MOLECULAR DIAGNOSIS OF MEDULLARY THYROID CARCINOMA BY NGS MUTATION DETECTION AND MICRORNA EXPRESSION PROFILING

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INTRODUCTION

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine cancer originating from parafollicular C-cells that produce calcitonin, and accounts for 2%-3% of all thyroid carcinomas. Sporadic forms represent 70% of cases and inherited forms (autosomal dominant) account for 30% of cases, often occurring in younger patients, and can be associated with MEN syndromes.[1] Despite well-established criteria for the diagnosis of MTC, the cytological diagnosis can often be challenging[2,3] and mutational analysis can serve as an important adjunct. The RET proto-oncogene and overexpression of miR-375 have been previously reported in association with MTC.[4,5] However, RAS mutations can also be present in some MTC cases,[6] are common to non-malignant thyroid disease, and can also show lack of detectable oncogenic mutational change. Here, we evaluated NGS mutational analysis, including RET proto-oncogene and 14 RET fusions transcripts, combined with microRNA expression profiling (Table 1) for the classification of MTC relative to other thyroid malignancies and benign lesions. Our analysis clearly demonstrates that MTCs show overexpression of miR-375 with or without the presence of RET mutations.

Table 1: ThyGeNEXT® and ThyraMIR® Panels

ThyGeNEXT DNA Panel		ThyGeNEXT RNA Panel		ThyraMIR miRNA Classifier Panel
Gene	**Thyroid Cancer Variants**	**Key Gene**	**Fusion Partner**	
ALK	L1198F, G1201E	*ALK*	*STRN, EML4*	*miR-29b-1-5p*
BRAF	V600E, K601E, A598V	*BRAF*	*AGK, AKAP9, SPTLC2*	*miR-31-5p*
GNAS	R201H, L203P, Q227E, R844C	*NTRK1*	*TPM3, TPR, TGF*	*miR-138-1-3p*
HRAS	G12V, G13R, Q61R, Q61K	*NTRK2*	*TERT*	*miR-139-5p*
KRAS	G12D, G12V, G13D, G13R, Q61K, Q61R, Q61H	*NTRK3*	*ETV6, SLC12A6*	*miR-146b-5p*
NRAS	G12D, G13D, G13R, Q61K, Q61R, Q61P	*PPARg*	*BMS1, PAX8, CREB3L2*	*miR-155*
PIK3CA	E542K, E545K, H1047L, H1047R	*RET*	*CCDC6, ELKS, GOLGA5, HOOK3, KTN1, NCO4*	*miR-204-5p*
PTEN	C124F, R130X	*RET*	*NCOA4, PCM1, PRKAR1A, RFG9, TRIM27*	*miR-222-3p*
RET	M918T, C630R, D631G, C634W, A883F, E921K	*THADA*	*IGF2BP3, TRA2A, LOC389473*	*miR-375*
TERT	-124C>T, -146C>T, -138_139CC>TT, -161C>T	Housekeeping genes - *NKX2.1, PAX8, TBP, USP33*		*miR-551b-3p*

ThyGeNEXT is an NGS test that interrogates hotspot regions in 10 genes for the presence of SNVs and 38 oncogenic fusion transcripts arising from 8 key thyroid oncogenes. The expression of four control mRNAs is also analyzed to determine thyroid cell content in the sample and to differentiate between thyroid and parathyroid tissue. ThyraMIR is a qPCR-based, 10-microRNA gene expression classifier for the diagnosis of thyroid malignancy.

METHODS

NGS results (ThyGeNEXT) and microRNA profiling (ThyraMIR) were reviewed in samples positive for the RET proto-oncogene as well as cases positive for RET/PTC1 (CCDC6_RET) fusions, or BRAF V600E mutations. Surgical histology outcomes for a subset of RET-positive resected specimens were obtained to confirm the presence of MTC. Relative expression of miR-375, based on qPCR Ct value, was evaluated in comparison to nine other microRNAs.

All testing was performed in a CAP accredited and CLIA certified laboratory.

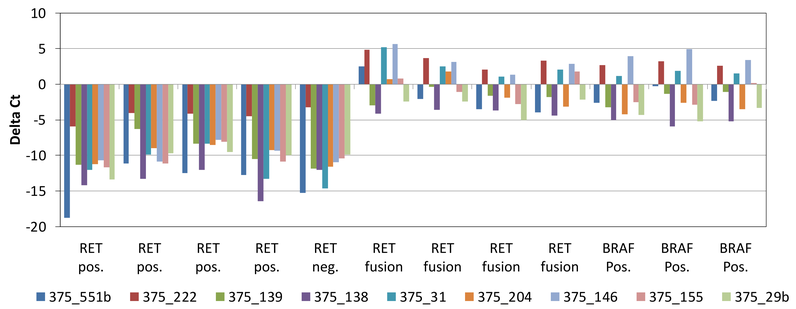
RESULTS

Because microRNAs play a critical role in the regulation of gene expression and cell-to-cell signaling, variations in microRNA expression may be able to identify MTC in the absence of mutational change and independently of limitations of tumor and/or sample heterogeneity. Although overexpression of miR-375 has been previously reported in MTC, a pairwise approach to microRNA analysis to identify the presence of MTC in thyroid nodule FNA has not been established.

