

Molecular genetics in the diagnosis and management of thyroid cancer

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Dr. McFadden has disclosed no financial relationships related to the content of this presentation. Dr. McFadden will not be discussing off-label use of FDA-approved drugs.

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Purpose:

Discuss clinical controversies in the diagnosis and management of thyroid cancers, including the overdiagnosis and overtreatment of indolent forms of thyroid cancer and the development of re-differentiation strategies for radioiodine-refractory advanced thyroid cancer.

Educational Objectives:

- 1) Review the spectrum of disease phenotypes for follicular cell derived thyroid cancers
- 2) Understand the somatic genetics of common forms of thyroid cancer
- 3) Present evidence supporting the current overdiagnosis and overtreatment of thyroid cancer
- 4) Discuss potential strategies to minimize overtreatment of indolent forms of thyroid cancer
- 5) Describe new approaches promoting the re-sensitization of radioiodine-refractory thyroid cancers to iodine-based therapies.

Introduction:

In 1947, the pathologist W.P. VanderLaan reviewed 35,000 autopsy reports from Boston City Hospital, the Peter Bent Brigham Hospital, and the Massachusetts General Hospital and identified a total of 20 deaths (0.06%) attributed to thyroid cancer (1). He presciently wrote, "In view of the relative infrequency with which carcinoma of the thyroid gland has been found as a cause of death in a large autopsy experience, it seems reasonable that the pathological criteria for this diagnosis should be reviewed in cases in which microscopic examination of thyroid nodules has led to the conclusion that carcinoma of the thyroid gland is of frequent occurrence."

Thyroid cancer is the most rapidly increasing cancer diagnosis in the United States, and in other countries, and is predicted to become the third most common cancer diagnosis in women with significant associated health care costs and patient morbidity (seer.cancer.gov) (2, 3). Autopsy pathological studies suggest the prevalence of disease is remarkably high, with up to 36% of examined thyroid glands harboring thyroid cancers, the majority of which are microscopic tumors (<1 cm) (4). However, few patients will die from thyroid cancer, and the mortality from thyroid cancer has remained stable over several decades despite the rapidly rising incidence (3).

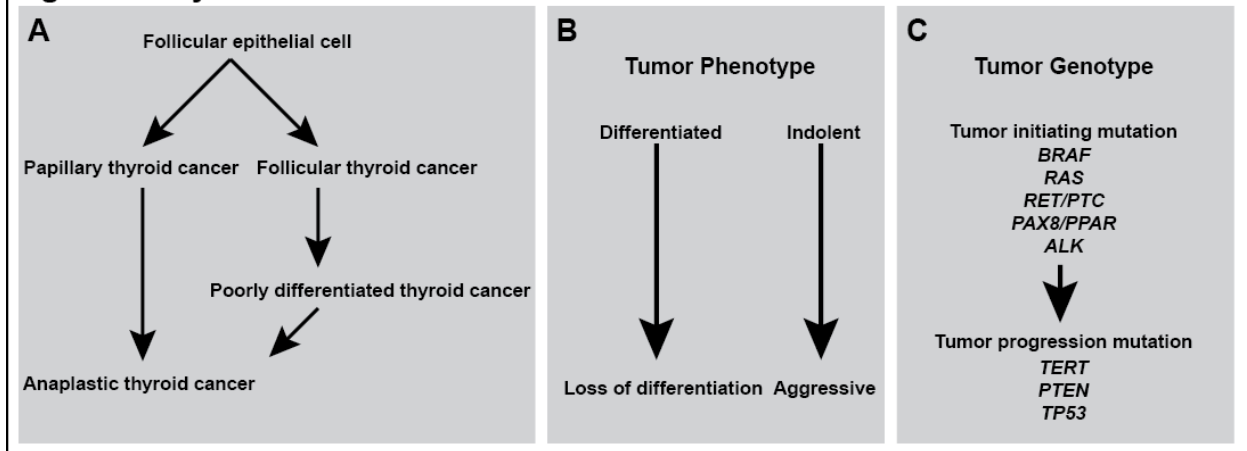
Thyroid cancer: A broad spectrum of disease phenotypes

Malignancies arising from the thyroid gland include the follicular epithelial cell derived neoplasms, C-cell derived medullary thyroid cancer, and less commonly thyroid lymphoma (5). Follicular cell neoplasms account for over 90% of thyroid cancer diagnoses, are responsible for the majority of deaths from thyroid cancer, and are the most rapidly increasing form of thyroid cancer (6). These neoplasms will be the focus of this UT Southwestern Internal Medicine Grand Rounds. Hereafter, use of the term thyroid cancer specifically refers to the follicular cell derived neoplasms.

