT0005591 | CCUCUUCCCCUUGUCUCUCCAG |
| hsa-miR-124-3p | MIMAT0000422 | UAAGGCACGCGGUGAAUGCC |
| hsa-miR-1244 | MIMAT0005896 | AAGUAGUUGGUUUGUAUGAGAUGGUU |
| hsa-miR-1245a | MIMAT0005897 | AAGUGAUCUAAAGGCCUACAU |
| hsa-miR-1245b-3p | MIMAT0019951 | UCAGAUGAUCUAAAGGCCUAUA |
| hsa-miR-1245b-5p | MIMAT0019950 | UAGGCCUUUAGAUCACUUAAA |
| hsa-miR-1246 | MIMAT0005898 | AAUGGAUUUUUGGAGCAGG |
| hsa-miR-1247-5p | MIMAT0005899 | ACCCGUCCCGUUCGUCCCCGGA |
| hsa-miR-1248 | MIMAT0005900 | ACCUUCUUGUAUAAGCACUGUGCUAAA |
| hsa-miR-1249-3p | MIMAT0005901 | ACGCCCUUCCCCCCCUUCUUCA |
| hsa-miR-1249-5p | MIMAT0032029 | AGGAGGGAGGAGAUGGGCCAAGUU |
| hsa-miR-1250-5p | MIMAT0005902 | ACGGUGCUGGAUGUGGCCUUU |

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| hsa-miR-1252-5p | MIMAT0005944 | AGAAGGAAAUUGAAUUCAUUUA |
| hsa-miR-1253 | MIMAT0005904 | AGAGAAGAAGAUCAGCCUGCA |
| hsa-miR-1254 | MIMAT0005905 | AGCCUGGAAGCUGGAGCCUGCAGU |
| hsa-miR-1255a | MIMAT0005906 | AGGAUGAGCAAAGAAAGUAGAUU |
| hsa-miR-1255b-5p | MIMAT0005945 | CGGAUGAGCAAAGAAAGUGGUU |
| hsa-miR-1257 | MIMAT0005908 | AGUGAAUGAUGGGUUCUGACC |
| hsa-miR-1258 | MIMAT0005909 | AGUUAGGAUUAGGUCGUGGAA |
| hsa-miR-125a-3p | MIMAT0004602 | ACAGGUGAGGUUCUUGGGAGCC |
| hsa-miR-125a-5p | MIMAT0000443 | UCCCUGAGACCCUUUAACCUGUGA |
| hsa-miR-125b-5p | MIMAT0000423 | UCCCUGAGACCCUAACUUGUGA |
| hsa-miR-1260a | MIMAT0005911 | AUCCCACCUCUGCCACCA |
| hsa-miR-1260b | MIMAT0015041 | AUCCCACCACUGCCACCAU |
| hsa-miR-1261 | MIMAT0005913 | AUGGAUAAGGCUUUGGCUU |
| hsa-miR-1262 | MIMAT0005914 | AUGGGUGAAUUUGUAGAAGGAU |
| hsa-miR-126-3p | MIMAT0000445 | UCGUACCGUGAGUAAUAAUGCG |
| hsa-miR-1264 | MIMAT0005791 | CAAGUCUUAUUUGAGCACCUGUU |
| hsa-miR-1266-5p | MIMAT0005920 | CCUCAGGGCUGUAGAACAGGGCU |
| hsa-miR-1268a | MIMAT0005922 | CGGGCGUGGUGGUGGGGG |
| hsa-miR-1268b | MIMAT0018925 | CGGGCGUGGUGGUGGGGGUG |
| hsa-miR-1269a | MIMAT0005923 | CUGGACUGAGCCGUGCUACUGG |
| hsa-miR-1269b | MIMAT0019059 | CUGGACUGAGCCAUGCUACUGG |
| hsa-miR-1270 | MIMAT0005924 | CUGGAGAUAUGGAAGAGCUGUGU |
| hsa-miR-1271-3p | MIMAT0022712 | AGUGCCUGCUAUGUGCCAGGCA |
| hsa-miR-1271-5p | MIMAT0005796 | CUUGGCACCUAGCAAGCACUCA |
| hsa-miR-1272 | MIMAT0005925 | GAUGAUGAUGGCAGCAAAUUCUGAAA |
| hsa-miR-1273c | MIMAT0015017 | GGCGACAAAACGAGACCCUGUC |
| hsa-miR-127-3p | MIMAT0000446 | UCGGAUCCGUCUGAGCUUGGCU |

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| hsa-miR-1275 | MIMAT0005929 | GUGGGGGAGAGGCUGUC |
| hsa-miR-127-5p | MIMAT0004604 | CUGAAGCUCAGAGGGCUCUGAU |
| hsa-miR-1276 | MIMAT0005930 | UAAAGAGCCCUGUGGAGACA |
| hsa-miR-1277-3p | MIMAT0005933 | UACGUAGAUAUAUAUGUAUUUU |
| hsa-miR-1278 | MIMAT0005936 | UAGUACUGUGCAUAUCAUCUAU |
| hsa-miR-1279 | MIMAT0005937 | UCAUAUUGCUUCUUUCU |
| hsa-miR-1281 | MIMAT0005939 | UCGCCUCCUCCUCUCCC |
| hsa-miR-128-1-5p | MIMAT0026477 | CGGGGCCGUAGCACUGUCUGAGA |
| hsa-miR-128-2-5p | MIMAT0031095 | GGGGGCCGAUACACUGUACGAGA |
| hsa-miR-1283 | MIMAT0005799 | UCUACAAAGGAAAGCGCUUUCU |
| hsa-miR-128-3p | MIMAT0000424 | UCACAGUGAACCGGUCUCUUU |
| hsa-miR-1285-3p | MIMAT0005876 | UCUGGGCAACAAAGUGAGACCU |
| hsa-miR-1285-5p | MIMAT0022719 | GAUCUCACUUUGUUGCCCAGG |
| hsa-miR-1286 | MIMAT0005877 | UGCAGGACCAAGAUGAGCCCU |
| hsa-miR-1287-3p | MIMAT0026738 | CUCUAGCCACAGAUGCAGUGAU |
| hsa-miR-1287-5p | MIMAT0005878 | UGCUGGAUCAGUGGUUCGAGUC |
| hsa-miR-1288-3p | MIMAT0005942 | UGGACUGCCCUGAUCUGGAGA |
| hsa-miR-1289 | MIMAT0005879 | UGGAGUCCAGGAAUCUGCAUUUU |
| hsa-miR-1290 | MIMAT0005880 | UGGAUUUUUGGAUCAGGGA |
| hsa-miR-1291 | MIMAT0005881 | UGGCCCUGACUGAAGACCAGCAGU |
| hsa-miR-129-2-3p | MIMAT0004605 | AAGCCCUUACCCCAAAAAGCAU |
| hsa-miR-1293 | MIMAT0005883 | UGGGUGGUCUGGAGAUUUGUGC |
| hsa-miR-1295a | MIMAT0005885 | UUAGGCCGCAGAUCUGGGUGA |
| hsa-miR-129-5p | MIMAT0000242 | CUUUUUGCGGUCUGGGCUUGC |
| hsa-miR-1296-3p | MIMAT0026637 | GAGUGGGGCUUCGACCCUAACC |
| hsa-miR-1296-5p | MIMAT0005794 | UUAGGGCCCUGGCUCCAUCUCC |
| hsa-miR-1297 | MIMAT0005886 | UUCAAGUAAUUCAGGUG |

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| hsa-miR-1298-5p | MIMAT0005800 | UUCAUUCGGCUGUCCAGAUGUA |
| hsa-miR-1299 | MIMAT0005887 | UUCUGGAAUUCUGUGUGAGGGA |
| hsa-miR-1301-3p | MIMAT0005797 | UUGCAGCUGCCUGGGAGUGACUUC |
| hsa-miR-1302 | MIMAT0005890 | UUGGGACAUACUUAUGCUAAA |
| hsa-miR-1303 | MIMAT0005891 | UUUAGAGACGGGGUCUUGCUCU |
| hsa-miR-1304-3p | MIMAT0022720 | UCUCACUGUAGCCUCGAACCCC |
| hsa-miR-1304-5p | MIMAT0005892 | UUUGAGGCUACAGUGAGAUGUG |
| hsa-miR-1305 | MIMAT0005893 | UUUUCAACUCUAAUGGGAGAGA |
| hsa-miR-1306-3p | MIMAT0005950 | ACGUUGGCUCUGGUGGUG |
| hsa-miR-1306-5p | MIMAT0022726 | CCACCUCCCCUGCAAACGUCCA |
| hsa-miR-1307-3p | MIMAT0005951 | ACUCGGCGUGGCGUCGGUCGUG |
| hsa-miR-1307-5p | MIMAT0022727 | UCGACCGGACCUCGACCGGCU |
| hsa-miR-130a-3p | MIMAT0000425 | CAGUGCAAUGUUAAAAGGGCAU |
| hsa-miR-130b-3p | MIMAT0000691 | CAGUGCAAUGAUGAAAGGGCAU |
| hsa-miR-1322 | MIMAT0005953 | GAUGAUGCUGCUGAUGCUG |
| hsa-miR-1323 | MIMAT0005795 | UCAAAACUGAGGGGCAUUUUCU |
| hsa-miR-132-3p | MIMAT0000426 | UAACAGUCUACAGCCAUGGUCG |
| hsa-miR-133a-3p | MIMAT0000427 | UUUGGUCCCCUUCAACCAGCUG |
| hsa-miR-133a-5p | MIMAT0026478 | AGCUGGUAAAAUGGAACCAAAU |
| hsa-miR-133b | MIMAT0000770 | UUUGGUCCCCUUCAACCAGCUA |
| hsa-miR-134-3p | MIMAT0026481 | CCUGUGGGCCACCUAGUCACCAA |
| hsa-miR-134-5p | MIMAT0000447 | UGUGACUGGUUGACCAGAGGGG |
| hsa-miR-135a-5p | MIMAT0000428 | UAUGGCUUUUUAUUCCUAUGUGA |
| hsa-miR-135b-5p | MIMAT0000758 | UAUGGCUUUUCAUUCCUAUGUGA |
| hsa-miR-136-5p | MIMAT0000448 | ACUCCAUUUGUUUUGAUGAUGGA |
| hsa-miR-137 | MIMAT0000429 | UUAUUGCUUAAGAAUACGCGUAG |
| hsa-miR-138-5p | MIMAT0000430 | AGCUGGUGUUGUGAAUCAGGCCG |

