The Genomic Landscape of Thyroid Nodules in Patients ≥75 Years Old

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INTRODUCTION

- Thyroid nodules are increasingly common in older adults; most are benign.[1]
- In patients ≥75 years, malignant nodules often behave more aggressively, making accurate risk stratification essential.
- The Afirma Genomic Sequencing Classifier (GSC) is an exome-enriched RNA-seq assay validated in adults ≥21 years that refines malignancy risk in Bethesda III/IV nodules, potentially avoiding unnecessary surgery.[2]
- Limited data exist on the genomic and transcriptomic landscape of thyroid nodules in patients ≥75 years.
- The goal of this study was to characterize the molecular and transcriptomic profiles of thyroid nodules in patients ≥75 years compared with younger cohorts.

METHODS

- The Afirma GSC database was queried from August 2017 – June 2024.
- Nodules that had adequate genomic material from follicular cells were identified to analyze expressed variants and fusions among Afirma GSC-(S)uspicious and nodules with Bethesda V/VI cytology.
  - Nodules from patients <20 yrs were excluded.
  - *TERT* promoter mutations were analyzed from March 2023 – June 2024.
- A subset of samples with available transcriptomic were analyzed for ERK and EMT signaling expression, the *BRAF-RAS* (BRS) score, and expression of the Human Molecular Signatures Database (MSigDB) hallmarks of cancer pathways.
- Patients were stratified into the following age cohorts:
  - 20-54 yrs
  - 55-74 yrs
  - ≥75 yrs

RESULTS

- 250,200 were included in the analysis. Expressed variants and fusions are reported amongst nodules that were Afirma GSC-(S)uspicious or that had Bethesda V/VI cytology.
  - 8,627 samples with *TERT* promoter mutation testing were analyzed.
  - A subset of 17,000 GSC-S or B V/VI samples with available transcriptomic were analyzed for ERK and EMT signaling expression, the *BRAF-RAS* (BRS) score, and expression of the Human Molecular Signatures Database (MSigDB) hallmarks of cancer pathways.

TABLE 1

Proportion of Bethesda cytology categories by age group

- Patients ≥75 yrs had a higher rate of BIII cytology compared to those 20-54 yrs.
- Patients ≥75 yrs had lower rates of BV/VI cytology compared to those ≤74 yrs.

	Age groups		
	20-54	**55-74**	**≥75**
Total	**99,671**	**116,788**	**32,141**
B-III	75,632 (76%)	94,472 (80.9%)	26,044 (81%)*
B-IV	16,847 (16.9%)	18,155 (15.5%)	5,136 (16%)
B-V	3,416 (3.4%)	2,182 (1.9%)	535 (1.7%)**
B-VI	3,779 (3.8%)	1,978 (1.7%)	426 (1.3%)**

*p <0.01 compared to 20-54 yrs; **p <0.01 compared to ≤74 yrs

- Patients ≥75 years had larger median nodule size (2.4 cm [1.7-3.3]) compared to 55-74 years (2.2 cm [1.6-3.1]) and <54 years (2.2 cm [1.5-3.3]) (p <0.01).

TABLE 2

Afirma GSC-B call rates by age group (a) and sex (b)

(a)

	Age groups		
	20-54	**55-74**	**≥75**
Total	**92,479**	**112,627**	**31,180**
GSC-B	56,015 (60.6%)	83,390 (74%)	23,648 (76%)*

*p <0.01 in ≥75 years compared to younger ages.

(b)

	Age groups (Male)		
	20-54	**55-74**	**≥75**
Total	**15,398**	**28,988**	**9,094**
GSC-B	8,832 (57.4%)	19,584 (67.5%)	6,237 (68.6%)

	Age groups (Female**)		
	20-54	**55-74**	**≥75**
Total	**77,058**	**83,613**	**22,084**
GSC-B	47,169 (61.2%)	63,789 (76.3%)	17,409 (79%)

**p <0.01 in females compared to males.

- Patients ≥75 years had a higher Afirma GSC-(B)enign rate compared to younger age groups (Table 2a).
  - Females had a higher rate of Afirma GSC-B than males in all age groups (Table 2b).