Neoplasms arising from the thyroid follicular epithelial cell are broadly divided into well-differentiated, poorly differentiated, and undifferentiated (anaplastic) lesions (Figure 1A). Well-differentiated thyroid cancers (WDC) account for the vast majority of new diagnoses of thyroid cancer and the majority of thyroid-cancer related deaths. WDC tumors consist of two primary subtypes including papillary thyroid cancer (PTC), and follicular thyroid cancer (FTC). Papillary thyroid carcinomas exhibit papillary histological growth pattern, and defined nuclear features including pseudoinclusions, nuclear folds/grooves, and nuclear clearing are part of the diagnostic criteria for the disease (7). These tumors exhibit a high frequency of metastasis to local cervical lymph nodes, yet distant metastases and death from PTC are relatively uncommon. Features that predict a poorer prognosis in PTC include old age at diagnosis, extrathyroidal extension of tumor, spread to lateral cervical lymph nodes, and specific histological variants including the tall cell, columnar and hobnail variants.

Follicular thyroid carcinomas exhibit a microfollicular growth pattern, and have largely normal appearing nuclei. Diagnosis of FTC requires surgical pathology to

Figure 1: Thyroid cancer classification



demonstrate tumor capsule invasion or vascular invasion, and follicular neoplasms without invasion are categorized as follicular adenomas - benign entities with a near zero recurrence risk. FTC metastasis occurs by hematogenous seeding, and local cervical lymph node metastases are rare. The most frequent sites of metastasis of FTC are lung and bone.

The follicular variant of papillary thyroid cancer (FVPTC), exhibits features of both PTC and FTC, including a microfollicular growth pattern reminiscent of FTC combined with the nuclear features of PTC. These tumors may be encapsulated by a fibrous band of tissue, or infiltrate the thyroid parenchyma (see below).

Thyroid cancers exhibit a very high diversity of clinical phenotypes. Microscopic papillary thyroid carcinoma rarely contributes to patient mortality, and likely exists as a subclinical disease reservoir in a large fraction of the population. Non-invasive encapsulated follicular tumors of the thyroid also exhibit nearly 0% mortality and a very low risk of recurrence. In contrast, anaplastic thyroid cancer is among the most aggressive of all malignancies, with a median survival of 9 weeks and a 5-year survival of less than 7% (8). Therefore, assessing disease risk for each individual thyroid cancer patients is a primary goal of the treating physician.

A Disequilibrium Between Disease and Therapy

The biological diversity of thyroid cancer exists in the context of the fact that overwhelming majority of thyroid cancers represent clinically indolent disease, and are unlikely to impact patient health, even if untreated. How do clinicians determine the subset of tumors warranting treatments? Approaches are needed to minimize diagnosis and unnecessary treatments for patients with tumors that would never cause clinical harm. Conversely, aggressive subsets of thyroid cancers respond poorly to standard

chemotherapeutic regimens, and despite recent successful clinical trials using multi-kinase inhibitors, disease mortality and morbidity remains largely unchanged. Therefore, a great need exists to both minimize treatments for indolent thyroid cancers, as well as improve treatments for clinically aggressive forms of the disease. Here, I review evidence for these problems, and discuss recently described approaches to begin to address these conundrums, in part using molecular genetics to refine diagnostic and therapeutic strategies.

The somatic genetics of thyroid cancer

A majority of thyroid cancers harbor oncogenic mutations leading to activation of the mitogen activated protein kinase (MAPK) pathway, and genetically engineered mouse models support the notion that these mutations are tumor initiating events for thyroid cancer (9-13). The *BRAF*^{V600E} mutation is the most frequently detected mutation in thyroid cancer, occurring in approximately 60% of PTCs, and 30-50% of ATCs. Mutations in the RAS family (*NRAS*, *HRAS*, and *KRAS*) are frequently detected in FTC, FVPTC, PDTC, and ATC. Translocations involving the *RET* receptor tyrosine kinase have been identified in approximately 5-15% of PTCs. The *PAX8-PPAR γ* translocation is also reported in approximately 30% of FTCs.

Novel oncogenes have been recently described in thyroid cancers, including gene rearrangements involving the oncogene *ALK*, point mutations in a translation initiating factor, *EIF1Ax*, and gene fusions protein of unknown function, *THADA* (9, 14, 15). The underlying mechanisms by which these novel candidates promote tumorigenesis in follicular epithelial cells will be important to characterize.

