

Are MicroRNA 190 and MicroRNA95-3P in the Circulation Can Be Used As Predictive Bioindicators in Differentiated Thyroid Cancer in Patients with Atypia of Undetermined Significance Based on Thyroid Fine Needle Aspiration Biopsy Results?

Özgün Arařtırma
Research Article

Tiroid İnce İğne Aspirasyon Biyopsisi Önemi Belirsiz Atipi Olan Hastalarda Dolařımdaki MikroRNA 190 ve MikroRNA95-3P Diferansiye Tiroid Kanserini Öngörebilen Birer Biyobelirteç Midir?

Mustafa Gökhan Ünsal[®], Erdem Barıř Çartı[®], Mustafa Ünübol[®], Elif Duygu Topan[®]
Zehra Erdemir[®], Engin Güney[®], Nesibe Kahraman Çetin[®], İbrahim Halil Erdoğan[®],
Ulař Utku řekerci[®]

ABSTRACT

Objective: It is not always possible to make a definitive diagnosis preoperatively based on thyroid fine needle aspiration biopsy results. Decision making is quite complicated in cases with atypia of undetermined significance (AUS). MicroRNAs have been shown to be associated with the development of neoplasia. For this purpose, we aimed to investigate if the circulating microRNA 190 and microRNA 95-3P can be used as biomarkers to distinguish preoperatively between postoperatively detected benign and malignant cases based on pathological evaluation of thyroid fine needle aspiration biopsy (FNAB) specimens which established the diagnosis of atypia of undetermined significance (AUS)

Method: Patients with diagnosis of atypia of undetermined significance based on preoperative fine needle aspiration biopsy results as malignant (n=29) or benign (29) were included in the study. Venous blood samples were isolated using a specific miRNA kit.

Results: According to the postoperative pathology results, statistically significant differences were detected between thyroid cancer, and benign cases as for circulating levels of miRNA 190 and miRNA 95-3p.

Conclusion: It has been thought that miRNA 95 and miRNA 190 assessment can help to differentiate thyroid cancer from benign thyroid nodules and may be useful in avoiding unnecessary surgery in patients with atypical cells of undetermined significance.

Keywords: miRNA, fine needle aspiration biopsy, atypical cells of undetermined significance

ÖZ

Amaç: TİAB'leri ile preoperatif olarak her zaman kesin tanı konulamamaktadır. Önemi belirsiz atipi saptanan olgularda karar vermek oldukça karmaşıktır. MikroRNA'ların neoplazi gelişimi ile ilişkili olduğu gösterilmiştir. Bu amaçla çalışmamızda, TİAB'ı ÖBA saptanıp post-operatif patoloji sonucu benign ve malignant olan olguları preoperatif dönemde birbirinden ayırt etmede bir biyobelirteç olarak dolařımdaki mikroRNA 190 ve mikroRNA 95-3P düzeylerinin araştırılması amaçlandı.

Yöntem: Çalışmaya preoperatif ince iğne aspirasyon biyopsisinde önemi belirsiz atipi saptanan hastalar dahil edildi. Operasyon sonrası patoloji sonucu malignant ve benign olan 29'ar hasta çalışmaya alındı. Hastalardan alınan venöz kan örnekler miRNA'ta özgü bir kit kullanılarak izole edildi.

Bulgular: Ameliyat sonrası patoloji sonuçlarına göre tiroid kanseri saptanan olgularla benign olan olgular arasında dolařımdaki miRNA 190 ve miRNA 95-3p arasında istatistiksel olarak anlamlı farklılık saptandı.

Sonuç: Dolařımdaki miRNA 95 ve miRNA 190'ın TİAB sonucu belirsiz olan hastalarda, tiroid kanserini benign tiroid nodüllerinden ayırmada yardımcı olabileceğini ve gereksiz cerrahiden kaçınmada faydalı olabileceği düşünülmektedir.

