Short Call Poster #46

The addition of microRNA expression testing increases the sensitivity and specificity to detect thyroid cancer in pediatric patients

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ABSTRACT

Thyroid cancer is the most common endocrine malignancy, and incidence is rising. MAP kinase (MAPK) signaling has been implicated in playing a critical role in the initiation and maintenance of thyroid cancer, as evidenced by the high incidence of non-overlapping mutations of the genes encoding *RET* and *TRK*, as well as of *NRAS*, *HRAS*, *KRAS*, and *BRAF*. Although pediatric and adult thyroid cancers have been found to share many of the same driver mutations, it is not yet known and validated in pediatric populations whether molecular testing can improve diagnostic accuracy, particularly in ruling out malignancy. Further, it is not yet clear in pediatric populations whether microRNA expression can improve diagnostic accuracy. We sought to determine whether a 10-microRNA classifier developed and optimized for adult thyroid lesions could improve the diagnostic accuracy of thyroid nodules in pediatric patients. In future studies we plan to use these preliminary findings to further understand the molecular differences that drive the differential pathogenesis of pediatric versus adult tumors, despite sharing identical driving mutations.

INTRODUCTION

Detection of thyroid mutations is associated with an increased risk of malignancy across a spectrum of predictability from highly predictive, including *BRAF V600E* and fusions in *RET*, *NTRK*, and *ALK*, to less predictive, including *RAS*, *DICER1*, *PTEN and PAX8/PPARG*. The inability to detect a mutation does not decrease the probability of malignancy. The aim of this study was to assess whether adding a 10-microRNA classifier could improve the diagnostic accuracy in pediatric patients.

METHODS

Residual surgical specimens collected from 113 patients at the Children's Hospital of Philadelphia (CHOP) between 1989 and 2012 were evaluated under a research protocol approved by CHOP's Institutional Review Board. All cases were reviewed by expert pathologists (L.S. and V.L.) to ensure accurate diagnosis based on standard histopathological criteria defined by the World Health Organization. There were 47 benign specimens: 29 follicular adenoma (FA), including 14 with hyperplastic/papillary changes (pFA); 11 diffuse hyperplasia (Graves' disease); three multinodular goiters (MNG); three chronic lymphocytic thyroiditis; and one infectious thyroiditis. The 66 malignant specimens consisted of 27 classic papillary thyroid carcinoma (cPTC), 23 follicular variant or PTC (fvPTC), 8 diffuse sclerosing variant of PTC (dsvPTC), 6 follicular thyroid carcinoma (FTC), 1 oncocytic PTC (oPTC), and 1 mixed PTC displaying classic papillary, follicular and solid-growth patterns (mixPTC). Molecular testing of residual total nucleic acids containing DNA, mRNA, and miRNA was carried out using the ThyGeNEXT® and ThyraMIR® tests (Interpace Diagnostics, Parsippany, NJ). For statistical analyses, binary molecular results (positive or negative) were compared to binary clinicopathologic parameters. 95% confidence intervals were calculated using the Clopper-Person method for proportions and the Armitage-Berry method for odds ratios. P-values for categorical variables were calculated using Fisher's exact test.

	Number of subject (n)	Age (yr.) Range	Age (yr.) Mean	Female (%)
Benign	47	2-18	14	87
FA	29	5-17	14	79
Other	18	2-18	13	100
Malignant	66	5-18	14	77
cPTC	27	8-18	15	81
fvPTC	23	10-18	14	75
Other	16	5-17	14	87
Overall	113	2-18	14	81

Table 1: Characteristics of study population according to surgical outcome.

