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RESEARCH

Thyroid differentiation profile for differentiated thyroid cancer

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Abstract

Radioactive iodine (RAI) treatment is an established therapeutic tool for 'differentiated thyroid cancers'. The therapeutic effectiveness is linked to the preservation of the iodine-concentrating ability of the neoplastic tissue, a unique, inherent quality of a normal thyroid gland. Iodine concentration is a function involving the expression of transport proteins and organification. Thyroid differentiation score (TDS) is an integrated quantity, first introduced by The Cancer Genome Atlas (TCGA), conveying the relative expression of proteins involved in histogenesis, morphologic and functional differentiation of thyroid tissue. The concept is well described for the expression of metabolic suppression of thyroid cancers associated with RAI-refractoriness. We evaluated the mRNA expressions of thyroid metabolomics-specific genes, comparing normal thyroid to neoplastic tissue in a cohort where patient-specific paired data were available. Fifty-nine papillary thyroid cancer samples from the TCGA project with matched tumor-normal tissue samples were analyzed. Of the 59 samples, 29 contained a $BRAF^{V600E}$ mutation, seven a RAS mutation, 10 a mutation other than BRAF or RAS, and 14 either no mutation or an unknown mutation. Our analysis demonstrated that there was a significant downregulation of the RAI theranostic transcriptome, much more significant in BRAF-initiated cancers vs RAS-initiated ones. There was also notable heterogeneity in respective mutational categories where individual assessment of the thyroid differentiation profile (TDP) would potentially be clinically relevant for RAI treatment planning. Determination of TDP and development of a theranostic TDS may have an impact on clinical decision making as to the extent of thyroidectomy and postoperative RAI therapy.

Keywords: radioactive iodine; thyroid cancer; papillary thyroid cancer; differentiated thyroid cancer; thyroid differentiation score; MAPK pathway; BRAF; RAS; ERK; thyrosine kinase; TKI; redifferentiation; RAI refractory

Introduction

Thyroid iodine physiology and its exploitation for metabolic/molecular radiotherapy is the earliest clinical application of the concept of 'theranostics'. The idea was developed in 1936 (Ehrhardt *et al.* 2020). It was first and most successfully applied for the treatment of Graves' disease. It is still considered a clinical standard for the treatment of this condition. Its use in thyroid cancer was first implemented in 1946 (Seidlin *et al.* 1946) and has been in use with variable success rate but with momentous controversy. The term that best defines the current state of radioactive iodine (RAI) use in thyroid cancer is '*equipoise*'. The root cause of this equipoise lies in the heterogeneities in (within and among) different genomic, transcriptomic and proteomic expression

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patterns of key molecules of iodine physiology in thyroid cancer. In general, most of the key molecule expressions are suppressed/depressed as part of oncopathophysiology, and this shows high degree of variability among individual cancer cases. A constitutive activation of the MAPK (mitogen-activated protein kinase) signaling pathway, and also the less vigorously studied PI3K signaling pathway, leads to the downregulation of thyroid function genes. As a result, cells undergo functional dedifferentiation and lose their uptake and processing capability for iodine.