TABLE 3

Proportion of expressed variants and fusions in Afirma GSC-S (a) and BV/VI nodules (b)

Molecular alterations were detected at different rates across age groups in indeterminate nodules (BIII/IV) that are Afirma GSC-S (Table 3a) and BV/VI nodules (Table 3b).

(a)

	Age groups	20-54	55-74	≥75	P values	
	Afirma GSC-S	**36,449**	**29,235**	**7,530**	**20-54 vs ≥75**	**≥75 vs 55-74**
VARIANTS	**Total variant alterations**	**14,861 (40.77%)**	**10,206 (34.91%)**	**2,515 (33.4%)**	<0.0001	0.01
	BRAFp.K601E	462 (1.27%)	285 (0.97%)	55 (0.73%)	0.0001	ns
	BRAFp.V600E	3,172 (8.7%)	1,969 (6.74%)	384 (5.1%)	<0.0001	<0.0001
	HRAS	3,257 (8.94%)	2,513 (8.6%)	607 (8.06%)	0.01	ns
	KRAS	637 (1.75%)	537 (1.84%)	129 (1.71%)	ns	ns
	NRAS	5,790 (15.89%)	3,578 (12.24%)	895 (11.89%)	<0.0001	ns
	DICER1	798 (2.19%)	293 (1%)	60 (0.8%)	<0.0001	ns
	EIF1AX	106 (0.29%)	204 (0.7%)	97 (1.29%)	<0.0001	<0.0001
	SPOP	193 (0.53%)	335 (1.14%)	88 (1.17%)	<0.0001	ns
	JAK2	22 (0.06%)	90 (0.3%)	66 (0.87%)	<0.0001	<0.0001
	PIK3CA	27 (0.07%)	50 (0.17%)	20 (0.26%)	<0.0001	ns
	TP53	47 (0.13%)	60 (0.2%)	27 (0.36%)	<0.0001	0.02
	TSHR	399 (1.09%)	377 (1.3%)	102 (1.35%)	ns	ns
FUSIONS	**Total fusion alterations**	**2,549 (6.99%)**	**1,282 (4.39%)**	**266 (3.53%)**	<0.0001	0.001
	RET	356 (0.98%)	127 (0.43%)	29 (0.39%)	<0.0001	ns
	NTRK3	541 (1.48%)	165 (0.56%)	31 (0.41%)	<0.0001	ns
	NTRK1	56 (0.15%)	29 (0.1%)	8 (0.11%)	ns	ns
	ALK	114 (0.31%)	50 (0.17%)	9 (0.12%)	0.005	ns
TERT	**Total**	**3,066**	**2,538**	**750**		
	TERT+	46 (1.5%)	138 (5.4%)	81 (10.8%)	<0.0001	<0.0001
	TERT+BRAFV600E	5 (0.2%)	32 (1.3%)	17 (2.3%)	<0.0001	0.06

(b)

