Editorial Commentary

Genetic molecular testing of thyroid nodules

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Introduction

The incidence of thyroid cancer has seen a significant increase in the United States over the past few decades. This rise can be attributed to both improved detection methods (1) as well as a genuine increase in the occurrence of the disease (2). Thyroid nodules are found to be malignant in only 5% to 15% of cases (3). To determine the malignancy potential of a thyroid nodule, various factors such as medical history, physical examination, radiographic assessment, and fine needle aspiration (FNA) are taken into consideration. The analysis of FNA specimens has long played a crucial role in assessing and characterizing thyroid nodules, complementing clinical and radiological criteria. This cytological evaluation has significantly impacted patient management by reducing the need for surgery by half and increasing the proportion of identified cancers among surgical patients (4). The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), introduced by the National Cancer Institute in 2009 and revised in 2017, is widely used to classify the risk of malignancy based on cytological findings. It has proven to be accurate in establishing a diagnosis (benign *vs.* malignant) in approximately 70–80% of cases (5).

However, a current limitation of cytological evaluation is that around 20–25% of cases are reported as indeterminate, falling under Bethesda III (atypia of undetermined significance or follicular lesion of undetermined significance) and Bethesda IV (follicular neoplasm or suspicious for a follicular neoplasm). Unilateral lobectomy (hemithyroidectomy) is currently considered the standard procedure to obtain a definitive diagnosis for cytologically indeterminate thyroid lesions. Nevertheless, recent guidelines from the American Thyroid Association (ATA) suggest alternative options such as repeat FNA cytology, molecular testing, or active surveillance, depending on clinical risk factors, sonographic pattern, and patient preference (6,7).

Prior to 2009, a significant number of patients with indeterminate thyroid cytology (ITC) underwent diagnostic surgery based on a malignancy probability ranging from 15% to 40% (7). However, recent advancements have introduced molecular genetic testing to study these indeterminate cases. In this review, we explore this concept along with some commonly used commercial molecular genetic panels in current thyroid neoplasia practice.

Molecular markers

Mutations

Genomic studies of thyroid cancer have revealed two signaling pathways that play a crucial role in thyroid growth and proliferation (8). These pathways are the

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mitogen-activating protein kinase (MAPK) pathway and the Phosphatidylinositol-3-kinase (PI3K)/AKT pathway. Mutations in kinases and transcription factors involved in these pathways initiate the development and progression of thyroid cancer. When these key targets in the respective pathway undergo mutations or genetic rearrangements, they become constitutively active, leading to the translocation of transcription factors into the nucleus. This translocation results in the upregulation of genes that promote tumorigenesis and cancer progression. Mutations in key regulators of the MAPK pathway are present in approximately 70% of all papillary thyroid carcinoma (PTC) cases (9).

One notable mutation is the BRAFV600E mutant protein, which was first described in thyroid cancer by Cohen *et al.* (10). This mutation leads to the activation of the BRAF kinase independently of its upstream target, RAS. The BRAFV600E kinase domain is approximately 500 times more active than the wild type BRAF kinase (11). This mutation is considered one of the fundamental initiating events in the development and progression of PTC. Additionally, the BRAFV600E mutation inhibits proapoptotic genes, further contributing to the survival and proliferation of cancer cells (12). A meta-analysis of 18 studies, encompassing 2,766 thyroid FNA samples, demonstrated that among the 581 cases that tested positive for the BRAF mutation, 580 were diagnosed as papillary thyroid cancers (13). Therefore, the presence of a BRAF V600E mutation in a thyroid nodule is highly indicative of the presence of papillary thyroid cancer.

The PI3K/AKT signaling pathway plays a vital role in regulating various cellular processes, including apoptosis, proliferation, cell cycle progression, angiogenesis, cytoskeleton integrity, and energy metabolism (14). Within this pathway, one of the frequently mutated targets is RAS, which acts as an upstream kinase of BRAF kinase. Mutations in RAS result in its continuous activation in a GTP-bound state. Among the three isoforms of RAS (NRAS, HRAS, and KRAS), NRAS is the most commonly mutated in human thyroid cancer (15).