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| hsa-miR-139-3p | MIMAT0004552 | UGGAGACGCGGCCCUGUUGGAGU |
| hsa-miR-139-5p | MIMAT0000250 | UCUACAGUGCACGUGUCUCCAG |
| hsa-miR-1-3p | MIMAT0000416 | UGGAAUGUAAAGAAGUAUGUAU |
| hsa-miR-140-3p | MIMAT0004597 | UACCACAGGGUAGAACCACGG |
| hsa-miR-140-5p | MIMAT0000431 | CAGUGGUUUUACCCUAUGGUAG |
| hsa-miR-141-3p | MIMAT0000432 | UAACACUGUCUGGUAAAGAUGG |
| hsa-miR-142-3p | MIMAT0000434 | UGUAGUGUUUCCUACUUUAUGGA |
| hsa-miR-142-5p | MIMAT0000433 | CAUAAAGUAGAAAGCACUACU |
| hsa-miR-143-3p | MIMAT0000435 | UGAGAUGAAGCACUGUAGCUC |
| hsa-miR-144-3p | MIMAT0000436 | UACAGUAUAGAUGAUGUACU |
| hsa-miR-145-5p | MIMAT0000437 | GUCCAGUUUUCCCAGGAAUCCCU |
| hsa-miR-1469 | MIMAT0007347 | CUCGGCGCGGGGCGCGGGCUCC |
| hsa-miR-146a-5p | MIMAT0000449 | UGAGAACUGAAUUCCAUGGGUU |
| hsa-miR-146b-3p | MIMAT0004766 | UGCCCUGUGGACUCAGUUCUGG |
| hsa-miR-146b-5p | MIMAT0002809 | UGAGAACUGAAUUCCAUAGGCU |
| hsa-miR-147a | MIMAT0000251 | GUGUGUGGAAAUGCUUCUGC |
| hsa-miR-147b | MIMAT0004928 | GUGUGCGGAAAUGCUUCUGCUA |
| hsa-miR-148a-3p | MIMAT0000243 | UCAGUGCACUACAGAACUUUGU |
| hsa-miR-148b-3p | MIMAT0000759 | UCAGUGCAUCACAGAACUUUGU |
| hsa-miR-149-5p | MIMAT0000450 | UCUGGCUCCGUGUCUUCACUCCC |
| hsa-miR-150-5p | MIMAT0000451 | UCUCCCAACCCUUGUACCAGUG |
| hsa-miR-151a-3p | MIMAT0000757 | CUAGACUGAAGCUCCUUGAGG |
| hsa-miR-151a-5p | MIMAT0004697 | UCGAGGAGCUCACAGUCUAGU |
| hsa-miR-151b | MIMAT0010214 | UCGAGGAGCUCACAGUCU |
| hsa-miR-152-3p | MIMAT0000438 | UCAGUGCAUGACAGAACUUGG |
| hsa-miR-152-5p | MIMAT0026479 | AGGUUCUGUGAUACACUCCGACU |
| hsa-miR-153-3p | MIMAT0000439 | UUGCAUAGUCACAAAAGUGAUC |

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| hsa-miR-1537-3p | MIMAT0007399 | AAAACCGUCUAGUUACAGUUGU |
| hsa-miR-154-5p | MIMAT0000452 | UAGGUUAUCCGUGUUGCCUUCG |
| hsa-miR-155-5p | MIMAT0000646 | UUAAUGCUAAUCGUGAUAGGGGU |
| hsa-miR-15a-5p | MIMAT0000068 | UAGCAGCACAUAAUGGUUUGUG |
| hsa-miR-15b-5p | MIMAT0000417 | UAGCAGCACAUCAUGGUUUACA |
| hsa-miR-1-5p | MIMAT0031892 | ACAUACUUCUUUAUAUGCCCAU |
| hsa-miR-16-5p | MIMAT0000069 | UAGCAGCACGUAAAUAUUGGCG |
| hsa-miR-17-5p | MIMAT0000070 | CAAAGUGCUUACAGUGCAGGUAG |
| hsa-miR-181a-2-3p | MIMAT0004558 | ACCACUGACCGUUGACUGUACC |
| hsa-miR-181a-3p | MIMAT0000270 | ACCAUCGACCGUUGAUUGUACC |
| hsa-miR-181a-5p | MIMAT0000256 | AACAUUCAACGCUGUCGGUGAGU |
| hsa-miR-181b-2-3p | MIMAT0031893 | CUCACUGAUCAAUGAAUGCA |
| hsa-miR-181b-5p | MIMAT0000257 | AACAUUCAUUGCUGUCGGUGGGU |
| hsa-miR-181c-5p | MIMAT0000258 | AACAUUCAACCUGUCGGUGAGU |
| hsa-miR-181d-3p | MIMAT0026608 | CCACCGGGGGAUGAAUGUCAC |
| hsa-miR-181d-5p | MIMAT0002821 | AACAUUCAUUGUUGUCGGUGGGU |
| hsa-miR-182-3p | MIMAT0000260 | UGGUUCUAGACUUGCCAACUA |
| hsa-miR-182-5p | MIMAT0000259 | UUUGGCAAUGGUAGAACUCACACU |
| hsa-miR-1827 | MIMAT0006767 | UGAGGCAGUAGAUUGAAU |
| hsa-miR-183-5p | MIMAT0000261 | UAUGGCACUGGUAGAAUUCACU |
| hsa-miR-184 | MIMAT0000454 | UGGACGGAGAACUGAUAAGGGU |
| hsa-miR-185-5p | MIMAT0000455 | UGGAGAGAAAGGCAGUUCCUGA |
| hsa-miR-186-5p | MIMAT0000456 | CAAAGAAUUCUCCUUUUGGGCU |
| hsa-miR-187-3p | MIMAT0000262 | UCGUGUCUUGUGUUGCAGCCGG |
| hsa-miR-188-3p | MIMAT0004613 | CUCCCACAUGCAGGGUUUGCA |
| hsa-miR-188-5p | MIMAT0000457 | CAUCCCUUGCAUGGUGGAGGG |
| hsa-miR-18a-5p | MIMAT0000072 | UAAGGUGCAUCUAGUGCAGAUAG |

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| hsa-miR-18b-5p | MIMAT0001412 | UAAGGUGCAUCUAGUGCAGUUAG |
| hsa-miR-1908-3p | MIMAT0026916 | CCGGCCGCCGGCUCCGCCCCG |
| hsa-miR-1908-5p | MIMAT0007881 | CGGCGGGGACGGCGAUUGGUC |
| hsa-miR-1909-3p | MIMAT0007883 | CGCAGGGGCCGGGUGCUCACCG |
| hsa-miR-190a-3p | MIMAT0026482 | CUAUAUAUCAAACAUAUUCCU |
| hsa-miR-190a-5p | MIMAT0000458 | UGAUAUGUUUGAUAUAUUAGGU |
| hsa-miR-190b | MIMAT0004929 | UGAUAUGUUUGAUAUUGGGUU |
| hsa-miR-1910-3p | MIMAT0026917 | GAGGCAGAAGCAGGAUGACA |
| hsa-miR-1910-5p | MIMAT0007884 | CCAGUCCUGUGCCUGCCGCCU |
| hsa-miR-1915-3p | MIMAT0007892 | CCCCAGGGCGACGCGGCGGG |
| hsa-miR-191-5p | MIMAT0000440 | CAACGGAAUCCCAAAAGCAGCUG |
| hsa-miR-192-5p | MIMAT0000222 | CUGACCUAUGAAUUGACAGCC |
| hsa-miR-193a-3p | MIMAT0000459 | AACUGGCCUACAAAGUCCCAGU |
| hsa-miR-193a-5p | MIMAT0004614 | UGGGUCUUUGCGGGCGAGAUGA |
| hsa-miR-193b-3p | MIMAT0002819 | AACUGGCCCUCAAAGUCCCGCU |
| hsa-miR-193b-5p | MIMAT0004767 | CGGGGUUUUGAGGGCGAGAUGA |
| hsa-miR-194-5p | MIMAT0000460 | UGUAACAGCAACUCCAUGUGGA |
| hsa-miR-195-5p | MIMAT0000461 | UAGCAGCACAGAAAUAUUGGC |
| hsa-miR-196a-3p | MIMAT0004562 | CGGCAACAAGAAACUGCCUGAG |
| hsa-miR-196a-5p | MIMAT0000226 | UAGGUAGUUUCAUGUUGUUGGG |
| hsa-miR-196b-5p | MIMAT0001080 | UAGGUAGUUUCCUGUUGUUGGG |
| hsa-miR-1972 | MIMAT0009447 | UCAGGCCAGGCACAGUGGCUCA |
| hsa-miR-1973 | MIMAT0009448 | ACCGUGCAAAGGUAGCAUA |
| hsa-miR-197-3p | MIMAT0000227 | UUCACCACCUUCUCCACCCAGC |
| hsa-miR-197-5p | MIMAT0022691 | CGGGUAGAGAGGGCAGUGGGAGG |
| hsa-miR-1976 | MIMAT0009451 | CCUCCUGCCCUCCUUGCUGU |
| hsa-miR-198 | MIMAT0000228 | GGUCCAGAGGGGAGAUAGGUUC |

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| hsa-miR-199a-3p | MIMAT0000232 | ACAGUAGUCUGCACAUUGGUUA |
| hsa-miR-199a-5p | MIMAT0000231 | CCCAGUGUUCAGACUACCUGUUC |
| hsa-miR-199b-3p | MIMAT0004563 | ACAGUAGUCUGCACAUUGGUUA |
| hsa-miR-199b-5p | MIMAT0000263 | CCCAGUGUUUAGACUAUCUGUUC |
| hsa-miR-19a-3p | MIMAT0000073 | UGUGCAAAUCUAUGCAAAACUGA |
| hsa-miR-19b-3p | MIMAT0000074 | UGUGCAAAUCCAUGCAAAACUGA |
| hsa-miR-200a-3p | MIMAT0000682 | UAACACUGUCUGGUAACGAUGU |
| hsa-miR-200b-3p | MIMAT0000318 | UAAUACUGCCUGGUAAUGAUGA |
| hsa-miR-200c-3p | MIMAT0000617 | UAAUACUGCCGGGUAAUGAUGGA |
| hsa-miR-202-3p | MIMAT0002811 | AGAGGUAUAGGGCAUGGGAA |
| hsa-miR-203a-3p | MIMAT0000264 | GUGAAAUGUUUAGGACCACUAG |
| hsa-miR-203a-5p | MIMAT0031890 | AGUGGUUCUUAACAGUUCAACAGUU |
| hsa-miR-204-5p | MIMAT0000265 | UUCCCUUUGUCAUCCUAUGCCU |
| hsa-miR-2053 | MIMAT0009978 | GUGUUAAUUAAACCUCUAUUUAC |
| hsa-miR-205-5p | MIMAT0000266 | UCCUUCAUUCCACCGGAGUCUG |
| hsa-miR-206 | MIMAT0000462 | UGGAAUGUAAGGAAGUGUGUGG |
| hsa-miR-208a-3p | MIMAT0000241 | AUAAGACGAGCAAAAAGCUUGU |
| hsa-miR-208b-3p | MIMAT0004960 | AUAAGACGAACAAAAGGUUUGU |
| hsa-miR-208b-5p | MIMAT0026722 | AAGCUUUUUGCUCGAAUUAUGU |
| hsa-miR-20a-5p | MIMAT0000075 | UAAAGUGCUUAUAGUGCAGGUAG |
| hsa-miR-20b-5p | MIMAT0001413 | CAAAGUGCUCAUAGUGCAGGUAG |
| hsa-miR-210-3p | MIMAT0000267 | CUGUGCGUGUGACAGCGGCUGA |
| hsa-miR-210-5p | MIMAT0026475 | AGCCCCUGCCCACCGCACACUG |
| hsa-miR-2110 | MIMAT0010133 | UUGGGGAAACGGCCGCUGAGUG |
| hsa-miR-2113 | MIMAT0009206 | AUUUGUGCUUGGCUCUGUCAC |
| hsa-miR-211-3p | MIMAT0022694 | GCAGGGACAGCAAAGGGGUGC |
| hsa-miR-211-5p | MIMAT0000268 | UUCCCUUUGUCAUCCUUCGCCU |

