Molecular Genetics and Diagnostics of Thyroid Cancer

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Introduction

In the age of personalized medicine, molecular markers are being increasingly utilized to provide diagnostic, prognostic, and therapeutic information. Thyroid cancer, in particular, is ideally suited to incorporating molecular markers into clinical management. Several factors contribute toward this: thyroid nodules are easily accessible for fine-needle aspiration (FNA) biopsy (which generates sufficient material for both diagnostic evaluation and ancillary testing on nearly all patients), a substantial proportion (20–30%) of thyroid nodules are diagnostically indeterminate by cytopathologic analysis, and thyroid cancer is well characterized with a relatively smaller number of genomic alterations (many of which are highly specific for malignancy).

Ultrasound and cytologic examination of thyroid nodules is standard in the diagnostic evaluation of thyroid nodules and reliably classifies the majority (70–80%) of thyroid nodules as benign or malignant [1, 2]. Those thyroid nodules classified as benign have a low risk (approximately 0–3%) of malignancy, while those nodules classified as malignant have a high risk of malignancy (97–99%) [3]. The remaining thyroid nodules are classified cytologically using the Bethesda reporting system as fitting into one of three indeterminate categories: atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), follicular or oncocytic (Hürthle cell) neoplasm/suspicious for a follicular or oncocytic (Hürthle cell) neoplasm (FN/SFN), and suspicious for malignant cells (SUSP) [4, 5]. The risk of malignancy for an indeterminate cytology thyroid nodule ranges from 5 to 75% (5–15% risk for AUS/FLUS nodules, 15–30% risk for FN/SFN nodules, and 60–75% risk for SUSP nodules) [3]. Based on the Bethesda classification, recommended management is repeat FNA biopsy for AUS/FLUS, diagnostic lobectomy for FN/SFN, and thyroidectomy or lobectomy for SUSP nodules [3].

The majority of surgically resected nodules are benign and the remaining 10–40% of nodules are malignant [4, 6, 7]. Thus, for many patients, surgery is unnecessary. Furthermore, in the patients with malignant thyroid nodules greater than 1 cm in size who have undergone diagnostic lobectomy, a completion lobectomy is typically performed to remove the remaining thyroid lobe. These patients could have benefitted from an upfront thyroidectomy rather than two separate

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surgeries. In addition, well-differentiated thyroid cancer is overall an indolent disease with a small proportion (5–10%) expected to have an aggressive course. Many patients can be spared more aggressive therapy if cancer is low-risk, and conversely, high-risk cancers need appropriate treatment. So, molecular markers may assist tumor prognostication.

To further refine the risk conferred by Bethesda classification, to reduce the need for diagnostic lobectomies and two-step surgeries, and to aid in tumor prognostication, several ancillary approaches have been pursued. These include the use of microRNAs, gene mutations/rearrangements, and gene expression panels [8–11]. Several of these ancillary studies are being used in clinical management and will be discussed below.

Molecular Alterations in Thyroid Cancer

The genomic alterations underlying thyroid cancer pathogenesis have been well characterized (Table 1). Studies from multiple laboratories have identified the driver mutations for the majority of thyroid tumors, and recent large scale sequencing projects have identified genomic alterations in many of the remaining thyroid tumors as well as provided an overview of the landscape of alterations. These findings have been important in shaping and evolving the classification of thyroid tumors to reflect histologic, molecular, and behavioral features.

Recently, papillary thyroid carcinoma was extensively studied through The Cancer Genome Atlas (TCGA) initiative [12]. Using data on single nucleotide variants, small indels, translocations, mRNA expression, miR expression, protein expression, DNA methylation, and copy number alterations from 496 papillary thyroid carcinomas, driver mutations were identified in 96.5% of cases [12]. Papillary thyroid carcinomas were found to have a low frequency of somatic variants, and most tumor genomes were "quiet," with few copy number gains or losses [12]. Most of the alterations seen in the TCGA study as well as in previous studies involved genes that function in the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K) pathways.

