MicroRNA Profiling Complements Microscopic/Mutational Analysis by Defining Alternative Pathways of Carcinogenesis

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Background

Thyroid follicular cell neoplasia is biologically heterogeneous ranging from benign to indolent malignancy to high grade cancer. Mutation genotyping is effective at predicting tumor aggressiveness. Aggressive disease can be seen in a significant minority of cases where common driver mutations are undetected. We performed a correlative analysis of microRNA profiling with specific patterns of thyroid follicular cell nodules using a large clinical case database (n=5,210) seeking discriminating information.

Design

Data was retrospectively mined from a large clinical testing cohort of FNA thyroid nodule cytology. Cytology was based on pathologist Bethesda diagnostic classification. Mutation analysis targeted common mutations (BRAF, ras, PIK3CA, PAX8/PPAR and RET/PTC translocations) by next generation (Illumina). RNA expression classifier utilized a 10 miRNA panel consisting of 5 miRs with increased expression in cancer and 5 miRs with hyperexpression in benign states.

Figure 1. ThyraMIR: microRNA gene expression based prediction of malignancy risk

- **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

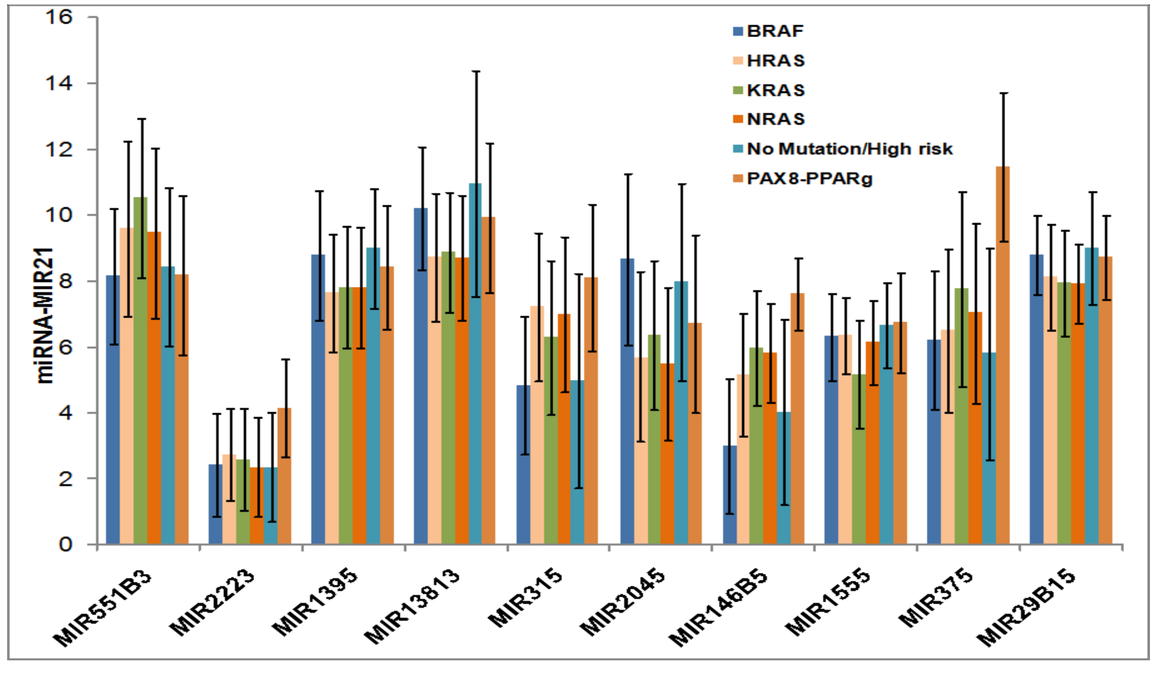
MicroRNA classifer testing is recommended for nodules that are mutation negative and nodules that show weak driver mutations

Data

Table 1. Comparison of ThyraMir microRNA gene expression between BRAF mutated versus no mutation /high risk specimens (quantitative PCR relative to comparator gene expression)

	MIR551B3	MIR2223	MIR1395	MIR13813	MIR315	MIR2045	MIR146B5	MIR1555	MIR375	MIR29B15
BRAF Average Ct	8.14	2.43	8.77	10.20	4.83	8.66	2.99	6.30	6.20	8.79
Std Dev	2.06	1.56	1.97	1.86	2.08	2.59	2.05	1.31	2.10	1.20
No Mutation/High risk Average Ct	8.43	2.35	8.99	10.95	4.99	7.96	4.03	6.66	5.80	9.00
Std Dev	2.41	1.66	1.82	3.43	3.25	3.00	2.81	1.29	3.22	1.71

Figure 2. ThyraMir microRNA gene expression in common mutations



Results

Mutation analysis defined six groups for correlative miR profiling: BRAF+ (aggressive), N/H/K ras+ (indolent to aggressive), PAX8/PPARgamma translocation+ (indolent to aggressive) and NO detectable mutation yet aggressive. BRAF+ compared to RAS+ groups showed distinct profiling differences for 7 of 10 Individual miRs. No detectable mutation/aggressive subset closely matched BRAF+ supporting common molecular pathway involvement (BRAF phenotype). Among the different ras+ nodules, NRAS and HRAS showed a nearly matching miR profile for all 10 miRs. KRAS profiling differences were detected for 5 miRs in keeping with potentially differing biology of this form of RAS mutation. PAX8/PPAR gamma displayed profiling differences for 8 miR distinct from both BRAF and ras groups. Cytology diagnoses were significant for B5 and B6 in BRAF+ cases but otherwise molecular patterns were not predicted by microscopic classification.

Conclusion

Oncogene driver mutations can predict biological aggressiveness including benign versus malignant status in a proportion of thyroid follicular nodules that are indeterminate after cytology evaluation. BRAF mutated nodules display a unique miR expression profile distinct from other common mutations. This BRAF profile is present in mutation negative aggressive disease suggesting BRAF mutational heterogeneity across the nodule with sampling variation. An approach combining RNA expression profiling to define distinct molecular pathways and mutational genotype is well suited to complement microscopic assessment of thyroid neoplasia.