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|---|---|---|
| hsa-miR-2116-5p | MIMAT0011160 | GGUUCUUAGCAUAGGAGGUCU |
| hsa-miR-2117 | MIMAT0011162 | UGUUCUCUUUGCCAAGGACAG |
| hsa-miR-212-3p | MIMAT0000269 | UAACAGUCUCCAGUCACGGCC |
| hsa-miR-214-3p | MIMAT0000271 | ACAGCAGGCACAGACAGGCAGU |
| hsa-miR-215-5p | MIMAT0000272 | AUGACCUAUGAAUUGACAGAC |
| hsa-miR-21-5p | MIMAT0000076 | UAGCUUAUCAGACUGAUGUUGA |
| hsa-miR-216a-5p | MIMAT0000273 | UAAUCUCAGCUGGCAACUGUGA |
| hsa-miR-216b-5p | MIMAT0004959 | AAAUCUCUGCAGGCAAAUGUGA |
| hsa-miR-217 | MIMAT0000274 | UACUGCAUCAGGAACUGAUUGGA |
| hsa-miR-218-5p | MIMAT0000275 | UUGUGCUUGAUCUAACCAUGU |
| hsa-miR-219a-1-3p | MIMAT0004567 | AGAGUUGAGUCUGGACGUCCCG |
| hsa-miR-219a-2-3p | MIMAT0004675 | AGAAUUGUGGCUGGACAUCUGU |
| hsa-miR-219a-5p | MIMAT0000276 | UGAUUGUCCAAACGCAAUUCU |
| hsa-miR-219b-3p | MIMAT0019748 | AGAAUUGCGUUUGGACAAUCAGU |
| hsa-miR-221-3p | MIMAT0000278 | AGCUACAUUGUCUGCUGGGUUUC |
| hsa-miR-221-5p | MIMAT0004568 | ACCUGGCAUACAAUGUAGAUUU |
| hsa-miR-222-3p | MIMAT0000279 | AGCUACAUCUGGCUACUGGGU |
| hsa-miR-223-3p | MIMAT0000280 | UGUCAGUUUGUCAAAUACCCCA |
| hsa-miR-22-3p | MIMAT0000077 | AAGCUGCCAGUUGAAGAACUGU |
| hsa-miR-224-5p | MIMAT0000281 | CAAGUCACUAGUGGUUCCGUU |
| hsa-miR-2278 | MIMAT0011778 | GAGAGCAGUGUGUGUUGCCUGG |
| hsa-miR-23a-3p | MIMAT0000078 | AUCACAUUGCCAGGGAUUUCC |
| hsa-miR-23b-3p | MIMAT0000418 | AUCACAUUGCCAGGGAUUACC |
| hsa-miR-23c | MIMAT0018000 | AUCACAUUGCCAGUGAUUACCC |
| hsa-miR-24-3p | MIMAT0000080 | UGGCUCAGUUCAGCAGGAACAG |
| hsa-miR-25-3p | MIMAT0000081 | CAUUGCACUUGUCUCGGUCUGA |
| hsa-miR-25-5p | MIMAT0004498 | AGGCGGAGACUUGGGCAAUUG |

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|---|---|---|
| hsa-miR-2682-5p | MIMAT0013517 | CAGGCAGUGACUGUUCAGACGUC |
| hsa-miR-26a-5p | MIMAT0000082 | UUCAAGUAAUCCAGGAUAGGCU |
| hsa-miR-26b-5p | MIMAT0000083 | UUCAAGUAAUUCAGGAUAGGU |
| hsa-miR-27a-3p | MIMAT0000084 | UUCACAGUGGCUAAGUUCCGC |
| hsa-miR-27b-3p | MIMAT0000419 | UUCACAGUGGCUAAGUUCUGC |
| hsa-miR-28-3p | MIMAT0004502 | CACUAGAUUGUGAGCUCCUGGA |
| hsa-miR-28-5p | MIMAT0000085 | AAGGAGCUCACAGUCUAUUGAG |
| hsa-miR-296-3p | MIMAT0004679 | GAGGGUUGGGUGGAGGCUCUCC |
| hsa-miR-296-5p | MIMAT0000690 | AGGGCCCCCCCUCAAUCCUGU |
| hsa-miR-297 | MIMAT0004450 | AUGUAUGUGUGCAUGUGCAUG |
| hsa-miR-298 | MIMAT0004901 | AGCAGAAGCAGGGAGGUUCUCCCA |
| hsa-miR-299-3p | MIMAT0000687 | UAUGUGGGAUGGUAAACCGCUU |
| hsa-miR-299-5p | MIMAT0002890 | UGGUUUACCGUCCCACAUACAU |
| hsa-miR-29a-3p | MIMAT0000086 | UAGCACCAUCUGAAAUCGGUUA |
| hsa-miR-29b-3p | MIMAT0000100 | UAGCACCAUUUGAAAUCAGUGUU |
| hsa-miR-29c-3p | MIMAT0000681 | UAGCACCAUUUGAAAUCGGUUA |
| hsa-miR-300 | MIMAT0004903 | UAUACAAGGGCAGACUCUCUCU |
| hsa-miR-301a-3p | MIMAT0000688 | CAGUGCAAUAGUAUUGUCAAAGC |
| hsa-miR-301a-5p | MIMAT0022696 | GCUCUGACUUUAUUGCACUACU |
| hsa-miR-301b-3p | MIMAT0004958 | CAGUGCAAUGAUAUUGUCAAAGC |
| hsa-miR-301b-5p | MIMAT0032026 | GCUCUGACGAGGUUGCACUACU |
| hsa-miR-302a-3p | MIMAT0000684 | UAAGUGCUUCCAUGUUUUGGUGA |
| hsa-miR-302a-5p | MIMAT0000683 | ACUUAAACGUGGAUGUACUUGCU |
| hsa-miR-302b-3p | MIMAT0000715 | UAAGUGCUUCCAUGUUUUAGUAG |
| hsa-miR-302c-3p | MIMAT0000717 | UAAGUGCUUCCAUGUUUCAGUGG |
| hsa-miR-302d-3p | MIMAT0000718 | UAAGUGCUUCCAUGUUUGAGUGU |
| hsa-miR-302e | MIMAT0005931 | UAAGUGCUUCCAUGCUU |

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|---|---|---|
| hsa-miR-302f | MIMAT0005932 | UAAUUGCUUCCAUGUUU |
| hsa-miR-3065-3p | MIMAT0015378 | UCAGCACCAGGAUAUUGUUGGAG |
| hsa-miR-3065-5p | MIMAT0015066 | UCAACAAAAUCACUGAUGCUGGA |
| hsa-miR-3074-3p | MIMAT0015027 | GAUAUCAGCUCAGUAGGCACCG |
| hsa-miR-30a-3p | MIMAT0000088 | CUUUCAGUCGGAUGUUUGCAGC |
| hsa-miR-30a-5p | MIMAT0000087 | UGUAAACAUCCUCGACUGGAAG |
| hsa-miR-30b-5p | MIMAT0000420 | UGUAAACAUCCUACACUCAGCU |
| hsa-miR-30c-1-3p | MIMAT0004674 | CUGGGAGAGGGUUGUUUACUCC |
| hsa-miR-30c-5p | MIMAT0000244 | UGUAAACAUCCUACACUCUCAGC |
| hsa-miR-30d-5p | MIMAT0000245 | UGUAAACAUCCCCGACUGGAAG |
| hsa-miR-30e-3p | MIMAT0000693 | CUUUCAGUCGGAUGUUUACAGC |
| hsa-miR-30e-5p | MIMAT0000692 | UGUAAACAUCCUUGACUGGAAG |
| hsa-miR-3127-5p | MIMAT0014990 | AUCAGGGCUUGUGGAAUGGGAAG |
| hsa-miR-3130-3p | MIMAT0014994 | GCUGCACCGGAGACUGGGUAA |
| hsa-miR-3131 | MIMAT0014996 | UCGAGGACUGGUGGAAGGGCCUU |
| hsa-miR-3136-5p | MIMAT0015003 | CUGACUGAAUAGGUAGGGUCAUU |
| hsa-miR-3140-3p | MIMAT0015008 | AGCUUUUGGGAAUUCAGGUAGU |
| hsa-miR-3140-5p | MIMAT0019204 | ACCUGAAUUACCAAAAGCUUU |
| hsa-miR-3144-3p | MIMAT0015015 | AUAUACCUGUUCGGUCUCUUUA |
| hsa-miR-3144-5p | MIMAT0015014 | AGGGGACCAAAGAGAUAUAUAG |
| hsa-miR-3147 | MIMAT0015019 | GGUUGGGCAGUGAGGAGGGUGUGA |
| hsa-miR-3150b-3p | MIMAT0018194 | UGAGGAGAUCGUCGAGGUUGG |
| hsa-miR-3151-5p | MIMAT0015024 | GGUGGGGCAAUGGGAUCAGGU |
| hsa-miR-3158-3p | MIMAT0015032 | AAGGGCUUCCUCUCUGCAGGAC |
| hsa-miR-31-5p | MIMAT0000089 | AGGCAAGAUGCUGGCAUAGCU |
| hsa-miR-3161 | MIMAT0015035 | CUGAUAAGAACAGAGGCCCAGAU |
| hsa-miR-3164 | MIMAT0015038 | UGUGACUUUAAGGGAAAUGGCG |

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| hsa-miR-3168 | MIMAT0015043 | GAGUUCUACAGUCAGAC |
| hsa-miR-3179 | MIMAT0015056 | AGAAGGGGUGAAAUUUAAACGU |
| hsa-miR-3180 | MIMAT0018178 | UGGGGCGGAGCUUCCGGAG |
| hsa-miR-3180-3p | MIMAT0015058 | UGGGGCGGAGCUUCCGGAGGCC |
| hsa-miR-3180-5p | MIMAT0015057 | CUUCCAGACGCUCCGCCCCACGUCG |
| hsa-miR-3182 | MIMAT0015062 | GCUUCUGUAGUGUAGUC |
| hsa-miR-3185 | MIMAT0015065 | AGAAGAAGGCGGUCGGUCUGCGG |
| hsa-miR-3190-3p | MIMAT0022839 | UGUGGAAGGUAGACGGCCAGAGA |
| hsa-miR-3192-5p | MIMAT0015076 | UCUGGGAGGUUGUAGCAGUGGAA |
| hsa-miR-3195 | MIMAT0015079 | CGCGCCGGGCCCGGGUU |
| hsa-miR-3196 | MIMAT0015080 | CGGGGCGGCAGGGGCCUC |
| hsa-miR-3202 | MIMAT0015089 | UGGAAGGGAGAAGAGCUUUAAU |
| hsa-miR-320a | MIMAT0000510 | AAAAGCUGGGUUGAGAGGGCGA |
| hsa-miR-320b | MIMAT0005792 | AAAAGCUGGGUUGAGAGGGCAA |
| hsa-miR-320c | MIMAT0005793 | AAAAGCUGGGUUGAGAGGGU |
| hsa-miR-320d | MIMAT0006764 | AAAAGCUGGGUUGAGAGGA |
| hsa-miR-320e | MIMAT0015072 | AAAGCUGGGUUGAGAAGG |
| hsa-miR-323a-3p | MIMAT0000755 | CACAUUACACGGUCGACCUCU |
| hsa-miR-323a-5p | MIMAT0004696 | AGGUGGUCCGUGGCGCGUUCGC |
| hsa-miR-323b-3p | MIMAT0015050 | CCCAAUACACGGUCGACCUCUU |
| hsa-miR-323b-5p | MIMAT0001630 | AGGUUGUCCGUGGUGAGUUCGCA |
| hsa-miR-324-3p | MIMAT0000762 | ACUGCCCCAGGUGCUGCUGG |
| hsa-miR-324-5p | MIMAT0000761 | CGCAUCCCCUAGGGCAUUGGUGU |
| hsa-miR-325 | MIMAT0000771 | CCUAGUAGGUGUCCAGUAAGUGU |
| hsa-miR-32-5p | MIMAT0000090 | UAUUGCACAUUACUAAGUUGCA |
| hsa-miR-326 | MIMAT0000756 | CCUCUGGGCCCUUCCUCCAG |
| hsa-miR-328-3p | MIMAT0000752 | CUGGCCCUCUCUGCCCUUCCGU |

