tRNA-Derived Fragments as Post-Transcriptional Regulators of Oncogenes and Tumor Suppressor Genes in Papillary Thyroid Carcinoma

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Simple Summary

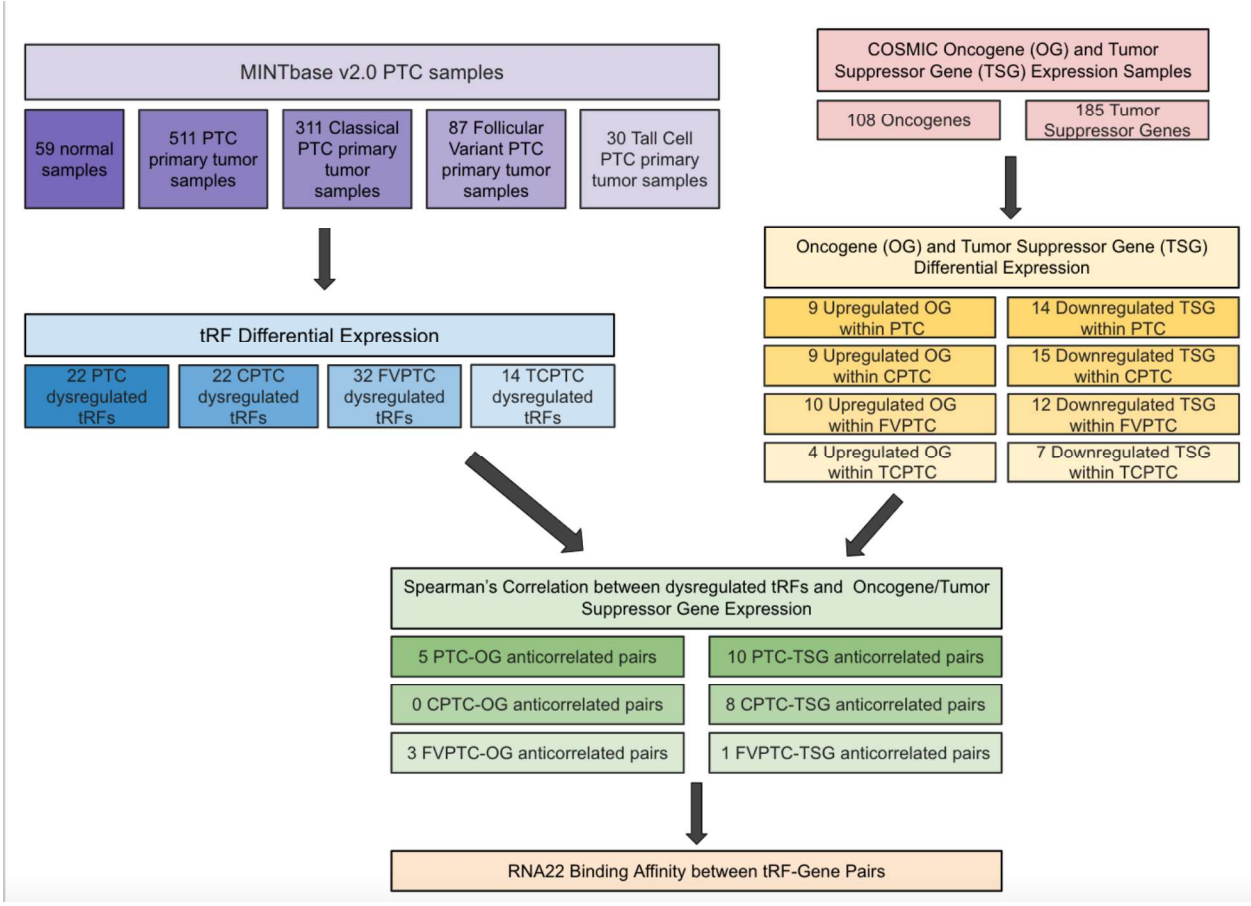
Papillary thyroid carcinoma (PTC) is a common cancer in which the role of transfer-RNA derived fragments (tRFs), a relatively novel class of small non-coding RNA, has yet to be widely explored. It has been proposed that tRFs influence cancer proliferation by binding to mRNA transcripts and inducing their degradation through the recruitment of cleaving proteins. This study aims to characterize the expression of tRFs, oncogenes, and tumor suppressor genes in PTC with respect to the following subtypes: classical PTC, follicular variant PTC, and tall cell PTC. Across all cohorts, we found that tRFs are significantly dysregulated ($p<0.05$), and that their expression is anticorrelated with dysregulated oncogenes and tumor suppressor genes. Binding affinity analysis further revealed that significant pairs of anticorrelated tRFs and genes have sufficient complementarity to form heteroduplexes, supporting the hypothesis that tRFs are mechanistically relevant to cancer and contribute to post-transcriptional regulation.