Table 1 Average frequency of main mutations and gene fusions in different types of thyroid cancer

Papillary thyroid carcinoma	
RAF	40–45%
RET/PTC	10–20%
RAS	10–20%
TERT	10%
NTRK	<5%
Follicular carcinoma	
RAS	40–50%
PAX8-PPARG	30–35%
TERT	10–20%
PIK3CA	<10%
PTEN	<10%
Poorly differentiated carcinoma	
TERT	40%
RAS	25–30%
CTNNB1	10–20%
TP53	20–30%
BRAF	10–15%
EIF1AX	10%
Anaplastic carcinoma	
TP53	70–80%
CTNNB1	60–70%
TERT	70%
RAS	40–50%
BRAF	20–30%
EIF1AX	10%
Medullary carcinoma	
RET	40–50%
RAS	20%
STK11	10–20%

BRAF, a serine threonine kinase, is a key player in the MAPK pathway. Activating mutations in *BRAF* are estimated to occur in approximately 40–45% of papillary thyroid cancers [13, 14]. Most *BRAF* mutations are the activating V600E mutation, although other mutations such as K601E mutation or small in-frame insertions or deletions have also been reported [15–18]. An association of the *BRAF* V600E mutation with conventional and tall cell variant of papillary thyroid carcinoma has been reported, while the *BRAF* K601E mutation has been reported to be associated with follicular variant of papillary

thyroid cancer [19–23]. Alternate activation of *BRAF* and MAPK signaling in papillary thyroid carcinoma occurs through the generation of BRAF fusion proteins. Reported fusions such as *AKAP9-BRAF*, *SND1-BRAF*, or *MKRN1-BRAF* preserve the C-terminal kinase domain of BRAF while removing and replacing the N-terminal regulatory domain of BRAF with a fusion partner [12, 24].

Oncogenic mutations in *NRAS*, *HRAS*, or *KRAS* are also seen in papillary thyroid carcinoma. These mutations most frequently occur at codon 61 in *NRAS* and *HRAS*, although mutations at codons 12 and 13 are also seen. *RAS* gene mutations are primarily seen in the follicular variant of papillary thyroid cancer [19, 25, 26]. The observation that *RAS* mutations primarily occur in noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) and invasive follicular variant of papillary thyroid carcinoma has led to the suggestion that NIFTP may represent a precursor to invasive follicular variant of papillary thyroid carcinoma [25].

Driver fusion genes are also important in papillary thyroid carcinoma pathogenesis. The most common rearrangements are RET/PTC1 (fusion of RET with CCDC6) and RET/PTC3 (fusion of 47\RET with NCOA4). These fusions were previously observed at approximately 20–30% frequency two decades ago and are now seen in approximately 10% of cases [27–29]. In 5% of papillary thyroid carcinomas, rearrangements involving *NTRK1* and *NTRK3* are seen [30–34], although a recent report suggests that the frequency of *NTRK* rearrangements in pediatric papillary thyroid carcinoma may be much higher [35]. Other fusions, such as those involving *THADA* and *ALK* genes, are observed in approximately 1% of papillary thyroid carcinomas [12, 36].

The TCGA study of papillary thyroid carcinoma identified a novel significantly mutated gene, *EIF1AX* [12]. This gene encodes an essential eukaryotic translational initiation factor. Recurrent mutations in *EIF1AX* were observed, primarily in tumors lacking known MAPK pathway driver mutations, suggesting a possible novel driver role for *EIF1AX* in papillary thyroid carcinoma [12]. However, a subsequent study found that although *EIF1AX* mutations were seen in approximately 2% of papillary thyroid carcinomas, mutations were also seen in two follicular adenomas and one hyperplastic nodule, possibly limiting the utility of *EIF1AX* as a highly specific marker of papillary thyroid carcinoma [37].

For follicular adenomas and follicular carcinomas, the *RAS* genes have been implicated as major driver genes [38–40]. Approximately 40–50% of follicular carcinomas and 20–40% of follicular adenomas have been reported to harbor *RAS* gene mutations [38–41]. Also seen at a significant frequency (30–40%) in follicular carcinoma is *PAX8/PPARG* rearrangement [42–44]. This rearrangement may also be seen, at lower frequencies, in follicular adenomas as well as in the follicular variant of papillary thyroid carcinoma [42–46]. Another alteration that has been reported in both follicular adenoma and follicular carcinoma is mutation of *PTEN*, a tumor suppressor gene that functions as a negative regulator of the PI3K/AKT pathway [34, 47–50].