Histology	Sex	Age (yr.)	ThyGeNEXT®	ThyraMIR®
cPTC	M	18	CCDC6-RET	Positive
cPTC	F	18	CCDC6-RET	Positive
cPTC	F	17	CCDC6-RET	Positive
dsvPTC	F	16	CCDC6-RET	Positive
dsvPTC	F	12	CCDC6-RET	Positive
cPTC	F	12	ETV6-NTRK3	Positive
dsvPTC	F	14	ETV6-NTRK3	Positive
cPTC	F	13	NCOA4-RET	Positive
cPTC	F	10	NCOA4-RET	Positive
dsvPTC	F	13	NCOA4-RET	Positive
fvPTC	F	13	NCOA4-RET	Positive
fvPTC	M	15	PAX8-PPARG	
fvPTC	M	11	PAX8-PPARG	
fvPTC	F	12	STRN-ALK	Positive
cPTC	M	12	STRN-ALK	
cPTC	F	18	BRAF V600E, 22%	Positive
cPTC	F	15	BRAF V600E, 24%	Positive
cPTC	F	15	BRAF V600E, 25%	Positive
cPTC	F	15	BRAF V600E, 26% PIK3CA M1043I, 12%	Positive
cPTC	F	15	BRAF V600E, 28%	Positive
cPTC	F	16	BRAF V600E, 34%	Positive
cPTC	F	16	BRAF V600E, 35%	Positive
cPTC	F	14	BRAF V600E, 40%	Positive
cPTC	M	16	BRAF V600E, 41%	Positive
cPTC	M	9	BRAF V600E, 41%	Positive
cPTC	F	17	BRAF V600E, 43%	Positive
cPTC	F	18	BRAF V600E, 7%	Positive
fvPTC	M	18	HRAS G13R, 23%	Positive
FTC	F	16	HRAS G13R, 31%	Positive
FTC	F	18	HRAS Q61R, 34%	Positive
FTC	F	14	KRAS G12V, 28%	
fvPTC	M	15	NRAS Q61R, 41%	Positive
fvPTC	F	17	NRAS Q61R, 17%	
fvPTC	F	11	NRAS Q61R, 42% PIK3CA H1047R, 38%	
fvPTC	F	16	TERT c.-124C>T, 34%	
cPTC	F	17		Positive
cPTC	F	17		Positive
cPTC	F	16		Positive
cPTC	F	15		Positive
cPTC	F	15		Positive
cPTC	M	12		Positive
dsvPTC	M	13		Positive
dsvPTC	F	6		Positive
fvPTC	M	10		Positive
fvPTC	F	14		Positive
mixPTC	F	18		Positive

Table 2: Malignant cases with positive molecular results (n=46).

RESULTS

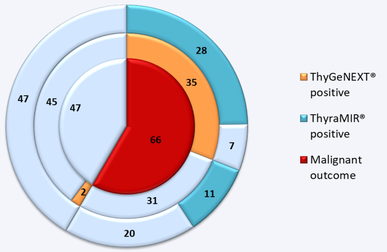

Figure 1: Distribution of negative and high-risk positive molecular results according to surgical outcome.

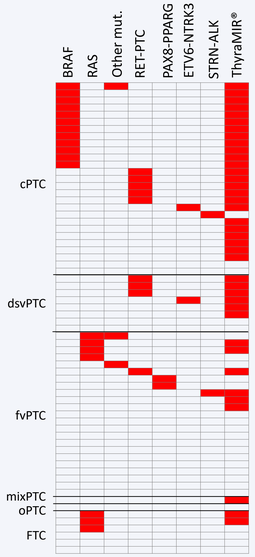

Figure 2: Distribution of molecular results in 66 malignant cases according to histopathologic classification. Each row represents a single case and positive molecular results are color coded.

Histology	Sex	Age (yr.)	ThyGeNEXT®	ThyraMIR®
MNG	F	15	GNAS R201H, 37%	Negative
pFA	M	15	GNAS R201H, 43%	Negative
FA	F	12	PTEN R130*, 9%	Negative
Diffuse hyperplasia	F	18	TERT c.-124C>T, 19%	Negative
pFA	F	13	PAX8-PPARG GNAS Q227H, 18%	Negative

Table 3: Benign cases with positive molecular results (n=5). Mutations that are reported by ThyGeNEXT® but not associated with high risk of cancer are highlighted in green (GNAS and PTEN).