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| hsa-miR-328-5p | MIMAT0026486 | GGGGGGGCAGGAGGGGCUCAGGG |
| hsa-miR-329-3p | MIMAT0001629 | AACACACCUGGUUAACCUCUUU |
| hsa-miR-329-5p | MIMAT0026555 | GAGGUUUUCUGGGUUUCUGUUUC |
| hsa-miR-330-3p | MIMAT0000751 | GCAAAGCACACGGCCUGCAGAGA |
| hsa-miR-330-5p | MIMAT0004693 | UCUCUGGGCCUGUGUCUUAGGC |
| hsa-miR-331-3p | MIMAT0000760 | GCCCCUGGGCCUAUCCUAGAA |
| hsa-miR-331-5p | MIMAT0004700 | CUAGGUAUGGUCCCAGGGAUCC |
| hsa-miR-335-5p | MIMAT0000765 | UCAAGAGCAAUAACGAAAAAUGU |
| hsa-miR-337-3p | MIMAT0000754 | CUCCUAUAUGAUGCCUUUCUUC |
| hsa-miR-337-5p | MIMAT0004695 | GAACGGCUUCAUACAGGAGUU |
| hsa-miR-338-5p | MIMAT0004701 | AACAAUAUCCUGGUGCUGAGUG |
| hsa-miR-339-3p | MIMAT0004702 | UGAGCGCCUCGACGACAGAGCCG |
| hsa-miR-339-5p | MIMAT0000764 | UCCCUGUCCUCCAGGAGCUCACG |
| hsa-miR-33a-5p | MIMAT0000091 | GUGCAUUGUAGUUGCAUUGCA |
| hsa-miR-33b-5p | MIMAT0003301 | GUGCAUUGCUGUUGCAUUGC |
| hsa-miR-340-5p | MIMAT0004692 | UUAUAAAGCAAUGAGACUGAUU |
| hsa-miR-342-3p | MIMAT0000753 | UCUCACACAGAAAUCGCACCCGU |
| hsa-miR-342-5p | MIMAT0004694 | AGGGGUGCUAUCUGUGAUUGA |
| hsa-miR-345-3p | MIMAT0022698 | GCCCUGAACGAGGGGUCUGGAG |
| hsa-miR-345-5p | MIMAT0000772 | GCUGACUCCUAGUCCAGGGCUC |
| hsa-miR-346 | MIMAT0000773 | UGUCUGCCCGCAUGCCUGCCUCU |
| hsa-miR-34a-5p | MIMAT0000255 | UGGCAGUGUCUUAGCUGGUUGU |
| hsa-miR-34b-3p | MIMAT0004676 | CAAUCACUAACUCCACUGCCAU |
| hsa-miR-34c-3p | MIMAT0004677 | AAUCACUAACCACACGGCCAGG |
| hsa-miR-34c-5p | MIMAT0000686 | AGGCAGUGUAGUUAGCUGAUUGC |
| hsa-miR-3605-3p | MIMAT0017982 | CCUCCGUGUUACCUGUCCUCUAG |
| hsa-miR-3605-5p | MIMAT0017981 | UGAGGAUGGAUAGCAAGGAAGCC |

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| hsa-miR-3613-3p | MIMAT0017991 | ACAAAAAAAAAAGCCCAACCCUUC |
| hsa-miR-3613-5p | MIMAT0017990 | UGUUGUACUUUUUUUUUUGUUC |
| hsa-miR-361-3p | MIMAT0004682 | UCCCCCAGGUGUGAUUCUGAUUU |
| hsa-miR-3614-3p | MIMAT0017993 | UAGCCUUCAGAUCUUGGUGUUUU |
| hsa-miR-3614-5p | MIMAT0017992 | CCACUUGGAUCUGAAGGCUGCCC |
| hsa-miR-3615 | MIMAT0017994 | UCUCUCGGCUCCUCGCGGCUC |
| hsa-miR-361-5p | MIMAT0000703 | UUAUCAGAAUCUCCAGGGGUAC |
| hsa-miR-362-3p | MIMAT0004683 | AACACACCUAUUCAAGGAUUCA |
| hsa-miR-362-5p | MIMAT0000705 | AAUCCUUGGAACCUAGGUGUGAGU |
| hsa-miR-363-3p | MIMAT0000707 | AAUUGCACGGUAUCCAUCUGUA |
| hsa-miR-363-5p | MIMAT0003385 | CGGGUGGAUCACGAUGCAAUUU |
| hsa-miR-365a-3p | MIMAT0000710 | UAAUGCCCCUAAAAAUCCUUAU |
| hsa-miR-365b-3p | MIMAT0022834 | UAAUGCCCCUAAAAAUCCUUAU |
| hsa-miR-365b-5p | MIMAT0022833 | AGGGACUUUCAGGGGCAGCUGU |
| hsa-miR-367-3p | MIMAT0000719 | AAUUGCACUUUAGCAAUGGUGA |
| hsa-miR-3690 | MIMAT0018119 | ACCUGGACCCAGCGUAGACAAAG |
| hsa-miR-369-3p | MIMAT0000721 | AAUAAUACAUGGUUGAUCUUU |
| hsa-miR-369-5p | MIMAT0001621 | AGAUCGACCGUGUUAUAUUCGC |
| hsa-miR-370-3p | MIMAT0000722 | GCCUGCUGGGGUGGAACCUGGU |
| hsa-miR-370-5p | MIMAT0026483 | CAGGUCACGUCUCUGCAGUUAC |
| hsa-miR-371a-5p | MIMAT0004687 | ACUCAAACUGUGGGGGCACU |
| hsa-miR-371b-5p | MIMAT0019892 | ACUCAAAAGAUGGCGGCACUUU |
| hsa-miR-372-3p | MIMAT0000724 | AAAGUGCUGCGACAUUUGAGCGU |
| hsa-miR-373-3p | MIMAT0000726 | GAAGUGCUUCGAUUUUGGGGUGU |
| hsa-miR-374a-3p | MIMAT0004688 | CUUAUCAGAUUGUAUUGUAAUU |
| hsa-miR-374a-5p | MIMAT0000727 | UUAUAAUACAACCUGAUAAGUG |
| hsa-miR-374b-5p | MIMAT0004955 | AUAUAAUACAACCUGCUAAGUG |

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| hsa-miR-374c-5p | MIMAT0018443 | AUAAUACAACCUGCUAAGUGCU |
| hsa-miR-375 | MIMAT0000728 | UUUGUUCGUUCGGCUCGCGUGA |
| hsa-miR-376a-2-5p | MIMAT0022928 | GGUAGAUUUUCCUUCUAUGGU |
| hsa-miR-376a-3p | MIMAT0000729 | AUCAUAGAGGAAAAUCCACGU |
| hsa-miR-376b-3p | MIMAT0002172 | AUCAUAGAGGAAAAUCCAUGUU |
| hsa-miR-376c-3p | MIMAT0000720 | AACAUAGAGGAAAUUCCACGU |
| hsa-miR-376c-5p | MIMAT0022861 | GGUGGAUAUUCCUUCUAUGUU |
| hsa-miR-377-3p | MIMAT0000730 | AUCACACAAAGGCAACUUUUGU |
| hsa-miR-378b | MIMAT0014999 | ACUGGACUUGGAGGCAGAA |
| hsa-miR-378c | MIMAT0016847 | ACUGGACUUGGAGUCAGAAGAGUGG |
| hsa-miR-378d | MIMAT0018926 | ACUGGACUUGGAGUCAGAAA |
| hsa-miR-378e | MIMAT0018927 | ACUGGACUUGGAGUCAGGA |
| hsa-miR-378f | MIMAT0018932 | ACUGGACUUGGAGCCAGAAG |
| hsa-miR-378g | MIMAT0018937 | ACUGGGCUUGGAGUCAGAAG |
| hsa-miR-378h | MIMAT0018984 | ACUGGACUUGGUGUCAGAUGG |
| hsa-miR-378i | MIMAT0019074 | ACUGGACUAGGAGUCAGAAGG |
| hsa-miR-379-5p | MIMAT0000733 | UGGUAGACUAUGGAACGUAGG |
| hsa-miR-380-3p | MIMAT0000735 | UAUGUAAUAUGGUCCACAUCUU |
| hsa-miR-381-3p | MIMAT0000736 | UAUACAAGGGCAAGCUCUCUGU |
| hsa-miR-381-5p | MIMAT0022862 | AGCGAGGUUGCCCUUUGUAUAU |
| hsa-miR-382-3p | MIMAT0022697 | AAUCAUUCACGGACAACACUU |
| hsa-miR-382-5p | MIMAT0000737 | GAAGUUGUUCGUGGUGGAUUCG |
| hsa-miR-383-5p | MIMAT0000738 | AGAUCAGAAGGUGAUUGUGGCU |
| hsa-miR-384 | MIMAT0001075 | AUUCCUAGAAAUUGUUCAUA |
| hsa-miR-3916 | MIMAT0018190 | AAGAGGAAGAAAUGGCUGGUUCUCAG |
| hsa-miR-3918 | MIMAT0018192 | ACAGGGCCGCAGAUGGAGACU |
| hsa-miR-3928-3p | MIMAT0018205 | GGAGGAACCUUGGAGCUUCGGC |

