

Introduction

Thyroid cancer is the fastest growing cancer diagnosis in the US [1, 2] with a total of 44,670 new cases and 1,690 deaths expected in 2010 (www.cancer.gov/cancertopics/types/thyroid). From nuclear disasters such as Chernobyl in 1986, it is clear that radiation exposure is a significant risk factor for thyroid cancer [3] but, the majority of thyroid cancers appear to be sporadic in nature. Thyroid cancers encompass a variety of lesions that range from benign adenoma to malignant tumors. They also span the spectrum from, well-differentiated, poorly differentiated or undifferentiated (anaplastic). More than 95% of thyroid cancers are derived from thyroid follicular cells, while 2-3% of thyroid tumors (medullary thyroid cancers) are derived from the calcitonin producing C-cells.

A number of genetic alterations have been shown to be involved in the development of follicular cell-derived cancers. These point mutations and translocations occur in genes for several important signaling pathways, in particular the mitogen-activated protein kinase (MAPK) pathway, and are required for transformation of well-differentiated follicular cell-derived thyroid cancers, i.e. papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) [4, 5], as described in Figure 1.

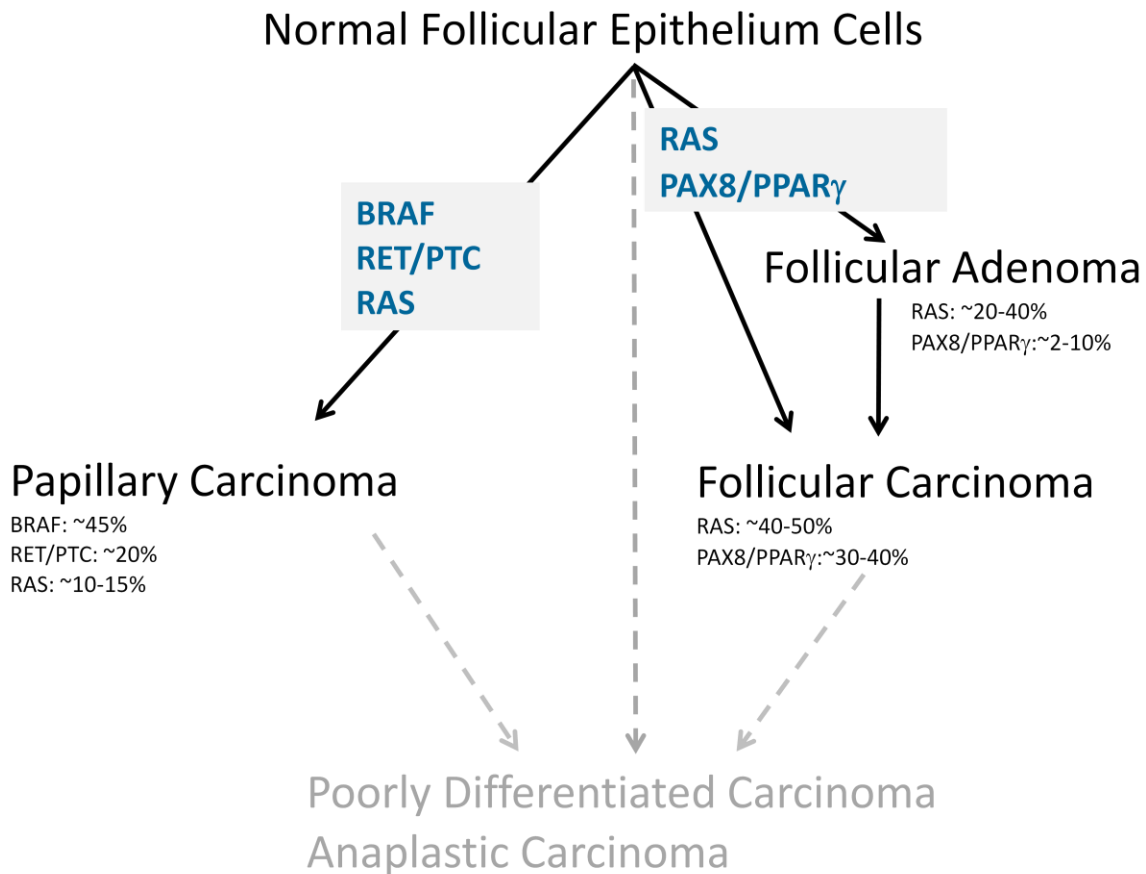


Figure 1: Mutations and translocations involved in the pathogenesis of papillary and follicular thyroid carcinomas, respectively. Variants of papillary and follicular carcinomas are not represented.

