Preoperative Thyroid Nodule mRNA Expression Signatures to Predict Postoperative Thyroid Histopathology

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INTRODUCTION

- The primary role for thyroid nodule molecular testing is to risk stratify nodules with indeterminate cytology (ITN – those with (B)ethesda III/IV cytology).
  - The primary objective is to find molecularly benign nodules and thus avoid unnecessary diagnostic surgery.
- Molecular testing may also provide prognostic information in ITN when suspicious for thyroid cancer.
  - For BV/VI thyroid nodules, the main goal of molecular testing is to obtain prognostic information.
- The Afirma Genomic Sequencing Classifier (GSC) is an exome-enriched RNA sequencing test that utilizes genomic classifiers to risk stratify ITN and reports on expressed variants and fusions in Afirma GSC-(S)uspicious nodules and in nodules with BV/VI cytology.
  - Expressed variants and fusions are reported as part of the Afirma Xpression Atlas (XA).
- The Afirma Genomic Resource for Intelligent Discovery (GRID) is a research use only tool that contains mRNA based molecular signatures and data points beyond expressed variants and fusions that may provide molecular data for research to improve upon the current prognostic information associated with clinically reported data.
- This study aimed to evaluate Afirma GRID for molecular signatures to preoperatively differentiate American Thyroid Association (ATA) low risk from intermediate/high risk thyroid cancers.

METHODS

- A retrospective cohort study of adult patients presenting to a community otolaryngology practice for evaluation of thyroid nodules between 2017-2025 was conducted.
- A total of 163 patients who had Afirma GSC testing (ordered at the discretion of the clinical provider) had final histopathology reports associated.
  - 119 had Afirma GSC-S result or had BV/VI cytology.
- Surgical pathology stratified by ATA risk of recurrence criteria was correlated with expressed mutations and Afirma GRID signatures.
- Of the 119 cases, 75 samples had risk assigned (cases that were histologically benign or NIFTP did not have ATA risk assigned).

METHODS—*CONT'D.*

- There were 49 ATA low risk cancers, 25 intermediate risk, and 1 high risk.
  - The 1 high risk case was included with the intermediate risk category for data analysis.
- The cohort with ATA risk assigned was evaluated for Afirma XA variant and fusion results.
- Of those patients with ATA risk assigned, 60 had Afirma GRID data available for analysis (Table 2).
- The expression level of GRID signatures relative to the median level of all GSC-S lesions, or those with BV/VI cytology, in the database was used to calculate odds ratios (OR) correlated with ATA low vs intermediate risk thyroid cancer using logistic regression.
  - A higher OR is consistent with ATA intermediate risk cancer.

RESULTS

TABLE 1

Expressed variants associated with ATA risk categories

- Of nodules with BV/VI cytology, 17 were intermediate risk and 23 were low risk.
- **XA results had no association with ATA risk.**
- One lesion with *TERT*p + *BRAF*p.K601E mutations was in an ATA low risk cancer.

		ATA risk		
		Low	Intermediate	High
	Total	**49**	**25**	**1**
Variants	***BRAF*p.V600E**	14 (28%)	7 (28%)	1
	HRAS	3 (6.1%)	2 (8%)	0
	NRAS	5 (10.2%)	1 (4%)	0
	EIF1AX	0	1 (4%)	0
	TSHR	1 (2%)	1 (4%)	0
	***BRAF*p.K601E+*TERT*p.C228T**	1 (2%)	0	0
Fusions	***AGK::BRAF***	1 (2%)	0	0
	CCDC6::RET	0	2 (8%)	0
	ETV6::NTRK3	2 (4.1%)	0	0
	PAX8::PPARG	3 (6.1%)	0	0
	CREB3L2::PPARG	1 (2%)	0	0

RESULTS—*CONT'D.*

TABLE 2

The data is presented as a continuous OR or relative to being above or below the 50th percentile of the signature