RAS mutations are considered premalignant mutations, meaning that additional mutations are required to initiate the development of cancer. In a recent meta-analysis (16), the specificity (the ability to accurately identify absence of disease) and positive predictive value (PPV) (the likelihood of correctly identifying the presence of disease) of RAS mutations were found to have average values of 23% and 82%, respectively. This suggests that while RAS mutations can provide useful diagnostic information, they are not highly specific indicators of thyroid cancer on their own.

A meta-analysis that combined data from seven studies revealed that RAS mutations had an overall sensitivity of 34.3% and a specificity of 93.5% in detecting malignancy among indeterminate FNA samples. Additionally, the positive likelihood ratio was 4.235, indicating a moderate increase in the risk of malignancy with a positive RAS mutation result, while the negative likelihood ratio was 0.775, suggesting only a slight reduction in the risk of malignancy with a negative RAS mutation result. Therefore, while a positive RAS mutation result moderately increases the likelihood of malignancy, a negative result only slightly reduces the risk (17).

In another study, differences in outcomes were observed when comparing RAS mutation subtypes. The probability of a malignant outcome followed a specific order among the mutation subtypes: KRAS (100%), NRAS (74%), and HRAS (56%). It is important to note that this study specifically focused on follicular adenoma and carcinoma cases, limiting its generalizability to other types of thyroid cancer (18).

Fusions/translocations

In addition to mutations, constitutive activation of both the MAPK and PI3K-AKT pathways can also result from gene translocations. One of the most common types of translocations observed in thyroid cancer is called RET/PTC, which was initially described in PTC by Fusco *et al.* in 1987 (19). These translocations involving the RET gene have shown average values of specificity (18%) and PPV (87%) (16). It is worth noting that these RET/PTC translocations are now commonly referred to as RET fusions.

The PAX8/peroxisome proliferator-activated receptor-γ (PPARγ) rearrangement is a result of a translocation known as (2;3)(q13;p25), which leads to the fusion of the PAX8 gene and the PPARγ gene. This specific translocation is observed in approximately 30–60% of follicular thyroid cancer (FTC) (16) cases and in 38% of the follicular variant of papillary thyroid cancer (FVPTC) cases (19,20).

MicroRNAs (miRNAs)

miRNAs are short non-coding RNA molecules that play a crucial role in regulating gene expression at the post-transcriptional level. They are involved in processes such as cell proliferation, apoptosis, and differentiation. The

deregulation of miRNA expression has been identified as an important factor in tumor development and progression. Numerous studies have highlighted the significance of miRNA abnormalities in PTC development (21). In PTCs, specific miRNAs exhibit distinct expression profiles compared to normal thyroid tissue and tumors with different biological properties. Notably, there is a significant increase in the expression of miR-221, miR-222, and miR-181b in PTC compared to normal thyroid tissue. When at least one of these miRNAs is overexpressed more than twofold, it has been shown to have a sensitivity of 100%, specificity of 94%, and an accuracy of 95% in detecting malignancy (21). These findings suggest that the assessment of miRNA expression profiles can serve as a valuable tool in the diagnosis and detection of thyroid cancer, particularly PTC. The altered expression of specific miRNAs can provide useful information for identifying malignancy in thyroid nodules.

Diagnostic implications

Although the aforementioned mutations and other mutations have been extensively studied, and some mutations and fusions are pathognomonic for malignancy in an indeterminate thyroid nodule (e.g., BRAF V600E mutation, RET fusion), none of these mutations can stand alone as a diagnostic test for cancer.

