Pathways and genetic events in papillary and follicular thyroid carcinomas:

During the last three decades, there have been significant advances in the understanding of the molecular events associated with thyroid neoplasia of follicular cell origin. In these tumors, the two main altered pathways are the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK-3-CA) pathways (1). MAPK is the most crucial pathway for tumor initiation and can harbor rearrangements of the RET protooncogene (RET/PTC) as well as mutations in the BRAF and RAS genes. The BRAF$^{V600E}$ mutation constitutes the vast majority of BRAF mutations in thyroid cancers (1). This mutation activates the MAPK cascade and consists of a T to A transversion at nucleotide 1799, which results in a valine to glutamate substitution at residue 600 (V600E) (2). This mutation which is virtually specific for carcinoma is easily detected in fine needle aspiration and paraffin embedded tissues using various DNA-based techniques such as single-strand conformation polymorphism, direct sequencing, mutation-specific polymerase chain reaction and mass spectrometry genotyping (3, 4). More recently, the mutated BRAFV600E protein has been specifically detected in paraffin-embedded papillary thyroid carcinoma (PTC) and malignant melanoma using immunohistochemistry (IHC) (5). Point mutations also occur at hotspots in the RAS protooncogene. NRAS and HRAS mutations are the most common RAS mutations in thyroid cancers and have been reported at codon 61 while KRAS at codon 12/13. In contrast to BRAF, RAS mutations are not restricted to thyroid carcinomas and can be found in 30% of follicular adenomas (1). The RET/PTC chromosomal rearrangement fuses the RET protooncogene to various partners. All rearranged genes contain the coding region for the intact tyrosine kinase domain of the RET protein fused to an active promoter of another gene that drives the expression of the RET/PTC protein (1). This leads to chronic stimulation of MAPK signaling and thyroid tumorigenesis. This alteration can be detected by RT PCR and FISH in FNA and paraffin embedded tissues. There is however a wide variation in the reported incidence and specificity of RET/PTC. This is partly due to the fact that RET/PTC can be a subclonal event present in non-neoplastic thyroid tissue such as chronic lymphocytic thyroiditis (6). Another important genetic event in follicular cell derived thyroid carcinomas is the PAX8/PPARγ rearrangement that fuses PAX8, a thyroid transcription factor that appear early in thyroid organogenesis with the PPARγ gene. This rearrangement that can be detected in FNA and paraffin embedded tissues by RT PCR and FISH is also not entirely specific for carcinomas and has been reported in follicular adenomas.

From the vast literature on the mutational profile of follicular derived thyroid carcinomas, two important concepts have emerged. First, the MAPK pathway alterations are mutually exclusive (i.e. BRAF are mutually exclusive with RAS or RET/PTC) in the large majority of thyroid carcinomas (2,7,8). This finding suggests that they are driver mutations responsible for tumor initiation. Secondly, there is a very good correlation between histologic phenotype and genotype. Indeed, papillary carcinomas harbor a high frequency of BRAF mutations (45%) with a lower
incidence of RAS (15%) mutations and RET/PTC fusions (10%) and no PAX8/PPARγ rearrangements (1). In contrast, follicular carcinomas have much higher rate of RAS (40%) but no BRAF (0%) mutations. Follicular carcinomas display PAX8/PPARγ (30%) rather than RET/PTC rearrangements (0%) (1).

Impact of genomics on reclassification of papillary thyroid carcinoma variants:

