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**American Society for Investigative Pathology**

***Sunday, March 25, 2007, 4:15 pm***

**4:15 Molecular Profiles of Follicular Cell Thyroid Tumors (Approx. 40 minutes with 5 minutes for questions)**

Thomas J. Giordano, University of Michigan, Ann Arbor, MI

**Abstract**

Well-differentiated thyroid carcinoma is a common type of thyroid cancer that is increasing in incidence. The cytologic and pathologic diagnosis of thyroid follicular tumors is often challenging, resulting in thousands of unnecessary surgeries for benign disease and disagreement amongst pathologists. More objective diagnostic tools would help reduce these difficulties. Further, in this age of targeted therapy, improved understanding of follicular cell thyroid cancer and its progression to aggressive forms of thyroid cancer would be beneficial for the development of appropriate novel therapies. In this presentation, some recent translational work using gene expression profiling approaches is presented.

**Introduction**

Follicular cell thyroid carcinoma is broadly divided into papillary (PTC) and follicular types (FTC). This distinction is based primarily on their morphologic overall features, i.e. tumor architecture and characteristic nuclear features (e.g., optical clearing). The classification is clouded by the recognition of variants such as the follicular variant of papillary carcinoma (FVPTC) and the subjectivity inherent in morphologic assessment. Consequently, there persists a high degree of diagnostic intra- and inter-observer variability<sup>1</sup>. One of the goals of genomic molecular pathology approaches is to reduce the diagnostic subjectivity of follicular cell tumors<sup>2</sup>. Over the past few years, several laboratories, including ours, have begun to use genomic approaches to address several issues in thyroid follicular cell pathology, such as understanding pathogenesis and improved diagnosis and prognosis. This presentation will highlight some work in this field.

**Gene expression profiling recapitulates the overall classification of thyroid tumors**

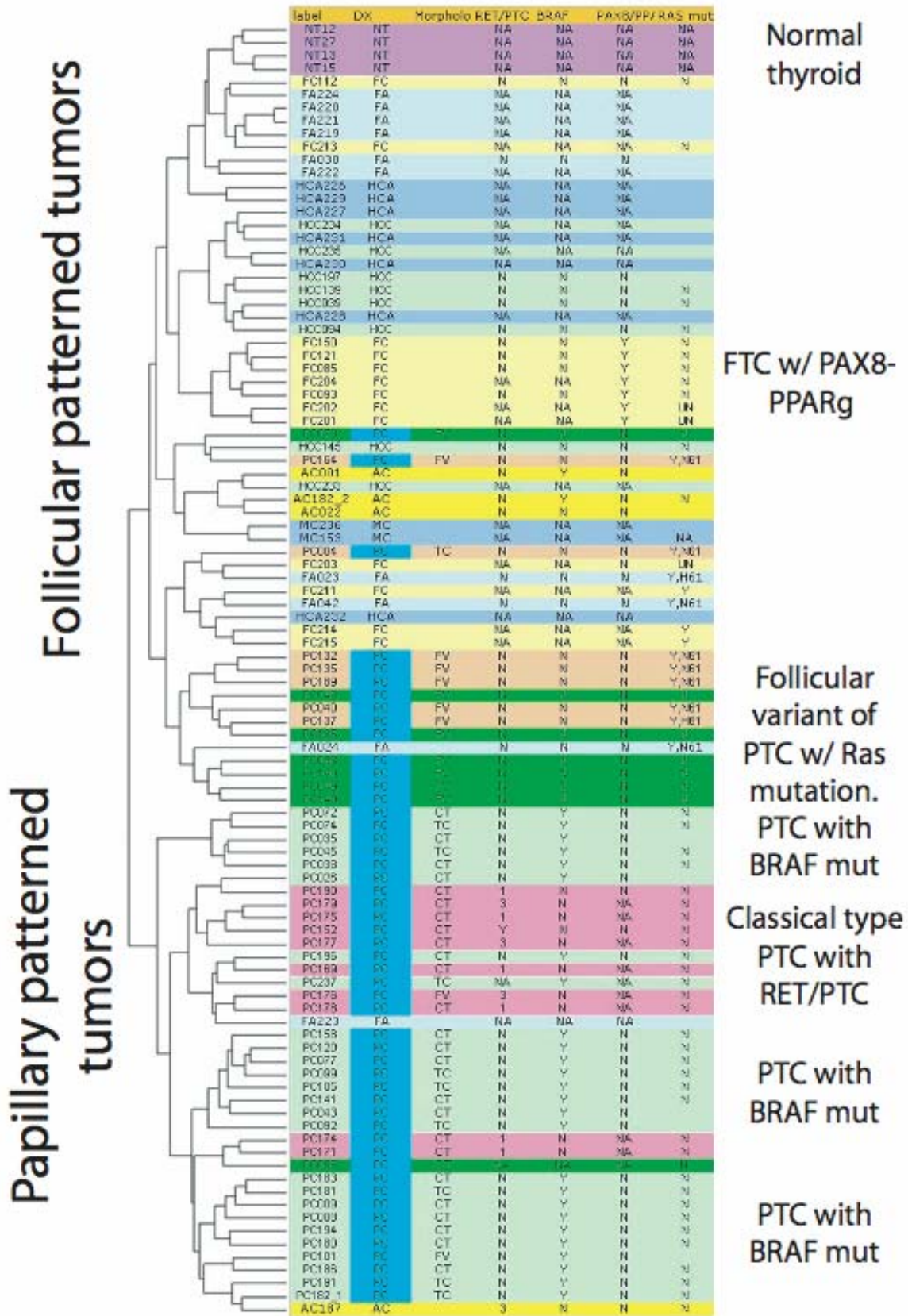
Using commercially-available oligonucleotide DNA microarrays (Affymetrix U133A), our laboratory has generated a gene expression dataset that corresponds to the common types of benign and malignant thyroid tumors. Various clustering methods have been applied to this dataset derived from 99 thyroid samples, resulting in a molecular classification that recapitulates the overall morphologic classification of thyroid tumors (Figures 1 and 2). This observation provides strong and compelling validation of the microarray data. The result from the first method, principal component analysis (PCA), is shown in Figure 1.