For instance, BRAF mutations are the most common genetic anomaly observed in PTC, occurring in approximately 45% of PTC cases (21). The BRAF V600E mutation specifically exhibits a high specificity for malignancy in indeterminate thyroid nodules. However, its utility as a diagnostic marker for indeterminate nodules is limited due to its low sensitivity for malignancy (15). Additionally, the presence of BRAF V600E mutation is infrequent in FNA samples of indeterminate nodules, which primarily consist of follicular patterned neoplasms and have a prevalence of less than 10% (22).

Indeed, considering multiple factors is crucial in the evaluation and diagnosis of thyroid nodules. While genetic mutations, such as RAS mutations, are present in a significant proportion of FTC and papillary thyroid carcinoma cases, their specificity for malignancy is limited. RAS mutations are observed in approximately 40–50% of FTC cases and 10–20% of PTC cases (22). However, it is important to note that RAS mutations are not specific indicators of malignancy, as they can also be found in 20–40% of follicular adenomas and are the predominant mutations in noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) (23).

The presence of RAS mutations alone cannot be relied upon as a definitive diagnostic marker for malignancy. The recent shift in the classification of NIFTP and the recalibration of the risk of malignancy in RAS FNA nodules further highlights the complexity of interpreting these mutations in relation to the true incidence of carcinomas, malignancies, adenomas, and NIFTP cases (24).

Therefore, it is essential to integrate genetic mutation analysis with other diagnostic criteria, including clinical history, physical examination, radiographic assessment, and cytological evaluation, to accurately assess the risk of malignancy in thyroid nodules. The combination of these factors allows for a comprehensive evaluation and diagnosis of thyroid nodules.

RET fusions (RET/PTC translocations) are detected in approximately 10% of patients with PTC. In a retrospective study, the presence of RET/PTC fusions in indeterminate thyroid nodules was associated with malignancy in 60% of cases, with no false positives (25). RET fusions are generally considered pathognomonic for malignancy in indeterminate thyroid nodules. Similarly, other fusions such as NTRK fusions found in indeterminate thyroid nodules are also typically indicative of malignancy. NTRK fusions can be observed in around 2–3% of sporadic PTC cases (26). Like RET fusions, NTRK fusions are a recombination of parts of NTRK genes with parts of any of a large number of genes leading to activation of RAS and the MAPK and PI3K/AKT pathways. All types of NTRK fusions seem to be associated with a 100% probability of malignancy, since they have not been found in benign nodules in large histopathological series (27).

Strong driver mutations such as BRAFV600E, RET fusions, and TERT promoter mutations have proven to be useful in surgical decision making due to their high predictability of malignancy. However, the most common mutations observed in indeterminate nodules are RAS mutations, which are considered weak driver mutations with a lower PPV. The PPV of RAS mutations in the Bethesda III and IV categories varies across different studies, leading to a lack of confidence in a single estimate of PPV (28,29).

In summary, while certain driver mutations like BRAFV600E, RET fusions, and NTRK fusions are highly indicative of malignancy, the predictive value of RAS mutations in indeterminate nodules is lower and can vary across different studies. Therefore, the interpretation of these mutations in the context of diagnosing malignancy

Table 1 Clinical performance of select genetic molecular tests for indeterminate thyroid nodules

Assay	Sensitivity, %	Specificity, %	Cancer prevalence, %	NPV, %	PPV, %	Adjusted NPV, % (pre-test 25%)	Adjusted PPV, % (pre-test 25%)
ThyGeNext - Thyamir	93	90	30	95	74	97	72
ThyroidPrint	91	88	32	95	78	97	71
Mir-THYpe	94	81	39	95	76	97	61
Afirma-GSC	91	68	24	96	47	95	48
ThyroSeq V3	94	82	28	97	66	97	63

NPV, negative predictive value; PPV, positive predictive value; GSC, Gene Sequencing Classifier.