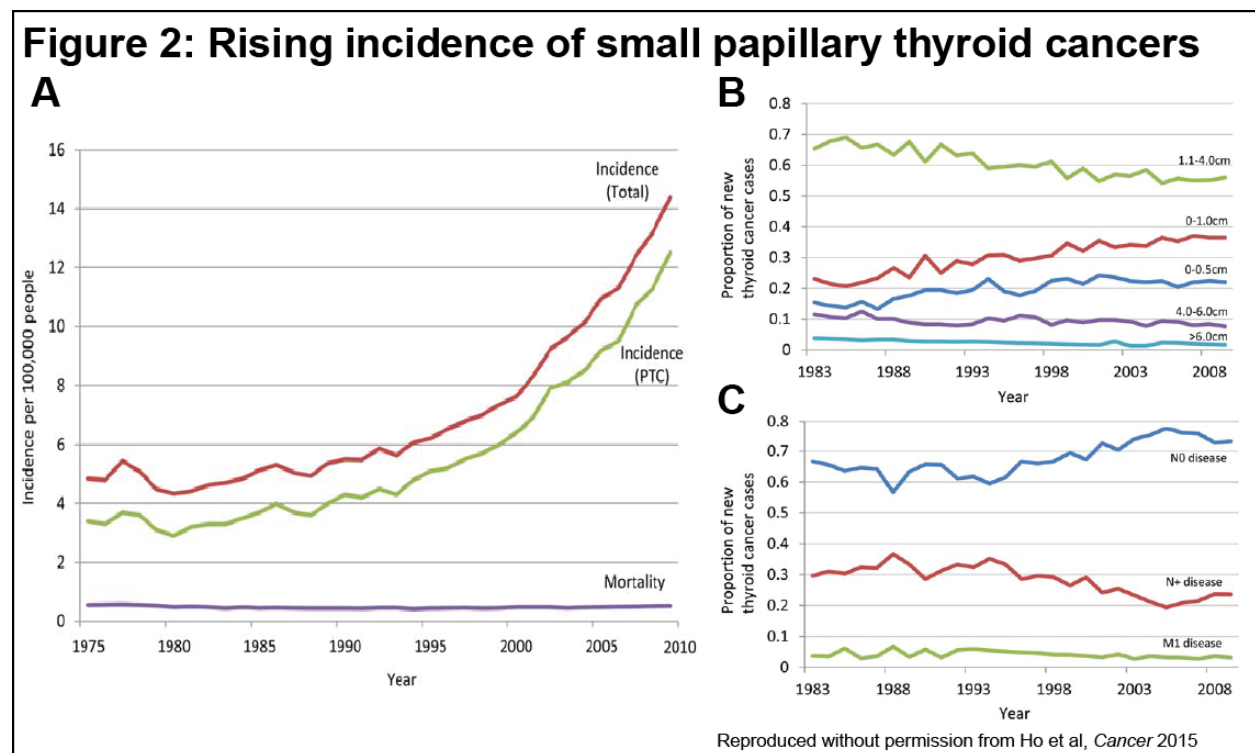
Clinically aggressive thyroid cancers harbor mutations in key tumor suppressor pathways that are believed to promote or enable tumor progression in the context of a tumor initiating mutation (12). Mutations impacting the promoter region of the *TERT* gene, which encodes the catalytic subunit of telomerase, are associated with clinically aggressive subsets of disease in PTC, FTC, and appear to be enriched in PDTC and ATC tumors (16-18). Poorly-differentiated thyroid cancers (PDTC) and ATC harbor recurrent mutations impacting the PTEN/PI3K pathway (19-21). The majority of ATCs harbor mutations in the p53 tumors suppressor. *BRAF* and *RAS* mutations are most commonly observed initiating MAPK pathway mutation in ATC.

The clinical implication of specific mutations in thyroid cancer remains controversial. Detection of a *TERT* promoter mutation, or p53 mutation might have prognostic relevance given the strong association with aggressive forms of thyroid cancer. However, this has not been validated in prospective trials. Identification of a specific targetable mutation (such as an *ALK* rearrangement) might also impact the eligibility of a patient for a clinical trial or treatment with a small molecule kinase inhibitor. Several clinical trials have examined efficacy of novel small molecules for treatment of advanced thyroid cancer, leading to FDA approval for the use of sorafenib and lenvatinib for RAI-refractory thyroid cancer (22, 23). Although promising results

have been obtained, these therapies are not curative, and have not been shown to prolong patient survival to date, except in subgroup analysis with lenvatinib. Nonetheless, the continued application of targeted chemotherapeutic drugs is likely to be an important avenue to improve patient outcomes, as well as impact the sensitivity of thyroid cancers to radioiodine therapy (see below).

The over-diagnosis problem: papillary thyroid cancer

Stable mortality in the face of rising incidence suggests overdiagnosis of a disease. Diagnosis of a disease which would otherwise never impact patient health leads to unnecessary tests, treatments and morbidity. The incidence of papillary thyroid cancer in the United States has risen from 4.9 cases per 100,000 individuals in 1975 to 14.3 per 100,000 in 2009 (3). Since 2009, the incidence has continued to climb, with thyroid cancer now becoming the most rapidly increasing cancer diagnosis (Figure 2A). Several studies suggest the increasing incidence is both a result of increased use of imaging studies such as thyroid ultrasound and computed tomography (24). In addition,