miR-375 Differential Expression

Initial efforts focused on re-analysis of ThyraMIR data in 12 specimens: 5 cases positive for RET proto-oncogene mutations or negative by mutation testing (representing MTC), and 7 cases positive for RET fusions or BRAF mutations (representing PTC). Pairwise analysis of miR-375 expression levels relative to the expression of the 9 other microRNAs (delta Ct) is presented in Figure 1. All MTC cases showed overexpression of miR-375 compared to all other microRNAs (negative delta Ct in Figure 1). In contrast, PTC had variable miR-375 expression in relation to the other markers.

Figure 1: miR-375 Expression Relative to 9 Other microRNAs in 12 Thyroid Nodules



miR-375 Pairwise Analysis in Specimens With Known Surgical Outcome

To further evaluate relative microRNA expression in various thyroid disease states, pairwise analysis was carried out in total nucleic acid samples isolated from FNA or FFPE tissues with confirmed histology and mutational status (Figure 2). miR-375 was overexpressed in MTC-positive or negative for RET mutations, but not in PTC or benign lesions positive or negative by mutation testing. These findings suggest that pairwise microRNA analysis along with the ThyGeNEXT and ThyraMIR test results can be effective at the detection of MTC in thyroid FNA specimens.

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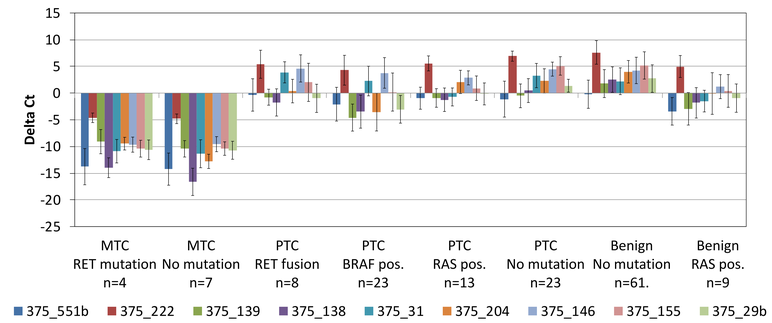
Figure 2: miR-375 Pairwise Analysis (Relative Expression) in 148 Thyroid Nodules



Confirmatory Sample Set

We conducted a retrospective analysis of clinical samples with Bethesda III, IV, and V cytological diagnosis that had been prospectively submitted to Interpace Diagnostics for routine testing with ThyGeNEXT and ThyraMIR. At the time of this analysis, the final surgical pathology diagnosis was available for 11 cases. These cases were suspicious for MTC based on overexpression of miR-375, with or without RET mutations, and all were evaluated using pairwise analysis. All cases with overexpression of miR-375 were confirmed to have MTC based on surgical pathology (Table 2).

Table 2: Correlation of miR-375 Pairwise Analysis and Histology of MTC

Unique Study ID	Bethesda Cytology Diagnosis	RET Mutation Status	ThyraMIR Result	microRNA Pairwise Analysis	Surgical Pathology Diagnosis
TT10-02	B-III	Negative	Highly positive	MTC	MTC
TT10-03	B-III	M918T	Highly positive	MTC	MTC
TT10-04	B-IV	M918T	Highly positive	MTC	MTC
TT10-05	B-V	Negative	Highly positive	MTC	MTC
TT10-08	B-III	Negative	Highly positive	MTC	MTC
TT10-11	B-III	Negative	Highly positive	MTC	MTC
TT10-24	B-V	Negative	Highly positive	MTC	MTC
TT10-27	B-V	Negative	Highly positive	MTC	MTC
TT10-29	B-IV	Negative	Highly positive	MTC	MTC
TT10-31	B-IV	Negative	Highly positive	MTC	MTC
TT10-33	B-III	Negative	Highly positive	MTC	MTC

CONCLUSIONS

- miR-375 is differentially expressed in MTC, independently from RET mutation status
- microRNA expression profiling and next generation sequencing for oncogenic mutations can be effectively used together to identify MTC in indeterminate thyroid FNA samples
- These findings illustrate the potential impact of ThyGeNEXT and ThyraMIR on the clinical work-up and optimal management of MTC