In 2014, The Cancer Genome Atlas (TCGA) Research Network published results of a comprehensive molecular multi-platform analysis of 496 papillary thyroid cancer (PTC) patients (Cancer Genome Atlas Research Network 2014). To date, it has been cited more than 2000 times. Since its publication, more samples have been added to the database. The main objective of the study was to define cancer molecular subtypes including the cadences of intracellular signaling and correlate oncopathophysiology with cancer morphology, differentiation state and clinical risk assessment. PTC is the most common morphologic subtype of thyroid cancers of follicular cell origin. The two most common and mutually exclusive driver mutations are BRAF and RAS. Other mutations, in terms of their transcriptomic and molecular outputs, are positioned in between these two categorical polarities and are referred to as BRAF-like and RAS-like. $BRAF^{V600E}$ mutation is the most common driver mutation for PTC, comprising 60% of all PTCs (Cancer Genome Atlas Research Network 2014). RAS mutations occur in about 13% of PTC cases and typically preserve some follicular structural differentiation. Follicular thyroid cancers (FTCs) typically are in the RAS-like category. Gene expression profiles among patients with $BRAF^{V600E}$ mutations are highly variable, possibly accounting for the wide range of differentiation observed, and may explain the uncertainty of the prognostic, predictive and theranostic power of the $BRAF^{V600E}$ mutation alone (Chakravarty *et al.* 2011, Cancer Genome Atlas Research Network 2014, Gulec *et al.* 2021). Papillary differentiation is associated with functional misdifferentiation which diminishes the theranostic power of RAI. The BRAF mutation ignites constitutive activation of the MAPK signal transduction and augments ERK output. ERK, through complex and intertwined transcription factor interactions, downregulates the expression of genes involved in iodine metabolism (Chakravarty *et al.* 2011, Boucai *et al.* 2024). Differentiated thyroid cancer refers to/encompasses thyroid cancers arising from thyroid follicular epithelial cells, excluding poorly differentiated and undifferentiated (anaplastic) varieties. PTC, FTC and oncocytic thyroid cancer are considered differentiated. The term strictly implies a distinct morphologic differentiation. The iodine metabolic functionality of this group is highly variable. The metabolic function of these cancers is determined by their genomic and transcriptomic expressions. RAS-mutation-initiated cancers (follicular cancers and follicular variant of PTC) have diminished capacity for iodine processing. BRAF-mutation-initiated cancers (classic and tall cell variant PTC) have significantly depressed iodine metabolic function. Oncocytic cancers have completely different genomic constructs and have absent iodine-processing capacity. The term 'misdifferentiated' refers to thyroid cancers with distinct morphologic differentiation, but functionally showing significant deviance in their iodine processing capacity, in the most part, missing the ability of full metabolic functionality (Gulec *et al.* 2024).

The term 'thyroid differentiation score (TDS)' was first used in the 2014 TCGA Research Network article (Cancer Genome Atlas Research Network 2014). The original (TCGA) TDS is a measure of the functional differentiation of thyroid cancer in general. TDS was defined as the mean of log2 fold change (FC) across 16 selected genes. The 16-gene TDS correlated with higher tumor grade and risk for recurrence. There is a need for defining a thyroid differentiation panel/score that has a theranostic value in terms of responsiveness to RAI therapy. The specific transcriptome that needs to be interrogated/investigated is that of gene expressions of iodine metabolism (Table 1).

We postulate that by using the patient as their own control, data variability is reduced and the credibility of interpretation of results when comparing normal to tumor tissues is increased. The goal of this study is to investigate a thyroid differentiation profile (TDP) derived from cancer tissue as well as the patient's normal thyroid tissue and to determine the level of depression in functional differentiation contributing to the theranostic power of RAI.

Materials and methods

A retrospective analysis of data from TCGA was performed. In our study, we compared the expressions of thyroid differentiation genes for iodine metabolism (the theranostic transcriptome) in cancer tissue vs normal tissue. The 'theranostic transcriptome' refers to the use of an mRNA expression level profile to diagnose the extent of disease and drive therapy decisions. Our study specifically interrogated 59 patients with PTC and their normal thyroid tissue samples for RAI theranostics transcriptome. Of the 59 normal-tumor paired samples in the TCGA project database, a $BRAF^{V600E}$ (n = 28), RAS (n = 7) or other mutations (n = 10) were identified in 45 samples. Either no mutation or an unknown mutation was found in 14 samples. The histological variant was available for 52 of the 59 samples. Forty-two samples were classified as 'classical/usual', seven as 'follicular (>=99% follicular patterned)' and three as 'tall cell (>=50% tall cell features)'. There was no difference among the groups based on

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Table 1 Name and function of the 19 genes evaluated in this study. The original TCGA list contained 16 genes; this study evaluated an additional three genes (IYD, SLC16A2 and SLC26A7).