	Age groups	20-54	55-74	≥75	P values	
	BV/VI nodules	**7,195**	**4,160**	**961**	**20-54 vs ≥75**	**≥75 vs 55-74**
VARIANTS	**Total variant alterations**	**4,934 (68.58%)**	**2,736 (65.77%)**	**599 (62.33%)**	0.0001	0.05
	BRAFp.K601E	4 (0.06%)	9 (0.22%)	2 (0.21%)	ns	nd
	BRAFp.V600E	4,561 (63.39%)	2,426 (58.32%)	509 (52.97%)	<0.0001	0.002
	HRAS	68 (0.95%)	47 (1.13%)	12 (1.25%)	ns	ns
	KRAS	39 (0.54%)	26 (0.63%)	12 (1.25%)	0.01	ns
	NRAS	131 (1.82%)	87 (2.09%)	31 (3.23%)	0.005	0.05
	DICER1	53 (0.74%)	24 (0.58%)	4 (0.42%)	ns	ns
	EIF1AX	7 (0.1%)	9 (0.22%)	8 (0.83%)	<0.0001	0.007
	SPOP	21 (0.29%)	27 (0.65%)	10 (1%)	0.001	ns
	JAK2	3 (0.04%)	4 (0.1%)	2 (0.2%)	ns	ns
	PIK3CA	19 (0.26%)	28 (0.67%)	13 (1.35%)	<0.0001	ns
	TP53	10 (0.14%)	13 (0.31%)	6 (0.6%)	0.005	ns
	TSHR	39 (0.54%)	58 (1.39%)	8 (0.83%)	ns	ns
FUSIONS	**Total fusion alterations**	**716 (9.95%)**	**241 (5.79%)**	**39 (4.06%)**	<0.0001	0.04
	RET	328 (4.56%)	84 (2.02%)	8 (0.83%)	<0.0001	0.02
	NTRK3	151 (2.1%)	33 (0.79%)	3 (0.31%)	<0.0001	ns
	NTRK1	35 (0.49%)	7 (0.17%)	1 (0.1%)	ns	ns
	ALK	29 (0.4%)	7 (0.17%)	1 (0.1%)	ns	ns
TERT	**Total**	**1,275**	**723**	**175**		
	TERT+	45 (3.6%)	107 (14.8%)	55 (31.4%)	<0.0001	<0.0001
	TERT+BRAFV600E	38 (3%)	81 (11.2%)	41 (23.4%)	<0.0001	<0.0001

FIGURE 1

The activity expression of ERK and EMT and the BRS score by age groups

ERK and EMT activity expression is significantly lower with advancing age groups while there is no change in the BRS score (Figure 1).

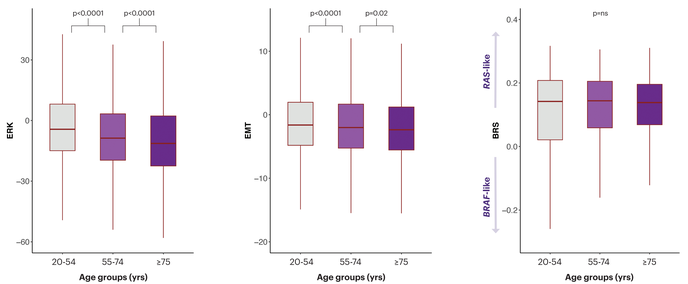
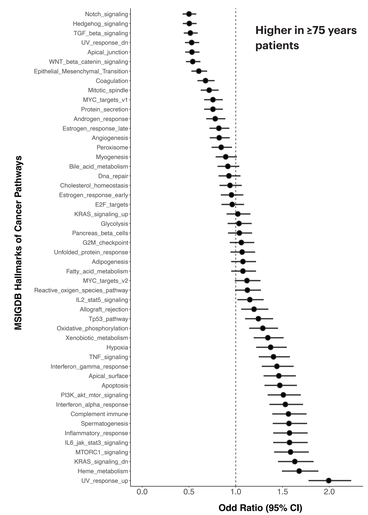


FIGURE 2

MSigDB Hallmarks of Cancer Pathways in patients ≥75 years vs younger patients

Hallmarks where the confidence intervals does not cross the reference line are statistically significant (p <0.05)

The expression of MSigDB hallmarks of cancer pathways are enriched in markers of UV response, *KRAS* signaling, *MTORC1* signaling and immune signaling, amongst others, in those ≥75 years vs younger patients. Hedgehog, TGFβ, and EMT signaling are lower (Figure 2).



CONCLUSION

- Molecularly tested nodules from patients ≥75 years have an overall higher benign call rate and generally favorable variant/fusion profiles.
- In BV/VI nodules, there are higher levels of alterations suggestive of aggressive thyroid cancer (e.g. *TERT*p).
- Differential gene expression analysis suggests nodules in patients ≥75 years have increased inflammatory changes, while younger patients demonstrate more neoplastic pathways.
- Future studies should be done with histopathology detail and longer-term oncologic outcomes to see if improved prognostic features from the above-described expression analysis can personalize treatment for thyroid cancer in older patients.
- These data suggest one can increase confidence in more conservative management in older patients and may decrease the need for unnecessary surgery in this population.

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