Anahtar kelimeler: miRNA, ince iğne aspirasyon biyopsisi, önemi belirsiz atipi

Received/Geliř: 13.07.2020
Accepted/Kabul: 18.08.2020
Published Online: 05.01.2021

Mustafa Gökhan Ünsal
Adnan Menderes Üniversitesi
Tıp Fakültesi, Genel Cerrahi
Anabilim Dalı,
Aydın - Türkiye

✉ mustafagokhanunsal@yahoo.com
ORCID: 0000-0002-6691-7511

E. B. Çartı 0000-0002-7139-0339
M. Ünübol 0000-0002-6444-3500
E. D. Topan 0000-0003-0097-1897
Z. Erdemir 0000-0001-8690-7612
E. Güney 0000-0001-6555-6729
N. K. Çetin 0000-0002-4549-1670
İ. H. Erdoğan 0000-0002-5445-2649
U. U. řekerci 0000-0002-2056-9590
Adnan Menderes Üniversitesi
Tıp Fakültesi, Genel Cerrahi
Anabilim Dalı,
Aydın Türkiye

Cite as: Ünsal MG, Çartı EB, Ünübol M, et al. Are MicroRNA 190 and MicroRNA95-3P in the circulation can be used as predictive bioindicators in differentiated thyroid cancer in patients with atypia of undetermined significance based on thyroid fine needle aspiration biopsy results?. Tepecik Eđit. ve Arařt. Hast. Dergisi. 2020;30(3):240-5.

© Telif hakkı T.C. Sađlık Bakanlıđı İzmir Tepecik Eđit. ve Arařt. Hastanesi. Logos Tıp Yayıncılık tarafından yayınlanmaktadır. Bu dergide yayınlanan bütün makaleler Creative Commons Atıf-GayriTicari 4.0 Uluslararası Lisansı ile lisanslanmıştır.

© Copyright Association of Publication of the T.C. Ministry of Health Izmir Tepecik Education and Research Hospital. This journal published by Logos Medical Publishing.

Licensed by Creative Commons Attribution-NonCommercial 4.0 International (CC BY)



INTRODUCTION

Thyroid cancers are the most common endocrine malignancy ⁽¹⁾. Papillary thyroid cancer is the most common among all thyroid cancers ⁽²⁾. The gold standard method for detecting thyroid cancers is thyroid fine needle aspiration biopsy (FNAB). However, definitive diagnosis cannot always be made preoperatively using FNAB. According to the Bethesda classification especially cases with atypical cells of undetermined significance (ACUS) detected in their FNAB specimens include the group of patients where decision making is a very complex process. The incidence of postoperative thyroid cancer in nodules with ACUS cytology is reported as 5-15%. For this group of patients, after the evaluation of clinical and sonographic features, it is recommended to perform FNA biopsy or molecular test again to support malignancy risk assessment instead of direct progression with a strategy for follow-up or diagnostic surgery. If recurrent FNAB, molecular test, or both are not performed or results cannot be obtained, it is stated that surgery or follow-up can be chosen depending on clinical risk factors, USG features and patient preference ⁽³⁾.

MicroRNAs (miRNA) are small, protein-free RNA molecules. They play a role in processes such as development, differentiation, proliferation and cell death by suppressing one or more target genes ⁽⁴⁾. More than 50% of miRNA genes are located in areas on the cancer-associated genome or in fragile regions which indicates that miRNAs have an important role in the pathogenesis of neoplasia ⁽⁵⁾. There are studies on patients with and without papillary thyroid cancer (PTC) comparing the levels of miRNA levels in cytology material, and pathology tissue samples. These studies have shown miRNA expressions in thyroid cancer tissue ⁽⁶⁻⁹⁾. However, limited number of studies have evaluated circulating miRNA levels in patients with thyroid cancer ⁽¹⁰⁻¹³⁾.

In this study, we aimed to investigate if microRNA

190 and microRNA 95-3P can be used as biomarkers to distinguish between cases with ACUS that received the diagnosis of benign or malignant lesions based on postoperative pathology results.

MATERIAL and METHOD

Patients determined as ACUS in the endocrinology outpatient clinic of Adnan Menderes University Medical Faculty and underwent thyroid surgery in the Department of General Surgery between December 2017 and December 2019 were included in the study. Patients with Postoperatively diagnosed 29 malignant (n=29) and 29 benign (n=29) cases with ACUS were included in the study.

