Introduction

Follicular cell thyroid tumors represent a challenging diagnostic area. Neoplastic follicular cells exhibit a wide degree of morphologic heterogeneity resulting in many growth patterns, i.e. follicular, papillary, solid, trabecular and insular, to name the most common. In addition, many tumors display admixtures of these growth patterns. The fundamental reasons for this morphologic heterogeneity are not entirely clear. However, we do know from numerous studies that the morphology of follicular cell thyroid tumors generally reflects their underlying genetic composition. Yet, even with the availability of genetic information, the narrative is somewhat cloudy. As was discussed in a recent editorial by Dr. Juan Rosai (1), different diagnoses can be derived for follicular cell thyroid tumors depending on the classification approach used (i.e. morphology, immunohistochemical profile, genotypic profile, and gene expression profile). Dr Rosai concluded it was time to go “Back to the Drawing Board” for the classification of some of these tumors, such as the encapsulated follicular variant of papillary carcinoma.

This presentation will present selected genetic research on encapsulated follicular cell tumors, with an emphasis on genotyping and genome-wide studies.

Genotyping Studies

By far, most of the genetic studies of follicular cell thyroid tumors have focused on defining mutations of specific genes known to be involved in the pathogenesis of these tumors and then examined their significance. As a consequence, much is known about a handful of genes in which mutations are thought to be dominant events in the initial development of tumors, so called driver mutations. (We now know that all mutations are not functionally equivalent. Thus, mutations are divided into two types: driver mutations and passenger mutations. See appendix). Driver mutations for follicular cell thyroid tumors include RET and NTRK1 rearrangements, RAS family point mutations, BRAF point mutations and rare rearrangements, PTEN mutations and likely PAX8/PPARY rearrangements. Mutations of CTNNB1 and TP53 are thought to be secondary mutations associated with histological progression to poorly differentiated and undifferentiated carcinoma. The Table below from Kondo et al. (2) summarizes the results from many genotyping studies.

<table>
<thead>
<tr>
<th>Genetic alteration</th>
<th>Well-differentiated thyroid carcinoma</th>
<th>Poorly differentiated thyroid carcinoma</th>
<th>Undifferentiated thyroid carcinoma</th>
<th>Post-Chernobyl childhood thyroid cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET rearrangement</td>
<td>13–43%</td>
<td>0%</td>
<td>0–13%</td>
<td>0%</td>
<td>50–90%</td>
</tr>
<tr>
<td>BRAF mutation</td>
<td>29–69%</td>
<td>0%</td>
<td>0–13%</td>
<td>10–35%</td>
<td>0–12%</td>
</tr>
<tr>
<td>BRAF rearrangement</td>
<td>1%</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>11%</td>
</tr>
<tr>
<td>NTRK1 rearrangement</td>
<td>5–13%</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>3%</td>
</tr>
<tr>
<td>Ras mutation</td>
<td>0–21%</td>
<td>40–53%</td>
<td>18–27%</td>
<td>20–60%</td>
<td>0%</td>
</tr>
<tr>
<td>PPARG rearrangement</td>
<td>0%</td>
<td>25–63%</td>
<td>0%</td>
<td>0%</td>
<td>Unknown</td>
</tr>
<tr>
<td>CTNNB1 mutation</td>
<td>0%</td>
<td>0%</td>
<td>0–25%</td>
<td>66%</td>
<td>Unkown</td>
</tr>
<tr>
<td>TP53 mutation</td>
<td>0–5%</td>
<td>0–9%</td>
<td>17–38%</td>
<td>67–88%</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

CTNNB1, β-catenin; NTRK1, neurotrophic tyrosine kinase receptor, type 1; PPARG, peroxisome-proliferator-activated-receptor-γ.
Some conclusions from these studies regarding driver mutations in follicular cell thyroid tumors include the following:

- Most classical type papillary thyroid carcinomas (PTC) contain \( \text{BRAF} \) mutations, unless radiation is involved, in which \( \text{RET} \) rearrangements become more common.
- Most tall cell variants of PTCs contain \( \text{BRAF} \) mutations.
- Most follicular variants of PTC contain \( \text{RAS} \) mutations.
- Most follicular thyroid carcinomas (FTC) contain either \( \text{RAS} \) mutations or \( \text{PAX8/PPAR} \gamma \) rearrangements.

Thus, it is quite apparent that there is a strong correlation between tumor morphology and its underlying genotype. This correlation has empowered recent studies from the Nikiforov group and others that demonstrated that genotyping improves the accuracy of thyroid FNA cytology diagnoses (3). Thus, genotyping of these genes is a powerful tool that will permit improved diagnosis and even imparts some prognostic information, as \( \text{BRAF} \) mutation is a risk factor for PTC recurrence (reviewed in (4)). However, the cancer genome is extremely complex and genotyping for these genes does not provide a complete picture of the genetics of these tumors. This fact is nicely illustrated in the following figure from the review of Xing (4) that shows secondary events in \( \text{BRAF} \)-mutant PTC that lead to tumor progression and invasiveness.