Symbol	Name	Functions
DIO1	Deiodinase, iodothyronine, type I	Activates thyroid hormone by converting the prohormone thyroxine (T4) by ORD to bioactive 3,3′,5-triiodothyronine (T3)
DIO2	Deiodinase, iodothyronine, type II	Activates thyroid hormone by converting the prohormone thyroxine (T4) by ORD to bioactive 3,3′,5-triiodothyronine (T3)
DUX01	Dual oxidase 1	Involved in synthesis of thyroid hormone
DUX02	Dual oxidase 2	Involved in synthesis of thyroid hormone
FOXE1	Forkhead box El	Thyroid transcription factor that likely plays a crucial role in thyroid morphogenesis
GLIS3	GLIS family zinc finger 3	Functions as both a repressor and an activator of transcription and is specifically involved in the development of pancreatic beta cells, the thyroid, eyes, liver and kidneys
IYD	Iodotyrosine deiodinase	Encodes an enzyme that catalyzes the oxidative NADPH-dependent deiodination of mono- and diiodotyrosine, which are the halogenated byproducts of thyroid hormone production
NKX2-1	NK2 homeobox l	Thyroid-specific transcription factor that binds to the thyroglobulin promoter and regulates the expression of thyroid specific genes
PAX8	Paired box 8	Transcription factor involved in thyroid follicular cell development and expression of thyroid specific genes
SLC16A2	Solute carrier family 16 member 2	Encoded protein facilitates the cellular importation of thyroxine (T4), triiodothyronine (T3), reverse triiodothyronine (rT3) and diidothyronine (T2)
SLC26A4	Solute carrier family 26 (anion exchanger), member 4	Iodide transmembrane transporter activity
SLC26A7	Solute carrier family 26 (anion exchanger), member 7	Member of a family of sulfate/anion transporter genes
SLC5A5 (NIS)	Solute carrier family 5 (sodium/iodide cotransporter), member 5	Sodium:iodide symporter activity, responsible for the uptake of iodine in the thyroid
SLC5A8	Solute carrier family 5 (sodium/ monocarboxylate cotransporter), member 8	Transport iodide by a passive mechanism
TG	Thyroglobulin	Substrate for the synthesis of thyroxine and triiodothyronine as well as the storage of the inactive forms of thyroid hormone and iodine
THRA	Thyroid hormone receptor, alpha	Nuclear hormone receptor for triiodothyronine
THRB	Thyroid hormone receptor, beta	Nuclear hormone receptor for triiodothyronine
TPO	Thyroid peroxidase	Iodination of tyrosine residues in thyroglobulin and phenoxy-ester formation between pairs of iodinated tyrosines to generate the thyroid hormones, thyroxine and triiodothyronine
TSHR	Thyroid-stimulating hormone receptor	Receptor for thyrotropin and a major controller of thyroid cell metabolism

TCGA, The Cancer Genome Atlas; ORD, outer ring deiodination.

histological variant, likely due to the small sample size and the few samples assigned to two of the three types. We generated a heat map that illustrates the down regulation of the RAI theranostic transcriptome in respective mutational categories compared to the normal thyroid tissue theranostic transcriptome. We calculated the FC between normal and neoplastic tissue RAI theranostic gene expressions. We then generated box plots for individual gene expressions comparing mutational categories. Expression values are RSEM transcripts per million (TPM) normalized counts (Li & Dewey, 2011) transformed by taking log2 (x + 1), where x is the RSEM count.

Results

The tumor tissue had significantly lower average expression of genes of iodine metabolism with a wider variation (Fig. 1). This wide degree of variability is likely to be associated with variable ERK output, the modulation/auto-regulation of which involves complex mechanisms. Some of the tumors had higher TDS, plausibly better preservation of iodine metabolism, whereas others with low TDS were expected to be RAI-indifferent. Figure 1A shows the TDS (as defined by TCGA) calculated for both normal and tumor tissue. Figure 1B shows the average log2 expression across the 16 TCGA TDS genes for normal and tumor tissue. The TDS defined in prior studies only included tumor specimens, thus there was no gauge of the magnitude of gene suppression in comparison with an individual patient's normal thyroid tissue. The differences between normal tissue and cancer tissue were expressed as FC. In this study, we looked at the FC of each theranostic gene expression (log2 TPM) in each patient. The heat map illustrates the downregulation of the RAI theranostic transcriptome in respective mutational categories