*Figure 1. Principal components analysis (PCA) of 99 thyroid samples (95 tumors and 4 normal thyroids). The PCA reveals separation of the papillary carcinomas (brown), the anaplastic carcinomas (red), and defines a follicular patterned cohort that includes normal thyroid (dark blue), follicular adenoma (black), follicular carcinoma (green). Further, the oncocyctic tumors (purple and yellow) also define a separate group. Two medullary carcinomas are distinctly different (light blue).*

The result from the second clustering method, hierarchical clustering (HC), is shown in Figure 2. In addition to morphologic assessment, the tumors were genotyped for their common mutations (e.g. BRAF, RET/PTC, RAS, PAX8-PPARgamma translocation). These results from the PCA and HC along with morphologic assessment reveals some interesting observations and confirms some previously known relationships, as outlined below:

1. There is a large degree of heterogeneity within the PTC cohort. This is consistent with the numerous morphologic variants associated with PTC and several different activating mutations (BRAF, RET/PTC, and RAS) (Figure 1).
2. Follicular patterned lesions (FA, FC and normal thyroid) share similar global patterns of gene expression.
3. Oncocytic tumors, benign and malignant, share global gene expression profiles.
4. Anaplastic carcinoma has a distinctly different gene expression pattern.
5. Medullary carcinoma has a distinctly different gene expression pattern.
6. Hierarchical clustering divides the cohort generally into tumors with papillary architecture and follicular architecture (Figure 2).]
7. PTC with BRAF activating mutations form several subclusters with the papillary architecture cluster (Figure 2).
8. PTC with RET/PTC mutations form 2 subclusters within the papillary architecture cluster (Figure 2).
9. PTC, follicular variants, form 2 subclusters within the follicular patterned tumors and have a predominance of RAS mutations.
10. FTC with the PAX8-PPARgamma translocation form a distinct subcluster within the follicular patterned lesions (Figure 2).
11. Normal thyroid shares a similar global gene expression profile within the follicular patterned samples (Figure 2).
12. Separation of many of the follicular patterned tumors is not straightforward (Figure 2).



## Activating mutations of the MAPK pathway are the primary determinant of gene expression variation within PTC

Examination of the PTC cohort by PCA revealed a molecular classification of PTC that was a reflection of both tumor morphology and underlying activation mutation<sup>3</sup>, as summarized in Figure 3.