Poorly differentiated and anaplastic thyroid carcinomas, as compared to well-differentiated follicular tumors, often harbor multiple driver mutations (Fig. 1). In addition to mutations in *BRAF* or *RAS*, these tumors typically acquire additional mutations in genes like *TP53*, *PIK3CA*,

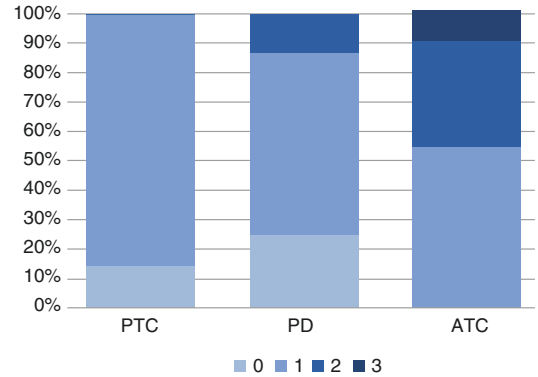

Fig. 1 Driver mutation/fusion frequency in thyroid cancer. Number of driver mutations (in *BRAF*, *NRAS*, *KRAS*, *HRAS*, *EIF1AX*, or *TP53* genes) or driver fusions across papillary thyroid carcinomas (PTC), poorly differentiated carcinomas (PD), and anaplastic thyroid carcinomas (ATC)

and *AKT1*. *TP53* is an important tumor suppressor and, in many tumor types, including thyroid cancer, is associated with aggressive behavior and tumor progression. Approximately 20–30% of poorly differentiated carcinomas and 70–80% of anaplastic thyroid carcinomas are reported to harbor *TP53* mutations [51–55]. Other genetic alterations that have been described in poorly differentiated and anaplastic thyroid carcinomas involve activating mutations in the *PIK3CA* and *AKT1* genes, both of which function in the PI3K/AKT pathway [49, 56, 57].

Recurrent mutations in the telomerase (*TERT*) promoter have been described in the last few years and have been described in a multitude of tumors including melanoma, glioblastoma, bladder, and thyroid cancers. These mutations, located 124 bp (C228T) and 146 bp (C250T) upstream of the initiating ATG, are thought to increase *TERT* promoter activity [58, 59]. *TERT* promoter mutations have been reported in follicular cell thyroid cancers, but have not been detected in benign thyroid lesions [60–63]. Although seen in well-differentiated papillary thyroid and follicular carcinomas, the frequency of *TERT* promoter mutations is significantly higher in aggressive tumors such as widely invasive oncocytic carcinoma, poorly differentiated carcinoma, and anaplastic thyroid carcinoma [60–63]. The presence of *TERT* promoter mutations is associated with increased risk for persistent disease, distant metastases, and disease-specific mortality for well-differentiated thyroid cancer [63].

Recently, two studies further characterized poorly differentiated and anaplastic thyroid carcinomas using either a 341-gene cancer panel or whole exome sequencing [64, 65]. Both studies confirmed previous findings of *BRAF* or *RAS* mutations, which often co-occurred with variants in *TP53*, *TERT*, or PI3K/AKT/mTOR pathway components. Interestingly, *EIF1AX* mutations were seen in 11% of poorly differentiated carcinomas and 9% of anaplastic carcinomas in one study and in 14% of anaplastic carcinomas in the other study [64, 65]. In both studies, a strong tendency toward co-occurrence of *EIF1AX* and *RAS* mutations was seen, in contrast to papillary thyroid carcinoma, where *EIF1AX* mutations were mostly mutually exclusive with other driver mutations [12, 64, 65]. These findings raise the possibility of a cooperative effect of *EIF1AX* and *RAS* mutations in poorly differentiated and anaplastic carcinomas.

Medullary thyroid carcinomas are also primarily driven by MAPK and PI3K/AKT pathway mutations. Mutation of *RET*, a receptor tyrosine kinase expressed in thyroid C cells, is seen in both familial and sporadic forms of medullary thyroid cancer. The activating tyrosine kinase domain M918T mutation in *RET* is the most common *RET* mutation seen in sporadic medullary thyroid carcinomas and accounts for greater than 75% of *RET* mutations [66, 67]. The M918T mutation is also commonly seen in tumors arising in MEN2B syndrome [68–70]. In MEN2A syndrome and familial medullary thyroid carcinoma, *RET* mutations typically do not occur in the tyrosine kinase domain and instead occur at one of five conserved cysteine residues in the extracellular domain [71, 72]. Mutation of the cysteine residues allows the mutant RET protein to undergo ligand-independent dimerization and activation. In addition to *RET* mutation, mutation of the *RAS* genes has been described in sporadic medullary thyroid carcinomas [73–77]. These mutations are mutually exclusive and account for up to 90% of sporadic medullary thyroid carcinomas [73].