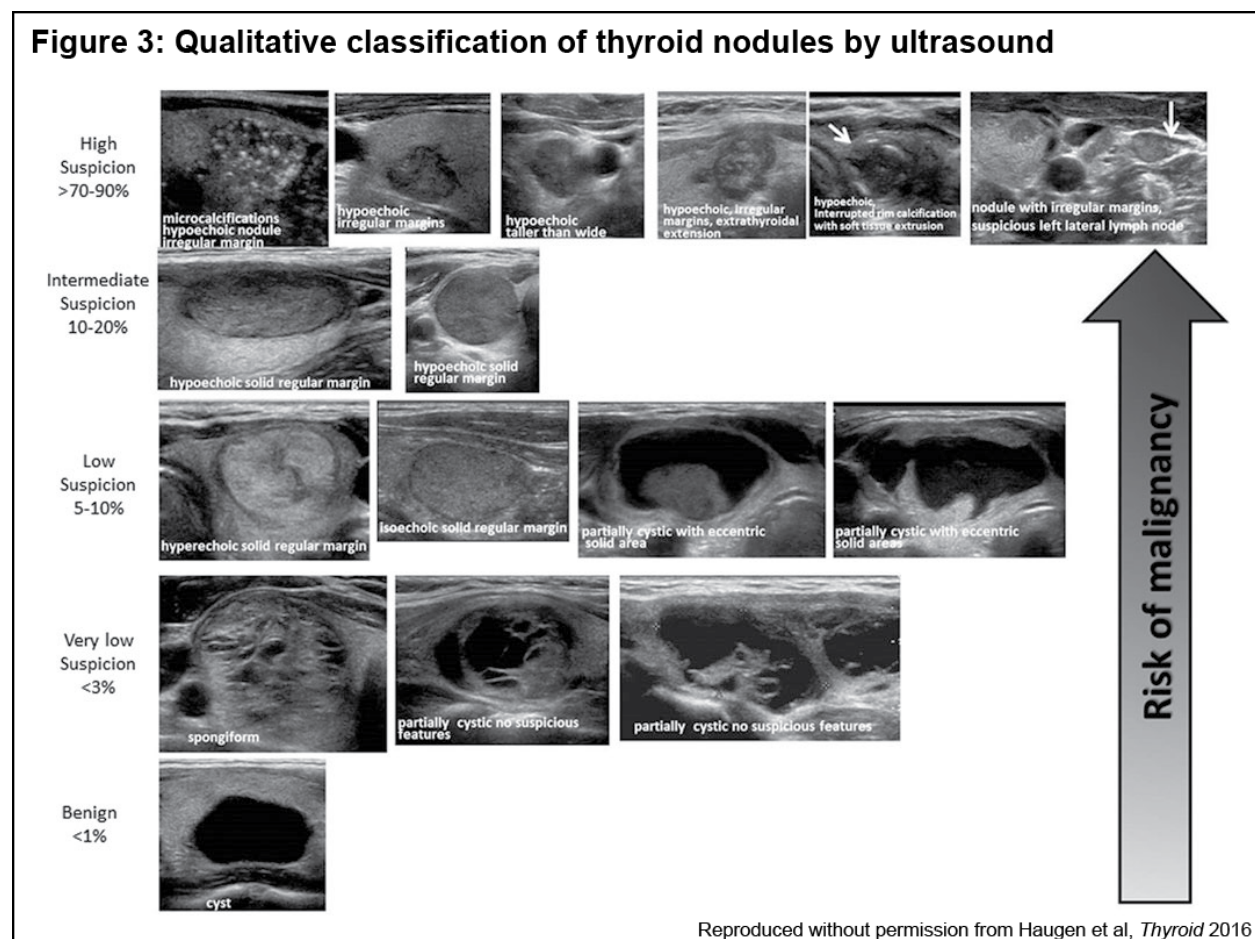
small thyroid cancers, without observable lymph node metastases, are responsible for most of the increased incidence of thyroid cancer within the past several decades (Figure 2B-C). In the face of this rising incidence, mortality from thyroid cancer has remained unchanged (25).

The recent South Korean experience is compelling (2). In 1999, the South Korean government initiated a national cancer screening program for early detection of

breast, cervical, colon, gastric and hepatic cancers. For a small \$30-50 add-on fee, a neck ultrasound was offered to screen for thyroid cancer. The incidence of thyroid cancer has increased 15-fold between 1993 and 2011. In addition, 25% of thyroid surgeries are now for tumors less than 5mm in diameter. Throughout this period, the number of deaths from thyroid cancer has remained unchanged, ranging between 300-400 per year.

If the increased detection of thyroid cancer identified tumors that have potential to become clinically aggressive, mortality (both in South Korea and the U.S.) would be expected to be decreasing. This is not the case. Therefore, it is unlikely that a large fraction of newly uncovered thyroid cancers have significant potential to cause morbidity or mortality if left undiagnosed.