Ethical approval was obtained from the Adnan Menderes University Faculty of Medicine Clinical Research Ethics Committee (protocol No: 2017/1094 and date of approval: 03.09. 2017. After the approval of the ethics committee, the study was carried out in patients who had a thyroid operation in the Department of General Surgery. The patients and control groups were informed about the purpose of the study and the procedures to be performed. Written informed consents were obtained by giving the pre-prepared informed consent forms to the patients.

Three-five cc venous blood samples drawn from patients who agreed to participate in the study were collected in EDTA and biochemistry tubes. Patients were grouped as having benign or malignant lesions according to the postoperative pathology results.

Isolation of Genomic miRNA from Blood Samples:

Venous blood samples drawn from patients were stored at -80 degrees. Samples were isolated using a miRNA-specific kit. Stem-loop primers specific to these miRNAs were designed which are considered as the most reliable primers because they bind specifically to the miRNA sequence when making cDNA synthesis from miRNA ⁽¹⁴⁾. cDNA synthesis was per-

formed using primers specific to each miRNA from the obtained miRNAs. Real time Polymerase Chain Reaction (PCR) protocol was applied after cDNA synthesis. Samples were studied in 2 replicates.

Statistical Analysis:

The suitability of quantitative data for normal distribution was evaluated by using Kolmogorov-Smirnov test while t test was used in independent groups after evaluating the distribution patterns. Descriptive statistics were shown as mean \pm standard deviation. Quantitative variables that did not distributed normally were analyzed using Mann-Whitney U or Kruskal-Wallis method according to the number of groups and descriptive statistics were given as median (25-75 percentile). In the analysis of qualitative data, chi-square test was used and the results were given as a percentage. $p < 0.05$ was considered as statistically significant.

RESULTS

Patients diagnosed as having ACUS based on FNA results in the Department of Endocrine Diseases, Adnan Menderes University Medical Faculty outpatient clinic and underwent thyroid surgery between December 2017 and December 2019 were included in the study. Twenty-nine patients with benign ACUS and 29 patients with malignant ACUS based on postoperative pathology results were included in the study.

According to the postoperative pathology results, papillary thyroid cancer was diagnosed in 7 (24.1%) and micropapillary thyroid cancer in 22 (75.9%) patients. All cases with thyroid cancer were stage 1. The mean tumor diameter was 10.1 ± 8.8 mm. Pathological LAP was detected in 2, lymphovascular invasion in 5, and capsular invasion in 1 patient. There were no patients with distant metastases.

Malignant ACUS group consisted of 22 female, and 7 male patients while benign ACUS group consisted of

24 female and 5 male patients. Both groups were similar in terms of gender ($p > 0.05$). The mean ages of the patients in the malignant, and benign ACUS groups were 52.31 ± 8.81 and 47.86 ± 14.58 , respectively. There was no statistically significant difference between the two groups ($p > 0.05$). In the malignant ACUS group, 4 patients had Graves disease and 5 patients had Hashimoto thyroiditis while in the benign ACUS group 4 patients had Graves disease and 3 patients had Hashimoto thyroiditis. In terms of autoimmune thyroid disease, both groups were similar ($p > 0.05$). The two groups were similar in terms of comorbid diseases (diabetes, hypertension, non-thyroid malignant disease, chronic heart disease, chronic kidney failure, chronic liver failure, and rheumatic diseases) ($p > 0.05$). TSH and fT4 values of both groups were similar ($p > 0.05$).

Circulating miRNA 190 and miRNA 95-3p levels were significantly different between cases with malignant and benign ACUS groups ($p < 0.001$, $p < 0.001$, respectively) (Table 1).

Table 1. Comparison of numerical data between the group with and without thyroid cancer.

	Thyroid Cancer (n=29)	Benign Thyroid Nodule (n=29)	p
Age	52.31 \pm 8.81	47.86 \pm 14.58	>0.05
miRNA 95-3p	14.22 \pm 8.15	21.94 \pm 6.64	<0.001
miRNA 190	13.46 \pm 1.57	16.38 \pm 1.69	<0.001
TSH (μ IU/mL)	2.18 \pm 4.5	1.19 \pm 1.31	>0.05
fT4 (ng/dl)	1.1 \pm 0.21	1.12 \pm 0.29	>0.05

DISCUSSION

In our study, circulating miRNA 190 and miRNA 95-3p levels were found to be significantly lower in the benign group compared to malignant group among patients with preoperative cytopathologic diagnosis of ACUS.