Table 2 Quality of evidence for select genetic molecular tests for indeterminate thyroid nodules

Assay	Diagnostic platform	Multicenter study?	Prospective trial?	Blinded?	Number of Bethesda III/IV cases in validation set	Reference
ThyGeNext - Thyamir	NGS/qPCR	Yes	No	Single	147*	Finkelstein, *Thyroid*, 2022
ThyroidPrint	qPCR	Yes	Yes	Double	270	Zafereo, *Thyroid*, 2020
Mir-THYpe	qPCR	No	No	Single	63	Tadeu dos Santos, *Thyroid*, 2018
Afirma-GSC	NGS	Yes	No**	Double	190	Kepal, *Surgery*, 2018
ThyroSeq V3	NGS	Yes	Yes	Double	247	Steward, *JAMA Oncology*, 2018

*, 147 of 178 Bethesda III and IV cases with a definitive result; **, prospectively collected samples of GEC trial were re-analyzed for the GSC study. NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction; GSC, Gene Sequencing Classifier; GEC, Gene Expression Classifier.

in thyroid nodules should be done cautiously, taking into account multiple diagnostic criteria and considering the specific mutation and its associated clinical significance.

Molecular marker panels

In order to address the challenges posed by the heterogeneous sensitivity and specificity of individual mutations in diagnosing thyroid cancer, genetic molecular panels have been developed as an efficient approach to test for a range of molecular markers. These panels have historically been categorized as either "rule out" or "rule in" tests. A "rule out" test aims to have high sensitivity and negative predictive value (NPV) to effectively exclude the presence of malignancy, while a "rule in" test aims to have high specificity and PPV to confidently indicate the presence of malignancy (30).

However, with advancements and newer generations of commercially available molecular tests for thyroid nodules, the NPV and PPV of various tests have become more aligned, making it challenging to easily classify a single test as a definitive "rule in" or "rule out" test. As a result, there are now several commercially available clinical tests, some of which are in their second or third generation, that provide risk stratification for indeterminate nodules, helping to predict the likelihood of malignancy. Detailed information on these tests can be found in *Tables 1*,*2*.

These molecular panels offer a comprehensive approach by assessing multiple genetic markers simultaneously, allowing for improved risk assessment and aiding in the diagnosis of thyroid nodules. By considering the collective information from these tests, healthcare professionals can make more informed decisions regarding patient management and treatment strategies.

Afirma

One of the molecular tests available for evaluating indeterminate thyroid nodules is Afirma GEC (Gene Expression Classifier). It is a microarray-based test that analyzes the mRNA expression of 167 genes. When initially introduced in 2012, it demonstrated high NPV for different

categories of indeterminate nodules. Specifically, the NPV for "atypia (or follicular lesion) of undetermined clinical significance", "follicular neoplasm or lesion suspicious for follicular neoplasm", and "suspicious cytologic findings" were reported to be 95%, 94%, and 85%, respectively (31).

More recently, a new version of the Afirma test has been developed, known as Afirma GSC (Gene Sequencing Classifier). This updated version incorporates gene sequencing and additional genomic analysis techniques, such as nuclear and mitochondrial RNA transcriptome gene expression and genomic copy number analysis, in addition to RNA sequencing. The aim of this enhanced version is to provide better prediction of thyroid cancers in indeterminate nodules while still being able to rule out cancer in benign nodules.

In a meta-analysis comparing the two methods, Afirma GSC was found to have several advantages over Afirma GEC. Afirma GSC showed higher risk of malignancy (60.1% *vs.* 37.6%), higher specificity (43.0% *vs.* 25.1%), and higher PPV (63.1% *vs.* 41.6%), while maintaining high sensitivity (94.3%) and high NPV (90.0%) (32). These findings indicate that Afirma GSC has improved accuracy in distinguishing between benign and malignant thyroid nodules compared to Afirma GEC.

Furthermore, the meta-analysis suggested that the use of Afirma GSC testing resulted in a relative reduction of surgical interventions by approximately 50% compared to Afirma GEC. Additionally, there was a relative increase in the malignancy rate by approximately 60% with the use of Afirma GSC. These results indicate the potential clinical impact of Afirma GSC in reducing unnecessary surgeries while effectively identifying malignancies among indeterminate thyroid nodules.