	n (%)	P-value	OR [95% CI]
Malignant outcome	39 (59%)	<0.0001	136 [8.1-2,310]
Benign outcome	0 (0%)		
Lymph node metastasis	27 (93%)	<0.0001	25.1 [4.6-138]
No lymph node metastasis	7 (35%)		
Extrathyroidal extension	15 (94%)	0.002	15.8 [1.9-131]
No extrathyroidal extension	20 (49%)		
Intrathyroidal spread	22 (85%)	0.003	6.2 [1.8-21.8]
No intrathyroidal spread	16 (47%)		

Table 4: Clinical parameters significantly associated with positive ThyraMIR® results.

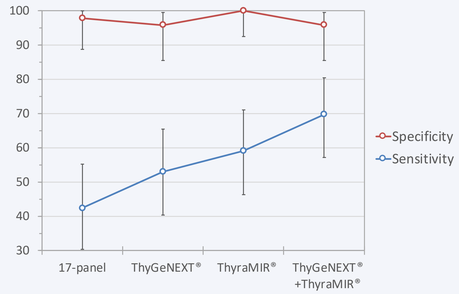

Figure 3: Sensitivity and specificity of different molecular panels to detect thyroid malignancy. The 17-marker panel corresponds to 14 single nucleotide substitutions in the BRAF, HRAS, KRAS, or NRAS genes and 3 fusion transcripts (PAX8-PPARG, RET-PTC1, and RETPTC3), as initially described by Beaudenon et al. (*Thyroid*, 2014). Error bars represent the calculated 95% confidence intervals.

RESULTS

- The study population consisted of 113 unique subjects aged 2-18 years, including 92 (81%) females (Table 1)
- Genetic alterations associated with an increased risk for malignancy were detected in 35 (53%) malignant cases (Table 2)
- 43% of the positive cases exhibited a gene rearrangement: RET/PTC (n=9), STRN/ALK (n=2), ETV6/NTRK3 (n=2), or PAX8/PPARG (n=2)
- The miRNA signature associated with a high risk for malignancy was detected in 11 (35%) malignant cases negative for the genetic alterations reported by ThyGeNEXT® (Figure 1)
- Among the 20 malignant cases negative with both ThyGeNEXT ® and ThyraMIR®, 10 out 12 fvPTC, 3 out of 3 FTC, and the single oPTC were encapsulated (Figure 2)
- A single molecular-negative case had documented extrathyroidal extension and lymph node metastasis (an aggressive dsvPTC)
- None of the benign cases were false positive with the ThyraMIR® miRNA classifier (Table 3)
- Overall, a positive ThyraMIR® result was strongly associated with malignant outcome, lymph node metastasis, extrathyroidal extension, and intrathyroidal spread (Table 4) but was not associated with age, sex, or histological tumor size (*P*>0.05)
- As previously reported by Wylie et al. (*J Pathol Clin Res*, 2016) for adult thyroid lesions, the combination of miRNA testing with a NGS panel interrogating only markers strongly associated with malignancy can improve test sensitivity without significantly affecting test specificity (Figure 3)
- ThyraMIR® combined with ThyGeNEXT® resulted in a sensitivity of 70% (95% CI: 57%-80%) and specificity of 96% (95% CI: 85%-99%) to detect a thyroid malignancy in pediatric lesions

CONCLUSIONS

These data suggest that the regulatory miRNA pathways underlying thyroid tumorigenesis may be similar in adults and children. Combined testing for well-established somatic gene alterations and miRNA gene expression improved the molecular classification of pediatric thyroid nodules. Incorporation of a miRNA expression classifier and oncogene testing into the preoperative assessment of indeterminate cytology nodules is predicted to improve the diagnostic accuracy for detecting malignancy as well as enhance and optimize risk stratification of thyroid surgery in pediatric patients.

ACKNOWLEDGEMENTS

We thank Interpace Diagnostics Inc. for generating the ThyGeNEXT® and ThyraMIR® test results. Molecular testing was performed blinded to patient information and histological outcome.