**Genome-Wide Studies**

Over the last decade, we have witnessed great advances in genomic science. The availability of genome-wide data is radically changing almost every field of biology, from cancer genetics to agriculture to human evolution. In addition, breakthroughs in high-throughput molecular biology techniques, such as DNA microarrays, and next-generation DNA sequencing, have leveraged genomic information to yield very interesting and informative studies. For instance, the genome of a single small cell neuroendocrine lung carcinoma was published recently, revealing literally thousands of DNA alterations and a mutational profile of tobacco smoking (5).

Genome-wide studies of follicular cell thyroid tumors have been more modest, but nonetheless informative. A full representation of the field is beyond the scope of this handout. This field was reviewed in 2008 (6). Selected studies will be presented.

Work from my research group at the University of Michigan in collaboration with Yuri Nikiforov has focused on genome-wide gene expression studies of follicular cell thyroid tumors using DNA microarrays.
We examined 97 follicular cell tumors and were able to recapitulate the overall established classification of thyroid tumors, thereby confirming the biologic significance of the gene expression measurements. Using the dataset, we showed that the tumors were broadly divided into 2 groups, those with follicular architecture and those with papillary architecture. Thus, with this approach, the follicular variant of PTC tumors (FV-PTC) were more closely related to the other follicular-patterned tumors (follicular adenoma (FTA) and follicular carcinoma (FTC)) than classical and tall cell type PTC. Thus, based solely on global gene expression, one would be tempted to classify the FV-PTCs within the umbrella of FTC rather than PTC.

Using this same dataset, we also showed that, within the group of PTC, global gene expression was strongly correlated to both tumor morphology and tumor genotype, as shown in the following principal components analysis (PCA) from (7):

![PCA images](image_url)

Also using this dataset, we showed that global gene expression in FTCs with the PAX8/PPAR\(\gamma\) translocation was distinctly different from other types of follicular cell tumors, demonstrating that this translocation drives expression of many PPAR\(\gamma\) target genes (8).

Collectively, these gene expression studies show a striking degree of correlation between a follicular cell tumor’s morphology, its genotype and its gene expression profile and offer a unifying scheme for the classification of these tumors.

Several studies have focused on gene expression profiling as a diagnostic aide using mRNA profiling of follicular tumors (9, 10). A more complete discussion of these studies can be found at (6).

**MicroRNA Studies**

At least 8 studies have examined expression of panels of miRNAs in thyroid tumors with the ultimate goal of defining specific miRNAs that possess diagnostic power (literature review can be found in (11). While several miRNAs with differential expression have been identified, no study has yet defined a panel that is ready for diagnostic use. Consistently identified differentially expressed miRNAs include 146b, 221, and 222. Over the next few years, it will become clearer what role miRNA profiling can play in the diagnosis of follicular cell thyroid tumors.

**What’s Still Needed**
Genomic evaluation of follicular cell tumors is still in its infancy. With the advances of next generation sequencing, it is now possible to sequence the genome of individual cancers (5, 12-14). Such studies have been applied to several tumor types and have greatly expanded the spectrum of mutations observed. It is fully anticipated that application of this approach to follicular cell tumors will lead to advances in the classification and diagnosis of these diagnostically challenging tumors.

Appendix

The paper by Stratton, Campbell and Futreal (Nature 458: 79-724) provides the following definitions of driver and passenger mutations:

“All cancers arise as a result of somatically acquired changes in the DNA of cancer cells. That does not mean, however, that all the somatic abnormalities present in a cancer genome have been involved in development of the cancer. Indeed, it is likely that some have made no contribution at all. To embody this concept, the terms ‘driver’ and ‘passenger’ mutation have been coined.

“A driver mutation is causally implicated in oncogenesis. It has conferred growth advantage on the cancer cell and has been positively selected in the microenvironment of the tissue in which the cancer arises. A driver mutation need not be required for maintenance of the final cancer (although it often is) but it must have been selected at some point along the lineage of cancer development.”

“A passenger mutation has not been selected, has not conferred clonal growth advantage and has therefore not contributed to cancer development. Passenger mutations are found within cancer genomes because somatic mutations without functional consequences often occur during cell division. Thus, a cell that acquires a driver mutation will already have biologically inert somatic mutations within its genome. These will be carried along in the clonal expansion that follows and therefore will be present in all cells of the final cancer.”

References

1. Rosai J 2010 The Encapsulated Follicular Variant of Papillary Thyroid Carcinoma: Back to the Drawing Board. Endocr Pathol