# The contribution of molecular pathology to the classification of thyroid tumors

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## Abstract

Significant molecular advances have been undertaken for the past two decades in the field of thyroid follicular neoplasms, including a detailed genomic profile of papillary thyroid carcinoma (PTC) by The Cancer Genome Atlas (TCGA) project. These molecular discoveries led to a better understanding of the pathogenesis of thyroid neoplasms and resulted in reclassification of certain types of thyroid tumors. This review discusses how, 1) the molecular profiles of follicular-patterned lesions led to the reclassification of the follicular variant of PTC into noninvasive follicular thyroid neoplasm with papillary like nuclei, 2) the genotyping of Hürthle cell neoplasm provided the rationale to classify these tumors independently from follicular adenomas and carcinomas, and 3) *BRAF* and *RAS* molecular signatures have the potential of subclassifying PTC and poorly differentiated thyroid carcinoma into clinically relevant molecular subtypes.

**Keywords** *BRAF*; follicular variant of papillary thyroid carcinoma; hürthle cell carcinoma; noninvasive follicular thyroid neoplasm with papillary-like nuclear features; *RAS*

## Follicular patterned lesions

In the 1950s, carcinomas were classified purely based on the predominant architecture: carcinomas making papillae were labeled as papillary thyroid carcinoma while those with follicles were diagnosed as follicular carcinoma (FTC) (Figure 1).[1] Lindsay was the first to describe the nuclear features of papillary thyroid carcinoma in follicular-pattered lesions and coin the term follicular variant of papillary thyroid carcinoma (FVPTC).[2] Chen and Rosai (1977) defined FVPTC as tumors that "resembled papillary carcinoma in its biologic behavior and all morphologic features with the exception that papillae were not present", thereby proving that it belonged to the papillary, as opposed to the follicular carcinoma family.[3] During the 1980s and 1990s the diagnosis of papillary carcinoma began to be more common than follicular carcinomas as nuclear features became the defining criterion for the diagnosis of PTC, regardless of architectural pattern and invasive status.[4] Based on that axiom and although FVPTCs first described by Lindsay and then by Chen and Rosai were infiltrative tumors with nodal metastasis, a new group of tumors began to appear, the noninvasive encapsulated follicular variant of PTC (NI-EFVPTC) defined as malignant solely on the basis of its nuclear characteristics (e.g. nuclear enlargement, nuclear membrane irregularity, and chromatin clearing) (Figure 2). As the threshold for nuclear alteration required to diagnose a tumor as PTC became lower,[5,6] this new entity, NI-EFVPTC, started to become a frequent diagnosis, accounting for nearly 15% of all PTCs diagnosed in Europe and United states.[7,8] It is estimated that 45,000 patients worldwide are diagnosed with NI-EFVPTC every year.[9] As most of the previous studies did not separate encapsulated from infiltrative FVPTC and some even included tumors with small proportion of papillae (e.g. >1%) as FVPTC, the reported rate of nodal metastasis in FVPTC overall was as high as 22%.[10,11] It was thus assumed for more than 30 years that the encapsulated FVPTC behaved and spread like its classical counterpart. The crisis in the over-diagnosis of FVPTC led to the proposal formulated in Europe in 2000 of the well differentiated tumor of uncertain malignant potential (WDT-UMP): An encapsulated tumor composed of well differentiated follicular cells with questionable papillary carcinoma type nuclei, blood vessel invasion and/or capsular invasion that is either absent or questionable.[12] This diagnostic term gained acceptance in several European Institutions but was not used in the vast majority of Hospitals in the US.[13]