Afirma XA is a molecular test designed for the evaluation of thyroid nodules that are cytologically indeterminate and have suspicious findings based on Afirma GSC testing, as well as nodules diagnosed as Bethesda V/VI (suspicious for malignancy or malignant). This test utilizes RNA sequencing of material collected through FNA to analyze expressed genomic variants and gene fusions associated with thyroid cancer. The purpose of Afirma XA is to provide additional insight into the risk of cancer, particularly for cytologically indeterminate nodules, by identifying specific genomic alterations. However, it is important to note that Afirma XA is not intended to be a definitive rule-out test for cancer. The identification of genomic alterations through Afirma XA can contribute to personalized care for biopsied lesions that are known or suspected to be malignant. By understanding the specific alterations present, healthcare providers can gain valuable information about the oncogenic drivers of the tumor. This knowledge can help inform treatment decisions, including the consideration of systemic targeted therapies, especially in cases where there are known or suspected thyroid cancer metastases (33).

Thyroseq

ThyroSeq (version 3) is a molecular test that utilizes next-generation sequencing to analyze the DNA and RNA of 112 thyroid-related genes. It is designed to detect various types of molecular alterations, including mutations, gene fusions, copy number alterations, and gene expression alterations.

The ThyroSeq v3 genomic classifier (GC) is the latest version of the test and was launched for clinical use in late 2017. It offers advanced sequencing technology, examining thousands of mutation hotspots and fusion types. The test can identify five different classes of genetic alterations, providing comprehensive genomic information. The sensitivity of ThyroSeq v3 GC for distinguishing benign from possibly malignant nodules is reported to be 98%, indicating its ability to detect true positive cases. The specificity is reported at 82%, indicating its ability to accurately identify true negative cases (34).

In a study involving 154 thyroid nodules and 93 nodules diagnosed as Bethesda categories III and IV, ThyroSeq v3 GC demonstrated a high NPV of 97%. The NPV represents the percentage of patients with indeterminate cytology nodules who can avoid unnecessary diagnostic surgeries and be managed similarly to patients with benign cytology results. The study reported five false-negative results, where the test missed low-risk papillary carcinomas and minimally invasive follicular carcinoma (35).

The benign call rate is an important factor for "rule-out tests" as it indicates the percentage of patients with indeterminate nodules who can be confidently classified as benign and avoid surgery. ThyroSeq v3 GC has shown a benign call rate of 61% based on multicenter studies, indicating that a significant proportion of histologically benign thyroid nodules can be accurately classified as negative by the test. Comparatively, the Afirma GSC has a benign call rate of 54%, with a lower percentage of histologically benign nodules classified as negative (35,36).

ThyGeNEXT/ThyraMIR

ThyGeNEXT/ThyraMIR uses a combination of two tests.

The first test (ThyGeNEXT) incorporates a mutation panel. If no mutation is found, a second test (ThyraMIR) is performed analyzing miRNA markers. miRNAs are short single-stranded non-coding RNA segments and abnormal expression has been found in thyroid cancers. Based on a threshold (positive or negative) the combination of both tests is able to provide a clinically actionable result in 79% of cases, where negative result on this combined test with Bethesda III and IV cytology was found to "rule-out" disease with 97% NPV (NPV; 95% CI: 91–99%) at a 30% disease prevalence, while a positive result "ruled-in" high risk disease with 75% PPV (95% CI: 60–86%) (37). The limitation of the test lies in a group of cases with a moderate threshold result which essentially remain indeterminate and would still potentially require diagnostic surgery. Thyramir™/ThyGenX™ currently does not have reported post-validation studies, which is a drawback compared to other tests available in the market. Estimation of the impact of the test must consider the number of surgeries being avoided, the number of misclassified cases with a malignancy and the overall cost of the molecular test (38). Recently, the second version of this test (MPTXv2) was tested on a training cohort of histopathology-proven benign nodules and then validation testing was carried out on a cohort of 178 intermediate nodules improving its specificity to 98%. Prospective trials for this method are still needed (39).

