Polyamine and Methionine Metabolism Gene Expression Analysis in Thyroid Tumors

Cody Huffman, MS,[1,2] Allan Golding, MD,[3] David Bimston, MD,[3] Mohammed Alshalalfa, PhD,[4] Yangyang Hao, PhD,[4] Joshua P. Klopper, MD,[4] Richard T. Kloos, MD,[4] Ryan S. Jackson, MD,[1,2] Patrik Pipkorn, MD,[1,2] Paul Zolkind, MD,[1,2] Jason Rich, MD,[1,2] Salma Ramadan, MD,[1,2] Wade Thorstad, MD,[1,2] Douglas Adkins, MD,[1,2] Peter Oppelt, MD,[1,2] Nikhil Rammohan, MD, PhD,[1,2] Anthony J. Apicelli, MD, PhD,[1,2] Michael J. Moravan, MD, PhD,[1,2] Guangyong Peng, MD, PhD,[1,2] Sidharth V. Puram, MD, PhD,[1,2] Sina Jasim, MD, MPH,[1] R. Alex Harbison, MD, MS[1,2]

1. Washington University in St Louis, Saint Louis, MO, USA. **2.** The Robert Ebert and Greg Stubblefield Head and Neck Tumor Center, Siteman Cancer Center, St. Louis MO, USA. **3.** Memorial Healthcare System, Hollywood, FL, USA. **4.** Veracyte, Inc, South San Francisco, CA, USA.

INTRODUCTION

- Polyamines are small organic cations that are essential for normal cell growth and development.[1]
- Polyamine levels are elevated in thyroid cancers and highest in poor prognostic subtypes including poorly differentiated and anaplastic thyroid cancers. Methionine, a methyl donor and nutrient important for nucleic acid synthesis, is an essential precursor for polyamine synthesis.
- We hypothesized that polyamine-methionine cycle gene expression may be directly correlated with thyroid tumor cytologic, molecular, and clinical risk (Figure 1).[2]

METHODS

- The Afirma Genomic Sequencing Classifier (GSC) exome-enriched RNAseq database was analyzed for polyamine and methionine metabolism gene expression from cytologically indeterminate ((B)ethesda III/IV - ITN) molecularly benign (GSC-B) (n=30,259), molecularly suspicious (GSC-S) (n=15,815), and malignant (BV/VI, n=1,621) fine needle biopsy specimens (Table 1).
- Patient samples with Afirma testing from an integrated interventional thyroid practice (n=464) were analyzed to assess the correlation between polyamine and methionine gene expression and histopathologic features including vascular invasion and lymph node metastasis (Table 1).

RESULTS

- Among malignant nodules, BVI and *BRAF*p.V600E-mutant (*BRAF*+) nodules exhibited high expression of genes involved in methionine salvage (*MTAP*), methyl group transfer (*PRMT5, NTMT1*) and folate metabolism (*MTHFR*). In contrast, high expression of polyamine catabolism genes (*SMOX, OAZ2, OAZ1*) and arginase expression (*ARG1*) were associated with a lower likelihood of *BRAF*+ or BVI cytopathology (p<0.001 for all) (Figure 2).
- High *MTAP* expression was directly associated with a higher likelihood of BV/VI cytology relative to ITN GSC-S, independent of *BRAF* status (p<0.001). *BRAF*+ samples had the highest expressed levels of *MTAP* (Figure 3) (p<0.001).
- Relative to GSC-B samples, *MTAP* expression varies relative to different genomic alterations in ITN GSC-S and BV/VI nodules. Relative to *RAS* family mutations, *BRAF*p.V600E and *PPARg* fusions have higher expression levels (Figure 4).
- High *MTAP* gene expression is associated with a higher likelihood of clinically significant lymph node metastases (Figure 5).

FIGURE 1
Polyamine metabolism

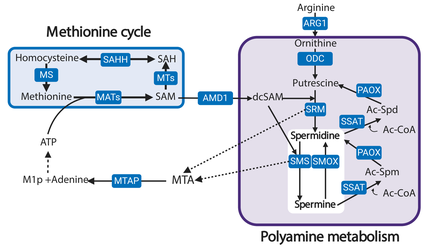


TABLE 1
Afirma database (CLIA) and Memorial Health cohorts

	CLIA	MH
Total	**47,695**	**464**
Age (median [IQR])	54 [40-66]	51 [39-60]
Bethesda		
III	37,133 (77.8%)	162 (34.9%)
IV	8,941 (18.7%)	90 (19.4%)
V	799 (1.7%)	51 (11%)
VI	822 (1.7%)	161 (34.7%)
GSC*		
Benign	30,259 (65.7%)	0
Suspicious	15,815 (34.3%)	251 (100%)
Outcome		
Vascular Invasion (inv)	NA	31 (6.7%)
LN metastasis (LNM)	NA	64 (13.8%)
inv or LNM	NA	83 (17.9%)

* within Bethesda III/IV

FIGURE 2
Odd ratios of genes to predict clinical and molecular endpoints in Bethesda V/VI subset

Genes to the right of the vertical line are positively associated with BVI or *BRAF*p.V600E and those to the left of the vertical line are negatively associated.