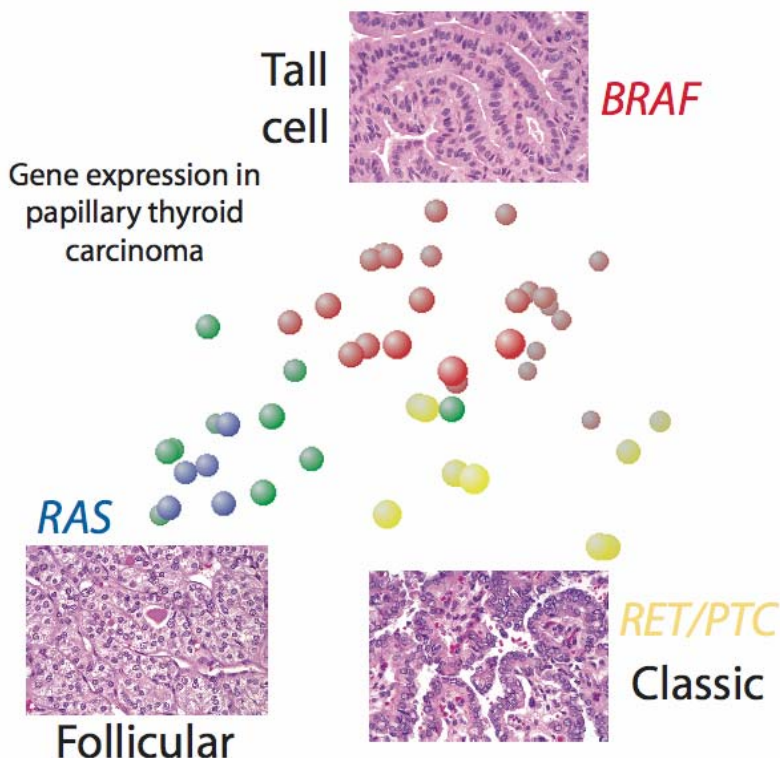


Figure 3. PCA of 51 PTC samples showing the relationships between tumor morphology and gene expression and mutation and gene expression. PTC with BRAf (red) tend to form a distinct cluster and have a tall cell morphology. PTC with RET/PTC rearrangements (yellow) tend to form a distinct cluster and have a classic papillary morphology. PTC with RAS mutations tend to form a distinct cluster (blue) and have a follicular morphology.

This striking observation means that these mutations are the primary determinants of gene expression variation in these tumors. The broader implication is that PTC is a relatively simpler carcinoma compared to many other common epithelial tumors, e.g. lung adenocarcinoma, in which it is much harder to define a relationship between mutation and gene expression. The implication of this observation is that PTC should be amenable to treatment with targeted therapies that block these activating mutations. Clinical trials are underway with specific BRAf inhibitors and it will be interesting to see their results. Animal studies suggest the BRAf inhibition will be effective<sup>4</sup>.

## Follicular carcinoma with the PAX8-PPARgamma translocation have distinct gene expression profiles

Included in our dataset are 7 FTCs with the PAX8-PPARgamma translocation. This molecular alteration was first described in 2000 in a subset of FTCs<sup>5</sup> and the observation has since been confirmed and extended by a number of studies<sup>6-9</sup>. Several groups have examined gene expression in these tumors<sup>10-12</sup>, with the 2 most recent studies in general agreement that these tumors display a characteristic gene expression profile. The latter 2 studies revealed that FTC with PAX8-PPARgamma translocation have a gene expression profile that is a function of the activity of the fusion protein, called PFP. These studies have implications for the role of this fusion protein in the pathogenesis of this type of FTC and by extension the treatment of this tumor. These aspects were recently reviewed<sup>13</sup>.

## Potential of molecular profiling for thyroid tumor diagnosis and prognosis

Many studies have examined the potential of molecular profiling for thyroid cancer. While none of the studies have resulted in a clinically available and validated diagnostic assay, the results are generally encouraging. For instance, a recent study by Lubitz et al. showed that gene expression profiling could be accurately employed at the cytology level<sup>14</sup>. Using a small cohort of 22 FNA specimens, they showed that examining 25 differentially expressed genes could correctly classify these lesions using hierarchical clustering. Although this study needs to be extended to a larger cohort and hierarchical clustering is not recommended for diagnostic assays<sup>15</sup>, it illustrates the potential of genomic approaches especially if they can be applied at the pre-operative level.