Figure 3: Qualitative classification of thyroid nodules by ultrasound



Minimizing unnecessary treatments: Biopsy fewer thyroid nodules

In this context of a large reservoir of small, clinically silent thyroid cancers in the population, recommendations endorsing biopsy of small thyroid nodules are likely to increase, rather than decrease, patient morbidity. As a result, several investigators and societies have called for a reduction in biopsy of small thyroid nodules. One study

suggests biopsy of thyroid nodules only over 2cm identified the vast majority of cancerous thyroid nodules (26). However, a one size fits all recommendation may not be adequate as a small fraction of microscopic PTC exhibit clinically aggressive behavior including metastasis to cervical lymph nodes and local (extra-thyroidal) invasion. Therefore, a proper thyroid ultrasound should include careful assessment of cervical nodes (including the lateral compartments) prior to a final recommendation regarding the decision for FNA. Recent revisions to the American Thyroid Association (ATA) Guidelines have also emphasized the sonographic features of benign and malignant thyroid nodules, and have tailored recommendations for FNA to include these qualitative features, in addition to the size of the nodule (Figure 3) (6).

Minimizing unnecessary treatments: Surveillance of microscopic PTC

The assumption that the majority of small papillary thyroid cancers will not impact patient health is implicit in recommendations against biopsy of these nodules. Otherwise, a strategy of early diagnosis and management should be standard of care, and lead to better outcomes, including lower thyroid cancer mortality. Although the epidemiologic and autopsy studies mentioned above strongly support this assumption, two Japanese studies have assessed the natural history of small papillary thyroid cancers in prospective clinical trials.

Ito et al. reported following over 300 patients with biopsy-proven microscopic (<1cm) PTC. Patients were offered surgery or observation by serial neck ultrasound. For the observation group, tumor growth (>3mm in any dimension) or detection of sonographically suspicious lymph nodes led to surgery. Over a 10-year observation period no patients in the surveillance group died of thyroid cancer, and new cervical nodal metastases were detected in approximately 3% of patients (27). Sugitani et al. also followed 244 patients with biopsy proven PTC for up to 17 years. None of these patients developed extrathyroidal invasion or distant metastases, and only 5% of patients underwent surgery for tumor growth or evidence of new cervical lymph node metastases (28).

Several challenges exist to implementation of a surveillance strategy for small thyroid nodules with suspicious ultrasound features, or small nodules with FNA cytology suspicious for, or diagnostic of, PTC. First, many patients will not be comfortable following, rather than acting upon, any lesion referred to as a cancer. Second, this strategy should likely only be considered at centers with expertise and appropriate clinical volume in thyroid cancer. Recommendations for implementation of an active surveillance approach have been proposed by Memorial Sloan Kettering Cancer Center, which take into account several patient and ultrasound characteristics in order to exclude nodules that have increased potential to exhibit clinically aggressive features (29). Results from prospective trials in the US will be needed to provide additional evidence supporting this strategy, and to improve patient awareness of the safety of a

surveillance approach. In addition, identifying biomarkers that predict tumor growth or metastasis in this patient/tumor population would be of significant benefit.

Minimizing unnecessary treatments: Renaming indolent neoplasms

The burden of responsibility of overtreatment of indolent thyroid cancers lies with physicians whose criteria for calling a lesion cancer overestimates the potential for the lesion to cause harm, as Dr. VanderLaan suggested in 1947. Therefore, another approach toward minimizing unnecessary treatments is the reclassification of indolent thyroid cancers as premalignant lesions. However, prediction of which thyroid cancers have potential for aggressive (malignant) features remains imperfect, complicating reclassification of all

small PTCs as premalignant lesions.

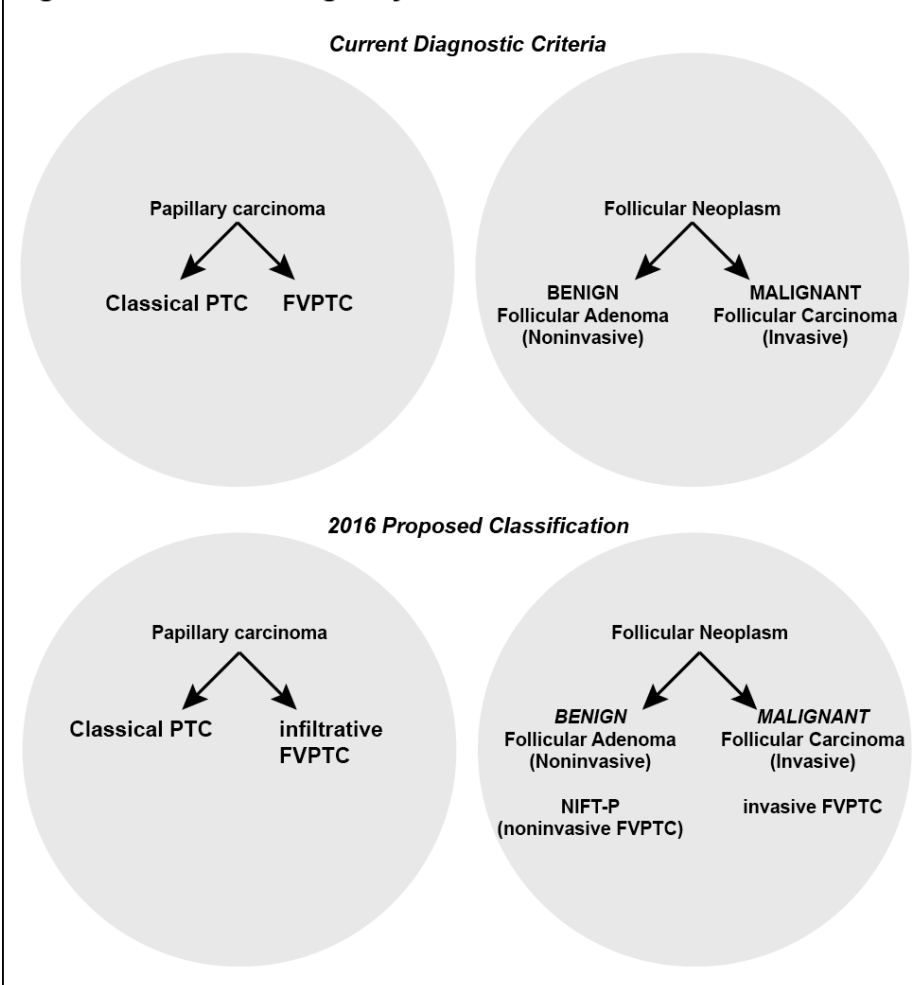
Reclassification of thyroid cancers must therefore be considered for only carefully selected tumors.