ThyGeNEXT/ThyraMIR is a combination test that consists of two components. The first component, ThyGeNEXT, is a mutation panel that analyzes specific genetic mutations associated with thyroid cancer. If no mutation is detected, the second component, ThyraMIR, is performed to analyze miRNA markers. Abnormal expression of miRNAs has been observed in thyroid cancers, making them valuable markers for diagnostic purposes.

In a study evaluating the combined ThyGeNEXT/ThyraMIR test in cases with Bethesda III and IV cytology, a negative result was found to have a high NPV of 97% at a disease prevalence of 30%. On the other hand, a positive "ruled-in" high risk disease with a PPV of 75% (37).

It's worth noting that ThyraMIR/ThyGenX currently lacks reported post-validation studies, which can be a limitation compared to other available tests in the market. The impact of the test should be evaluated considering factors such as the number of avoided surgeries, the possibility of misclassifying cases with malignancy, and the overall cost of the molecular test (38).

A newer version of the test, MPTXv2, has been developed and tested on a training cohort of histopathology-proven benign nodules. Validation testing on a cohort of 178 intermediate nodules showed improved specificity, reaching 98%. However, prospective trials are still needed to further evaluate the performance and effectiveness of this method (39).

ThyroidPrint

ThyroidPrint is a relatively new thyroid genetic classifier that utilizes quantitative polymerase chain reaction (qPCR) measurement of a 10 genes signatures, followed by neural network algorithm analysis. The test was developed with the potential of becoming a future diagnostic kit for broader laboratory access. In the discovery phase of its development, the expression of 18 genes was determined in a diverse set of 114 fresh-tissue biopsies, including 43 cancer samples and 71 benign samples. The expression data were used to train several classifiers using supervised machine learning approaches. These classifiers were then tested on an independent set of 139 samples. The best-performing classifier was selected to develop a multiplexed qPCR prototype kit assay.

The prospective multicenter cohort study using fine-needle aspiration biopsies showed promising results, with ThyroidPrint demonstrating a sensitivity of 96% and specificity of 87% (40). This means that the test correctly identified 96% of malignant nodules and 87% of benign nodules.

Subsequently, ThyroidPrint underwent two independent prospective multi-center validation trials. In these trials, the test accurately predicted 78 out of 86 malignant nodules and 162 out of 184 benign nodules. This resulted in a sensitivity of 91% and specificity of 88%, with positive and NPV of 95% and 78%, respectively (41).

mir-THYpe

mir-THYpe is a molecular classifier test that analyzes the expression profiles of 11 miRNAs. In the FNA smear slide validation set of 67 Bethesda III and IV samples, the mir-THYpe test reached 95% sensitivity, 81% specificity, 96% NPV, and 76% PPV (42). In a multicenter study, 435 patients with 440 Bethesda III/IV thyroid nodules were followed and compared to mir-THYpe test results. The rate of avoided surgeries was 53% for all surgeries and 75% for "potentially unnecessary" surgeries. The test

achieved 89% sensitivity, 82% specificity, 66% PPV, and 95% NPV (43).

Conclusions

The use of genetic molecular testing has been demonstrated to aid in the categorization of indeterminate thyroid nodules. Active surveillance, diagnostic lobectomy, and further evaluation with genetic molecular testing are all reasonable strategies for Bethesda III and IV nodules, dependent upon clinical and radiographic features, patient preference, and socioeconomic factors which differ across the globe. While some studies have suggested that these tests may reduce the rate of unnecessary surgeries, there is a significant cost and technology involved with genetic molecular testing, which limit their current global utility. Recently, minimally invasive technologies such as ultrasound, alcohol, and thermal ablation have been studied for benign (e.g., Bethesda II) thyroid nodules. As this field of nonsurgical intervention for thyroid nodules continues to expand, so also will the demand for genetic molecular testing to definitively diagnose a nodule as benign. Furthermore, as these technologies become cheaper, better, and more well studied, it is expected that the need for diagnostic lobectomies for indeterminate thyroid nodules will continue to decline.