Recent work has shown the presence of *ALK* gene fusions in medullary thyroid carcinoma [75]. An *EML4-ALK* fusion, as well as a novel *GFPT1-ALK* fusion, was reported [75]. ALK fusions have not been previously observed in medullary thyroid carcinoma, but have been observed in approximately 1–2% of papillary thyroid carcinomas, 4–9% of poorly differentiated carcinomas, and 4% of anaplastic thyroid carcinomas [36, 65].

Finally, other genetic alterations may be seen in benign lesions and may be of utility in differentiating between benign and malignant lesions. Somatic activating mutations in *TSHR* have been reported to occur in approximately 50–80% of hyperfunctioning nodules. [78, 79] Activating mutations of *GNAS* occur in approximately 3–6%

of hyperfunctioning nodules [80–82]. Mutations in either gene are seen primarily in benign hyperfunctioning nodules and have only rarely been seen in follicular carcinomas [34].

Gene Mutation/Rearrangement Testing

One approach is single-gene mutational testing of thyroid nodules. Several groups have reported experiences with the use of *BRAF* V600E mutational analysis preoperatively. *BRAF* V600E mutation is seen in approximately 45% of papillary thyroid cancers and is not seen in benign thyroid nodules [13, 14]. *BRAF* V600E mutation is detectable by a variety of molecular technologies, such as real-time PCR, sequencing (Sanger and next generation), or single-base (primer) extension assays, which contribute to ease of adoption and incorporation into routine diagnostics and clinical management. Testing for *BRAF* V600E mutation has been reported to result in increased sensitivity in papillary thyroid cancer detection [83, 84]. In a recent meta-analysis of *BRAF* V600E mutation testing in thyroid FNA specimens, the addition of *BRAF* V600E testing to FNA cytology increased the sensitivity from 81.4 to 87.4% [85]. However, although the specificity of *BRAF* V600E mutation testing is very high (86.1–99.7%), the sensitivity is low (19.5–59.4%) [85]. Use of ultrasensitive techniques to detect *BRAF* V600E mutation may lead to false-positive results [86]. Preoperative *BRAF* V600E mutation testing may also have utility in predicting disease persistence and recurrence [87]. However, although *BRAF* V600E testing offers some utility in increasing sensitivity and predicting disease recurrence, as a stand-alone test, it offers insufficient sensitivity and specificity for thyroid cancer.

To address this, a seven-gene panel of genetic mutations and gene rearrangements was developed. This panel includes the genes and rearrangements most frequently implicated in thyroid cancer (*BRAF*, *NRAS*, *HRAS*, *KRAS*, *RET/PTC1*, *RET/PTC3*, and *PAX8/PPARG*), which together account for driver genes of approximately 70% of thyroid cancers. Each of these genes and rearrangements shows a high specificity and positive predictive value (PPV) for cancer, although the positive predictive value for *NRAS*, *HRAS*, or *KRAS* mutations is lower at 74–87% [11, 88, 89]. This seven-gene panel, or a similar eight-gene panel that also includes *TRK* rearrangements, was initially described and validated at two institutions in three prospective studies [11, 88, 89]. These studies all showed this gene panel to have high specificity (97–100%) and high PPV (86–100%) for cancer in indeterminate thyroid nodules [11, 88, 89].

Subsequent validation of similar seven-gene mutational tests, either in one retrospective study at a single institution or in two prospective studies at multiple institutions of the commercially available offering of a seven-gene panel, the ThyGenX test (formerly the miRInform test) offered by Interpace Diagnostics, has shown similar results [90–92]. In FN/SFN thyroid nodules, these studies showed a specificity of 86–92% and PPV of 71–80% [90–92].