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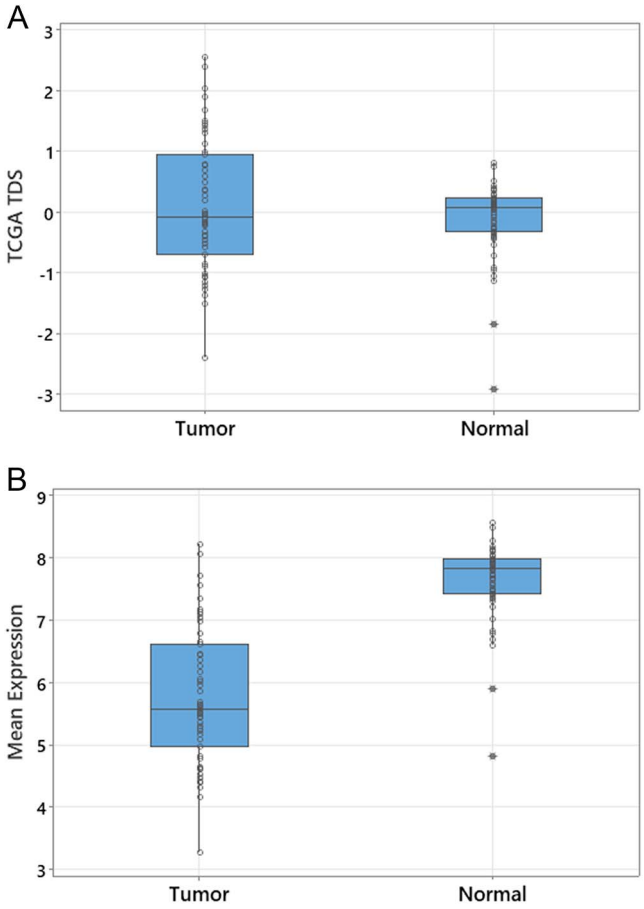

Figure 1

(A) Box plots of the TCGA TDS (median-centered) of the 16 TDS genes comparing normal to tumor tissue. (B) Box plots of the average log2-transformed mRNA expression of the 16 TDS genes comparing normal to tumor tissue. The data point is represented by a circle. The circle with the asterisk represents an outlier, defined as at least 1.5× the interquartile range (Q3–Q1) from the edge of the box. $P < 10–15$ by paired *t*-test.

compared to normal thyroid tissue (Fig. 2). The mean normal expression of different genes has different values and variances (Fig. 3).

Discussion

Molecular theranostics represents a new paradigm in thyroid cancer diagnosis and treatment. Genomics and molecular pathology relate to the true biologic nature of the different cancer types, which have been more traditionally defined exclusively by morphology. Molecular imaging, on the other hand, remains to be reflective of the oncophysiology of cancers. The tumor differentiation and dedifferentiation, as well as the biological behavior of an individual cancer, can be defined best by carefully investigated molecular markers. The RNA-Seq data from TCGA project for PTCs identified distinct molecular subtypes according to their cell signaling and state of differentiation. The term 'TDS' was first used in the 2014 TCGA Research Network article (Cancer Genome Atlas Research Network 2014). The original (TCGA) TDS is a measure of functional differentiation of thyroid cancer in general. TDS provides a continuous number (centered at zero) derived from the average of the log2 transformed FC of the mRNA expression level of 16 thyroid metabolism and function genes. The change in expression level of each gene in a particular tumor is relative to the median expression level (median-centered) of that gene across all of the available tumor samples in the cohort. Therefore, the TDS of TCGA is a comparison of the tumor differentiation in a patient against the tumor differentiation within a specific patient cohort and not against normal thyroid tissue. In fact, TDS calculated from a normal cohort would, by the same method, also be centered around zero and therefore, strictly speaking, a comparison of tumor TDS against normal thyroid tissue cannot be made. Since the tumor and normal expressions calculated by the original TDS method are both centered at the median, they both range, at least theoretically, from negative to positive infinity. Since the TDS value depends on the distribution of the mRNA expression of the 16 genes in the tumors within the specific cohort, it does not possess theranostic power for an individual patient.

The TCGA group associated the expression of the 16 genes of thyroid differentiation with BRAF-RAS score. The findings showed RAS and $BRAF^{V600E}$ mutations to have different genomic, transcriptomic and phenomic characteristics, which also highly correlated with TDS. The RAS-like PTCs have relatively high TDS values. The $BRAF^{V600E}$ PTCs, however, have a wider range of TDS values, with majority being on the lower end. As many as 20% of the $BRAF^{V600E}$ mutated tumors present a relatively normal level of differentiation with preserved RAI avidity (Sabra *et al.* 2013, Cancer Genome Atlas Research Network 2014, Liu *et al.* 2020, Boucai *et al.* 2022, Mu *et al.* 2024). So, for at least a subset of PTC patients, the $BRAF^{V600E}$ mutation and BRAF-RAS score may not be sufficient to rule out the potential efficacy of RAI therapy. In a study of 124 PTC patients, $BRAF^{V600E}$ mutation did not impact the prediction of patient outcomes (Scheffel *et al.* 2020). In that cohort, 32.8% carried the $BRAF^{V600E}$ mutation. Notably, nearly the same percentage of patients with the mutation received RAI therapy as those without the mutation.