Epidemiology

Papillary thyroid cancer (PTC) is the most common form of thyroid cancer, representing approximately 80% of all thyroid malignancies. PTC is usually indolent and curable but this cancer can spread early to local lymph nodes and disease persistence and/or recurrence are common and associated with increased mortality [6-8]. **Follicular thyroid cancer (FTC)** is the second most common thyroid cancer, representing approximately 15% of all thyroid malignancies. This cancer can develop from a pre-existing benign follicular adenoma or directly and it is characterized by hematogenous spread. **Poorly differentiated (PDC) and anaplastic (ATC) thyroid cancers** are rare, representing 2-5% of all thyroid cancers [4, 5]. These are very aggressive tumors that can develop de novo or from the progression of pre-existing papillary or follicular carcinomas. All the cancers described above are derived from the transformation of follicular cells.

The most common genetic abnormalities found in PTC are the point mutations of BRAF and RAS genes as well as RET/PTC rearrangements. These mutations are found in more than 70% of PTCs and tend to be mutually exclusive. The more common genetic alterations in FTCs are RAS point mutations or PAX8/PPAR γ rearrangements, respectively, which are also usually mutually exclusive. RAS and PAX8/PPAR γ rearrangements are found in ~80% of FTC. RAS and to a lesser extent PAX8/PPAR γ translocations, are also associated with follicular adenoma, with frequencies of 20-40% and of 2-10%, respectively [4, 5]. Up to 20% of PTCs and up to 30% of FTCs do not carry any of the mutations or translocations described above and therefore cannot be detected using an assay based on these gene alterations alone.

Clinical Overview

Although thyroid cancer is relatively rare, thyroid nodules are very common, present in 5% to 7% of the US adult population, representing 10 to 18 million individuals [26]. Because the vast majority of thyroid nodules are benign and because most cases of thyroid cancer are curable by surgery if detected early, it is challenging to identify those nodules that are malignant among the vast majority of nodules that are benign [27]. Fine-needle-aspiration (FNA) combined with cytological evaluation is currently the standard preoperative diagnostic tool for thyroid cancer. However, in 10-40% of cases, the cytological diagnosis remains indeterminate for malignancy [28]. Since 2008, the general category of indeterminate FNAs has been divided into three subcategories, that is follicular lesion of undetermined significance (FLUS), follicular or oncocytic (Hürthle cell) neoplasm, and suspicious for malignancy, with a predicted probability for malignancy of 5-10%, 20-30%, and 50-75% for each subcategory, respectively [29].

A number of studies have now shown that molecular testing of FNA biopsies, especially for BRAF, but also for a combination of markers, including BRAF, RAS, RET/PTC and PAX8/PPAR γ , is not only feasible but, can significantly improve the accuracy of the preoperative FNA diagnosis from cytology [7, 8, 30-38]. Recent large prospective studies have confirmed the ability of genetic markers (BRAF, RAS, RET/PTC and PAX8/PPAR γ) and protein markers (galectin-3) to improve the preoperative diagnostic accuracy for patients with indeterminate thyroid nodules [32-34, 39-41]. Furthermore, the use of molecular markers (e.g.,

BRAF, RAS, RET/PTC, and PAX8/PPAR γ) is now formally recommended in the 2009 Revised ATA Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer (Recommendation rating: C) [42]. In one of the prospective studies using BRAF, RAS, RET/PTC and PAX8/PPAR γ as a marker panel for testing FNAs [34], the sensitivity of malignant diagnosis in FNA thyroid nodules increased from 44% to 80%, when comparing cytology alone to cytology combined with molecular testing for the markers described above. The presence of any mutation was a strong predictor of malignancy, as 31 (97%) out of 32 of mutation-positive nodules were diagnosed as malignant after surgery while only one nodule (3%) out of 32 was a RAS-positive follicular adenoma [34]. In the indeterminate group of FNA samples, in particular, molecular testing helped identify 15 (70%) out of 21 malignant nodules after surgery, strongly indicating that molecular testing may help to improve the diagnosis of indeterminate FNAs [34]. In another study [40], the sensitivity of malignant diagnosis in FNA increased from 60%, when cytology was used alone, to 90% when cytology was combined with molecular testing for BRAF, RAS, RET, TRK, and PPAR γ mutations [40]. Similarly to the previous study, the presence of a mutation was a strong predictor of malignancy, as mutations were associated with cancers in 91% of the cases (61 out of 67 mutation-positive cases) and with benign follicular adenoma in 9% of the cases (6 out of 67 mutation-positive cases). Prospective evaluation of the clinical utility of preoperative testing for these markers is on-going.

