ENDO TOPICAL CATEGORY
THYROID BIOLOGY AND DISEASE

MON-380

Development of a Prognostic Cell Cycle Progression Score in Preoperative Thyroid Tumor Samples to Predict Prognosis

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INTRODUCTION

- The American Thyroid Association (ATA) thyroid nodule management guidelines follow a clinicopathological risk stratification system based on risk factors, which subdivides patients into high-risk, intermediate-risk and low-risk cohorts, providing prognostic and predictive information to facilitate clinical decisions.[1] However, many prognostic signatures lack reproducibility due to individual heterogeneity. Given the increasing use of lobectomy in thyroid cancer, as well as even less invasive approaches (e.g. active surveillance) there is a need for more comprehensive pre-operative classifying tools.
- While *BRAF*V600E and *RAS* mutations have an established role in diagnosis of thyroid cancer, their use for prognostication in thyroid cancer is more limited due to tumor heterogeneity and presence of other factors. Secondary mutations have been shown to confer worse prognosis in thyroid cancer, and especially mutations in *TERT* promoter and genes in the *PI3K/AKT/mTOR* pathway.[2,3]
- Alterations in cell cycle regulatory genes may lead to abnormal cellular proliferation, tumor development, and malignancy. Disruptions in the cell cycle can contribute to the development and progression of the disease.
- By leveraging the whole transcriptome derived Afirma Genomic Sequencing Classifier (GSC) thyroid nodule molecular testing platform,[4] a mRNA expression-based signature on genes implicated in cell cycle progression was developed.
- The objective of this study was to characterize cell cycle proliferation genes in thyroid nodules/cancer and assess the molecular and clinical associations of their expression.

METHODS

- A set of 47 genes implicated in cell cycle progression (CCP) (i.e. *TOP2A*, *MKI67*) was identified from The Cancer Genome Atlas (TCGA) Thyroid.
- A CCP activity z-score was derived from the expression of the 47 genes and subsequently associated with genomic alterations and outcome data in TCGA.
- 2,205 fine needle aspiration (FNA) samples with (B)ethesda V/VI cytology sent for Afirma testing were extracted from the Afirma thyroid nodule database.
  - The CCP score was analyzed in reference to *TERT* promoter mutation status and common oncogenic alterations reported by the Afirma Xpression Atlas (XA – the variant and fusion panel), as well as other molecular markers of tumor aggressiveness.
  - Fisher's exact test was used to assess statistical differences.

RESULTS

- TCGA samples were stratified based on the CCP score and then grouped into 4 quartiles (Q4: top 25%, Q3: 50-75%, Q2: 25-50%, Q1: low 25%) (Figure 1).
- Comparing Q4 to Q1, Q4 was enriched with *TERT* promoter mutations (14.4% vs 2.4%, p<0.001), disease progression (20% vs 8%, p=0.001), MACIS (Metastases, Age, Completeness of resection, Invasion, and Size) score > 8 (9.6% vs 3.2%, p=0.06), and stage IV disease (13.6% vs 5.6%, p=0.05). The Q4 group is associated with a shorter time to disease progression (HR: 2.56, 95% CI [1.23-5.3], p=0.01) relative to the Q1 group (Table 1).
- In Afirma B V/VI samples, Q4 CCP score was more enriched with *TERT* promoter mutations (11.2% vs 4.9%, p<0.001) compared to Q1, but less enriched with *BRAF*V600E (40% vs 69%, p<0.001), and *RAS* family variants (1.8% vs 6.4%, p<0.001) (Figure 2).

CONCLUSIONS

- The CCP score was associated with *TERT* promoter mutations and more advanced disease state in the TCGA cohort, and similarly with *TERT* promoter mutations in Afirma B V/VI samples.
- In the pre-operative setting, the CCP score may serve as proxy of tumor aggressiveness, especially in thyroid tumors lacking distinct oncogenic alterations, such as *BRAF*V600E, and with variable histologic phenotype.
- Further studies integrating histopathology and recurrence information are necessary to better characterize the applicability of CCP score in the pre-operative evaluation of thyroid tumors.

References

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FIGURE 1.
Quartiles of CCP scores in TCGA showing differential gene expression and associated histology, mutation, TCGA disease progression, MACIS, and high risk category

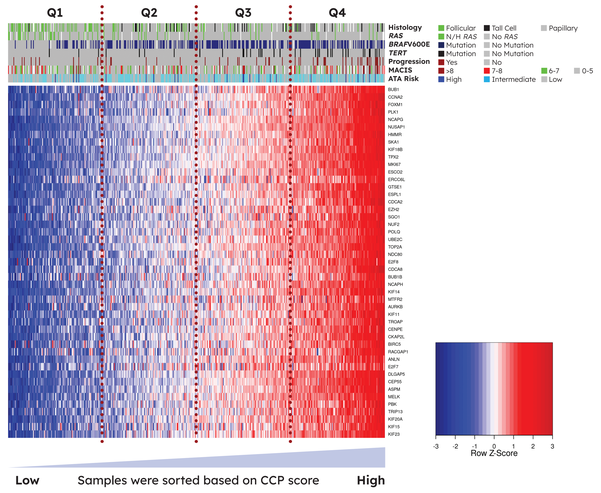

TABLE 1.
Relative proportion of mutations, risk of progression, histology, cancer risk, MACIS score, and disease stage by CCP quartile in TCGA data