Other studies have addressed the issue of genomic prediction of prognosis, a difficult task given the overall excellent prognosis of thyroid carcinoma. One such study used a genomic approach to delineate the MUC1 gene as an independent prognostic factor in PTC in a multivariate analysis<sup>16</sup>.

## MicroRNA profiling

MicroRNA profiling represents a complementary approach to messenger RNA profiling. MicroRNAs are small RNA molecules that are thought to function as negative regulators of gene expression<sup>17</sup>. They possess significant diagnostic potential<sup>18-25</sup> and have begun to be examined in thyroid cancer<sup>26-29</sup>. In addition to a role in PTC, our study of FTC with PAX8-PPARgamma translocation showed altered expression microRNA target genes, suggesting that microRNAs may play a role across the full spectrum of thyroid tumors. Much work still needs to be done in this exciting and promising area.

## Conclusion

The potential of using molecular profiling for thyroid neoplasia is significant. Such approaches have and should results in:

1. Improved understanding of thyroid tumor pathogenesis.
2. Improved understanding of thyroid cancer progression to aggressive forms.
3. Refinement of thyroid tumor classification.
4. Identification of novel diagnostic markers.
5. Identification of novel prognostic markers.

Collectively, these advances should result in improved management and care of patient with thyroid nodules and cancer.

## References

1. Lloyd RV, Erickson LA, Casey MB, Lam KY, Lohse CM, Asa SL, Chan JK, DeLellis RA, Harach HR, Kakudo K, LiVolsi VA, Rosai J, Sebo TJ, Sobrinho-Simoes M, Wenig BM, Lae ME: Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma, *Am J Surg Pathol* 2004, 28:1336-1340
2. Giordano TJ: Gene expression profiling of endocrine tumors using DNA microarrays: progress and promise, *Endocr Pathol* 2003, 14:107-116
3. Giordano TJ, Kuick R, Thomas DG, Misek DE, Vinco M, Sanders D, Zhu Z, Ciampi R, Roh M, Shedden K, Gauger P, Doherty G, Thompson NW, Hanash S, Koenig RJ, Nikiforov YE: Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis, *Oncogene* 2005, 24:6646-6656
4. Salvatore G, De Falco V, Salerno P, Nappi TC, Pepe S, Troncone G, Carlomagno F, Melillo RM, Wilhelm SM, Santoro M: BRAF is a therapeutic target in aggressive thyroid carcinoma, *Clin Cancer Res* 2006, 12:1623-1629
5. Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, Fletcher JA: PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma [corrected], *Science* 2000, 289:1357-1360