Abstract

tRNA-derived fragments (tRFs) are a relatively novel class of small non-coding RNA that has been implicated in the pathogenesis of certain cancers. It has been proposed that tRFs act as post-transcriptional regulators of mRNA by binding to complementary regions of the transcript and recruiting proteins that cleave the mRNA. Accordingly, this project identifies tRFs that are differentially expressed between papillary thyroid carcinoma (PTC) tissue samples (n=511) and normal tissue samples (n=59). We found 22 tRFs to be differentially expressed between these groups, then identified dysregulated tRFs that exhibited anticorrelated expression with upregulated oncogenes (OG) and downregulated tumor suppressor genes (TSG). Notably, tRF-oncogene pairs ArgCCG 3'-tRF-MACC1 and GlnTTG i-tRF-CYSLTR2 and tRF-tumor suppressor gene pairs AsnGTT 3'-tRF-FAT4, ProCGG 3'-tRF, and AsnGTT i-tRF-SFRP4. Binding affinity analysis further predicted that tRFs can bind to OG and TSG transcripts with sufficient complementarity to induce cleavage, supporting the hypothesis that tRFs mechanistically regulate cancer pathogenesis by promoting the degradation of mRNA transcripts. Overall, this study aims to elucidate the

role of tRFs in papillary thyroid carcinoma by analyzing their potential to regulate genes that are critical to disease progression.

Graphical Abstract



Keywords

tRNA-derived Fragments, tRNA-derived small RNA, Papillary Thyroid Carcinoma, Oncogenes, Tumor Suppressor Genes

1) Introduction

The incidence rate for thyroid cancer has more than tripled in the United States from 1975 to 2013 [1] and ranked 9th out of 36 cancers in a global survey conducted in 2020 [2]. The majority of these cases are attributed to papillary thyroid carcinoma (PTC), which accounts for over 80% of patient diagnoses [1]. PTC is a highly diverse cancer and can be classified into subtypes based on histology and prognosis, with three salient forms being classical papillary thyroid carcinoma (CPTC), follicular variant papillary thyroid carcinoma (FVPTC), and tall cell papillary thyroid carcinoma (TCPTC) [3]. Of these three, CPTC is the most common and least aggressive subtype; in comparison, FVPTC has higher mortality and distant metastases rates [4], while TCPTC is the rarest and most aggressive [5], with a worse average 5-year survival rate. However, PTC is generally considered to be a well-differentiated and indolent cancer, with most patients experiencing positive prognosis [6].

Meanwhile, transfer RNA-derived fragments (tRFs) are a class of microRNA that are derived from pre-tRNA and mature tRNA molecules [7]. The naming convention for tRFs used in this study is based on the source location on the parent tRNA molecule and the associated amino acid and anticodon—for example, AsnGTT 3'-tRF describes a tRF that is derived from the 3’ end of a tRNA molecule, has the anticodon GTT, and is associated with asparagine. tRFs have been found to be significantly dysregulated in several human cancers , and in some cases, have been found to be directly involved in cancer development; in one study, a specific class of tRFs was found to exhibit tumor suppression in response to hypoxic stress caused by breast cancer neoplasms [8,9]. Additionally, our lab has previously identified tRFs that were significantly dysregulated between lung squamous cell carcinoma tumor samples and normal tissue samples [10].

tRF-mediated gene regulation may be achieved through highly specific binding to complementary regions of mRNA transcripts, resulting in the formation of an RNA-induced silencing complex (RISC) that recruits Argonaute (AGO) proteins to degrade mRNA prior to translation [9, 11-13]. Of particular interest is tRF-mediated regulation of genes that direct cancer proliferation. Accordingly, this study aims to determine the relationship between tRNA-derived fragments (tRFs) and their target genes in PTC. To begin, we obtained from MINTbase v2.0 a dataset of tRF abundance for 511 primary PTC tumor samples (includes 311 CPTC, 87 FVPTC, and 30 TCPTC specimens) and 59 normal samples. All patient samples are derived from The Cancer Genome Atlas; cross-referencing patient IDs yielded corresponding gene expression data for tumor and normal samples. Using a list of known oncogenes (OGs) and tumor suppressor genes (TSGs) from the Catalogue of Somatic Mutations in Cancer (COSMIC), we then identified upregulated oncogenes and downregulated tumor suppressor genes. Subsequent analysis revealed a correlation

between dysregulated tRFs and the expression levels of OGs and TSGs; specifically, upregulated tRFs exhibited a correlation with decreased TSG expression, while downregulated tRFs exhibited a correlation with increased OG expression. Binding analysis was performed on tRF-gene pairs demonstrating significant anticorrelation ($r<0$, $p<0.05$) with RNA22 to identify pairs with sufficient complementarity to bind and induce Ago-mediated cleavage [14].