In regard to the subtypes of papillary carcinomas, many studies have shown that classical and tall cell variant PTC harbor a high frequency of BRAFV600E mutations while the BRAFV600E positivity rate is low in the follicular variant of papillary carcinomas (9). The data emanating from the ongoing cancer genome atlas project (TCGA) on 200 PTC patients confirmed these findings with a 79% and 89% BRAFV600E frequency in classical and tall cell PTC respectively. In contrast, the follicular variant PTC harbored BRAFV600E in 15% of cases and RAS mutations in 42%. Expression array studies performed by Giordano et al in 2005 and most recently mRNA sequencing data on over 200 PTC generated by the TCGA have clearly shown that the follicular variant of PTC clusters separately from classical and tall cell PTC (10). Furthermore, methylation and microRNA sequencing analysis on more than 300 PTC performed as part of the TCGA revealed that follicular variant PTC has different microRNA and methylation gene profiles than classical and tall cell PTC. From the above, it is clear that the molecular profile of the follicular variant of PTC is different from that of classical PTC and more similar to the one seen in the follicular adenoma/carcinoma class of tumors. The follicular variant of papillary thyroid carcinoma is almost exclusively arranged in follicles lined by cells with characteristic papillary carcinoma nuclei and very rare papillary formations (10). When defined by such strict criteria, follicular variant of papillary thyroid carcinoma has an overall low metastatic nodal rate (13-22%) compared to papillary thyroid carcinoma in general (11). Furthermore, some encapsulated follicular variant with extensive angioinvasion travel to distant sites bypassing the regional lymph nodes in a manner similar to follicular carcinomas (12). Follicular variant of papillary thyroid carcinoma can be subdivided into two histological subtypes: An encapsulated tumor and an infiltrative subvariant where tumor follicles invade in between non-neoplastic ones (11). Studies from Memorial Sloan-Kettering Cancer Center have shown that the encapsulated form rarely metastasize to lymph node (5% of cases) whereas infiltrative follicular variant has a nodal metastatic rate (65% of patients) similar to the one encountered in classical PTC (11). The encapsulated follicular variant is therefore closer in its invasive and nodal metastatic pattern to the follicular adenoma/carcinoma group of tumors, while infiltrative follicular variant behaves like classical papillary thyroid carcinoma (2). The molecular profile of each papillary thyroid carcinoma follicular variant subtype mirrors their histopathologic features and metastatic behavior. Indeed, we found BRAF V600E mutation in 26% of the infiltrative follicular variant and in none of the encapsulated follicular variant (p=0.007). By contrast, RAS mutations were seen in 10 of 28 (36%) of the encapsulated group and in only 2 of 19 (10%) of infiltrative tumors. This strongly argues for papillary thyroid carcinoma follicular variant being two diseases: 1) Encapsulated papillary thyroid carcinoma follicular variant with a genotypic, invasive and behavioral profile very close to follicular adenoma/carcinoma 2) Infiltrative papillary thyroid carcinoma follicular variant with invasive and behavioral features very close to classical papillary carcinoma and a molecular profile in between follicular adenoma/carcinoma and classical papillary carcinoma albeit closer to the latter.
These findings may have important clinical implications. Since encapsulated papillary thyroid carcinoma follicular variant has a genotypic profile as well as invasive and behavioral features very similar to follicular adenoma/carcinoma, one may reconsider reclassifying it as an entity close the follicular adenoma/carcinoma group. The same criteria that are used to decide whether follicular tumors are biologically benign or malignant (i.e., capsular and vascular invasion) would be applied to the evaluation of encapsulated papillary thyroid carcinoma follicular variant. In practical terms, a lack of capsular and vascular invasion should denote an extremely indolent clinical behavior in encapsulated papillary thyroid carcinoma follicular variant. If this reclassification is achieved, it will have a major impact on the diagnosis and management of patients with papillary thyroid carcinoma follicular variant. In noninvasive, encapsulated papillary thyroid carcinoma follicular variant, pathologists will be spared the frustrating and very subjective exercise of deciding whether a tumor has the nuclear features of papillary carcinoma. More importantly, countless numbers of patients with noninvasive, encapsulated papillary thyroid carcinoma follicular variant will be treated conservatively akin minimally invasive follicular carcinoma. These individuals will be spared unnecessary and aggressive surgical and radioactive iodine therapy with their attached morbidity and financial costs.

Impact of genomics on the diagnosis of papillary and follicular carcinomas:

The inability to rule out carcinoma in FNA interpreted as indeterminate nodules leads to diagnostic lobectomy for most of these patients, although 60–90% of the surgically removed thyroid nodules are found to be benign (14). This prompted the use of diagnostic molecular markers in the hope of better stratifying patients for surgery. Using a PCR based assay aimed at detecting B\textit{RAF} V600E, R\textit{AS} point mutations, R\textit{ET}/P\textit{TC} and P\textit{AX}8/PP\textit{AR}\text{γ} rearrangements, Nikiforov et al were able to refine the risk of malignancy in FNA specimens. For example, patients with a cytologic diagnosis of atypia/follicular lesion of undetermined significance (AUS/FLUS) that carries a 14% risk of carcinomas in their series were separated into a mutation positive group with 88% cancer risk and a mutation negative subset that has a 5.9% risk of malignancy (15). More recently, Alexander et al conducted a large prospective studies of 577 cytologically indeterminate aspirates using a multi-gene expression classifier (16). The latter assay consists of 167 genes previously identified as genetic signatures for benign and malignant nodules (17). The negative predictive value of this classifier in those lesions who were cytologically classified as AUS/FLUS or "follicular neoplasm or lesion suspicious for follicular neoplasm," were 95% and 94% respectively (16). In contrast, the malignant signature of this classifier lacked specificity. Additional studies will give us a better estimate of the practical value of these assays. It is important to realize that the added benefit of these molecular approaches probably depends on the skills and the criteria used by the cytopathologist to classify a lesion as indeterminate. Indeed, these assays will have higher values if the pathologist is very cautious in diagnosing malignancy using the indeterminate category as a “waste basket”.