6. Cheung L, Messina M, Gill A, Clarkson A, Learoyd D, Delbridge L, Wentworth J, Philips J, Clifton-Bligh R, Robinson BG: Detection of the PAX8-PPAR gamma fusion oncogene in both follicular thyroid carcinomas and adenomas, *J Clin Endocrinol Metab* 2003, 88:354-357
7. Dwight T, Thoppe SR, Foukakis T, Lui WO, Wallin G, Hoog A, Frisk T, Larsson C, Zedenius J: Involvement of the PAX8/peroxisome proliferator-activated receptor gamma rearrangement in follicular thyroid tumors, *J Clin Endocrinol Metab* 2003, 88:4440-4445
8. Marques AR, Espadinha C, Catarino AL, Moniz S, Pereira T, Sobrinho LG, Leite V: Expression of PAX8-PPAR gamma 1 rearrangements in both follicular thyroid carcinomas and adenomas, *J Clin Endocrinol Metab* 2002, 87:3947-3952
9. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW, 2nd, Tallini G, Kroll TG, Nikiforov YE: 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, 88:2318-2326
10. Giordano TJ, Au AY, Kuick R, Thomas DG, Rhodes DR, Wilhelm KG, Jr., Vinco M, Misek DE, Sanders D, Zhu Z, Ciampi R, Hanash S, Chinnaiyan A, Clifton-Bligh RJ, Robinson BG, Nikiforov YE, Koenig RJ: Delineation, functional validation, and bioinformatic evaluation of gene expression in thyroid follicular carcinomas with the PAX8-PPARG translocation, *Clin Cancer Res* 2006, 12:1983-1993
11. Lacroix L, Lazar V, Michiels S, Ripoche H, Dessen P, Talbot M, Caillou B, Levillain JP, Schlumberger M, Bidart JM: Follicular thyroid tumors with the PAX8-PPARGgamma1 rearrangement display characteristic genetic alterations, *Am J Pathol* 2005, 167:223-231
12. Lui WO, Foukakis T, Liden J, Thoppe SR, Dwight T, Hoog A, Zedenius J, Wallin G, Reimers M, Larsson C: Expression profiling reveals a distinct transcription signature in follicular thyroid carcinomas with a PAX8-PPAR(gamma) fusion oncogene, *Oncogene* 2005, 24:1467-1476
13. Reddi HV, McIver B, Grebe SK, Eberhardt NL: The Paired Box-8/Peroxisome Proliferator-Activated Receptor- $\{\gamma\}$  Oncogene in Thyroid Tumorigenesis, *Endocrinology* 2007, 148:932-935
14. Lubitz CC, Ugras SK, Kazam JJ, Zhu B, Scognamiglio T, Chen YT, Fahey TJ, 3rd: Microarray analysis of thyroid nodule fine-needle aspirates accurately classifies benign and malignant lesions, *J Mol Diagn* 2006, 8:490-498; quiz 528
15. Simon R: Roadmap for developing and validating therapeutically relevant genomic classifiers, *J Clin Oncol* 2005, 23:7332-7341
16. Wreesmann VB, Sieczka EM, Socci ND, Hezel M, Belbin TJ, Childs G, Patel SG, Patel KN, Tallini G, Prystowsky M, Shaha AR, Kraus D, Shah JP, Rao PH, Ghossein R, Singh B: Genome-wide profiling of papillary thyroid cancer identifies MUC1 as an independent prognostic marker, *Cancer Res* 2004, 64:3780-3789
17. Pasquinelli AE, Hunter S, Bracht J: MicroRNAs: a developing story, *Curr Opin Genet Dev* 2005, 15:200-205
18. Calin GA, Croce CM: MicroRNA signatures in human cancers, *Nat Rev Cancer* 2006, 6:857-866
19. Calin GA, Croce CM: MicroRNA-cancer connection: the beginning of a new tale, *Cancer Res* 2006, 66:7390-7394
20. Cummins JM, Velculescu VE: Implications of micro-RNA profiling for cancer diagnosis, *Oncogene* 2006, 25:6220-6227
21. Garzon R, Fabbri M, Cimmino A, Calin GA, Croce CM: MicroRNA expression and function in cancer, *Trends Mol Med* 2006, 12:580-587
22. Meltzer PS: Cancer genomics: small RNAs with big impacts, *Nature* 2005, 435:745-746
23. Voorhoeve PM, Agami R: Classifying microRNAs in cancer: The good, the bad and the ugly, *Biochim Biophys Acta* 2006,
24. Wu W, Sun M, Zou GM, Chen J: MicroRNA and cancer: Current status and prospective, *Int J Cancer* 2007, 120:953-960
25. Zhang L, Coukos G: MicroRNAs: a new insight into cancer genome, *Cell Cycle* 2006, 5:2216-2219
26. Cahill S, Smyth P, Finn SP, Denning K, Flavin R, O'Regan EM, Li J, Potratz A, Guenther SM, Henfrey R, O'Leary JJ, Sheils O: Effect of ret/PTC 1 rearrangement on transcription and post-transcriptional regulation in a papillary thyroid carcinoma model, *Mol Cancer* 2006, 5:70
27. He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A: The role of microRNA genes in papillary thyroid carcinoma, *Proc Natl Acad Sci U S A* 2005, 102:19075-19080
28. Pallante P, Visone R, Ferracin M, Ferraro A, Berlingieri MT, Troncone G, Chiappetta G, Liu CG, Santoro M, Negrini M, Croce CM, Fusco A: MicroRNA deregulation in human thyroid papillary carcinomas, *Endocr Relat Cancer* 2006, 13:497-508
29. Weber F, Teresi RE, Broelsch CE, Frilling A, Eng C: A limited set of human MicroRNA is deregulated in follicular thyroid carcinoma, *J Clin Endocrinol Metab* 2006, 91:3584-3591