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References

1. Davies L, Welch HG. Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg 2014;140:317-22.
2. Lim H, Devesa SS, Sosa JA, et al. Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013. JAMA 2017;317:1338-48.
3. Mazzaferri EL. Management of a solitary thyroid nodule. N Engl J Med 1993;328:553-9.
4. Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med 2004;351:1764-71.
5. Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. Thyroid 2009;19:1159-65.
6. Cibas ES, Ali SZ. The 2017 Bethesda System for Reporting Thyroid Cytopathology. Thyroid 2017;27:1341-6.
7. Haugen BR, Alexander EK, Bible KC, 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;26:1-133.
8. Nylén C, Mechera R, Maréchal-Ross I, et al. Molecular

Markers Guiding Thyroid Cancer Management. Cancers (Basel) 2020;12:2164.
9. Adeniran AJ, Zhu Z, Gandhi M, et al. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol 2006;30:216-22.
10. Cohen Y, Xing M, Mambo E, et al. BRAF mutation in papillary thyroid carcinoma. J Natl Cancer Inst 2003;95:625-7.
11. Fagin JA. Challenging dogma in thyroid cancer molecular genetics--role of RET/PTC and BRAF in tumor initiation. J Clin Endocrinol Metab 2004;89:4264-6.
12. Lee SJ, Lee MH, Kim DW, et al. Cross-regulation between oncogenic BRAF(V600E) kinase and the MST1 pathway in papillary thyroid carcinoma. PLoS One 2011;6:e16180.
13. Nikiforov YE. Molecular diagnostics of thyroid tumors. Arch Pathol Lab Med 2011;135:569-77.
14. Shinohara M, Chung YJ, Saji M, et al. AKT in thyroid tumorigenesis and progression. Endocrinology 2007;148:942-7.
15. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 2013;13:184-99.
16. Ferrari SM, Fallahi P, Ruffilli I, et al. Molecular testing in the diagnosis of differentiated thyroid carcinomas. Gland Surg 2018;7:S19-29.
17. Clinkscales W, Ong A, Nguyen S, et al. Diagnostic Value of RAS Mutations in Indeterminate Thyroid Nodules. Otolaryngol Head Neck Surg 2017;156:472-9.
18. Radkay LA, Chiosea SI, Seethala RR, et al. Thyroid nodules with KRAS mutations are different from nodules with NRAS and HRAS mutations with regard to cytopathologic and histopathologic outcome characteristics. Cancer Cytopathol 2014;122:873-82.
19. Fusco A, Grieco M, Santoro M, et al. A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. Nature 1987;328:170-2.
20. Castro P, Rebocho AP, Soares RJ, et al. PAX8-PPARgamma rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. J Clin Endocrinol Metab 2006;91:213-20.
21. Nikiforova MN, Tseng GC, Steward D, et al. MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab 2008;93:1600-8.
22. Nikiforov YE. Role of molecular markers in thyroid nodule management: then and now. Endocr Pract 2017;23:979-88.
23. Esapa CT, Johnson SJ, Kendall-Taylor P, et al. Prevalence of Ras mutations in thyroid neoplasia. Clin Endocrinol (Oxf) 1999;50:529-35.
24. D'Cruz AK, Vaish R, Vaidya A, et al. Molecular markers in well-differentiated thyroid cancer. Eur Arch Otorhinolaryngol 2018;275:1375-84.
25. Cheung CC, Carydis B, Ezzat S, et al. Analysis of ret/PTC gene rearrangements refines the fine needle aspiration diagnosis of thyroid cancer. J Clin Endocrinol Metab 2001;86:2187-90.
26. Elisei R, Romei C, Vorontsova T, et al. RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated, malignant and benign thyroid lesions in children and adults. J Clin Endocrinol Metab 2001;86:3211-6.
27. Pekova B, Sykorova V, Mastnikova K, et al. NTRK Fusion Genes in Thyroid Carcinomas: Clinicopathological Characteristics and Their Impacts on Prognosis. Cancers (Basel) 2021;13:1932.
28. Nabhan F, Porter K, Lupo MA, et al. Heterogeneity in Positive Predictive Value of RAS Mutations in Cytologically Indeterminate Thyroid Nodules. Thyroid 2018;28:729-38.
29. Guan H, Toraldo G, Cerda S, et al. Utilities of RAS Mutations in Preoperative Fine Needle Biopsies for Decision Making for Thyroid Nodule Management: Results from a Single-Center Prospective Cohort. Thyroid 2020;30:536-47.
30. Vargas-Salas S, Martínez JR, Urra S, et al. Genetic testing for indeterminate thyroid cytology: review and meta-analysis. Endocr Relat Cancer 2018;25:R163-77.
31. Alexander EK, Kennedy GC, Baloch ZW, et al. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med 2012;367:705-15.
32. Vuong HG, Nguyen TPX, Hassell LA, et al. Diagnostic performances of the Afirma Gene Sequencing Classifier in comparison with the Gene Expression Classifier: A meta-analysis. Cancer Cytopathol 2021;129:182-9.
33. Krane JF, Cibas ES, Endo M, et al. The Afirma Xpression Atlas for thyroid nodules and thyroid cancer metastases: Insights to inform clinical decision-making from a fine-needle aspiration sample. Cancer Cytopathol 2020;128:452-9.
34. Nikiforov YE, Baloch ZW. Clinical validation of the ThyroSeq v3 genomic classifier in thyroid nodules with indeterminate FNA cytology. Cancer Cytopathol 2019;127:225-30.
35. Steward DL, Carty SE, Sippel RS, et al. Performance