According to Bethesda system, the estimated frequency of cancer is estimated to be 5-15% in patients whose FNA biopsy results are reported as ACUS⁽³⁾. The risk of malignancy after surgery is reported to be

14% (6-48) ⁽¹⁵⁾. Mileva et al. ⁽¹⁶⁾ reported that, thyroid cancer was detected in 35 (31.2%) of 112 patients whose FNA biopsy results were reported as ACUS, and 77 (68.8%) of them were found to be benign. Kuru et al. ⁽¹⁷⁾ reported that 22.9% of 179 patients whose FNA biopsy was reported as ACUS were diagnosed as having malign pathology after the surgery. Turkyilmaz et al. ⁽¹⁸⁾ reported that 139 (14.2%) of 976 patients with pathologic diagnosis of ACUS underwent surgery and 518 (53.1%) patients had undergone additional FNA biopsy. A total of 305 (31%) patients were operated at different times. Thyroid cancer was found in 34.5% of the patients operated after the first FNA biopsy and in 37.9% of the patients in whom FNA had been repeated. In the study of Kuru et al. ⁽¹⁹⁾ thyroid cancer was found in 153 (22.8%) of 485 patients who were diagnosed as ACUS in the first FNA biopsy. The malignancy rates for the patients with ACUS with and without second FNA were 37.5% and 16.2%, respectively. It is also understood from different studies that postoperative thyroid cancer detection rates are low in patients with cytopathologic diagnosis of AUS. Therefore, decision of surgery based on a cytopathologic diagnosis of ACUS with single biopsy is not recommended in different guidelines. It is emphasized that ultrasonographic properties and molecular markers can be helpful. Therefore, it is clear that noninvasive biomarkers that may predict postoperative thyroid cancer are required in patients diagnosed with ACUS ⁽³⁾. Although there are many different studies on this subject, there are no clear-cut biomarkers that can be used in practical application ⁽²⁰⁾. miRNAs have also been used in studies performed in cases with undetermined cytologies especially detected in FNAB samples, but their use has not been fully confirmed for diagnostic purposes ^(21,22). In a meta-analysis, it was indicated that circulating miRNAs have a good diagnostic value for thyroid cancer and they may be helpful in separating benign from malignant thyroid nodules. It was also stated that miRNAs can help in the diagnosis of malignancy and may be helpful in avoiding unnecessary surgery. It was stated that cir-

culating miRNAs should be added in current thyroid nodule evaluations ⁽²³⁾.

In a study, cost estimates for gene expression classification by using standard approach and miRNA tests were studied. The gene expression classification has reduced the rate of unnecessary surgeries by 32% compared to the standard approach, with an additional cost of \$ 1008 per patient, preventing 5070 \$ for unnecessary surgeries. miRNA tests reduced the rate of surgery by 67%, but preventing \$ 3170 of cost for unnecessary surgeries but having an additional cost of \$ 1384 per patient. miRNA tests resulted in 52% lesser unnecessary surgeries compared to gene expression classification and these tests were found to be 70% superior in detecting benign nodules ⁽²⁴⁾. In a study combining expressions of 10 miRNA genes and a seven-gene mutation test, data of 109 ACUS or follicular neoplasia patients were investigated and the malign cases were detected with a 89% specificity, 85% sensitivity, 73% oPPV and 94% NPV ⁽²⁵⁾. While overexpression of specific miRNAs inhibits the expression of tumor suppressor genes, downregulation of different miRNAs can reduce inhibition of the expression of oncogenes, which leads to increased cell proliferation in both cases ⁽²⁶⁾. Meta-analyses investigating thyroid cancer and miRNAs reported that overexpression of miRNA-146b, miRNA-221, miRNA-222 and miRNA-181b are the most common ones in thyroid cancer compared to normal thyroid tissues ⁽²⁷⁻³¹⁾. In the study of Cantara et al, 8 different miRNA evaluations were performed on 12 healthy individuals, 12 nodular goiter and 12 papillary thyroid carcinoma (PTC) patients. In PTC patients, miRNA579, -95, -29b, 5-01-3p, -548d-5p were downregulated and miRNA190, -362-3p, -518a-5p were upregulated compared to healthy control group and benign nodular goiter patients. These miRNAs were also validated in a second cohort including 79 PTC, 80 NG and 41 healthy control group patients. Multivariable risk model including miRNA95 together with miRNA190 revealed a diagnostic sensitivity of 94.9%, reaching up to 100% ⁽³²⁾. The study of Pilli