Improved diagnosis from molecular testing of thyroid FNA biopsies

Positive Result/s	Probability of Malignancy
Cytology	44-60%
Cytology plus Molecular testing	80-90%
BRAF testing	99.8%

Table 1: Percentages above are based on a combination of publications [5, 7, 34, 40].

Markers

BRAF: The BRAF mutation (V600E) is the most common mutation in PTC, occurring with a prevalence of ~45% (range 27%-87%) [8, 9]. It is mostly found in conventional PTC and the tall cell variant of PTC and less frequently in the follicular variant of PTC. BRAF point mutation is not found in follicular thyroid cancer and benign thyroid nodules. BRAF V600E mutation leads to the constitutive activation of the BRAF protein kinase of the MAPK pathway. Recent studies have established BRAF V600E as a marker of disease aggressiveness, disease recurrence, and poor prognosis [8, 10-15], although these findings have not been confirmed in some other studies [16, 17]. Other BRAF activating point mutations have been described at positions 598, 599, and 601, but these mutations are very rare compared to the activating mutation at position 600. BRAF V600E is the target for research of new therapies [18, 19].

RET/PTC rearrangements: Rearrangements of the RET gene, called RET/PTC rearrangements, are the second most common genetic alteration described in PTC. They occur in ~20% of sporadic PTC, although their prevalence has been shown variable among studies, mostly due to variations in the geographical distribution, the different methodologies used for its detection and tumor heterogeneity [20]. These rearrangements are specific for PTC and PTC variants, such as the oncocytic (Hürthle cell) variant, and are usually not found in benign tumors. RET/PTC rearrangements are more prevalent in radiation-induced PTC. At least 11 different RET/PTC rearrangements have been described to date, the two most common in sporadic (i.e. non-radiation induced) PTC being RET/PTC1 (60-70% of positive cases) and RET/PTC3 (20-30% of positive cases).

RAS: Point mutations within RAS genes involve codons 12, 13, and 61 of NRAS, HRAS and KRAS, with mutations of NRAS and HRAS at codon 61 and of KRAS at codon 12/13 being the most common. Mutant RAS proteins constitutively activate the MAPK and PI3K/AKT pathways. In contrast to the other markers, RAS mutations are not restricted to a particular histological subtype of thyroid tumor. RAS mutations are found in ~ 10-15% PTCs (higher in follicular variant of PTC) but are more prevalent in FTC, where they are associated with 40%-50% of the cancers. RAS mutations are also found in ~35% of poorly differentiated and ~50% of anaplastic thyroid cancers, where the presence of RAS mutations seems to correlate with more aggressive tumor behavior and poor prognosis [4, 21, 22]. RAS mutations are also found in 20%-40% of follicular adenoma, but it remains unclear whether these tumors represent pre-invasive follicular carcinomas.

PAX8/PPAR γ rearrangements: PAX8/PPAR γ rearrangements are found in 30-40% of conventional FTC and in ~5% of oncocytic carcinomas [10, 23]. Tumors associated with PAX8/PPAR γ usually carry a favorable prognosis. Tumors with PAX8/PPAR γ rearrangement do not usually carry any RAS mutation, suggesting that the development of FTC involves two independent pathways associated with either PAX8/PPAR γ translocation or RAS mutation [23]. PAX8/PPAR γ rearrangements are also found in 2-10% of follicular adenomas, and in the follicular variant of PTC [23-25]. PAX8/PPAR γ translocations have been reported in a very low percentage (0%-1%) of PTC [24].

Conclusion

Currently, the best preoperative tool for thyroid cancer is the cytological examination of FNA biopsies from thyroid nodules. Unfortunately, FNA cytology lacks specificity and in up to 40% of cases, the diagnosis of thyroid nodules remains indeterminate [39]. The markers described in this paper are well characterized and have been demonstrated to improve the diagnosis of thyroid nodules when used in conjunction with traditional cytology.