To test variants that encompass a greater percentage of thyroid cancers and to further increase the sensitivity of mutational testing, next-generation sequencing (NGS) testing—either pan-cancer or thyroid specific panels—can be utilized [93, 94]. NGS technology is suited for high-throughput, massively parallel sequencing needs and can interrogate multiple genes simultaneously. A large, thyroid cancer-specific next-generation sequencing-based assay was recently developed and characterized (ThyroSeq v2). The genes on the ThyroSeq v2 panel include the seven genes in the other mutational panels but additionally include mutational hotspots in *AKT1*, *PTEN*, *TP53*, *TSHR*, *GNAS*, *CTNNB1*, *RET*, *PIK3CA*, *EIF1AX*, and *TERT*, as well as rearrangements of *RET*, *BRAF*, *NTRK1*, *NTRK3*, *PPARG*, and *THADA* [94]. Mutations or rearrangements involving the majority of these additional genes are primarily seen in thyroid carcinomas. A subset of these genes, such as *PTEN* and *EIF1AX*, are mutated in both benign and malignant lesions [12, 34, 37, 47–50], and activating mutations of *TSHR* and *GNAS* are mostly seen in hyperfunctioning nodules [78–82]. In the validation study

of ThyroSeq v2, a combined retrospective and prospective study at a single institution of 143 FN/SFN thyroid nodules, the test performed well with good specificity (93%) and PPV (83%) and additionally, showed good sensitivity (90%) and NPV (96%) [94].

Expression Classifier Testing

mRNA Gene Expression Classifier

Another methodology widely used in testing indeterminate thyroid nodules is gene expression profiling. The mRNA expression profiles of thyroid nodules were used to train a molecular classifier [95]. By examining the pattern of expression of 142 genes, which are involved in diverse processes such as energy metabolism or cell differentiation/development, thyroid nodules are classified into benign or suspicious categories [8, 95]. This test is currently offered commercially as the Afirma gene expression classifier (Veracyte).

The Afirma test was initially validated in a multi-institutional study of 265 indeterminate thyroid nodules and was found to have a high sensitivity (90%) and NPV (94%). The validation study was somewhat limited by small sample size, and some subsequent studies performed in institutions with higher disease prevalence, reported lower NPVs for the Afirma test [96–99]. Recently, a meta-analysis of seven studies of the Afirma test was performed [100]. In these studies, true negative and false negative rates were somewhat difficult to ascertain as many patients with benign results by the Afirma gene expression classifier did not undergo surgery. The prevalence of malignancy in the pooled cohort was 37.1% [100]. The meta-analysis found a pooled sensitivity of 95.7% and pooled specificity of 30.5% [100].

miRNA Expression Classifier

The differential expression of miRNAs has also been used in the classification of indeterminate thyroid nodules. miRNAs are small, noncoding RNAs. miRNAs regulate gene expression by binding to the 3′ untranslated region of target mRNAs and result in mRNA degradation or translation inhibition. Many miRNAs have been characterized in thyroid carcinoma, and the expression of a subset has been associated with not only the presence of carcinoma but additionally with prognostic features such as advanced disease or extrathyroidal extension [101, 102].

A panel of 10 miRNAs (miR-29-b-1-5p, miR-31-5p, miR-138-1-3p, miR-139-5p, miR-146b-5p, miR-155, miR-204-5p, miR-222-3p, miR-375, and miR-551-3p) is used to classify nodules as “positive” or “negative.” This testing is currently available commercially as the ThyraMIR test (Interpace Diagnostics) and is offered as reflex testing on thyroid nodules that are negative by the ThyGenX panel [92]. In the initial validation study of this miRNA classifier, the reported sensitivity was 57%, specificity 92%, NPV 82%, and PPV 77% [92].

Test Performance Comparisons and Potential Improvements

Evaluation of diagnostic tests typically involves comparisons of specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV). Tests with clinical utility in ruling out malignancy should have high sensitivity and NPV, and tests with clinical utility in ruling in malignancy should have high specificity and PPV. Sensitivity and specificity reflect test performance characteristics, but NPV and PPV may vary significantly depending on the prevalence of disease. In the context of thyroid nodules, this may reflect differences in patient population demographics or institutional differences in the malignancy rates in each indeterminate cytology category.