	Q1 (n=125)	Q2 (n=124)	Q3 (n=124)	Q4 (n=125)	p-value (Q4 vs Q1)
*BRAF*p.V600E	31 (24.8%)	61 (49.2%)	72 (58.1%)	70 (56%)	**p<0.001**
N/H *RAS*	27 (21.6%)	13 (10.5%)	6 (4.8%)	7 (5.6%)	**p<0.001**
TERT	3 (2.4%)	4 (3.2%)	10 (8.1%)	18 (14.4%)	**p<0.001**
Progression	10 (8%)	6 (4.8%)	10 (8.1%)	25 (20%)	**p=0.001**
Histology					**p<0.001**
Tall cell	1 (0.8%)	11 (8.9%)	9 (7.3%)	13 (10.4%)	
PTC	67 (53.6%)	82 (66.1%)	85 (68.5%)	87 (69.6%)	
FTC	48 (38.4%)	23 (18.5%)	17 (13.7%)	11 (8.8%)	
Risk					**p<0.001**
High	4 (3.2%)	5 (4%)	8 (6.5%)	7 (5.6%)	
Intermediate	51 (40.8%)	69 (55.6%)	61 (49.2%)	75 (60%)	
Low	59 (47.2%)	42 (33.9%)	42 (33.9%)	28 (22.4%)	
MACIS					
MACIS>8	4 (3.2%)	4 (3.2%)	6 (4.8%)	12 (9.6%)	
MACIS7-8	11 (8.8%)	11 (8.9%)	4 (3.2%)	12 (9.6%)	
MACIS6-7	21 (16.8%)	13 (10.5%)	15 (12.1%)	14 (11.2%)	
MACIS<6	75 (60%)	85 (68.5%)	86 (69.4%)	68 (54.4%)	
Disease Stage					**p=0.05**
I	60 (48%)	65 (52%)	74 (60%)	67 (54%)	
II	18 (14.4%)	16 (13%)	12 (10%)	3 (2.5%)	
III	30 (24%)	23 (18.5%)	21 (17%)	27 (21.6%)	
IV	7 (5.6%)	13 (11%)	7 (6%)	17 (13.6%)	

FIGURE 2.
CCP quartiles by genomic alterations in Afirma Bethesda V/VI samples

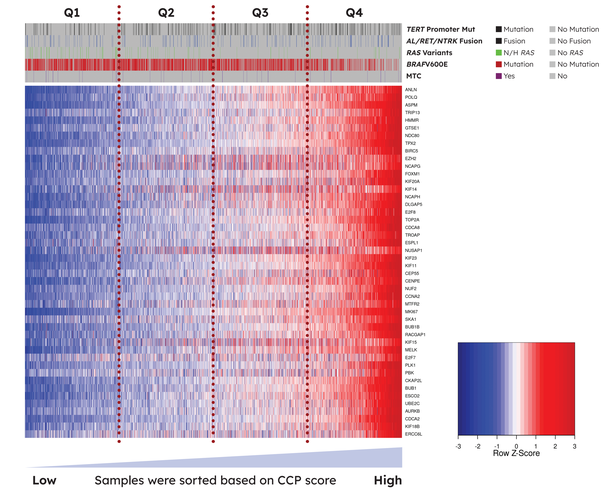

TABLE 2.
Relative proportion of mutations, sex, nodules size, and age by CCP quartile

	Q1	Q2	Q3	Q4	p-value (Q4 vs Q1)
Total	**551**	**552**	**550**	**552**	
Key Genomic Alterations					
TERT+	27 (4.9%)	56 (10.1%)	61 (11.1%)	62 (11.2%)	**p<0.001**
*BRAF*V600E+	382 (69.3%)	393 (71.2%)	350 (63.6%)	223 (40.4%)	**p<0.001**
PTEN/ *PIK3* variants	0 (0%)	0 (0%)	9 (1.6%)	5 (0.9%)	
RAS	35 (6.4%)	21 (3.8%)	8 (1.5%)	10 (1.8%)	**p<0.001**
RET/ALK/NTRK fusions	33 (6%)	37 (6.7%)	29 (5.3%)	40 (7.2%)	
Sex					
Male	156 (28.3%)	163 (29.5%)	133 (24.2%)	155 (28.1%)	
Female	395 (71.7%)	389 (70.5%)	416 (75.6%)	397 (71.9%)	
Nodule Size (cm)					
<1	27 (4.9%)	23 (4.2%)	29 (5.3%)	41 (7.4%)	
1-1.99	288 (52.3%)	257 (46.6%)	255 (46.4%)	277 (50.2%)	
2-2.99	130 (23.6%)	153 (27.7%)	138 (25.1%)	121 (21.9%)	
3-3.99	59 (10.7%)	60 (10.9%)	64 (11.6%)	52 (9.4%)	
≥4	42 (7.6%)	43 (7.8%)	54 (9.8%)	54 (9.8%)	
Age (years)					
<21	13 (2.4%)	20 (3.6%)	23 (4.2%)	19 (3.4%)	
21-39	135 (24.5%)	167 (30.3%)	165 (30%)	156 (28.3%)	
40-59	219 (39.7%)	196 (35.5%)	195 (35.5%)	217 (39.3%)	
60-74	145 (26.3%)	135 (24.5%)	120 (21.8%)	119 (21.6%)	
≥75	39 (7.1%)	34 (6.2%)	47 (8.5%)	41 (7.4%)	