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| hsa-miR-3934-5p | MIMAT0018349 | UCAGGUGUGGAAACUGAGGCAG |
| hsa-miR-409-3p | MIMAT0001639 | GAAUGUUGCUCGGUGAACCCCU |
| hsa-miR-409-5p | MIMAT0001638 | AGGUUACCCGAGCAACUUUGCAU |
| hsa-miR-410-3p | MIMAT0002171 | AAUAUAACACAGAUGGCCUGU |
| hsa-miR-411-3p | MIMAT0004813 | UAUGUAACACGGUCCACUAACC |
| hsa-miR-411-5p | MIMAT0003329 | UAGUAGACCGUAUAGCGUACG |
| hsa-miR-412-3p | MIMAT0002170 | ACUUCACCUGGUCCACUAGCCGU |
| hsa-miR-421 | MIMAT0003339 | AUCAACAGACAUUAAUUGGGCGC |
| hsa-miR-422a | MIMAT0001339 | ACUGGACUUAGGGUCAGAAGGC |
| hsa-miR-423-3p | MIMAT0001340 | AGCUCGGUCUGAGGCCCCUCAGU |
| hsa-miR-423-5p | MIMAT0004748 | UGAGGGGCAGAGAGCGAGACUUU |
| hsa-miR-424-5p | MIMAT0001341 | CAGCAGCAAUUCAUGUUUUGAA |
| hsa-miR-425-5p | MIMAT0003393 | AAUGACACGAUCACUCCCGUUGA |
| hsa-miR-4284 | MIMAT0016915 | GGGCUCACAUCACCCCAU |
| hsa-miR-4286 | MIMAT0016916 | ACCCCACUCCUGGUACC |
| hsa-miR-429 | MIMAT0001536 | UAAUACUGUCUGGUAAAACCGU |
| hsa-miR-431-5p | MIMAT0001625 | UGUCUUGCAGGCCGUCAUGCA |
| hsa-miR-432-5p | MIMAT0002814 | UCUUGGAGUAGGUCAUUGGGUGG |
| hsa-miR-433-3p | MIMAT0001627 | AUCAUGAUGGGCUCCUCGGUGU |
| hsa-miR-433-5p | MIMAT0026554 | UACGGUGAGCCUGUCAUUAUUC |
| hsa-miR-4421 | MIMAT0018934 | ACCUGUCUGUGGAAAGGAGCUA |
| hsa-miR-4425 | MIMAT0018940 | UGUUGGGAUUCAGCAGGACCAU |
| hsa-miR-4431 | MIMAT0018947 | GCGACUCUGAAAACUAGAAGGU |
| hsa-miR-4435 | MIMAT0018951 | AUGGCCAGAGCUCACACAGAGG |
| hsa-miR-4443 | MIMAT0018961 | UUGGAGGCGUGGGUUUU |
| hsa-miR-4448 | MIMAT0018967 | GGCUCCUUGGUCUAGGGGUA |
| hsa-miR-4451 | MIMAT0018973 | UGGUAGAGCUGAGGACA |

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| hsa-miR-4454 | MIMAT0018976 | GGAUCCGAGUCACGGCACCA |
| hsa-miR-4455 | MIMAT0018977 | AGGGUGUGUGUGUUUUU |
| hsa-miR-4458 | MIMAT0018980 | AGAGGUAGGUGUGGAAGAA |
| hsa-miR-4461 | MIMAT0018983 | GAUUGAGACUAGUAGGGCUAGGC |
| hsa-miR-448 | MIMAT0001532 | UUGCAUAUGUAGGAUGUCCCAU |
| hsa-miR-4485-3p | MIMAT0019019 | UAACGGCCGCGGUACCCUAA |
| hsa-miR-4488 | MIMAT0019022 | AGGGGGCGGGCUCCGGCG |
| hsa-miR-449a | MIMAT0001541 | UGGCAGUGUAUUGUUAGCUGGU |
| hsa-miR-449b-5p | MIMAT0003327 | AGGCAGUGUAUUGUUAGCUGGC |
| hsa-miR-449c-5p | MIMAT0010251 | UAGGCAGUGUAUUGCUAGCGGCUGU |
| hsa-miR-450a-1-3p | MIMAT0022700 | AUUGGGAACAUUUUGCAUGUAU |
| hsa-miR-450a-2-3p | MIMAT0031074 | AUUGGGGACAUUUUGCAUUCAU |
| hsa-miR-450a-5p | MIMAT0001545 | UUUUGCGAUGUGUUCCUAAUAU |
| hsa-miR-450b-3p | MIMAT0004910 | UUGGGAUCAUUUUGCAUCCAUA |
| hsa-miR-450b-5p | MIMAT0004909 | UUUUGCAAUAUGUUCCUGAAUA |
| hsa-miR-4516 | MIMAT0019053 | GGGAGAAGGGUCGGGGC |
| hsa-miR-451a | MIMAT0001631 | AAACCGUUACCAUUACUGAGUU |
| hsa-miR-4521 | MIMAT0019058 | GCUAAGGAAGUCCUGUGCUCAG |
| hsa-miR-4524a-5p | MIMAT0019062 | AUAGCAGCAUGAACCUGUCUCA |
| hsa-miR-452-5p | MIMAT0001635 | AACUGUUUGCAGAGGAAACUGA |
| hsa-miR-4531 | MIMAT0019070 | AUGGAGAAGGCUUCUGA |
| hsa-miR-4532 | MIMAT0019071 | CCCCGGGGAGCCCGGCG |
| hsa-miR-4536-3p | MIMAT0020959 | UCGUGCAUAUAUCUACCACAU |
| hsa-miR-4536-5p | MIMAT0019078 | UGUGGUAGAUAUAUGCACGAU |
| hsa-miR-454-3p | MIMAT0003885 | UAGUGCAAUAUUGCUUAUAGGGU |
| hsa-miR-455-3p | MIMAT0004784 | GCAGUCCAUGGGCAUAUACAC |
| hsa-miR-455-5p | MIMAT0003150 | UAUGUGCCUUUGGACUACAUCG |

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| hsa-miR-4647 | MIMAT0019709 | GAAGAUGGUGCUGUGCUGAGGAA |
| hsa-miR-4707-3p | MIMAT0019808 | AGCCCGCCCCAGCCGAGGUUCU |
| hsa-miR-4707-5p | MIMAT0019807 | GCCCCGGCGCGGGCGGGUUCUGG |
| hsa-miR-4741 | MIMAT0019871 | CGGGCUGUCCGGAGGGGUCGGCU |
| hsa-miR-4755-5p | MIMAT0019895 | UUUCCCUUCAGAGCCUGGCUUU |
| hsa-miR-4787-3p | MIMAT0019957 | GAUGCGCCGCCCACUGCCCCGCGC |
| hsa-miR-4787-5p | MIMAT0019956 | GCGGGGGUGGCGGCGGCAUCCC |
| hsa-miR-4792 | MIMAT0019964 | CGGUGAGCGCUCGCUGGC |
| hsa-miR-483-3p | MIMAT0002173 | UCACUCCUCUCCUCCCGUCUU |
| hsa-miR-483-5p | MIMAT0004761 | AAGACGGGAGGAAAGAAGGGAG |
| hsa-miR-484 | MIMAT0002174 | UCAGGCUCAGUCCCCUCCCGAU |
| hsa-miR-485-3p | MIMAT0002176 | GUCAUACACGGCUCUCCUCUCU |
| hsa-miR-485-5p | MIMAT0002175 | AGAGGCUGGCCGUGAUGAAUUC |
| hsa-miR-486-3p | MIMAT0004762 | CGGGGCAGCUCAGUACAGGAU |
| hsa-miR-487a-3p | MIMAT0002178 | AAUCAUACAGGGACAUCCAGUU |
| hsa-miR-487b-3p | MIMAT0003180 | AAUCGUACAGGGUCAUCCACUU |
| hsa-miR-487b-5p | MIMAT0026614 | GUGGUUAUCCCUGUCCUGUUCG |
| hsa-miR-488-3p | MIMAT0004763 | UUGAAAGGCUAUUUCUUGGUC |
| hsa-miR-489-3p | MIMAT0002805 | GUGACAUCACAUAUACGGCAGC |
| hsa-miR-490-3p | MIMAT0002806 | CAACCUGGAGGACUCCAUGCUG |
| hsa-miR-490-5p | MIMAT0004764 | CCAUGGAUCUCCAGGUGGGU |
| hsa-miR-491-3p | MIMAT0004765 | CUUAUGCAAGAUUCCCUUCUAC |
| hsa-miR-491-5p | MIMAT0002807 | AGUGGGGAACCCUUCCAUGAGG |
| hsa-miR-492 | MIMAT0002812 | AGGACCUGCGGGACAAGAUUCUU |
| hsa-miR-493-3p | MIMAT0003161 | UGAAGGUCUACUGUGUGCCAGG |
| hsa-miR-494-3p | MIMAT0002816 | UGAAACAUACACGGGAAACCUC |
| hsa-miR-494-5p | MIMAT0026607 | AGGUUGUCCGUGUUGUCUUCUCU |

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| hsa-miR-495-3p | MIMAT0002817 | AAACAAACAUGGUGCACUUCUU |
| hsa-miR-495-5p | MIMAT0022924 | GAAGUUGCCCAUGUUAUUUUCG |
| hsa-miR-496 | MIMAT0002818 | UGAGUAUUACAUGGCCAAUCUC |
| hsa-miR-497-5p | MIMAT0002820 | CAGCAGCACACUGUGGUUUGU |
| hsa-miR-498 | MIMAT0002824 | UUUCAAGCCAGGGGGCGUUUUUC |
| hsa-miR-499a-3p | MIMAT0004772 | AACAUCACAGCAAGUCUGUGCU |
| hsa-miR-499a-5p | MIMAT0002870 | UUAAGACUUGCAGUGAUGUUU |
| hsa-miR-499b-3p | MIMAT0019898 | AACAUCACUGCAAGUCUUAACA |
| hsa-miR-499b-5p | MIMAT0019897 | ACAGACUUGCUGUGAUGUUCA |
| hsa-miR-5001-3p | MIMAT0021022 | UUCUGCCUCUGUCCAGGUCCUU |
| hsa-miR-5001-5p | MIMAT0021021 | AGGGCUGGACUCAGCGGCGGAGCU |
| hsa-miR-500a-5p | MIMAT0004773 | UAAUCCUUGCUACCUGGGUGAGA |
| hsa-miR-5010-3p | MIMAT0021044 | UUUUGUGUCUCCCAUUCCCCAG |
| hsa-miR-5010-5p | MIMAT0021043 | AGGGGGAUGGCAGAGCAAAAUU |
| hsa-miR-501-3p | MIMAT0004774 | AAUGCACCCGGGCAAGGAUUCU |
| hsa-miR-501-5p | MIMAT0002872 | AAUCCUUUGUCCCUGGGUGAGA |
| hsa-miR-502-3p | MIMAT0004775 | AAUGCACCUGGGCAAGGAUUCA |
| hsa-miR-502-5p | MIMAT0002873 | AUCCUUGCUAUCUGGGUGCUA |
| hsa-miR-503-3p | MIMAT0022925 | GGGGUAUUGUUUCCGCUGCCAGG |
| hsa-miR-503-5p | MIMAT0002874 | UAGCAGCGGGAACAGUUCUGCAG |
| hsa-miR-504-3p | MIMAT0026612 | GGGAGUGCAGGGCAGGGUUUC |
| hsa-miR-504-5p | MIMAT0002875 | AGACCCUGGUCUGCACUCUAUC |
| hsa-miR-505-3p | MIMAT0002876 | CGUCAACACUUGCUGGUUUCCU |
| hsa-miR-506-3p | MIMAT0002878 | UAAGGCACCCUUCUGAGUAGA |
| hsa-miR-506-5p | MIMAT0022701 | UAUUCAGGAAGGUGUUACUUAA |
| hsa-miR-507 | MIMAT0002879 | UUUUGCACCUUUUGGAGUGAA |
| hsa-miR-508-3p | MIMAT0002880 | UGAUUGUAGCCUUUUGGAGUAGA |