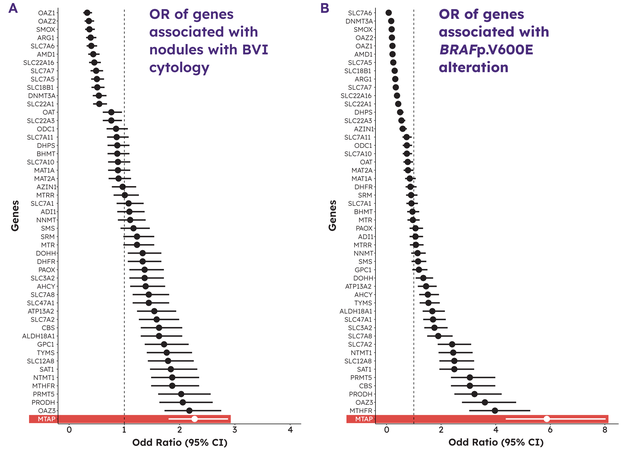


FIGURE 3
***MTAP* expression across cytology and *BRAF*p.V600E categories**

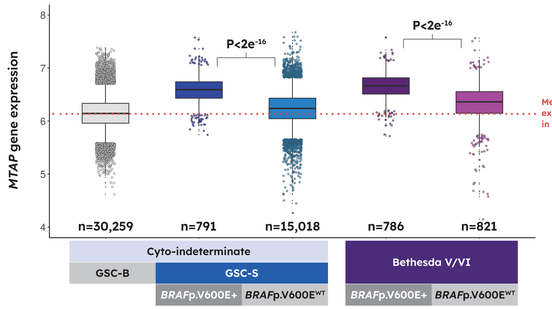


FIGURE 4
***MTAP* expression across genomic alterations in ITN GSC-S and BV/VI thyroid nodules**

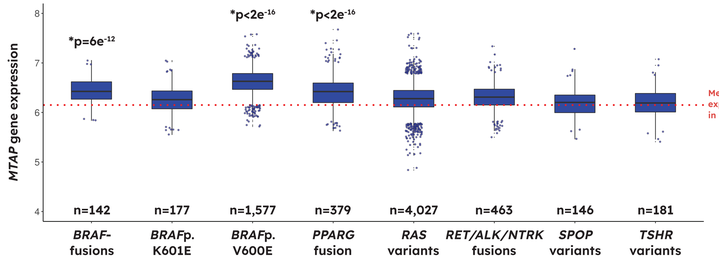
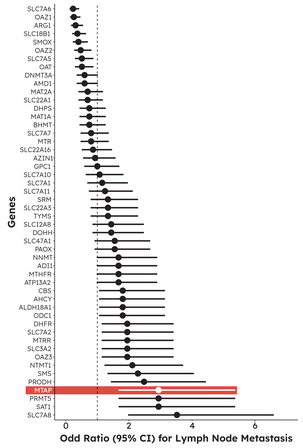


FIGURE 5
Association of genes with regional lymph node metastases

Genes to the right of the vertical line are positively associated with clinically relevant lymph node metastases and those to the left of the vertical line are negatively associated.



CONCLUSION

- Higher expression of methionine salvage pathway genes is associated with greater cytologic risk and clinically aggressive behavior whereas polyamine catabolism gene expression confers less risk.
- Future work seeks to leverage these data to further risk stratify those patients with ITN and molecularly suspicious tumors who may be more likely to present with clinically aggressive thyroid cancer warranting closer surveillance.

REFERENCES

1. Nowotarski SL, Woster PM, Casero RA Jr. Polyamines and cancer: implications for chemotherapy and chemoprevention Expert Rev Mol Med. 2013 Feb 22;15:e3. doi: 10.1017/erm.2013.3. PMID: 23432971.
2. Created in BioRender. Harbison, R. (2024) BioRender.com/u08f754.