The Expression of Cell-Surface Targets as Potential Prognostic and Therapeutic Markers in Thyroid Tumors

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INTRODUCTION

Cell surface targets (CSTs) are increasingly important in oncology, with the evolution of cancer immunotherapies. Recognising cell surface targets that are specific to malignant cells has had significant impact in haematological malignancies and has expanded more recently to solid malignancies. One limiting factor in the application of these novel therapies is the lack of suitable targets, in part because these have not all been identified. While there is a well-developed pathway of surgery and radioactive iodine for differentiated thyroid cancer, the challenge of radioiodine refractory disease persists. Characterizing CSTs in thyroid cancer (TC) is currently understudied. We aimed to evaluate the baseline expression of CSTs in molecularly benign thyroid nodules, to compare this to thyroid cancer, and to identify targets that are associated with potentially aggressive forms of TC. These would have potential to be used in prognostication and as a therapeutic target.

METHODS

- The Afirma Genomic Sequencing Classifer (GSC) utilizes exome-enriched next generation RNA sequencing (RNAseq) as the platform to develop classifiers and report on expressed variants and fusions for thyroid nodule clinical management.[1]
- The expression level of 78 CSTs was characterized across thyroid nodules with Bethesda III-VI cytology sent for Afirma Genomic Sequencing Classifier (GSC) molecular testing. The testing was requested at the discretion of the ordering clinician.
- CSTs that have FDA approved drugs to target them are denoted in red.
- We evaluated the "baseline" expression of CSTs in the Bethesda III/IV-GSC-(B)enign nodules (n=30,259).
- We then evaluated their expression of these CSTs in Bethesda III/IV GSC-(S)uspicious (n=15,815) nodules relative to the expression in GSC-B nodules.
- Finally, we evaluated the expression of these CSTs in Bethesda V/VI (n=1,621) nodules relative to the expression in GSC-S nodules.
- Within Bethesda V/VI samples with *TERT* promoter (*TERT*p) mutation profiling ordered (n=2,240), we evaluated the association of CST expression with *BRAF*p.V600E+ (n=1,348), *TERT*p+ (n=206) and *TERT*p+*BRAF*p.V600E+ (n=160).
- To associate the CST expression with histopathology outcomes, we evaluated a cohort of Afirma GSC tested nodules that had surgery and final histopathology available (n=463).
  - The primary outcome of interest was loco-regional lymph node metastases.
- Logistic regression was used to calculate odd ratios (OR) to associate expression (top 25% vs others) with adverse features.
- Pathology reports were collected under WCG IRB DHF 005-077.

RESULTS

FIGURE 1

The baseline expression of CSTs in Afirma GSC-B samples.

- *FN1, CD46, ADAM9, EPCAM, ERBB2, ALCAM* are highly expressed in these nodules.
- Most FDA approved targets have low expression (*TACSTD2*: TROP2, *PVRL4*: NECTIN4, *DLL3*, *FOLH1*: PSMA, *SSTR2*)

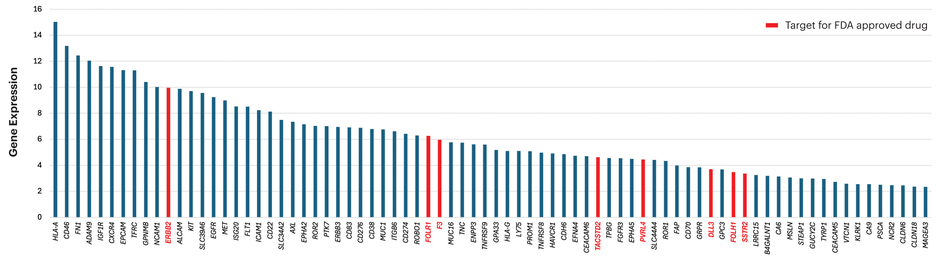
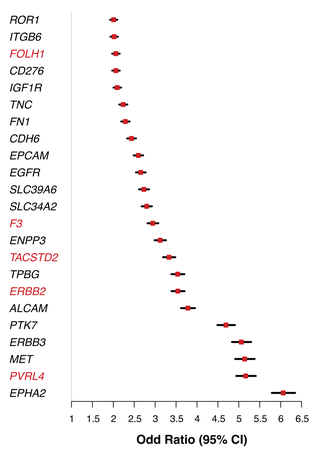


FIGURE 2

A. The odds ratio of CSTs associated with Afirma GSC-S samples relative to the GSC-B cohort (OR>2, p<0.001 for all).

23 genes were strongly associated with GSC-S compared to GSC-B.