The incidence of the FVPTC, described above, has increased dramatically, and currently accounts for approximately 20-30% of new PTC diagnoses in the U.S. More recently, several studies have suggested that current diagnostic criteria for FVPTC captures two biologically distinct

entities (Figure 4). Pathological examination of FVPTC has identified two general subtypes - tumors with a fibrous capsule ("encapsulated"), and FVPTC that infiltrate the surrounding thyroid parenchyma ("infiltrative").

Genetic and clinical studies have supported this bifurcation of FVPTC. Several studies have shown that infiltrative FVPTC frequently metastasize to cervical lymph

Figure 4: FVPTC - Biologically distinct entities



nodes, and are enriched for the *BRAF*^{V600E} mutation (15, 30). This suggests infiltrative are biologically similar to classical PTC. In contrast, encapsulated FVPTC rarely metastasize to cervical lymph nodes, and recurrence and metastasis are only observed in highly invasive tumors (with capsular, and generally vascular invasion). In concordance with the clinical behavior associated with FTC, encapsulated FVPTC harbor frequent *RAS* mutations, and occasionally FTC-type rearrangements including translocations involving *PPARγ* and *ALK*.

An important extrapolation of these observations impacts an encapsulated, non-invasive FVPTC (31). Until this month (April 2016), this tumor was categorized as a carcinoma. Given the above genetic and clinical associations, as well as multiple studies demonstrating a very low risk of recurrence for noninvasive, encapsulated FVPTC, the entity was renamed as “noninvasive follicular thyroid neoplasm papillary-like nuclear features” (NIFT-P) (32). This effectively places encapsulated FVPTC back into the follicular neoplasm category (Figure 4, lower panel). Future studies will be required to validate this reclassification. In addition, several criteria will be important including careful pathological examination of the entire tumor capsule, as is currently standard of care for follicular patterned lesions. Nonetheless, reclassifying tumors with a predicted recurrence risk of less than 1% over 15 years should significantly reduce unnecessary treatments, as an estimated 45,000 patients per year worldwide are diagnosed with noninvasive encapsulated FVPTC (32).

Molecular analysis of thyroid nodules

FNA cytology of thyroid nodules is a highly accurate medical test, with approximately a 1-3% false negative rate for nodules called benign (6). Likewise, for nodules called papillary thyroid cancer, the test is nearly 100% accurate, due to the nuclear features diagnostic of this neoplasm. However, approximately 15-30% percent of biopsies fall into categories considered “cytologically indeterminate.” This is in large part due to the biology of follicular thyroid neoplasms discussed above. Follicular carcinomas exhibit normal nuclear features, and the diagnosis requires surgical pathology to demonstrate tumor capsule invasion and/or vascular invasion. In addition, FVPTC sometimes exhibits only foci of nuclear atypia, which may not be adequately sampled by needle biopsy.

Two recently described approaches attempt to leverage molecular genetics to refine FNA cytological diagnosis. Veracyte diagnostics developed a 167 gene expression classifier (GEC) that predicted benignity of a thyroid nodule in several studies (33). Nodules were reported as “benign” or “suspicious” by the GEC. Nodules with a benign GEC were highly likely to be benign on surgical pathology, with an approximated 95% negative predictive value (NPV), but an overall low (~52%) positive predictive value (PPV) of cancer in nodules called suspicious. Therefore, the Veracyte test is considered a “rule out” diagnostic test. Although studies have demonstrated the effectiveness of the Afirma GEC in reducing the number of thyroid surgeries for benign

nodules, the rate of malignancy of suspicious GEC has been shown to vary widely between institutions (34). Therefore, the overall utility and cost effectiveness of the test remains under debate.