1. Hayat, M.J., et al., *Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance, Epidemiology, and End Results (SEER) Program*. *Oncologist*, 2007. **12**(1): p. 20-37.
2. Jemal, A., et al., *Cancer statistics, 2007*. *CA Cancer J Clin*, 2007. **57**(1): p. 43-66.
3. Detours, V., et al., *Absence of a specific radiation signature in post-Chernobyl thyroid cancers*. *Br J Cancer*, 2005. **92**(8): p. 1545-52.
4. Kondo, T., S. Ezzat, and S.L. Asa, *Pathogenetic mechanisms in thyroid follicular-cell neoplasia*. *Nat Rev Cancer*, 2006. **6**(4): p. 292-306.
5. Nikiforova, M.N. and Y.E. Nikiforov, *Molecular diagnostics and predictors in thyroid cancer*. *Thyroid*, 2009. **19**(12): p. 1351-61.
6. Sherman, S.I., et al., *Prospective multicenter study of thyroid carcinoma treatment: initial analysis of staging and outcome*. *National Thyroid Cancer Treatment Cooperative Study Registry Group*. *Cancer*, 1998. **83**(5): p. 1012-21.
7. Xing, M., et al., *BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer*. *J Clin Oncol*, 2009. **27**(18): p. 2977-82.
8. Xing, M., *BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications*. *Endocr Rev*, 2007. **28**(7): p. 742-62.
9. Lee, J.H., E.S. Lee, and Y.S. Kim, *Clinicopathologic significance of BRAF V600E mutation in papillary carcinomas of the thyroid: a meta-analysis*. *Cancer*, 2007. **110**(1): p. 38-46.
10. Placzkowski, K.A., et al., *The Role of the PAX8/PPARGamma Fusion Oncogene in Thyroid Cancer*. *PPAR Res*, **2008**: p. 672829.
11. Nikiforova, M.N., et al., *BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas*. *J Clin Endocrinol Metab*, 2003. **88**(11): p. 5399-404.
12. O'Neill, C.J., et al., *BRAF(V600E) mutation is associated with an increased risk of nodal recurrence requiring reoperative surgery in patients with papillary thyroid cancer*. *Surgery*, 2010. **148**(6): p. 1139-45; discussion 1145-6.
13. Xing, M., *BRAF mutation in papillary thyroid microcarcinoma: the promise of better risk management*. *Ann Surg Oncol*, 2009. **16**(4): p. 801-3.
14. Soares, P., et al., *BRAF mutations typical of papillary thyroid carcinoma are more frequently detected in undifferentiated than in insular and insular-like poorly differentiated carcinomas*. *Virchows Arch*, 2004. **444**(6): p. 572-6.
15. Elisei, R., et al., *BRAF(V600E) mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study*. *J Clin Endocrinol Metab*, 2008. **93**(10): p. 3943-9.
16. Trovisco, V., et al., *Type and prevalence of BRAF mutations are closely associated with papillary thyroid carcinoma histotype and patients' age but not with tumour aggressiveness*. *Virchows Arch*, 2005. **446**(6): p. 589-95.
17. Fugazzola, L., et al., *Correlation between B-RAFV600E mutation and clinico-pathologic parameters in papillary thyroid carcinoma: data from a multicentric Italian study and review of the literature*. *Endocr Relat Cancer*, 2006. **13**(2): p. 455-64.
18. Santoro, M. and F. Carlomagno, *Drug insight: Small-molecule inhibitors of protein kinases in the treatment of thyroid cancer*. *Nat Clin Pract Endocrinol Metab*, 2006. **2**(1): p. 42-52.
19. Woyach, J. and M. Shah, *New therapeutic advances in the management of progressive thyroid cancer*. *Endocr Relat Cancer*, 2009. **16**(3): p. 715-31.
20. Zhu, Z., et al., *Prevalence of RET/PTC rearrangements in thyroid papillary carcinomas: effects of the detection methods and genetic heterogeneity*. *J Clin Endocrinol Metab*, 2006. **91**(9): p. 3603-10.