Meanwhile, significant advances have taken place in the molecular understanding of thyroid neoplasms since the late 1980s (Figure 1). *RAS* point mutations were first discovered in follicular carcinoma in 1988 (Figures 1 and 2),[14] and soon after in follicular adenoma (FA).[15] Subsequent studies have also reported *PAX8-PPARγ* fusion in a subset of FTC/FA.[16,17] On the other hand, a significant proportion of PTCs, in particular the classical variant, has been shown to harbor *RET-PTC* fusion and $BRAF^{V600E}$ mutation.[18–20] It was not until 2003 that molecular studies began to indicate that a significant proportion (approximately 43%) of FVPTC harbor *RAS* mutation and a pattern of chromosome gain/loss akin to FA/FTC and in contrast to classical variant of PTC.[21–23] Furthermore, the expression profile of the follicular variant of PTC was shown to be different from that of classical and tall cell variant.[23] Additional studies showed that, as currently diagnosed, the encapsulated/well demarcated FVPTC has molecular alterations similar to follicular adenoma and carcinoma, characterized by the lack of *BRAF* mutations, a high prevalence of *RAS* mutations, and in some instances the presence of *PAX8-PPARγ* rearrangement.[24,25] In 2014, the analysis of approximately 500 papillary carcinomas by The Cancer Genome Atlas (TCGA) research network confirmed all previous molecular studies demonstrating that the FVPTC group of tumors, invasive and non-invasive, have a high frequency of *RAS* mutation and a RAS-like molecular signature, unlike the classical variant and tall cell variant of papillary carcinoma that features *BRAF* mutations and a $BRAF^{V600E}$-like profile.[26]

Inspired by these molecular findings, Liu et al. in 2006 re-examined the clinical behaviors of EFVPTC. In that study, EFVPTC behaved like the FA/FTC group of tumors, and no recurrence or nodal metastasis developed in their NI-EFVPTC

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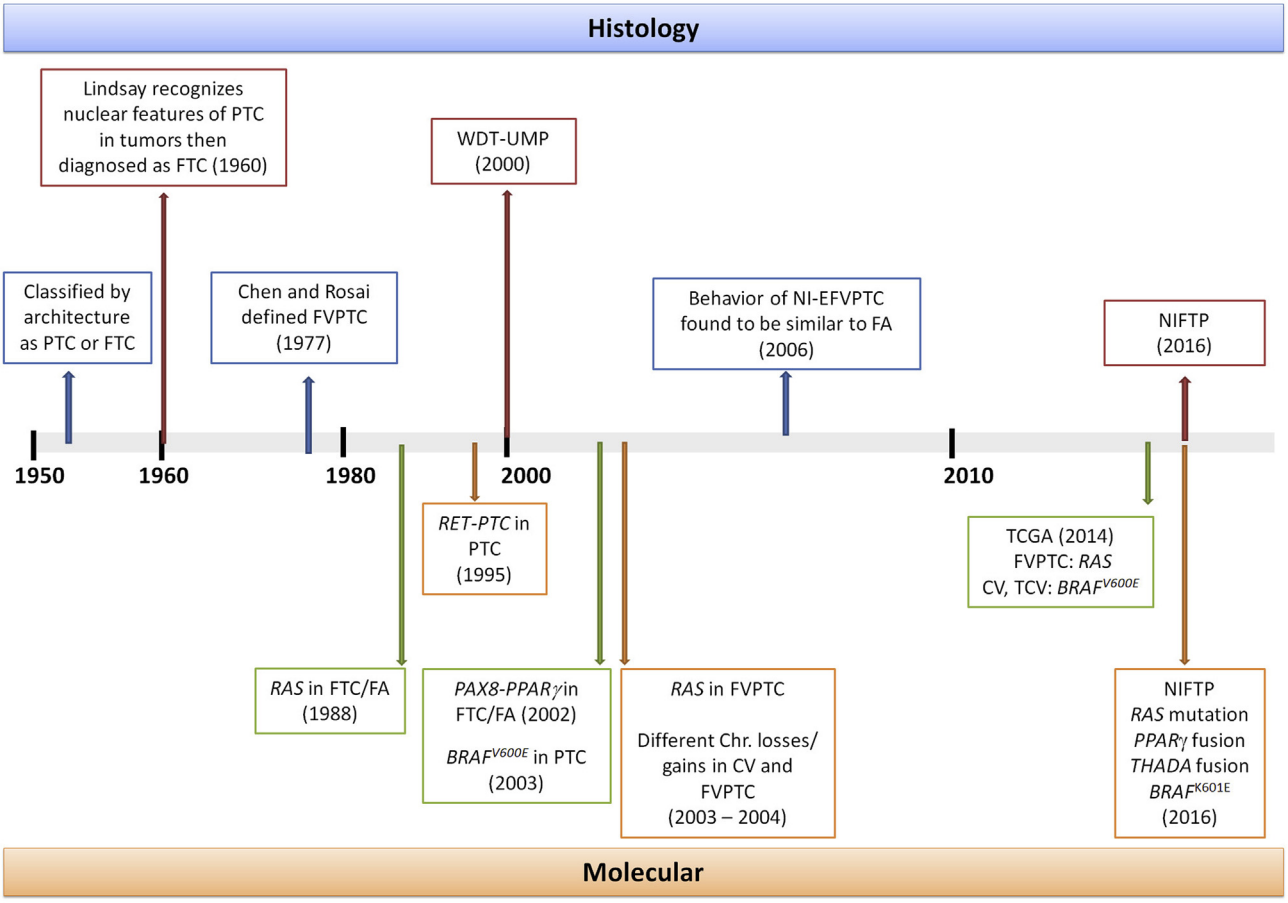


