Clinical and Biological Significance of Common Mutational Genotypes of Thyroid Follicular Neoplasia

Joseph F Annunziata[1], Anna Banizs[1], Christina M Narick[2], Sara Jackson[2], Jan F Silverman[1], Sydney Finkelstein[2]. [1]Allegheny General Hospital, Pittsburgh, PA; [2]Interpace Diagnostics, Pittsburgh, PA

Background

Somatically acquired oncogene mutational change is a fundamental to thyroid neoplasia. While certain mutations confer highly predictable degrees of biological aggressiveness, other mutation types are less predictable. We report on our large experience thyroid nodule mutational change correlated with cytology and RNA expression level determination to better understand neoplastic progression according to specific mutation type.

Design

5,210 indeterminate thyroid nodule needle aspirates underwent mutational analysis for common mutations (BRAF, HRAS, KRAS, NRAS, PIK3CA, PAX8/PPAR and RET/PTC translocation) by next generation sequencing (Illumina). Cytology diagnosis was based on pathology report diagnoses (Bethesda diagnostic categories). As an independent predictor of neoplastic behavior, a panel of 10 microRNAs was developed. Mutation type was correlated with cytology diagnosis and microRNA prediction of clinical aggressiveness. (Wylie et al. (2016), Molecular classification of thyroid lesions by combined testing for miRNA gene expression and somatic gene alterations. *J Path: Clin Res*, 2: 93–103. doi:10.1002/cjp2.38)

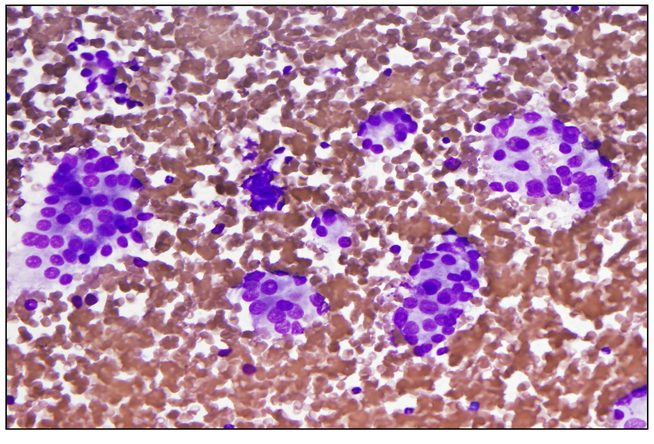

Figure 1. Follicular Variant of Papillary Carcinoma

Data

Table 1. Relationship of Mutation Genotype to Bethesda Diagnostic Category Classification

	Total cases n=5210							
	TOTAL	B-I	B-II	B-III	B-IV	B-V	B-VI	No Cytology
BRAF	300	4	3	92	38	83	35	45
RAS	741	9	18	462	135	26	3	88
PAX8-PPARg	38	0	0	24	9	1	0	4
PIK3CA	6	0	1	3	1	1	0	0
RET/PTC1	12	0	0	7	2	3	0	0
RET/PTC3	3	0	0	2	1	0	0	0

Cytology classification was obtained from original cytologist reporting of the Bethesda Diagnostic Category

Table 2. Relationship of Mutation Genotype to Risk of Malignancy Based on MicroRNA Classifier Status

		All Mutations	BRAFV600E	All RAS	KRAS	HRAS	NRAS
Benign/extreme low risk	0.0-0.4	17	0	14	4	3	7
Low risk	0.4-0.7	292	11	253	47	59	147
Moderate risk	0.7-0.9	238	32	186	22	52	112
Malignant/high risk	0.9-1.0	132	104	25	2	10	13

MicroRNA classifier quantitative risk assessment is based on an algorithm using the expression level of 10 microRNAs resulting in a value between 0 and 1.0. The closer the value to 1.0, the more likely malignancy is present. Conversely, the closer the value to 0, the more likely the nodule is benign.

Results

Oncogenic mutations were detected in 1100/5,210 (21.1%) of the cases. The majority were point mutations (95.3%) and the remainder were translocations (4.7%). Virtually all nodules displayed a single genotype for this mutation panel. RAS genes accounted for most point mutations (n=736) distributed as NRAS (57.0%), HRAS (26.7%) and KRAS (16.3%). Mutations were seen in across cytology categories, the relative Distribution varied according to specific genotype. B-V was dominated by BRAF V600E (40.9%) but included all genotypes. B-III and B-IV manifested all genotypes with relatively greater content of ras gene mutations (16.7% and 15.6 %) and all BRAF point mutations outside of V600E. MicroRNA (miR) expression classifier yielded a four level quantitative measure of increasing malignancy risk (very low 99+% NPV, low 94% NPV, moderate 74% PPV, high 99+% PPV). Individual mutational genotypes within specific oncogenes displayed unique malignancy risk profiles correlating with cytology.

Conclusion

Cytology prediction of malignancy risk is challenging in the earlier stages of neoplastic progression. RAS gene mutations, the most common oncogene alteration, are diverse and not equivalent. While certain mutations are highly predictive of cancer, the majority of individual mutation genotypes show biological heterogeneity. Combining RNA expression profiling with mutation determination leads to more information to base risk assessment.