et al showed that the combination of miRNA-190 and miRNA-95 in patients with differentiated thyroid cancer can be used with great accuracy for the differential diagnosis of thyroid nodules, especially those that are not diagnosed by cytology⁽³³⁾. Recent evidence suggests that miRNA-190-5p may play a dual role in tumor formation and progression. miRNA-190-5p has been reported to function as both a tumor suppressor and an oncogene in multiple human cancers. Upregulation of miRNA-190-5p was found in pancreatic cancer, bladder cancer, meningioma and stomach cancer while down-regulation of miRNA-190-5p was found in breast cancer, hepatocellular carcinoma, glioma, prostate cancer, rectal cancer and cervical cancer. These observations suggest that miR-190-5p can target multiple genes related to tumor development and progression⁽³⁴⁾. Vascular endothelial growth factor contributes significantly to angiogenesis, a vital process in tumor metastasis. It has been shown that miRNA-190-5p significantly suppress tumor metastasis and angiogenesis by managing a large group of angiogenic effectors including TCF4, SMAD2, SMAD4, RAS2, JAK2, IGF1 and HGF⁽³⁵⁾. In our study, we found that miRNA 95 was downregulated, similar to the literature. miRNA 190 was found to be downregulated in thyroid cancer cases although there is a limited number of data in the literature. However, given that miRNA 190 functions both as tumor suppressor and as oncogene in multiple human cancers, it is expected that it will be downregulated in thyroid cancer patients. Therefore, our data suggest that miRNA 190 is downregulated in thyroid cancer patients.

In our study, we found that levels of circulating miRNA 190 and miRNA95-3p were significantly lower in thyroid cancer patients with preoperatively diagnosed as having ACUS compared to patients with benign thyroid nodules. In conclusion, we think that circulating miRNA 95 and miRNA 190 can help in differentiating thyroid cancer from benign thyroid nodules and may be useful in avoiding unnecessary surgery in patients with uncertain FNA biopsy results.

We assert that the assessment of circulating miRNAs in daily practice in current thyroid nodule evaluations should be used. We think it would be beneficial to support our study in larger series.

Ethics Committee Approval: Approval was obtained from Adnan Menderes University Faculty of Medicine Clinical Research Ethics Committee (Protocol No: 2017/1094 Approval Date: 09.03.2017).

Conflict of Interest: The authors declare that there is no conflict of interest about the manuscript.

Funding: The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

Informed Consent: An informed consent form was obtained from the patients.