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| hsa-miR-508-5p | MIMAT0004778 | UACUCCAGAGGGCGUCACUCAUG |
| hsa-miR-509-3-5p | MIMAT0004975 | UACUGCAGACGUGGCAAUCAUG |
| hsa-miR-509-3p | MIMAT0002881 | UGAUUGGUACGUCUGUGGGUAG |
| hsa-miR-509-5p | MIMAT0004779 | UACUGCAGACAGUGGCAAUCA |
| hsa-miR-510-3p | MIMAT0026613 | AUUGAAACCUCUAAGAGUGGA |
| hsa-miR-510-5p | MIMAT0002882 | UACUCAGGAGAGUGGCAAUCAC |
| hsa-miR-511-5p | MIMAT0002808 | GUGUCUUUUGCUCUGCAGUCA |
| hsa-miR-512-3p | MIMAT0002823 | AAGUGCUGUCAUAGCUGAGGUC |
| hsa-miR-512-5p | MIMAT0002822 | CACUCAGCCUUGAGGGCACUUUC |
| hsa-miR-513a-3p | MIMAT0004777 | UAAAUUUCACCUUUCUGAGAAGG |
| hsa-miR-513a-5p | MIMAT0002877 | UUCACAGGGAGGUGUCAU |
| hsa-miR-513b-5p | MIMAT0005788 | UUCACAAGGAGGUGUCAUUUAU |
| hsa-miR-513c-3p | MIMAT0022728 | UAAAUUUCACCUUUCUGAGAAGA |
| hsa-miR-513c-5p | MIMAT0005789 | UUCUCAAGGAGGUGUCGUUUAU |
| hsa-miR-514a-3p | MIMAT0002883 | AUUGACACUUCUGUGAGUAGA |
| hsa-miR-514a-5p | MIMAT0022702 | UACUCUGGAGAGUGACAAUCAUG |
| hsa-miR-514b-3p | MIMAT0015088 | AUUGACACCUCUGUGAGUGGA |
| hsa-miR-514b-5p | MIMAT0015087 | UUCUCAAGAGGGAGGCAAUCAU |
| hsa-miR-515-3p | MIMAT0002827 | GAGUGCCUUCUUUUGGAGCGUU |
| hsa-miR-515-5p | MIMAT0002826 | UUCUCCAAAAGAAAGCACUUUCUG |
| hsa-miR-516a-3p | MIMAT0006778 | UGCUUCCUUUCAGAGGGU |
| hsa-miR-516a-5p | MIMAT0004770 | UUCUCGAGGAAAGAAGCACUUUC |
| hsa-miR-516b-3p | MIMAT0002860 | UGCUUCCUUUCAGAGGGU |
| hsa-miR-516b-5p | MIMAT0002859 | AUCUGGAGGUAAGAAGCACUUU |
| hsa-miR-517a-3p | MIMAT0002852 | AUCGUGCAUCCCUUUAGAGUGU |
| hsa-miR-517b-3p | MIMAT0002857 | AUCGUGCAUCCCUUUAGAGUGU |
| hsa-miR-517c-3p | MIMAT0002866 | AUCGUGCAUCCUUUUAGAGUGU |

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| hsa-miR-518a-5p | MIMAT0005457 | CUGCAAAGGGAAGCCCUUUC |
| hsa-miR-518b | MIMAT0002844 | CAAAGCGCUCCCCUUUAGAGGU |
| hsa-miR-518c-3p | MIMAT0002848 | CAAAGCGCUUCUCUUUAGAGUGU |
| hsa-miR-518c-5p | MIMAT0002847 | UCUCUGGAGGGAAGCACUUUCUG |
| hsa-miR-518d-3p | MIMAT0002864 | CAAAGCGCUUCCCUUUGGAGC |
| hsa-miR-518d-5p | MIMAT0005456 | CUCUAGAGGGAAGCACUUUCUG |
| hsa-miR-518e-3p | MIMAT0002861 | AAAGCGCUUCCCUUCAGAGUG |
| hsa-miR-518e-5p | MIMAT0005450 | CUCUAGAGGGAAGCGCUUUCUG |
| hsa-miR-518f-3p | MIMAT0002842 | GAAAGCGCUUCUCUUUAGAGG |
| hsa-miR-5196-3p | MIMAT0021129 | UCAUCCUCGUCUCCCUCCCAG |
| hsa-miR-5196-5p | MIMAT0021128 | AGGGAAGGGGACGAGGGUUGGG |
| hsa-miR-519a-3p | MIMAT0002869 | AAAGUGCAUCCUUUUAGAGUGU |
| hsa-miR-519a-5p | MIMAT0005452 | CUCUAGAGGGAAGCGCUUUCUG |
| hsa-miR-519b-3p | MIMAT0002837 | AAAGUGCAUCCUUUUAGAGGUU |
| hsa-miR-519b-5p | MIMAT0005454 | CUCUAGAGGGAAGCGCUUUCUG |
| hsa-miR-519c-3p | MIMAT0002832 | AAAGUGCAUCUUUUUAGAGGAU |
| hsa-miR-519c-5p | MIMAT0002831 | CUCUAGAGGGAAGCGCUUUCUG |
| hsa-miR-519d-3p | MIMAT0002853 | CAAAGUGCCUCCCUUUAGAGUG |
| hsa-miR-519e-3p | MIMAT0002829 | AAGUGCCUCCUUUUAGAGUGUU |
| hsa-miR-520a-3p | MIMAT0002834 | AAAGUGCUUCCCUUUGGACUGU |
| hsa-miR-520a-5p | MIMAT0002833 | CUCCAGAGGGAAGUACUUUCU |
| hsa-miR-520b | MIMAT0002843 | AAAGUGCUUCCUUUUAGAGGG |
| hsa-miR-520c-3p | MIMAT0002846 | AAAGUGCUUCCUUUUAGAGGGU |
| hsa-miR-520c-5p | MIMAT0005455 | CUCUAGAGGGAAGCACUUUCUG |
| hsa-miR-520d-3p | MIMAT0002856 | AAAGUGCUUCUCUUUGGUGGGU |
| hsa-miR-520d-5p | MIMAT0002855 | CUACAAAGGGAAGCCCUUUC |
| hsa-miR-520e | MIMAT0002825 | AAAGUGCUUCCUUUUUGAGGG |

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| hsa-miR-520f-3p | MIMAT0002830 | AAGUGCUUCCUUUUAGAGGGUU |
| hsa-miR-520g-3p | MIMAT0002858 | ACAAAGUGCUUCCCUUUAGAGUGU |
| hsa-miR-520h | MIMAT0002867 | ACAAAGUGCUUCCCUUUAGAGU |
| hsa-miR-521 | MIMAT0002854 | AACGCACUUCCCUUUAGAGUGU |
| hsa-miR-522-3p | MIMAT0002868 | AAAAUGGUUCCCUUUAGAGUGU |
| hsa-miR-522-5p | MIMAT0005451 | CUCUAGAGGGAAGCGCUUUCUG |
| hsa-miR-523-3p | MIMAT0002840 | GAACGCGCUUCCCUAUAGAGGGU |
| hsa-miR-523-5p | MIMAT0005449 | CUCUAGAGGGAAGCGCUUUCUG |
| hsa-miR-524-3p | MIMAT0002850 | GAAGGCGCUUCCCUUUGGAGU |
| hsa-miR-525-3p | MIMAT0002839 | GAAGGCGCUUCCCUUUAGAGCG |
| hsa-miR-525-5p | MIMAT0002838 | CUCCAGAGGGAUGCACUUUCU |
| hsa-miR-526a | MIMAT0002845 | CUCUAGAGGGAAGCACUUUCUG |
| hsa-miR-526b-5p | MIMAT0002835 | CUCUUGAGGGAAGCACUUUCUGU |
| hsa-miR-527 | MIMAT0002862 | CUGCAAAGGGAAGCCCUUUC |
| hsa-miR-532-3p | MIMAT0004780 | CCUCCCACACCCAAGGCUUGCA |
| hsa-miR-532-5p | MIMAT0002888 | CAUGCCUUGAGUGUAGGACCGU |
| hsa-miR-539-3p | MIMAT0022705 | AUCAUACAAGGACAAUUUCUUU |
| hsa-miR-539-5p | MIMAT0003163 | GGAGAAAUUAUCCUUGGUGUGU |
| hsa-miR-541-3p | MIMAT0004920 | UGGUGGGCACAGAAUCUGGACU |
| hsa-miR-542-3p | MIMAT0003389 | UGUGACAGAUUGAUAACUGAAA |
| hsa-miR-542-5p | MIMAT0003340 | UCGGGGAUCAUCAUGUCACGAGA |
| hsa-miR-543 | MIMAT0004954 | AAACAUUCGCGGUGCACUUCUU |
| hsa-miR-544a | MIMAT0003164 | AUUCUGCAUUUUUAGCAAGUUC |
| hsa-miR-545-3p | MIMAT0003165 | UCAGCAAACAUUUAUUGUGUGC |
| hsa-miR-548a-3p | MIMAT0003251 | CAAAACUGGCAAUUACUUUUGC |
| hsa-miR-548a-5p | MIMAT0004803 | AAAAGUAAUUGCGAGUUUUACC |
| hsa-miR-548aa | MIMAT0018447 | AAAAACCACAAUUACUUUUGCACCA |

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| hsa-miR-548ad-3p | MIMAT0018946 | GAAAACGACAAUGACUUUUGCA |
| hsa-miR-548ah-3p | MIMAT0020957 | CAAAAACUGCAGUUACUUUUGC |
| hsa-miR-548ah-5p | MIMAT0018972 | AAAAGUGAUUGCAGUGUUUG |
| hsa-miR-548ai | MIMAT0018989 | AAAGGUAAUUGCAGUUUUUCCC |
| hsa-miR-548ak | MIMAT0019013 | AAAAGUAACUGCGGUUUUUGA |
| hsa-miR-548al | MIMAT0019024 | AACGGCAAUGACUUUUGUACCA |
| hsa-miR-548am-5p | MIMAT0022740 | AAAAGUAAUUGCGGUUUUUGCC |
| hsa-miR-548ar-3p | MIMAT0022266 | UAAAACUGCAGUUAUUUUUGC |
| hsa-miR-548ar-5p | MIMAT0022265 | AAAAGUAAUUGCAGUUUUUGC |
| hsa-miR-548av-3p | MIMAT0022304 | AAAACUGCAGUUACUUUUGC |
| hsa-miR-548b-3p | MIMAT0003254 | CAAGAACCUCAGUUGCUUUUGU |
| hsa-miR-548c-5p | MIMAT0004806 | AAAAGUAAUUGCGGUUUUUGCC |
| hsa-miR-548d-3p | MIMAT0003323 | CAAAAACCACAGUUUCUUUUGC |
| hsa-miR-548d-5p | MIMAT0004812 | AAAAGUAAUUGUGGUUUUUGCC |
| hsa-miR-548e-3p | MIMAT0005874 | AAAAACUGAGACUACUUUUGCA |
| hsa-miR-548e-5p | MIMAT0026736 | CAAAAGCAAUCGCGGUUUUUGC |
| hsa-miR-548g-3p | MIMAT0005912 | AAAACUGUAAUUACUUUUGUAC |
| hsa-miR-548h-3p | MIMAT0022723 | CAAAAACCGCAAUUACUUUUGCA |
| hsa-miR-548h-5p | MIMAT0005928 | AAAAGUAAUCGCGGUUUUUGUC |
| hsa-miR-548i | MIMAT0005935 | AAAAGUAAUUGCGGAUUUUGCC |
| hsa-miR-548j-3p | MIMAT0026737 | CAAAAACUGCAUUACUUUUGC |
| hsa-miR-548j-5p | MIMAT0005875 | AAAAGUAAUUGCGGUCUUUGGU |
| hsa-miR-548k | MIMAT0005882 | AAAAGUACUUGCGGAUUUUGCU |
| hsa-miR-548l | MIMAT0005889 | AAAAGUAUUUGCGGGUUUUGUC |
| hsa-miR-548m | MIMAT0005917 | CAAAGGUAUUUGUGGUUUUUG |
| hsa-miR-548n | MIMAT0005916 | CAAAAGUAAUUGUGGAUUUUGU |
| hsa-miR-548o-3p | MIMAT0005919 | CCAAAACUGCAGUUACUUUUGC |

