MicroRNA Profiling Addresses Mutational Heterogeneity in Individual Thyroid Nodules

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88th Annual Meeting of the American Thyroid Association®
October 3-7, 2018 ✳ Washington, DC
Marriott Marquis
AMERICAN THYROID ASSOCIATION
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INTRODUCTION:

- Acquisition of cancer-associated mutational change is fundamental to thyroid carcinogenesis however up to 30% of thyroid follicular lining cell malignancies can lack detection of mutational change.
- Our study uses a combined mutation detection and microRNA (miRNA) classifier determination to evaluate the role of mutational heterogeneity in thyroid nodules.

METHODS:

- Cytologically indeterminate thyroid nodules (n=48) from aspirates or microdissected cytology slides underwent molecular testing (oncogene point mutation/fusion detection [ThyGenX] and miRNA profiling using a 10 marker algorithmic classifier determination as well as pair-wise analysis of individual miRNA expression differences [ThyraMIR]).
- Malignant outcome was based on surgical pathology.

Table 1. Markers in Mutation panel and microRNA classifier panel

Next Generation Sequencing Mutation Panel	
BRAF	BRAF_V600E, BRAF_K601E, BRAF_A598V
HRAS	HRAS_G12V, HRAS_G13R, HRAS_Q61K, HRAS_Q61L,HRAS_Q61R
NRAS	NRAS_G12D, NRAS_G13R, NRAS_Q61R, NRAS_Q61K, NRAS_Q61P
KRAS	KRAS_G12D, KRAS_G12V, KRAS_G13D, KRAS_Q61R
PIK3CA	PIK3CA_E542K, PIK3CA_H1047L, PIK3CA_H1047R
RNA Fusion Transcripts	RET-PTC1
	RET-PTC3
	PAX8-PPARγ
10 MicroRNA (MiR) Classifier Panel	
Down-regulated	miR-204-5p, miR-139-5p, miR-29b-1-5p, miR-155-5p, miR-138-1-3p
Up-regulated	miR-375, miR-551-b-3p, miR-146b-5p, miR-31-5p, miR-222-3p

Table 2. Mutation variant content

- Mutation variant content ranged from 2- 47.5% in malignant nodules.
- No nodules showed 100% mutated cell content.

		Mutation Variant	
Gene	n	mean	standard deviation
BRAF V600E	21	19.3%	13.7%
RAS	19	19.3%	11.8%

- Pair-wise miRNA marker analysis among the 10 miRNAs yielded distinctly different miRNA profiles for *BRAF V600E* positive nodules and *RAS* positive nodules, enabling these two forms of thyroid follicular lining cell neoplasia to be identified using pair-wise miRNA comparisons
- 75% (6/8) of nodules that **lacked detectable mutations** but had miRNA classifier levels consistent with malignancy, showed two distinct pair-wise miRNA profiles
  1. Consistent with that observed in BRAF V600E positive nodules
  2. Consistent with that observed in RAS positive nodules
- Detection of these two distinct miRNA profiles in nodules that **lack detectable mutational change** is supportive of mutational heterogeneity in nodules, where BRAF and RAS mutations are not detected in the sample but distinct miRNA profiles consistent with either mutation is present.

CONCLUSIONS:

- **Thyroid cancers in cytologically indeterminate nodules are composed of a chimeric mixture of mutated and non-mutated cells with respect to common mutational genotypes (*BRAF V600E, RAS*).**
- **While the topographic distribution of mutated cells can be heterogeneous, the more homogeneous character of the miRNA profile can confirm the acquisition of common forms of mutational change.**
- **This work supports the concept that the mutated cell subset is able to recruit non-mutated cells to be phenotypically malignant, a function ideally suited to miRNA serving as a complementary component of combined molecular testing.**