Although there were institutional differences because of variability in disease prevalence, in general, available follow-up studies have supported the findings in the initial validation studies. Seven-gene mutation/rearrangement studies have high specificity and PPV and show utility in

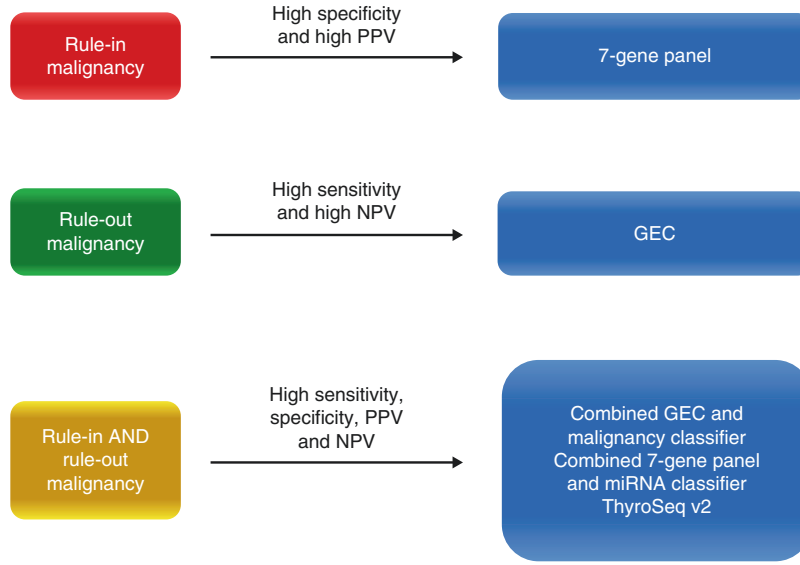

Fig. 2 Utility of currently available diagnostic tests and their performance characteristics

"ruling in" malignancy, while the gene expression classifier has high sensitivity and NPV and shows utility in "ruling out" malignancy (Fig. 2). The ideal diagnostic test, however, would have high PPV, and high NPV would be able to both rule in and rule out malignancy. One possible approach would be to add-on or combine testing. Afirma, for example, in addition to the gene expression classifier, also offers two malignancy classifiers for nodules suspicious by GEC or cytopathology, Afirma MTC and Afirma BRAF. These are mRNA gene expression classifiers specific for genes differentially expressed in either medullary thyroid cancer or *BRAF* V600E mutation-positive thyroid cancer. A positive result for the Afirma MTC or Afirma BRAF test may add additional specificity to the Afirma GEC, although data regarding this has not yet been published. Interpace Diagnostics combines the miRNA-based classifier (ThyraMIR) in thyroid nodules that are negative by the seven-gene mutational panel (ThyGenX). In their validation studies, they report that by combining tests, they are able to achieve a sensitivity of 89%, specificity of 85%, NPV of 94%, and PPV of 74%. Further studies of this test are needed to explore this test.

Of the currently available tests, ThyroSeq v2 with a sensitivity of 90%, specificity of 93%, NPV of 96%, and PPV of 83% in FN/SFN nodules currently shows much promise as a potential test to both rule in and rule out malignancy. Potential increases in specificity and sensitivity may be both from further expanding the panel and from increased understanding of thyroid pathogenesis and "cooperating" genes that drive malignancy. For example, whereas *BRAF* mutation and *RET/PTC* rearrangement are seen virtually exclusively in thyroid cancer, *RAS* mutations are also seen in benign or indolent neoplasms such as follicular adenomas or NIFTP, and thus the PPV of *RAS* mutations for malignancy ranges from 74 to 87% [11, 88, 89]. Recent studies, however, suggest that coexisting *RAS* and *TP53* or *RAS* and *EIF1AX* mutation may, with further study, prove to be associated with increased risk.

Clinical Utility of Molecular Testing of Indeterminate Thyroid Nodules

Based on the performance characteristics of seven-gene mutation/rearrangement panels (high specificity and high PPV) and gene expression classifiers (high sensitivity and high NPV), clinical algorithms have been suggested to guide perioperative decision-making [103]. With seven-gene mutation/rearrangement panels, the suggested management for a positive result for AUS/FLUS, FN/SFN, or SUSP nodules is oncologic thyroidec-

tomy. Negative results for AUS/FLUS nodules may be managed by observation or diagnostic thyroid lobectomy, whereas negative results for FN/SFN or SUSP nodules should be managed by at least a diagnostic thyroid lobectomy. For gene expression classifier testing results, suspicious results for AUS/FLUS or FN/SFN nodules should be managed by at least a diagnostic thyroid lobectomy, and benign results may be managed by observation or diagnostic thyroid lobectomy. Testing of SUSP nodules by gene expression classifier is generally not recommended as both benign and suspicious results should still be managed with at least a diagnostic thyroid lobectomy.