Several FNA cytology tumor genotyping tests have also been developed. These tests identify mutations in known thyroid cancer oncogenes and tumor suppressors from DNA isolated from FNA (35). These tests initially reported a high specificity for cancer (and PPV), but a low sensitivity (and NPV) to exclude malignancy (so called “rule in” test). However, expanded versions of the tests, which utilize next-generation DNA sequencing methods and assess a greater number of genes, have been demonstrated to exhibit both high NPV and PPV (36).

Many thyroid centers and clinics currently utilize one of these tests, although the clinical value and cost effectiveness, and the best approach to utilizing the test (at initial FNA or upon repeat FNA for indeterminate cytology) continue to be debated.

Clinically aggressive thyroid cancers: differentiation therapy

Following a 1936 presentation at Harvard Medical School from Karl Compton, President of the Massachusetts Institute of Technology (MIT) entitled, “What physics can do for biology and medicine,” Dr. Saul Hertz from the Massachusetts General Hospital (MGH) Thyroid Clinic contacted Dr. Compton with the question, “Could iodine be made artificially radioactive?” This led to the study of radioisotopes of iodine for the treatment of Graves’ disease at MGH by Drs. James Howard Means, Hertz, and Earle Chapman in collaboration with Dr. Robley Evans and Arthur Roberts at MIT (37). Subsequent to these descriptions, Dr. Samuel Seidlin and colleagues at the Montefiore and Memorial Hospitals in New York pioneered the use of ¹³¹I in the treatment of thyroid cancer. Radioiodine has remained a mainstay of thyroid cancer therapy (38).

Iodine entry into thyroid follicular cells is stimulated by the action thyroid stimulating hormone (TSH), secreted from the anterior pituitary. TSH, acting through a seven transmembrane spanning G-protein coupled receptor (TSH receptor) increases mRNA transcription of the sodium iodide symporter (NIS, encoded by the *SLC5A5* gene), and promotes NIS protein transport to the basolateral plasma membrane. NIS mediates entry of iodide into the follicular cell, which is then transported across the apical membrane by transporters including pendrin. In the thyroid follicular lumen, iodine is conjugated to tyrosyl side chains of the protein thyroglobulin by the action of the rate-limiting enzyme, thyroid peroxidase (5).

Many well-differentiated thyroid cancers maintain the ability to concentrate iodine, including ¹³¹I, which emits β -radiation, leading to cell death and therapeutic responses. However, virtually all patients who die from thyroid cancer become or present with disease refractory to RAI. Therefore, understanding the mechanisms underlying sensitivity and resistance to RAI is an important area of active research.

TSH stimulation is an important regulator of iodine entry into normal and cancerous thyroid cells. However, thyroid cancers express lower levels of markers of

terminal differentiation, including *SLC5A5* and other components of the iodine handling machinery. Therefore, supraphysiological levels of TSH are required to promote adequate ¹³¹I uptake into thyroid cancer cells. Such levels are achieved following total thyroidectomy, which renders the patient hypothyroid, or by injections of recombinant human TSH after levothyroxine replacement therapy following surgery (6).