A potential utility of TDS as a predictor for I-131 avidity in a clinical trial of the MAPK pathway inhibitor vemurafenib for potential redifferentiation in RAI-refractory and BRAF mutant thyroid cancer patients was examined by Dunn *et al.* (2019). The group expanded the original 16-gene set to include 29 genes positively (eTDS (+)) and 19 genes negatively correlated with TDS in the $BRAFV^{600E}$ cohort. The study recruited 12 differentiated thyroid cancer patients to receive I-124 imaging to predict I-131 avidity. Eight patients with low

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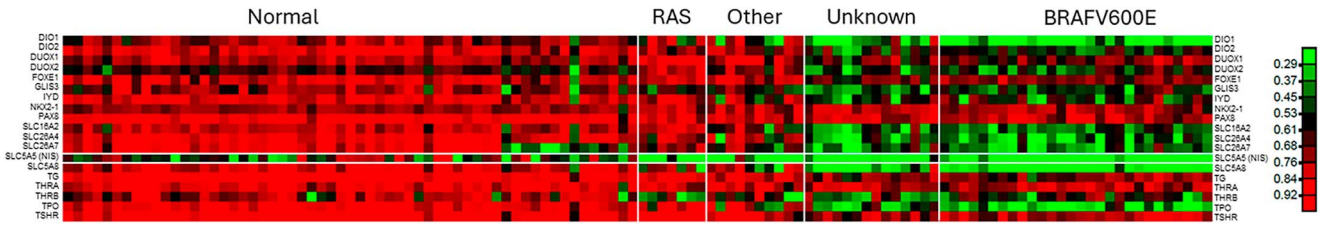

Figure 2

Heatmap of the normalized log2 (TPM+1) expression of each gene from normal tissue and from tumor tissue from each of the four mutation status groups. The NIS gene (SLC5A5) is outlined in a white box.

tumor I-124 tumor uptake received vemurafenib for 4 weeks, followed by a 2nd I-124 scan to check for tumor redifferentiation. In three of the patients, biopsies were performed before and near the end of vemurafenib treatment, and eTDS values increased in all three tumors, with the patients experiencing the highest eTDS change also exhibiting the highest change in tumor I-124 update SUV following vemurafenib treatment compared to before treatment. Thus, at least in this small study, the degree of differentiation score (eTDS) and RAI avidity was supported.

Taking advantage of the paired (tumor and normal tissue) data available from 59 patients in the TCGA database, comparisons were made for RNA-Seq expression of genes in the 16 genes in the TCGA TDS and SLC16A2, IYD and SLC26A7. FC tumor relative to normal tissue mRNA expression levels were compared among the genes (H_1, FC < 0) using a Wilcoxon signed-rank test for paired samples at α = 0.05. Rather than calculate TDS as formally defined, which will naturally center both groups around zero, the log2 transform of normalized mRNA expression levels were subtracted {log2 (tumor) – log2 (normal) or equivalently log2 (tumor/normal)} for each gene. Figure 1A compares the TCGA TDS calculated from the tumors and normal tissue in the cohort of the 59 patients in which biopsies from both were obtained. As is obvious, the TDS of both tumor and normal are centered around zero, with the tumors having a much greater spread in mRNA expression of the 16 genes. Figure 1B presents the comparison between the tumor and normal tissues based on the FC (tumor relative to normal). Clearly, the difference that is lost when represented by