Impact of genomics on the prognosis of papillary carcinomas:

The B\textit{RAF}V600E was found to be associated with aggressive clinico-pathologic features such as extra-thyroid extension, nodal metastasis and advanced stage in P\textit{TC} in most studies. Based on a compilation of 28 publications, Xing found a higher frequency of extra-thyroid extension( 47%)
in BRAF V600E PTC compared to 26% in non-mutated tumors (18). BRAFV600E also has been shown in most (18, 19), but not all (20) studies to be a marker of poor outcome in PTC. Whether BRAFV600E is a surrogate marker for the conventional clinicopathological factors or an independent marker of tumor aggressiveness is still subject to considerable debate. While some studies have shown an independent prognostic value for BRAFV600E in multivariate analysis (21), others found that the correlation between BRAFV600E and aggressiveness is lost when PTC is stratified by subtypes. In the study of Cheng et al, BRAFV600E association with aggressive features (defined in their publication as extra-thyroid extension, nodal metastases, vascular invasion) is explained by its association with classical variant PTC and its absence in follicular variant PTC. Within each morphologic subtype, BRAF mutation alone did not predict aggressive features (22). More studies are needed to assess the added predictive value of BRAF mutation in PTC.

Pathways and genetic events in poorly differentiated and anaplastic thyroid carcinomas and their impact on therapy:

In addition to alterations in the MAPK cascade, poorly differentiated and anaplastic carcinomas develop genetic alterations in the PIK3CA pathway as well as p53 mutations. The latter has been reported in 20-30% of poorly differentiated carcinomas and 50-80% of anaplastic tumors (1). Poorly differentiated carcinomas have a lower BRAFV600E mutation rate (12%) than anaplastic carcinomas (44%) (3). In contrast, RAS mutations are more frequent in poorly differentiated (43%) than anaplastic tumors (23%) (3). In our series, we found RET/PTC rearrangement in poorly differentiated carcinomas (18%) and none in anaplastic tumors. In regard to PIK3CA and AKT1 mutations, they are found at a similar low rate (5-10%) in poorly differentiated carcinomas. In anaplastic tumors, PIK3CA and AKT1 mutations were reported in 10-30% and 5-10% of cases respectively (1). Interestingly, we found that radioactive iodine refractory poorly differentiated carcinomas are enriched in BRAF mutations (39%) compared to poorly differentiated carcinoma in general (12%). The high frequency of BRAF mutation in advanced radioactive iodine refractory tumors and its known inhibitory effect in vitro on iodine uptake in tumor cells, makes BRAF an attractive target for therapy to induce cell death and/or to restore radioactive iodine uptake (3). The putative success of such therapeutic strategies requires that all metastatic or recurrent tumors in the same patient harbor the same genetic defect. We genotyped radioactive iodine refractory thyroid carcinoma patients with multiple carcinoma deposits and found high concordance of BRAF mutations between multiple tumor sites within the same patient (3). Moreover, immunostaining with the VE1 directed specifically against the mutated BRAFV600E protein revealed tumor homogeneity for BRAFV600E in the vast majority of thyroid carcinomas of follicular cell origin. (23).

In conclusion, the vast knowledge acquired in the molecular analysis of follicular cell derived thyroid carcinoma can be used directly to develop useful diagnostic assays (in FNA) and design target therapy as well as indirectly to help reclassify thyroid carcinomas into clinically relevant histopathological entities (e.g. PTC follicular variant). The challenge now resides in 1) refining the current markers for diagnosis and prognosis by comparing them with meticulous histopathologic examination 2) search for crucial molecular initiating events in the 25% of thyroid carcinomas who lack driver mutations in the hope of finding new therapeutic targets.
References:


of BRAF, RET and NTRK1 are associated with similar but distinct gene expression patterns in papillary thyroid cancer. Oncogene 23:7436-7440.