REFERENCES

1. Durante C, Grani G, Lamartina L, Filetti S, Mandel SJ, Cooper DS. The diagnosis and management of thyroid nodules: a review. *Jama*. 2018;319(9):914-24. [\[CrossRef\]](#)
2. Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL. Immunohistochemical diagnosis of papillary thyroid carcinoma. *Modern Pathology*. 2001;14(4):338. [\[CrossRef\]](#)
3. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*. 2016. 2016;26:1-133. [\[CrossRef\]](#)
4. Ambros V. The functions of animal microRNAs. *Nature*. 2004;431(7006):350. [\[CrossRef\]](#)
5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281-97. [\[CrossRef\]](#)
6. Wang Y, Gong W, Yuan Q. Effects of miR-27a upregulation on thyroid cancer cells migration, invasion, and angiogenesis. *Genetics and molecular research: GMR*. 2016;15(4). [\[CrossRef\]](#)
7. Liang W, Xie Z, Cui W, Guo Y, Xu L, Wu J, et al. Comprehensive gene and microRNA expression profiling reveals a role for miRNAs in the oncogenic roles of SphK1 in papillary thyroid cancer. *Journal of Cancer Research and Clinical Oncology*. 2017;143(4):601-11. [\[CrossRef\]](#)
8. Boufraquech M, Klubo-Gwiedzinska J, Kebebew E. MicroRNAs in the thyroid. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2016;30(5):603-19. [\[CrossRef\]](#)
9. Zhao H, Tang H, Huang Q, Qiu B, Liu X, Fan D, et al. MiR-101 targets USP22 to inhibit the tumorigenesis of papillary thyroid carcinoma. *American Journal of Cancer Research*. 2016;6(11):2575.
10. Milas M, Shin J, Gupta M, Novosel T, Nasr C, Brainard J, et al. Circulating thyrotropin receptor mRNA as a novel marker of thyroid cancer: clinical applications learned from 1758 sam-