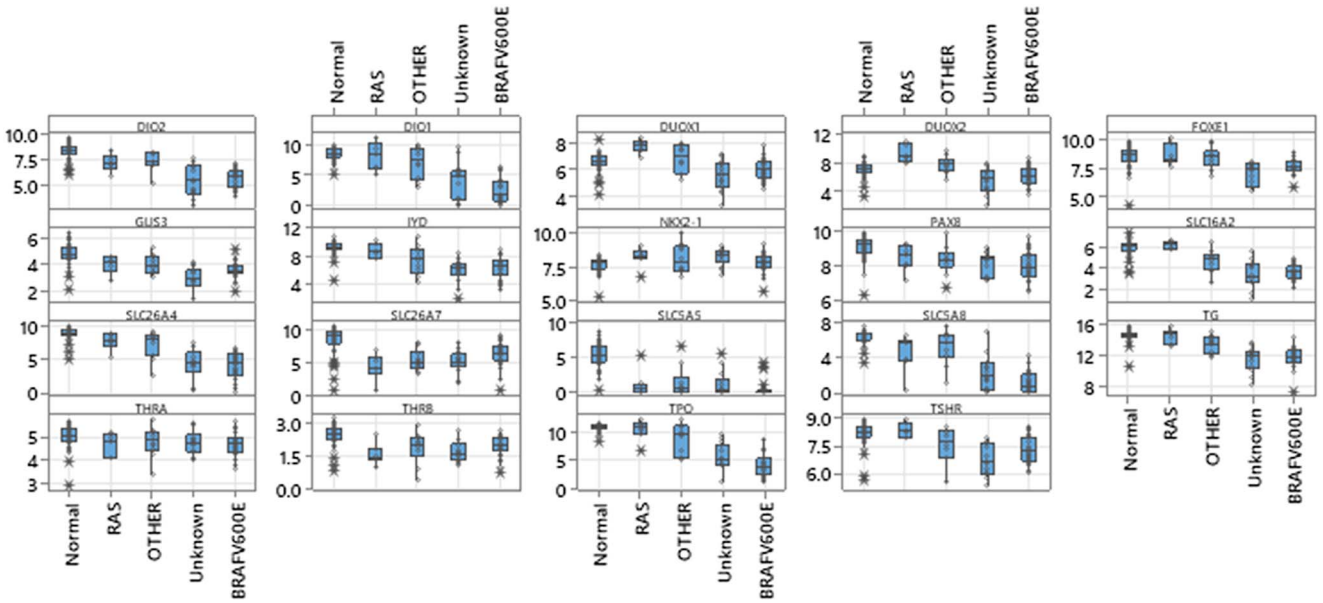

Figure 3

Box plots of gene expression in log2 (TPM+1). Normal tissue (n = 59), tumor tissue with RAS mutation (n = 7), other mutations (n = 10), unknown (or no) mutation (n = 14) and $BRAF^{V600E}$ mutation (n = 28). The asterisk represents an outlier, defined as at least 1.5× the interquartile range (Q3–Q1) from the edge of the box.

the TCGA TDS is recovered by the FC comparison method. Table 2 shows the FC of the 19 TDS genes. Figure 2 provides a heat map of each gene, normalized to its maximum over all 59 subjects. An interesting observation is that expression of the NIS gene is relatively low across all mutation groups but also low in several normal thyroid tissue samples.

The original TCGA TDS was demonstrated to be a predictor of the aggressiveness of thyroid cancer (Cancer Genome Atlas Research Network 2014) based on the degree of mutations in the $BRAF^{V600E}$ and RAS oncogenes. The $BRAF^{V600E}$ mutations are highly correlated with loss of differentiation and function of follicular cells, more so than those with RAS mutations. The driver genomic alteration(s) are suggestive of, but not accurately predictive of, the degree of functional differentiation of individual tumors. Genomic profiling provides insight into the potential theranostic power of RAI. However, the proteomics, metabolomics and phenomics (RAI theranostics) may remain variable compared to the normal cells for individual patients, particularly when the natural variations of gene expressions in each patient's normal thyroid tissue are taken into consideration.

The choice of the 16 genes to include in the original TCGA TDS was apparently selected based on an *a priori* selection of genes already known to be related to thyroid metabolism and function. As explained above, an expanded list includes 29 genes positively (eTDS (+)) and 19 genes negatively correlated with TDS in the $BRAF^{V600E}$ cohort. However, while correlated to cancer, the genes were not selected based on their theranostic potential. That is, their selection was based on their correlation to the extent of disease but not with consideration of their value to predict response to any therapy. Our analysis is of 19 genes, which includes the 16 TDS genes proposed by TCGA, two addition genes, IYD and SLC16A2, known to be directly involved in iodine uptake and handling (organification) and SLC26A7. SLC26A7 is a member of a family of sulfate/anion transporter genes not specific to the thyroid but has been implicated in thyroid iodine handling (Ishii *et al.* 2019, Yamaguchi *et al.* 2022) and was also included in the eTDS (+) list (Dunn *et al.* 2019). It is important to emphasize that the context of TDS in the original TCGA paper is different than the goal in our study, that introduces the concept of TDP which can lead to a theranostic thyroid differentiation score (T-TDS).