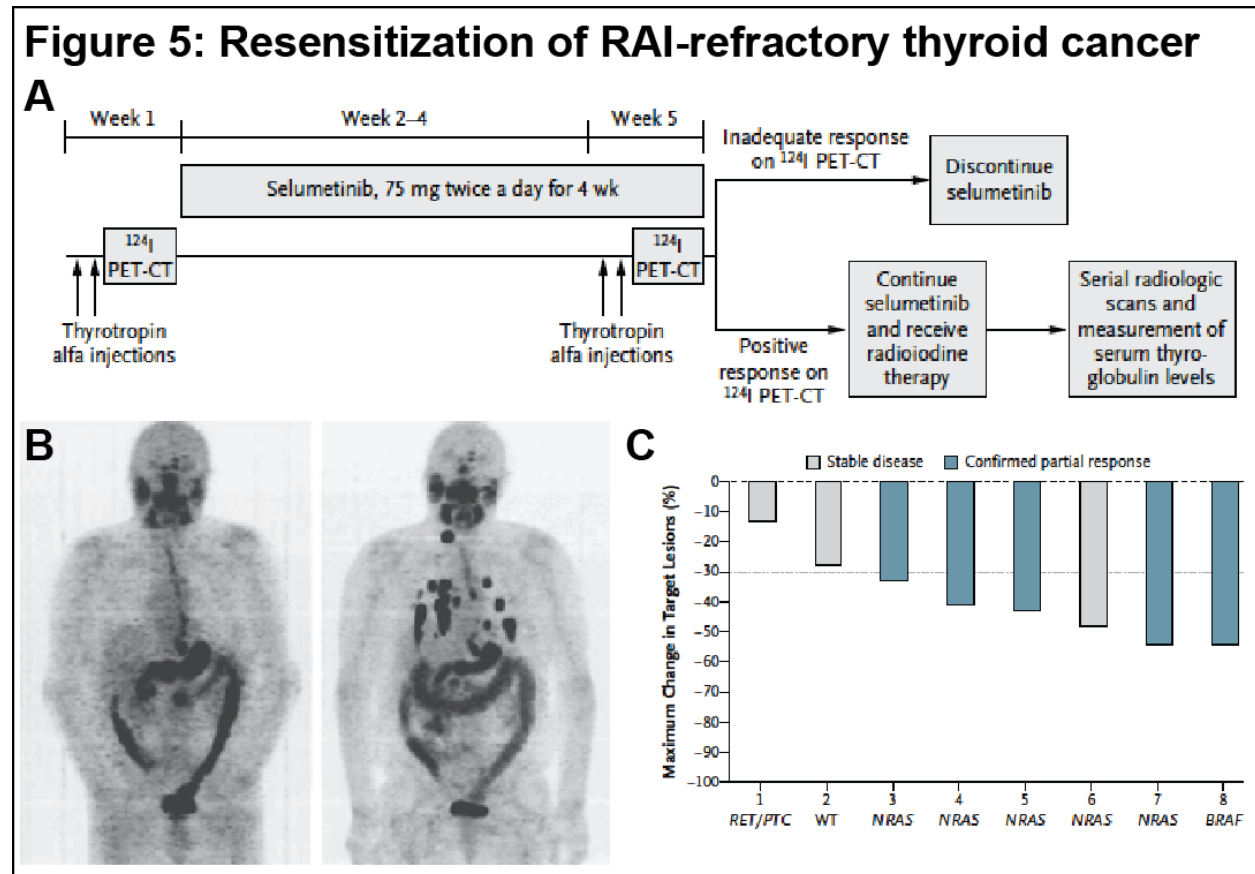
Another determinant of radioiodine sensitivity is signaling flux through the MAPK pathway. Correlative studies support the role of the MAPK signaling pathway in regulation of responsiveness to radioiodine. *BRAF*^{V600E} mutations potentially activate signaling through the MAPK pathway, and *BRAF*-mutant thyroid cancers express lower levels of NIS compared to *BRAF*-wild-type tumors (39). This concept was corroborated and validated in the Cancer Genome Atlas study, which revealed that *BRAF*-mutant PTCs both exhibit higher signaling through the MAPK pathway, and express lower levels of several markers of thyroid follicular cell terminal differentiation, including key genes involved in iodine transport and organification (9).

An extension of this concept is the hypothesis that MAPK pathway inhibitors might improve sensitivity to radioiodine in RAI-refractory thyroid cancers. Proof of principle studies using a genetically-engineered mouse model of *BRAF*-mutant PTC demonstrated that NIS levels decreased upon activation of the *BRAF*-mutant transgene (40). Conversely, inactivation of the *BRAF*^{V600E} transgene by genetic approaches restored expression of NIS in the thyroid follicular epithelium, and small molecule BRAF and MEK inhibitors also increased NIS expression and radioiodine uptake.

Two proof of concept clinical trials have extended this concept into patients with radioiodine-refractory disease. First, Ho et al. showed in a panel of RAI-refractory tumors, that four weeks treatment with selumetinib led to restoration of ¹³¹I uptake in 12/20 patients, including 8/20 patients demonstrating sufficient increase to warrant treatment with a therapeutic dose of ¹³¹I (41). Five of the 8 patients exhibited a partial structural response, and the other three shows stable disease during the trial period. Consistent with high-level activation of MAPK signaling in response to the *BRAF*^{V600E} mutation, 4/9 *BRAF*-mutant tumors exhibited an increase in RAI uptake, compared to 5/5 responses in the *NRAS*-mutant cohort.

Rothenberg et al. subsequently used a small molecule BRAF inhibitor (dabrafenib) to treat a cohort of 10 patients with *BRAF*-mutant RAI-refractory PTC (42). Following four weeks of treatment, new iodine uptake was observed in metastases 6/10 patients. This suggests that *BRAF*-mutant tumors may respond to these re-differentiation protocols, though the optimal small molecule or combination of treatments remains to be determined.

These early stage clinical trials have spawned several clinical trials investigating the use of MAPK pathway inhibitors for resensitization of RAI-refractory thyroid cancers. Optimal application of these approaches remains to be determined. For example, should a short course of a MAPK inhibitor be used in all *BRAF*-mutant patients who are treated with adjuvant RAI therapy at initial diagnosis? Should patients with initial surgical pathology predictors of aggressive disease be treated at initial diagnosis? Alternatively, should treatment be withheld until the disease demonstrated bona-fide



resistance to RAI? Although it is clear that MAPK pathway plays a key role in this process, other cellular mechanisms responsible for iodine uptake and responsiveness in thyroid cells could also be targeted in order to optimize these strategies. Harnessing, and restoring, thyroid cancer cells unique ability to concentrate iodine has again become an exciting avenue of research, similar to the initial efforts to use iodine radioisotopes in the 1930s and 1940s.

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