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| hsa-miR-548o-5p | MIMAT0022738 | AAAAGUAAUUGCGGUUUUUGCC |
| hsa-miR-548q | MIMAT0011163 | GCUGGUGCAAAAGUAAUGGCGG |
| hsa-miR-548t-3p | MIMAT0022730 | AAAAACCACAAUUACUUUUGCACCA |
| hsa-miR-548v | MIMAT0015020 | AGCUACAGUUACUUUUGCACCA |
| hsa-miR-548y | MIMAT0018354 | AAAAGUAAUCACUGUUUUUGCC |
| hsa-miR-548z | MIMAT0018446 | CAAAAACCGCAAUUACUUUUGCA |
| hsa-miR-549a | MIMAT0003333 | UGACAACUAUGGAUGAGCUCU |
| hsa-miR-550a-5p | MIMAT0004800 | AGUGCCUGAGGGAGUAAGAGCCC |
| hsa-miR-551a | MIMAT0003214 | GCGACCCACUCUUGGUUUCCA |
| hsa-miR-551b-3p | MIMAT0003233 | GCGACCCAUACUUGGUUUCAG |
| hsa-miR-552-3p | MIMAT0003215 | AACAGGUGACUGGUUAGACAA |
| hsa-miR-553 | MIMAT0003216 | AAAACGGUGAGAUUUUGUUUU |
| hsa-miR-554 | MIMAT0003217 | GCUAGUCCUGACUCAGCCAGU |
| hsa-miR-555 | MIMAT0003219 | AGGGUAAGCUGAACCUCUGAU |
| hsa-miR-556-3p | MIMAT0004793 | AUAUUACCAUUAGCUCAUCUUU |
| hsa-miR-556-5p | MIMAT0003220 | GAUGAGCUCAUUGUAAUAUGAG |
| hsa-miR-561-3p | MIMAT0003225 | CAAAGUUUAAGAUCCUUGAAGU |
| hsa-miR-561-5p | MIMAT0022706 | AUCAAGGAUCUUAAACUUUGCC |
| hsa-miR-562 | MIMAT0003226 | AAAGUAGCUGUACCAUUUGC |
| hsa-miR-563 | MIMAT0003227 | AGGUUGACAUACGUUUCCC |
| hsa-miR-564 | MIMAT0003228 | AGGCACGGUGUCAGCAGGC |
| hsa-miR-566 | MIMAT0003230 | GGGCGCCUGUGAUCCCAAC |
| hsa-miR-567 | MIMAT0003231 | AGUAUGUUCUUCCAGGACAGAAC |
| hsa-miR-568 | MIMAT0003232 | AUGUAUAAAUGUAUACACAC |
| hsa-miR-570-3p | MIMAT0003235 | CGAAAACAGCAAUUACCUUUGC |
| hsa-miR-570-5p | MIMAT0022707 | AAAGGUAAUUGCAGUUUUUCCC |
| hsa-miR-571 | MIMAT0003236 | UGAGUUGGCCAUCUGAGUGAG |

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| hsa-miR-572 | MIMAT0003237 | GUCCGCUCGGCGGUGGCCCA |
| hsa-miR-573 | MIMAT0003238 | CUGAAGUGAUGUGUAACUGAUCAG |
| hsa-miR-574-3p | MIMAT0003239 | CACGCUCAUGCACACACCCACA |
| hsa-miR-574-5p | MIMAT0004795 | UGAGUGUGUGUGUGUGAGUGUGU |
| hsa-miR-575 | MIMAT0003240 | GAGCCAGUUGGACAGGAGC |
| hsa-miR-576-3p | MIMAT0004796 | AAGAUGUGGAAAAAUUGGAAUC |
| hsa-miR-576-5p | MIMAT0003241 | AUUCUAAUUUCUCCACGUCUUU |
| hsa-miR-577 | MIMAT0003242 | UAGAUAAAAUAUUGGUACCUG |
| hsa-miR-578 | MIMAT0003243 | CUUCUUGUGCUCUAGGAUUGU |
| hsa-miR-579-3p | MIMAT0003244 | UUCAUUUGGUAUAAACCGCGAUU |
| hsa-miR-579-5p | MIMAT0026616 | UCGCGGUUUGUGCCAGAUGACG |
| hsa-miR-580-3p | MIMAT0003245 | UUGAGAAUGAUGAAUCAUUAGG |
| hsa-miR-582-3p | MIMAT0004797 | UAACUGGUUGAACAACUGAACC |
| hsa-miR-582-5p | MIMAT0003247 | UUACAGUUGUUCAACCAGUUACU |
| hsa-miR-584-3p | MIMAT0022708 | UCAGUUCCAGGCCAACCAGGCU |
| hsa-miR-584-5p | MIMAT0003249 | UUAUGGUUUGCCUGGGACUGAG |
| hsa-miR-585-3p | MIMAT0003250 | UGGGCGUAUCUGUAUGCUA |
| hsa-miR-587 | MIMAT0003253 | UUUCCAUAGGUGAUGAGUCAC |
| hsa-miR-589-5p | MIMAT0004799 | UGAGAACCACGUCUGCUCUGAG |
| hsa-miR-590-3p | MIMAT0004801 | UAAUUUUAUGUAUAAGCUAGU |
| hsa-miR-590-5p | MIMAT0003258 | GAGCUUAUUCAUAAAAGUGCAG |
| hsa-miR-591 | MIMAT0003259 | AGACCAUGGGUUCUCAUUGU |
| hsa-miR-592 | MIMAT0003260 | UUGUGUCAAUAUGCGAUGAUGU |
| hsa-miR-593-3p | MIMAT0004802 | UGUCUCUGCUGGGGUUUCU |
| hsa-miR-595 | MIMAT0003263 | GAAGUGUGCCGUGGUGUGUCU |
| hsa-miR-596 | MIMAT0003264 | AAGCCUGCCCGGCUCCUCGGG |
| hsa-miR-597-5p | MIMAT0003265 | UGUGUCACUCGAUGACCACUGU |

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| hsa-miR-598-3p | MIMAT0003266 | UACGUCAUCGUUGUCAUCGUCA |
| hsa-miR-599 | MIMAT0003267 | GUUGUGUCAGUUUAUCAAAC |
| hsa-miR-600 | MIMAT0003268 | ACUUACAGACAAGAGCCUUGCUC |
| hsa-miR-601 | MIMAT0003269 | UGGUCUAGGAUUGUUGGAGGAG |
| hsa-miR-603 | MIMAT0003271 | CACACACUGCAAUUACUUUUGC |
| hsa-miR-604 | MIMAT0003272 | AGGCUGCGGAAUUCAGGAC |
| hsa-miR-605-5p | MIMAT0003273 | UAAAUCCCAUGGUGCCUUCUCCU |
| hsa-miR-606 | MIMAT0003274 | AAACUACUGAAAAUCAAAGAU |
| hsa-miR-607 | MIMAT0003275 | GUUCAAAUCCAGAUCUAUAAC |
| hsa-miR-608 | MIMAT0003276 | AGGGGUGGUGUUGGGACAGCUCCGU |
| hsa-miR-610 | MIMAT0003278 | UGAGCUAAAUGUGUGCUGGGA |
| hsa-miR-612 | MIMAT0003280 | GCUGGGCAGGGCUUCUGAGCUCCUU |
| hsa-miR-613 | MIMAT0003281 | AGGAAUGUUCCUUCUUUGCC |
| hsa-miR-614 | MIMAT0003282 | GAACGCCUGUUCUUGCCAGGUGG |
| hsa-miR-615-3p | MIMAT0003283 | UCCGAGCCUGGGUCUCCCUCUU |
| hsa-miR-615-5p | MIMAT0004804 | GGGGGUCCCCGGUGCUCGGAUC |
| hsa-miR-616-3p | MIMAT0004805 | AGUCAUUGGAGGGUUUGAGCAG |
| hsa-miR-617 | MIMAT0003286 | AGACUUCCCAUUUGAAGGUGGC |
| hsa-miR-619-3p | MIMAT0003288 | GACCUGGACAUGUUUGUGCCCAGU |
| hsa-miR-620 | MIMAT0003289 | AUGGAGAUAGAUAUAGAAAU |
| hsa-miR-624-3p | MIMAT0004807 | CACAAGGUAUUGGUAUUACCU |
| hsa-miR-625-5p | MIMAT0003294 | AGGGGGAAAGUUCUAUAGUCC |
| hsa-miR-626 | MIMAT0003295 | AGCUGUCUGAAAAUGUCUU |
| hsa-miR-627-3p | MIMAT0026623 | UCUUUUCUUUGAGACUCACU |
| hsa-miR-627-5p | MIMAT0003296 | GUGAGUCUCUAAGAAAAGAGGA |
| hsa-miR-628-3p | MIMAT0003297 | UCUAGUAAGAGUGGCAGUCGA |
| hsa-miR-628-5p | MIMAT0004809 | AUGCUGACAUAUUUACUAGAGG |