Previous research (Dunn *et al.* 2019) demonstrated that inhibition of the MAPK pathway using vemurafenib resulted in higher levels of RAI uptake, which resulted in an eTDS. Though the sample size was very small, the results showed that the thyroid differentiation state likely plays a major role in the regulation of iodine metabolism genes. The TCGA suggested oncogenic $BRAF^{V600E}$ mutations to be associated with the loss of iodine avidity and likely responsible for the development of RAI indifferent tumors. RAI avidity and genomic profile correlation were not within the scope of that study. However, this particular correlation was specifically looked at in a transgenic mouse model by the Fagin group (Chakravarty *et al.* 2011). From our results, one can observe that the loss of differentiation is most striking in the BRAF-mutated cancers. The downregulation of the

Table 2 FC (tumor relative to normal) for the 19 genes studied in this analysis.

	$BRAF^{V600E}$ (n = 28)				RAS (n = 7)				Others (n = 10)				Unknown (n = 14)			
Gene	**Mean**	**SD**	**Median**	***P***	**Mean**	**SD**	**Median**	***P***	**Mean**	**SD**	**Median**	***P***	**Mean**	**SD**	**Median**	***P***
DIO1	−5.97	1.85	−6.12	2E-06*	−0.24	1.64	−0.49	0.400	−1.98	2.99	−1.77	0.051	−3.90	3.60	−4.25	0.003*
DIO2	**−2.62**	**1.02**	**−2.59**	**2E-06***	**−1.35**	**0.78**	**−1.45**	**0.017***	**−1.43**	**1.10**	**−1.38**	**0.004***	**−2.27**	**1.61**	**−2.36**	**0.001***
DUOX1	−0.52	0.76	−0.48	8E-04*	0.82	0.64	0.81	0.989	0.05	1.00	0.06	0.658	−0.64	1.26	−0.71	0.030*
DUOX2	−1.04	1.13	−0.95	6E-05*	1.60	1.02	1.62	0.993	0.43	1.30	0.52	0.889	−0.98	2.05	−0.98	0.051
FOXE1	−0.94	0.80	−1.01	3E-05*	−0.30	0.76	−0.31	0.176	−0.25	0.92	−0.34	0.207	−0.87	1.41	−1.01	0.010*
GLIS3	**−1.25**	**0.75**	**−1.24**	**3E-06***	**−1.05**	**1.09**	**−0.91**	**0.011***	**−1.03**	**0.83**	**−1.09**	**0.007***	**−1.26**	**1.12**	**−1.50**	**0.004***
IYD	−2.94	1.69	−2.71	2E-06*	−0.90	1.38	−0.99	0.075	−1.93	2.20	−1.73	0.016*	−2.70	2.16	−2.61	0.001*
NKX2-1	0.13	0.83	0.18	0.900	0.40	0.48	0.39	0.962	0.41	0.97	0.41	0.889	0.61	0.86	0.49	0.998
PAX8	−0.97	0.71	−0.92	4E-06*	−0.51	0.65	−0.50	0.075	−1.03	0.66	−1.07	0.005*	−0.94	1.09	−1.09	0.007*
SLC16A2	−2.35	0.84	−2.36	2E-06*	−0.08	0.52	−0.11	0.400	−1.34	1.16	−1.30	0.004*	−2.14	1.63	−2.43	0.002*
SLC26A4	**−4.77**	**2.06**	**−4.72**	**2E-06***	**−1.65**	**1.17**	**−1.44**	**0.011***	**−1.90**	**2.41**	**−1.68**	**0.021***	**−3.92**	**2.82**	**−3.91**	**0.001***
SLC26A7	**−3.13**	**2.39**	**−2.90**	**4E-06***	**−5.62**	**2.02**	**−5.46**	**0.011***	**−3.84**	**2.15**	**−4.15**	**0.003***	**−3.37**	**2.37**	**−3.41**	**0.001***
SLC5A5 (NIS)	**−4.36**	**1.56**	**−4.34**	**2E-06***	**−4.39**	**3.15**	**−4.44**	**0.011***	**−4.82**	**2.51**	**−4.98**	**0.003***	**−3.56**	**3.10**	**−3.74**	**0.002***
SLC5A8	−5.28	1.39	−5.41	2E-06*	−2.13	2.22	−1.80	0.011*	−1.37	2.16	−1.23	0.051	−3.75	2.24	−3.91	0.001*
TG	−2.96	1.48	−2.82	2E-06*	−0.41	1.39	−0.15	0.336	−1.19	1.35	−0.97	0.007*	−2.52	2.06	−2.39	0.001*
THRA	−0.29	0.57	−0.29	9E-03*	−0.33	0.38	−0.25	0.017*	−0.39	0.40	−0.42	0.012*	−0.26	0.73	−0.34	0.058
THRB	**−0.44**	**0.56**	**−0.42**	**2E-04***	**−0.96**	**0.69**	**−0.98**	**0.017***	**−0.74**	**0.74**	**−0.75**	**0.010***	**−0.76**	**0.67**	**−0.82**	**0.002***
TPO	−6.71	1.99	−6.89	2E-06*	−0.75	1.93	−0.37	0.223	−2.30	2.95	−2.63	0.051	−4.65	2.91	−4.63	0.001*
TSHR	−0.94	0.66	−0.89	3E-06*	−0.11	0.62	−0.08	0.336	−0.63	0.74	−0.55	0.016*	−0.98	1.20	−1.12	0.009*