- ples. *Annals of Surgery*. 2010;252(4):643-51.
11. Lee JC, Zhao JT, Clifton-Bligh RJ, Gill A, Gundara JS, Ip JC, et al. MicroRNA-222 and MicroRNA-146b are tissue and circulating biomarkers of recurrent papillary thyroid cancer. *Cancer*. 2013;119(24):4358-65. [[CrossRef](#)]
 12. Yu S, Liu Y, Wang J, Guo Z, Zhang Q, Yu F, et al. Circulating microRNA profiles as potential biomarkers for diagnosis of papillary thyroid carcinoma. *The Journal of Clinical Endocrinology & Metabolism*. 2012;97(6):2084-92. [[CrossRef](#)]
 13. Cantara S, Pilli T, Sebastiani G, Cevenini G, Busonero G, Cardinale S, et al. Circulating miRNA95 and miRNA190 are sensitive markers for the differential diagnosis of thyroid nodules in a Caucasian population. *The Journal of Clinical Endocrinology & Metabolism*. 2014;99(11):4190-8. [[CrossRef](#)]
 14. Varkonyi-Gasic E, Hellens RP. Quantitative stem-loop RT-PCR for detection of microRNAs. *RNAi and Plant Gene Function Analysis*: Springer; 2011. p. 145-57. [[CrossRef](#)]
 15. Bongiovanni M, Crippa S, Baloch Z, Piana S, Spitale A, Pagni F, et al. Comparison of 5-tiered and 6-tiered diagnostic systems for the reporting of thyroid cytopathology: a multiinstitutional study. *Cancer Cytopathology*. 2012;120(2):117-25. [[CrossRef](#)]
 16. Mileva M, Stoilovska B, Jovanovska A, Ugrinska A, Petrushevska G, Kostadinova-Kunovska S, et al. Thyroid cancer detection rate and associated risk factors in patients with thyroid nodules classified as Bethesda category III. *Radiology and Oncology*. 2018.
 17. Kuru B, Atmaca A, Kefeli M. Malignancy rate associated with Bethesda category III (AUS/FLUS) with and without repeat fine needle aspiration biopsy. *Diagnostic Cytopathology*. 2016;44(5):394-8. [[CrossRef](#)]
 18. Turkyilmaz S, Ulusahin M, Celebi B, Cekic A, Mungan S, Kucuktulu U, et al. Thyroid nodules classified as atypia or follicular lesions of undetermined significance deserve further research: Analysis of 305 surgically confirmed nodules. *Cytopathology*. 2017;28(5):391-9. [[CrossRef](#)]
 19. Kuru B, Atmaca A, Tarim I, Kefeli M, Topgul K, Yoruker S, et al. Risk factors associated with malignancy and with triage to surgery in thyroid nodules classified as Bethesda category III (AUS/FLUS). *European Journal of Surgical Oncology (EJSO)*. 2016;42(1):87-93. [[CrossRef](#)]
 20. Prasad NB, Somervell H, Tufano RP, Dackiw AP, Marohn MR, Califano JA, et al. Identification of genes differentially expressed in benign versus malignant thyroid tumors. *Clinical Cancer Research*. 2008;14(11):3327-37. [[CrossRef](#)]
 21. Chen Y-T, Kitabayashi N, Zhou XK, Fahey III TJ, Scognamiglio T. MicroRNA analysis as a potential diagnostic tool for papillary thyroid carcinoma. *Modern Pathology*. 2008;21(9):1139. [[CrossRef](#)]
 22. Mazeh H, Mizrahi I, Halle D, Ilyayev N, Stojadinovic A, Trink B, et al. Development of a microRNA-based molecular assay for the detection of papillary thyroid carcinoma in aspiration biopsy samples. *Thyroid*. 2011;21(2):111-8. [[CrossRef](#)]
 23. Shi-Lin X, Yu-Yang T, Zhou Y, Li-Qiao L. Diagnostic value of circulating microRNAs in thyroid carcinoma: a systematic review and meta-analysis. *Clin Endocrinol (Oxf)*. 2020 May 7. [[CrossRef](#)]
 24. Labourier E. Utility and cost-effectiveness of molecular testing in thyroid nodules with indeterminate cytology. *Clinical Endocrinology*. 2016;85(4):624-31. [[CrossRef](#)]
 25. Labourier E, Shifrin A, Busseniers AE, Lupo MA, Manganelli ML, Andruss B, et al. Molecular testing for miRNA, mRNA, and DNA on fine-needle aspiration improves the preoperative diagnosis of thyroid nodules with indeterminate cytology. *The Journal of Clinical Endocrinology & Metabolism*. 2015;100(7):2743-50. [[CrossRef](#)]
 26. Nikiforova MN, Chiosea SI, Nikiforov YE. MicroRNA expression profiles in thyroid tumors. *Endocr Pathol*. 2009 Summer;20(2):85-91. [[CrossRef](#)]
 27. Chou C-K, Chen R-F, Chou F-F, Chang H-W, Chen Y-J, Lee Y-F, et al. miR-146b is highly expressed in adult papillary thyroid carcinomas with high risk features including extrathyroidal invasion and the BRAFV600E mutation. *Thyroid*. 2010;20(5):489-94. [[CrossRef](#)]
 28. Zhou Y-L, Liu C, Dai X-x, Zhang X-H, Wang O-C. Overexpression of miR-221 is associated with aggressive clinicopathologic characteristics and the BRAF mutation in papillary thyroid carcinomas. *Medical Oncology*. 2012;29(5):3360-6. [[CrossRef](#)]
 29. Wang Z, Zhang H, He L, Dong W, Li J, Shan Z, et al. Association between the expression of four upregulated miRNAs and extrathyroidal invasion in papillary thyroid carcinoma. *Oncotargets and Therapy*. 2013;6:281. [[CrossRef](#)]
 30. Stokowy T, Gawel D, Wojtas B. Differences in miRNA and mRNA profile of papillary thyroid cancer variants. *International Journal of Endocrinology*. 2016;2016. [[CrossRef](#)]
 31. Rosignolo F, Memeo L, Monzani F, Colarossi C, Pecce V, Verrienti A, et al. MicroRNA-based molecular classification of papillary thyroid carcinoma. *International Journal of Oncology*. 1899;50(5):1767-77. [[CrossRef](#)]
 32. Cantara S, Pilli T, Sebastiani G, Cevenini G, Busonero G, Cardinale S, Dotta F, Pacini F. Circulating miRNA95 and miRNA190 are sensitive markers for the differential diagnosis of thyroid nodules in a Caucasian population. *J Clin Endocrinol Metab*. 2014;99(11):4190-8. [[CrossRef](#)]
 33. Pilli T, Cantara S, Marzocchi C, Cardinale S, Santini C, Cevenini G, Pacini F. Diagnostic Value of Circulating microRNA-95 and -190 in the Differential Diagnosis of Thyroid Nodules: A Validation Study in 1000 Consecutive Patients Thyroid. 2017;27(8):1053-7. [[CrossRef](#)]
 34. Yu Y, Cao XC. miR-190-5p in human diseases. *Cancer Cell Int*. 2019;19:257. [[CrossRef](#)]