21. Nikiforova, M.N. and Y.E. Nikiforov, *Molecular genetics of thyroid cancer: implications for diagnosis, treatment and prognosis*. Expert Rev Mol Diagn, 2008. **8**(1): p. 83-95.
22. Ruggeri, R., Campenni, A, Baldari, S, Trimarchi, F, and Trovato, M, *What is new on thyroid cancer biomarkers*. Biomarker Insights, 2008. **3**: p. 237-252.
23. Nikiforova, M.N., et al., *RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma*. J Clin Endocrinol Metab, 2003. **88**(5): p. 2318-26.
24. Marques, A.R., et al., *Expression of PAX8-PPAR gamma 1 rearrangements in both follicular thyroid carcinomas and adenomas*. J Clin Endocrinol Metab, 2002. **87**(8): p. 3947-52.
25. Castro, P., et al., *PAX8-PPARgamma rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma*. J Clin Endocrinol Metab, 2006. **91**(1): p. 213-20.
26. Frates, M.C., et al., *Prevalence and distribution of carcinoma in patients with solitary and multiple thyroid nodules on sonography*. J Clin Endocrinol Metab, 2006. **91**(9): p. 3411-7.
27. Mahar, S.A., A. Husain, and N. Islam, *Fine needle aspiration cytology of thyroid nodule: diagnostic accuracy and pitfalls*. J Ayub Med Coll Abbottabad, 2006. **18**(4): p. 26-9.
28. Gharib, H. and E. Papini, *Thyroid nodules: clinical importance, assessment, and treatment*. Endocrinol Metab Clin North Am, 2007. **36**(3): p. 707-35, vi.
29. Baloch, Z.W., et al., *Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference*. Diagn Cytopathol, 2008. **36**(6): p. 425-37.
30. Cheung, C.C., et al., *Analysis of ret/PTC gene rearrangements refines the fine needle aspiration diagnosis of thyroid cancer*. J Clin Endocrinol Metab, 2001. **86**(5): p. 2187-90.
31. Eszlinger, M. and R. Paschke, *Molecular fine-needle aspiration biopsy diagnosis of thyroid nodules by tumor specific mutations and gene expression patterns*. Mol Cell Endocrinol, 2010. **322**(1-2): p. 29-37.
32. Moses, W., et al., *Molecular testing for somatic mutations improves the accuracy of thyroid fine-needle aspiration biopsy*. World J Surg, 2010. **34**(11): p. 2589-94.
33. Musholt, T.J., et al., *Detection of papillary thyroid carcinoma by analysis of BRAF and RET/PTC1 mutations in fine-needle aspiration biopsies of thyroid nodules*. World J Surg, 2010. **34**(11): p. 2595-603.
34. Nikiforov, Y.E., et al., *Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules*. J Clin Endocrinol Metab, 2009. **94**(6): p. 2092-8.
35. Ohori, N.P., et al., *Contribution of molecular testing to thyroid fine-needle aspiration cytology of "follicular lesion of undetermined significance/atypia of undetermined significance"*. Cancer Cytopathol, 2010. **118**(1): p. 17-23.
36. Salvatore, G., et al., *Analysis of BRAF point mutation and RET/PTC rearrangement refines the fine-needle aspiration diagnosis of papillary thyroid carcinoma*. J Clin Endocrinol Metab, 2004. **89**(10): p. 5175-80.
37. Zatelli, M.C., et al., *BRAF V600E mutation analysis increases diagnostic accuracy for papillary thyroid carcinoma in fine-needle aspiration biopsies*. Eur J Endocrinol, 2009. **161**(3): p. 467-73.
38. Guo, F., P. Hou, and B. Shi, *Detection of BRAF mutation on fine needle aspiration biopsy specimens: diagnostic and clinical implications for papillary thyroid cancer*. Acta Cytol, 2010. **54**(3): p. 291-5.
39. Bartolazzi, A., et al., *Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fine-needle aspiration cytology: a prospective multicentre study*. Lancet Oncol, 2008. **9**(6): p. 543-9.

-
40. Cantara, S., et al., *Impact of Proto-Oncogene Mutation Detection in Cytological Specimens from Thyroid Nodules Improves the Diagnostic Accuracy of Cytology*. J Clin Endocrinol Metab, 2010.
 41. Franco, C., et al., *Molecular markers in thyroid fine-needle aspiration biopsy: a prospective study*. Appl Immunohistochem Mol Morphol, 2009. **17**(3): p. 211-5.
 42. Cooper, D.S., et al., *Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer*. Thyroid, 2009. **19**(11): p. 1167-214.