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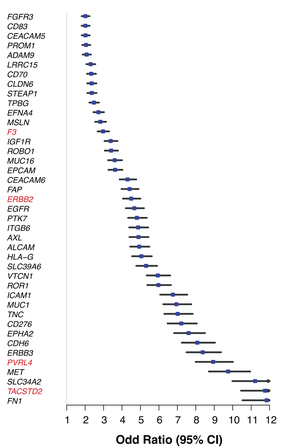
B. The odds ratio of CSTs associated with thyroid nodules with BV/VI cytology relative to the GSC-S cohort (OR>2, p<0.001 for all) .

41 genes had OR>2, with *FN1* having the highest OR.

- *TACSTD2* (TROP2), a tumor associated calcium signal transducer 2, that is a target for antibody-drug conjugates that has FDA approval (Sacituzumab govitecan) in triple negative breast cancer.
- *PVRL4* (NECTIN4), has an antibody-drug conjugate (ADC) that has been FDA approved for urothelial carcinoma (Enfortumab vedotin).



CSTs that have FDA approved drugs to target them are denoted in red.

RESULTS—*CONT'D.*

TABLE 1

The odds ratio of CSTs associated with thyroid nodules with BV/VI cytology with *TERT*p testing ordered.

- Samples that are *BRAF*p.V600E +.
  - *TACSTD2, PVRL4, ERBB2* are CSTs with FDA approved targeted drugs and high OR in this cohort.
- Samples that are *TERT*p+ or *TERT*p +*BRAF*p.V600E+.
  - *FN1* has the highest association with these adverse molecular findings.

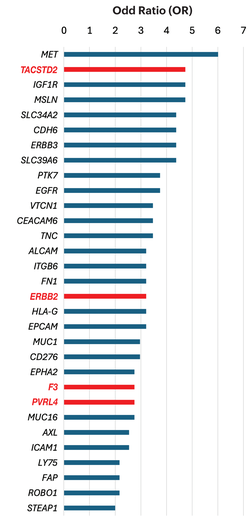
Gene	*BRAF*p.V600E+	*TERT*p+	*TERT*p+*BRAF*p.V600E+
SLC34A2	**46.2**	**3.1**	**5.4**
TACSTD2	**39.7**	1.4	**2.1**
ERBB3	**22.8**	**2.3**	**3.7**
MET	**20.1**	**2.7**	**4.5**
PVRL4	**16.7**	**2.5**	**3.9**
FN1	**15.1**	**3.9**	**5.9**
IGF1R	**10.8**	0.8	1
EPHA2	**8.2**	1.2	1.6
MUC1	**7.8**	**3.3**	**4.9**
HLA-G	**6.7**	0.9	1.1
VTCN1	**6.1**	1.2	1.7
ERBB2	**5.2**	1.1	1.4
ALCAM	**4.3**	1.4	1.7
ITGB6	**4.2**	1.9	**2.4**
MSLN	**4.2**	1	1.2
FGFR3	**3.8**	1	1.3
EGFR	**3.7**	1.1	1.4
ROR1	**3.5**	1.6	1.8
CDH6	**3.3**	1	1.2
FAP	**3.2**	**2.8**	**2.7**
TNC	**3**	**2.6**	**2.6**
LRRC15	1.7	**2.5**	**2.5**
TPBG	1.2	**2.3**	**2.1**
SLC39A6	**2.8**	**2.1**	**2.6**
FOLR1	2	1.7	**2.3**
FOLH1	1.2	1.9	1.7

CSTs that have FDA approved drugs to target them are denoted in red.

RESULTS—*CONT'D.*

FIGURE 3

When evaluating the association of CSTs with lymph node metastases, *MET*, TROP2, *SLC34A2* and *FN1* were significantly associated with lateral disease, >2mm central node deposits, or a high lymph node ratio (≥40%) (OR>2, p<0.001).



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CONCLUSION

- TROP2 and NECTIN4 are targets for FDA approved antibody-drug conjugates in triple negative breast cancer and urothelial carcinoma, respectively. These were more highly expressed in Afirma GSC-S nodules compared to GSC-B.
- These and other expressed CSTs may represent novel prognostic markers in lieu of, or associated with, classic canonical driver mutations in aggressive thyroid carcinoma.
- Additionally, these CSTs may represent novel molecular targets for new therapies in thyroid carcinomas that do not express a targetable mutation, or which develop resistance to currently approved targeted therapies.
- As further immunotherapies arise, expanded knowledge of the cancer cell surface proteome is critical for applying new therapies in TC.

REFERENCE

1. Walsh PS, Hao Y, Ding J, Qu J, Wilde J, Jiang R, Kloos RT, Huang J, Kennedy GC. Maximizing Small Biopsy Patient Samples: Unified RNA-Seq Platform Assessment of over 120,000 Patient Biopsies. J Pers Med. 2022 Dec 22;13(1):24. doi: 10.3390/jpm13010024. PMID: 36675685