Initial results on application of molecular testing results into the management of indeterminate thyroid nodules have been reported for both seven-gene mutation/rearrangement panels and gene expression classifiers [104, 105]. For the seven-gene mutation/rearrangement panel, in a series of 471 patients with AUS/FLUS or FN/SFN nodules at a single institution, patients who did not undergo seven-gene mutation/rearrangement panel testing were found to be 2.5-fold more likely to require a two-step (initial lobectomy followed by completion thyroidectomy) surgery [105]. For gene expression classifier testing, a study of 273 patients at a single institution reported a change in clinical management in 8.4% of patients who underwent testing [104]. Further studies are needed to more fully assess the impact of molecular testing on clinical management.

Prognostic Applications of Molecular Markers

Molecular profiling of mutations and gene rearrangements not only provides helpful diagnostic information that can help rule in malignancy but can also simultaneously identify molecular alterations with prognostic and therapeutic applications. Molecular profiling may inform surgical management as some patient may benefit from a more extensive initial surgery, may affect post-surgical surveillance for disease recurrence, and may provide therapeutic targets for metastatic or recurrent disease.

The *BRAF* V600E mutation has been extensively characterized as a possible prognostic marker. Multiple studies have found an association in papillary thyroid cancer between the *BRAF* V600E mutation and factors such as extrathyroidal invasion, metastatic disease, and disease recurrence. However, other studies did not show a strong association [106–108]. In a meta-analysis of 14 studies, the *BRAF* V600E mutation was found to be associated with tumor recurrence and persistent disease (25% in *BRAF* mutation-positive tumors vs. 13% in mutation-negative tumors). Furthermore, in a large, multicenter study, the *BRAF* V600E mutation was shown to be significantly associated with mortality (5% in mutation-positive patients vs. 1% in mutation-negative patients) [109]. For both tumor recurrence and mortality, the increases were small but statistically significant, suggesting that *BRAF* V600E mutation alone is a relatively sensitive, but not specific marker of tumor recurrence and tumor-related mortality.

TP53 has been described as a prognostic marker in several tumors and, in thyroid cancer, is a well characterized genetic event governing thyroid tumor dedifferentiation. *TP53* mutations occur in well-differentiated tumors but occur at highest frequency in poorly differentiated and anaplastic thyroid cancers [51, 52]. Further studies are needed, but *TP53* mutations in well-differentiated tumors may herald the potential for dedifferentiation or a more aggressive clinical course.

The recurrent mutations of the *TERT* promoter are seen more frequently in aggressive thyroid tumors such as widely invasive oncocytic carcinoma and anaplastic thyroid carcinoma [60–63]. *TERT* promoter mutations have been reported to be an independent risk factor for poor prognostic factors such as persistent disease, distant metastases, and disease-specific mortality for well-differential thyroid cancer [63]. In addition, *TERT* promoter mutations were found to frequently co-occur with *BRAF* V600E mutation, which suggested a possible interplay between MAPK pathway and telomerase activation in aggressive tumors [60, 62]. Indeed, in a recent study of 551 patients with differentiated thyroid cancer, the coexistence of *BRAF* or *RAS* mutations with

TERT promoter mutations was found to be associated with increased recurrence and mortality [110].

As we continue to elucidate the genomic landscape of thyroid cancer, it is likely that more markers of aggressive tumor behavior will be found. It is becoming clear that rather than the presence of a single biomarker, a profile of genomic alterations may be more useful in predicting tumor behavior. Coexisting mutations in driver genes such as *BRAF* or *RAS* with mutations in *PIK3CA*, *AKT1*, or *TP53* occur in poorly differentiated and anaplastic tumors [49, 56, 111]. Multiple mutations have also been seen in a small number of well-differentiated tumors, which were aggressive and presented with distant metastases [112]. Detection of multiple mutations can be achieved in FNA samples of even very small tumors, allowing for both diagnosis and prognostication prior to surgery [113].

Summary

Improved diagnostic accuracy of thyroid nodules and clinical management decision support is achievable by incorporating molecular mutation/rearrangement or gene expression information. Currently available tests excel in ruling in or ruling out malignancy, and further improvements are expected with expanded panels that include more thyroid cancer markers. Gene mutation/rearrangement panels additionally offer prognostic information that can guide the extent of the initial surgical management as well as postsurgical management. With further improvements in technology and decreased costs of testing, routine molecular profiling of thyroid tumors can help achieve a personalized treatment and management plan for every patient.

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