A. OR of the evaluated samples relative to the median GSC-S percentile score of GRID signatures

	Continuous		Category (>50% vs ≤50%)	
Signature	**OR**	**p value**	**OR**	**p value**
Angiogenesis hallmark	1.9	0.54	0.9	0.86
Apical junction hallmark	2.3	0.41	1.9	0.31
Apical surface hallmark	0.4	0.39	0.7	0.58
Apoptosis hallmark	2.8	0.34	1.4	0.58
DNA repair hallmark	1	0.96	1.1	0.86
***E2F* targets hallmark**	**6.8**	**0.05**	2.7	0.09
Epithelial mesenchymal transition hallmark	1.1	0.91	0.8	0.71
Estrogen response early hallmark	0.3	0.32	0.7	0.54
Estrogen response late hallmark	0.4	0.35	0.5	0.24
Hedgehog signaling pathway	1.2	0.88	0.9	0.82
Hypoxia hallmark	**0.1**	**0.01**	**0.2**	**0.02**
Inflammatory response hallmark	2.1	0.55	1	0.99
mTOR complex1 signaling hallmark	0.7	0.76	1	0.94
TP53 pathway hallmark	0.3	0.15	**0.3**	**0.05**
Pi3k akt *mtor* signaling hallmark	1.2	0.86	1.6	0.43
TGF beta signaling hallmark	0.9	0.89	1.2	0.71
Interferon gamma response hallmark	4.7	0.25	1.8	0.33
Activated CD4	**17.8**	**0.02**	1.6	0.43
Activated CD8	4.8	0.15	1.6	0.43
Immune content estimation	3.5	0.3	2.5	0.12
Immunomodulators	0.5	0.52	0.6	0.42
M2 to M1 macrophage ratio	0.3	0.39	0.4	0.13
PDL1 signaling	0.6	0.64	1	0.94
T cell exhaustion	0.4	0.42	0.9	0.87
T cell regulatory	0.4	0.37	0.6	0.3
Cancer associated fibroblast	0.6	0.68	0.7	0.55
T cell accumulation	**13.9**	**0.03**	**3.3**	**0.04**
NIS expression	0.8	0.79	0.8	0.65
Hürthle Oncocytic cell index score	0.8	0.89	2.1	0.39
Invasion signature score	0.8	0.82	0.8	0.69
Lymphnode metastasis signature score	6.3	0.12	7.4	0.06

B. OR of the evaluated samples relative to the median BV/VI percentile score of GRID signatures

	Continuous		Category (>50% vs ≤50%)	
Signature	**OR**	**p value**	**OR**	**p value**
Angiogenesis hallmark	3.2	0.25	1.7	0.32
Apical junction hallmark	1.7	0.65	0.7	0.63
Apical surface hallmark	0.6	0.6	1.1	0.84
Apoptosis hallmark	2.5	0.33	1.4	0.53
DNA repair hallmark	0.8	0.84	0.9	0.9
E2F targets hallmark	6.2	0.06	2.7	0.09
Epithelial mesenchymal transition hallmark	2.7	0.34	1.3	0.68
Estrogen response early hallmark	0.3	0.29	0.8	0.67
Estrogen response late hallmark	0.5	0.57	0.8	0.67
Hedgehog signaling pathway	1.3	0.77	1.2	0.78
Hypoxia hallmark	**0**	**0.01**	**0.2**	**0.01**
Inflammatory response hallmark	2.6	0.41	2	0.23
mTOR complex1 signaling hallmark	0.7	0.75	0.6	0.43
TP53 pathway hallmark	0.1	0.08	0	0.99
Pi3k akt *mtor* signaling hallmark	0.6	0.61	0.6	0.43
TGF beta signaling hallmark	0.8	0.86	1.4	0.56
Interferon gamma response hallmark	4.9	0.2	**4.1**	**0.02**
Activated CD4	**22.6**	**0.02**	**6.2**	**0.01**
Activated CD8	4.3	0.21	2.1	0.21
Immune content estimation	3.4	0.31	2	0.26
Immunomodulators	0.5	0.51	0.7	0.56
M2 to M1 macrophage ratio	0.3	0.32	0.6	0.32
PDL1 signaling	1	0.97	2.5	0.2
T cell exhaustion	0.3	0.18	0.4	0.11
T cell regulatory	0.4	0.35	0.6	0.32
Cancer associated fibroblast	1.4	0.77	1	0.98
T cell accumulation	7.1	0.09	1.9	0.38
NIS expression	0.8	0.78	0.8	0.68
Hürthle Oncocytic cell index score	0.7	0.8	1.3	0.73
Invasion signature score	1	0.99	1.3	0.67
Lymphnode metastasis signature score	2.9	0.26	1.6	0.43

ATA intermediate risk thyroid cancers appear to have higher immune activity and lower hypoxia hallmark signature compared to low risk thyroid cancers.

CONCLUSION

- *BRAF*p.V600E and other classically described canonical mutations did not differentiate low from intermediate risk thyroid cancers in this cohort.
- The mRNA expression of immune activation, cell cycle progression, and hypoxia hallmark signatures were significantly different between thyroid cancers with ATA low and intermediate risk categorization.
- If validated for clinical use, GRID signatures may represent novel preoperative prognostic markers for molecularly suspicious ITN or nodules with BV/VI cytology.