**Figure 1 Timeline depicting the evolution of histologic classification and the identification of common molecular events in well differentiated thyroid carcinoma.** The references pertaining to the above time points are as follows: Histology in chronological order: Lindsay S,[2] Chen and Rosai,[3] WDT-UMP,[12] behavior of non-invasive PTC-EFV similar to follicular adenoma,[27] and NIFTP [9]; molecular studies: *RAS* in FTC,[14] *RET-PTC* fusion in PTC,[18] *PPARγ-PAX8* in FTC/FA,[16,17] $BRAF^{V600E}$ in PTC,[19,20] *RAS* in FVPTC, pattern of chromosomal (chr.) gains/losses in FVPTC different than classical PTC,[21,22] the Cancer Genome Atlas Research Network molecular analysis of PTC.[26] PTC, papillary thyroid carcinoma; FTC, follicular carcinoma; WDT-UMP, well-differentiated tumor of uncertain malignant potential; FVPTC, follicular variant of papillary thyroid carcinoma; NI-EFVPTC, noninvasive encapsulated follicular variant of papillary thyroid carcinoma; TCGA, the cancer genome atlas research network; NIFTP, noninvasive follicular thyroid neoplasms with papillary-like nuclear features.

cohort treated by surgery alone with a median follow up of 11-years.[27] This indolent behavior has subsequently been confirmed by multiple studies.[8,28–34] In 2016, the problems generated by the over-diagnosis and treatment of the NI-EFVPTC brought a working group of the Endocrine Pathology Society to critically re-examine this entity.[9] As a result of this endeavor, Nikiforov et al. advocated a revision of diagnostic terminology to noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) with the fundamental goal of avoiding the term carcinoma, and the consequent risk of overtreatment for non-invasive tumors that are clinically benign, with a recurrence rate <1% (Table 1).[9] Molecular profiling was performed in 27 tumors from the consensus NIFTP cohort, which again demonstrated a high frequency of *RAS* mutation (8/27, 30%) and *PAX8-PPARγ* fusion (6/27, 22%), as well as the absence of $BRAF^{V600E}$ in this group of tumor.[9]

In conclusion, the analysis of the molecular profile of follicular-patterned lesion was the key step that prompted a better histopathological appraisal and clinical correlation of different forms of FVPTC, which eventually led to proper re-classification of a subgroup of these lesions as NIFTP. The fourth edition of the World Health Organization (WHO) classification for endocrine tumors (2017)[35] has adopted these new concepts and classified follicular patterned lesion based on both invasive status and nuclear characteristics (Figure 3). Tumors with invasion are classified as carcinoma, while encapsulated lesions without evidence of capsular or vascular invasion are follicular adenomas or NIFTPs. In addition, a third category of tumors, named as "tumors of uncertain malignant potential", was added to address encapsulated lesions with questionable capsular or vascular invasion. Therefore, whenever we encounter a follicular patterned nodule, regardless of the presence and extent of the alterations of nuclear morphology in neoplastic cells, pathologists have to carefully analyze and report whether the tumor is invasive or not. If possible, the entire nodule/adjacent tissue interface should be submitted and examined microscopically.




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