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| hsa-miR-629-5p | MIMAT0004810 | UGGGUUUACGUUGGGAGAACU |
| hsa-miR-630 | MIMAT0003299 | AGUAUUCUGUACCAGGGAAGGU |
| hsa-miR-631 | MIMAT0003300 | AGACCUGGCCCAGACCUCAGC |
| hsa-miR-637 | MIMAT0003307 | ACUGGGGGCUUUCGGGCUCUGCGU |
| hsa-miR-638 | MIMAT0003308 | AGGGAUCGCGGGCGGGUGGCGGCCU |
| hsa-miR-639 | MIMAT0003309 | AUCGCUGCGGUUGCGAGCGCUGU |
| hsa-miR-640 | MIMAT0003310 | AUGAUCCAGGAACCUGCCUCU |
| hsa-miR-641 | MIMAT0003311 | AAAGACAUAGGAUAGAGUCACCUC |
| hsa-miR-642a-3p | MIMAT0020924 | AGACACAUUUGGAGAGGGAACC |
| hsa-miR-642a-5p | MIMAT0003312 | GUCCCUCUCCAAAUGUGUCUUG |
| hsa-miR-643 | MIMAT0003313 | ACUUGUAUGCUAGCUCAGGUAG |
| hsa-miR-644a | MIMAT0003314 | AGUGUGGCUUUCUUAGAGC |
| hsa-miR-648 | MIMAT0003318 | AAGUGUGCAGGGCACUGGU |
| hsa-miR-649 | MIMAT0003319 | AAACCUGUGUUGUUCAAGAGUC |
| hsa-miR-650 | MIMAT0003320 | AGGAGGCAGCGCUCUCAGGAC |
| hsa-miR-6503-3p | MIMAT0025463 | GGGACUAGGAUGCAGACCUCC |
| hsa-miR-6503-5p | MIMAT0025462 | AGGUCUGCAUUCAAAUCCCCAGA |
| hsa-miR-6511a-3p | MIMAT0025479 | CCUCACCAUCCCUUCUGCCUGC |
| hsa-miR-6511a-5p | MIMAT0025478 | CAGGCAGAAGUGGGGCUGACAGG |
| hsa-miR-651-3p | MIMAT0026624 | AAAGGAAAGUGUAUCCUAAAAG |
| hsa-miR-651-5p | MIMAT0003321 | UUUAGGAUAAGCUUGACUUUUG |
| hsa-miR-652-3p | MIMAT0003322 | AAUGGCGCCACUAGGGUUGUG |
| hsa-miR-652-5p | MIMAT0022709 | CAACCCUAGGAGAGGGUGCCAUUCA |
| hsa-miR-654-3p | MIMAT0004814 | UAUGUCUGCUGACCAUCACCUU |
| hsa-miR-654-5p | MIMAT0003330 | UGGUGGGCCGCAGAACAUGUGC |
| hsa-miR-655-3p | MIMAT0003331 | AUAAUACAUGGUUAACCUCUUU |
| hsa-miR-656-3p | MIMAT0003332 | AAUAUUAUACAGUCAACCUCU |

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|---|---|---|
| hsa-miR-660-3p | MIMAT0022711 | ACCUCCUGUGUGCAUGGAUUA |
| hsa-miR-660-5p | MIMAT0003338 | UACCCAUUGCAUAUCGGAGUUG |
| hsa-miR-661 | MIMAT0003324 | UGCCUGGGUCUCUGGCCUGCGCGU |
| hsa-miR-663a | MIMAT0003326 | AGGCGGGGCGCCGCGGGACCGC |
| hsa-miR-664a-3p | MIMAT0005949 | UAUUCAUUUAUCCCCAGCCUACA |
| hsa-miR-664b-3p | MIMAT0022272 | UUCAUUUGCCUCCCAGCCUACA |
| hsa-miR-664b-5p | MIMAT0022271 | UGGGCUAAGGGAGAUGAUUGGGUA |
| hsa-miR-665 | MIMAT0004952 | ACCAGGAGGCUGAGGCCCCU |
| hsa-miR-671-3p | MIMAT0004819 | UCCGGUUCUCAGGGCUCCACC |
| hsa-miR-671-5p | MIMAT0003880 | AGGAAGCCCUGGAGGGGCUGGAG |
| hsa-miR-6720-3p | MIMAT0025851 | CGCGCCUGCAGGAACUGGUAGA |
| hsa-miR-6721-5p | MIMAT0025852 | UGGGCAGGGGCUUAUUGUAGGAG |
| hsa-miR-6724-5p | MIMAT0025856 | CUGGGCCCGCGGCGGGCGUGGGG |
| hsa-miR-6728-5p | MIMAT0027357 | UUGGGAUGGUAGGACCAGAGGGG |
| hsa-miR-6732-3p | MIMAT0027366 | UAACCCUGUCCUCUCCCUCCCAG |
| hsa-miR-675-5p | MIMAT0004284 | UGGUGCGGAGAGGGCCCACAGUG |
| hsa-miR-708-5p | MIMAT0004926 | AAGGAGCUUACAAUCUAGCUGGG |
| hsa-miR-744-5p | MIMAT0004945 | UGCGGGGCUAGGGCUAACAGCA |
| hsa-miR-758-3p | MIMAT0003879 | UUUGUGACCUGGUCCACUAACC |
| hsa-miR-758-5p | MIMAT0022929 | GAUGGUUGACCAGAGAGCACAC |
| hsa-miR-7-5p | MIMAT0000252 | UGGAAGACUAGUGAUUUUGUUGU |
| hsa-miR-760 | MIMAT0004957 | CGGCUCUGGGUCUGUGGGGA |
| hsa-miR-761 | MIMAT0010364 | GCAGCAGGGUGAAACUGACACA |
| hsa-miR-764 | MIMAT0010367 | GCAGGUGCUCACUUGUCCUCCU |
| hsa-miR-765 | MIMAT0003945 | UGGAGGAGAAGGAAGGUGAUG |
| hsa-miR-766-3p | MIMAT0003888 | ACUCCAGCCCCACAGCCUCAGC |
| hsa-miR-766-5p | MIMAT0022714 | AGGAGGAAUUGGUGCUGGUCUU |

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| hsa-miR-767-3p | MIMAT0003883 | UCUGCUCAUACCCCAUGGUUUCU |
| hsa-miR-767-5p | MIMAT0003882 | UGCACCAUGGUUGUCUGAGCAUG |
| hsa-miR-769-3p | MIMAT0003887 | CUGGGAUCUCCGGGGUCUUGGUU |
| hsa-miR-769-5p | MIMAT0003886 | UGAGACCUCUGGGUUCUGAGCU |
| hsa-miR-770-5p | MIMAT0003948 | UCCAGUACCACGUGUCAGGGCCA |
| hsa-miR-7975 | MIMAT0031178 | AUCCUAGUCACGGCACCA |
| hsa-miR-802 | MIMAT0004185 | CAGUAACAAAGAUUCAUCCUUGU |
| hsa-miR-873-3p | MIMAT0022717 | GGAGACUGAUGAGUUCCCGGGA |
| hsa-miR-873-5p | MIMAT0004953 | GCAGGAACUUGUGAGUCUCCU |
| hsa-miR-874-3p | MIMAT0004911 | CUGCCCUGGCCCGAGGGACCGA |
| hsa-miR-874-5p | MIMAT0026718 | CGGCCCCACGCACCAGGGUAAGA |
| hsa-miR-875-3p | MIMAT0004923 | CCUGGAAACACUGAGGUUGUG |
| hsa-miR-876-3p | MIMAT0004925 | UGGUGGUUUACAAAGUAAUUCA |
| hsa-miR-876-5p | MIMAT0004924 | UGGAUUUCUUUGUGAAUCACCA |
| hsa-miR-877-5p | MIMAT0004949 | GUAGAGGAGAUGGCGCAGGG |
| hsa-miR-885-3p | MIMAT0004948 | AGGCAGCGGGGUGUAGUGGAUA |
| hsa-miR-885-5p | MIMAT0004947 | UCCAUUACACUACCCUGCCUCU |
| hsa-miR-887-3p | MIMAT0004951 | GUGAACGGGCGCCAUCCCGAGG |
| hsa-miR-887-5p | MIMAT0026720 | CUUGGGAGCCCUGUUAGACUC |
| hsa-miR-888-5p | MIMAT0004916 | UACUCAAAAAGCUGUCAGUCA |
| hsa-miR-889-3p | MIMAT0004921 | UUAAUAUCGGACAACCAUUGU |
| hsa-miR-890 | MIMAT0004912 | UACUUGGAAAGGCAUCAGUUG |
| hsa-miR-891a-5p | MIMAT0004902 | UGCAACGAACCUGAGCCACUGA |
| hsa-miR-891b | MIMAT0004913 | UGCAACUUACCUGAGUCAUUGA |
| hsa-miR-892a | MIMAT0004907 | CACUGUGUCCUUUCUGCGUAG |
| hsa-miR-892b | MIMAT0004918 | CACUGGCUCCUUUCUGGGUAGA |
| hsa-miR-922 | MIMAT0004972 | GCAGCAGAGAAUAGGACUACGUC |

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|---|---|---|
| hsa-miR-924 | MIMAT0004974 | AGAGUCUUGUGAUGUCUUGC |
| hsa-miR-92a-1-5p | MIMAT0004507 | AGGUUGGGAUCGGUUGCAAUGCU |
| hsa-miR-92a-3p | MIMAT0000092 | UAUUGCACUUGUCCCGGCCUGU |
| hsa-miR-92b-3p | MIMAT0003218 | UAUUGCACUCGUCCCGGCCUCC |
| hsa-miR-933 | MIMAT0004976 | UGUGCGCAGGGAGACCUCUCCC |
| hsa-miR-934 | MIMAT0004977 | UGUCUACUACUGGAGACACUGG |
| hsa-miR-935 | MIMAT0004978 | CCAGUUACCGCUUCCGCUACCGC |
| hsa-miR-93-5p | MIMAT0000093 | CAAAGUGCUGUUCGUGCAGGUAG |
| hsa-miR-936 | MIMAT0004979 | ACAGUAGAGGGAGGAAUCGCAG |
| hsa-miR-937-3p | MIMAT0004980 | AUCCGCGCUCUGACUCUCUGCC |
| hsa-miR-939-5p | MIMAT0004982 | UGGGGAGCUGAGGCUCUGGGGGUG |
| hsa-miR-940 | MIMAT0004983 | AAGGCAGGGCCCCCGCUCCCC |
| hsa-miR-941 | MIMAT0004984 | CACCCGGCUGUGUGCACAUGUGC |
| hsa-miR-942-3p | MIMAT0026734 | CACAUGGCCGAAACAGAGAAGU |
| hsa-miR-942-5p | MIMAT0004985 | UCUUCUCUGUUUUGGCCAUGUG |
| hsa-miR-944 | MIMAT0004987 | AAAUUAUUGUACAUCGGAUGAG |
| hsa-miR-95-3p | MIMAT0000094 | UUCAACGGGUAUUUAUUGAGCA |
| hsa-miR-9-5p | MIMAT0000441 | UCUUUGGUUAUCUAGCUGUAUGA |
| hsa-miR-96-5p | MIMAT0000095 | UUUGGCACUAGCACAUUUUUGCU |
| hsa-miR-98-3p | MIMAT0022842 | CUAUACAACUUACUACUUUCCC |
| hsa-miR-98-5p | MIMAT0000096 | UGAGGUAGUAAGUUGUAUUGUU |
| hsa-miR-99a-5p | MIMAT0000097 | AACCCGUAGAUCCGAUCUUGUG |
| hsa-miR-99b-5p | MIMAT0000689 | CACCCGUAGAACCGACCUUGCG |

## Non-Mammalian Spike In miRNA probes

| | |
|---|---|
| ath-miR159a | MIMAT0000177 |
| cel-miR-248 | MIMAT0000304 |
| cel-miR-254 | MIMAT0000310 |
| osa-miR414 | MIMAT0001330 |
| osa-miR442 | MIMAT0001605 |

## Internal Reference Genes

| | |
|---|---|
| ACTB | NM_001101.2 |
| B2M | NM_004048.2 |
| GAPDH | NM_002046.3 |
| RPL19 | NM_000981.3 |
| RPLP0 | NM_001002.3 |

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