*Genes with $P < 0.05$ for FC depression (FC < 0) in tumor vs normal by the one-sided Wilcoxon signed rank test for dependent (paired) samples. Genes highlighted in **bold** are significantly depressed in tumors of all four mutation groups.
FC, fold change.

genes involved in iodide uptake and processing showed variability in each mutational category, justifying individual assessment of the theranostic transcriptome.

Presumably, the intent of the original TCGA TDS was to demonstrate a correlation of the BRAF-RAS score with thyroid differentiation. Figure 1 demonstrates why TDS, as defined by TCGA, does not have theranostic power. Variability in TDS among tumor samples is high, and thus, a score based on the median of the expression of tumor samples fails to accurately reflect the degree of actual differentiation (or dedifferentiation) of the tumor. Without consideration of the mRNA expression from the paired normal tissue, the true differentiation (compared to normal) may be obscured. The intent of a new T-TDS is to define a single metric with true theranostic power. A T-TDS should exclude FOXE1, GLIS3, NKX2-1 and PAX8 of the original TDS set of 16 genes,since they are thyroid-specific transcription factors that have the priming role in the expression of a multitude of functionally and morphologically relevant genes. The FC of these genes was small (Table 2). The mean FC expression of several of the other genes is also small (e.g., DUOX1, DUOX2, THRA, THRB and TSHR). Importantly, the contribution of each proteomic expression on the metabolomics and overall T-TDS is yet to be determined.

Conclusion

A complete index of thyroid functional differentiation should reflect the preservation of the iodine machinery, that is, iodine uptake and organification, which can be quantified by *in vitro* thyroid theranostic differentiation determined by a molecular analysis and RAI imaging following complete removal of the thyroid gland. To our knowledge, this paper is the first published analysis of the comparison between normal and tumor tissues of the expression of the 16 genes used in the TCGA cohort. Clearly, the relative contribution of each gene to overall thyroid iodine metabolism is quite different and some experience little or no depression in tumor tissue. A new T-TDS is needed that is directly related to iodine uptake and metabolism and is based on a FC between normal thyroid and thyroid cancer tissues. The T-TDS is predicted to provide greater theranostic power compared to the original TDS, which is based on 16 genes, not all of which are specifically related to iodine uptake and handling, and does not account for natural variability in gene expression associated with normal thyroid tissue. A prospective study is being designed to correlate expression levels with actual RAI uptake, which is necessary to finalize the formula for the T-TDS.

Declaration of interest

Anthony McGoron, Jose Garcia, Burulca Uluvar and Seza Gulec have no disclosures to report.

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Author contribution statement

AJM and SAG helped in conceptualization. JMG and BU helped in data curation. AJM and SAG helped in formal analysis. SAG helped in funding acquisition. AJM helped in methodology. AJM and SAG helped in supervision. AJM and BU helped in visualization. AJM and JMG helped in the writing of the original draft. AJM and SAG helped in writing, reviewing and editing.

Ethics statement

The authors confirm that the research meets the ethics guidelines, including adherence to the legal requirements of the United States of America. The data used are anonymized and publicly available from TCGA program (https://portal.gdc.cancer.gov/).

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