of a Multigene Genomic Classifier in Thyroid Nodules With Indeterminate Cytology: A Prospective Blinded Multicenter Study. JAMA Oncol 2019;5:204-212. Erratum in: JAMA Oncol. 2019;5:271.
36. Patel KN, Angell TE, Babiarz J, et al. Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules. JAMA Surg 2018;153:817-24.
37. Lupo MA, Walts AE, Sistrunk JW, et al. Multiplatform molecular test performance in indeterminate thyroid nodules. Diagn Cytopathol 2020;48:1254-64.
38. Garcia LP, Bello F. Outcomes of molecular testing in cytologically indeterminate thyroid nodules. Endocrine Practice 2019;25:314.
39. Finkelstein SD, Sistrunk JW, Malchoff C, et al. A Retrospective Evaluation of the Diagnostic Performance of an Interdependent Pairwise MicroRNA Expression Analysis with a Mutation Panel in Indeterminate Thyroid Nodules. Thyroid 2022;32:1362-71.
40. González HE, Martínez JR, Vargas-Salas S, et al. A 10-Gene Classifier for Indeterminate Thyroid Nodules: Development and Multicenter Accuracy Study. Thyroid 2017;27:1058-67.
41. Hershman JM. A 10-Gene Classifier Can Accurately Diagnose Malignant Versus Benign Cytologically Indeterminate Thyroid Nodules. Clinical Thyroidology 2017;29:375-7.
42. Santos MTD, Buzolin AL, Gama RR, et al. Molecular Classification of Thyroid Nodules with Indeterminate Cytology: Development and Validation of a Highly Sensitive and Specific New miRNA-Based Classifier Test Using Fine-Needle Aspiration Smear Slides. Thyroid 2018;28:1618-26.
43. Santos MT, Rodrigues BM, Shizukuda S, et al. Clinical decision support analysis of a microRNA-based thyroid molecular classifier: A real-world, prospective and multicentre validation study. EBioMedicine 2022;82:104137.

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