2) Results

2a) Differentially Expressed tRFs

Differential expression analysis on the tRF abundance of PTC primary tumor samples and normal tissue samples revealed statistically significant differences in the tRF landscape of cancer samples ($p < 0.05$). In the PTC cohort, 22 differentially expressed tRFs were identified **(Figure 2b)**, with 11 upregulated tRFs and 11 downregulated tRFs. Among CPTC, FVPTC, and TCPTC subtype cohorts, 22, 32, and 14 differentially expressed tRFs were found, respectively. Of the 22 tRFs identified in CPTC cohort, 9 were downregulated and 13 were upregulated. In the FVPTC cohort, 19 tRFs were downregulated and 13 were upregulated. In TCPTC, 3 tRFs were downregulated and 11 tRFs were upregulated. Notably, each subtype had unique dysregulated tRFs, with the majority of dysregulated tRFs not being shared in common with another subtype; ArgCCG 3'-tRF and GluTTC 5'-tRF were the only 2 tRFs found to be dysregulated across all groups. Characteristic of each subtype were GlyGCC 3'-tRF and ThrAGT i-tRF in CPTC, AsnGTT 3'-tRF and AsnGTT 5'-tRF in FVPTC, and AlaAGC i-tRF and AlaCGC 5'-tRF in TCPTC ($p < 0.001$).

The negative logarithm of the test statistic was plotted against the $\log_2 FC$ of tRF expression between each PTC cohort and normal samples **(Figure 1).** While most tRFs that overlapped between cohorts followed similar trends (upregulated vs downregulated), ProAGG i-tRF was found to be downregulated in FVPTC patients but upregulated in CPTC patients. (**Figure 2a)** provides a holistic overview of identified dysregulated tRfs stratified by cohort and their subsequent overlap.

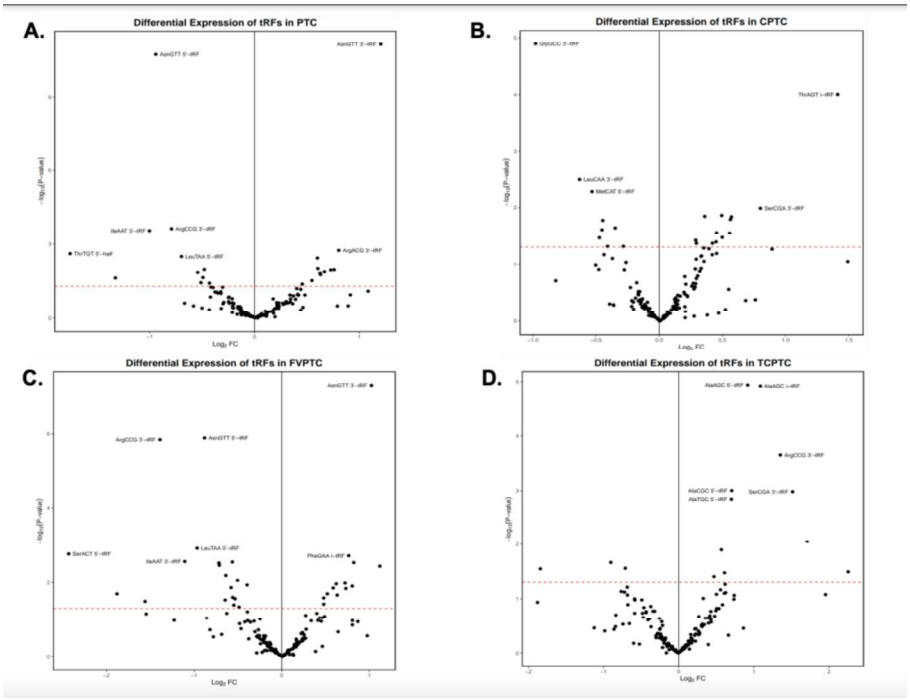
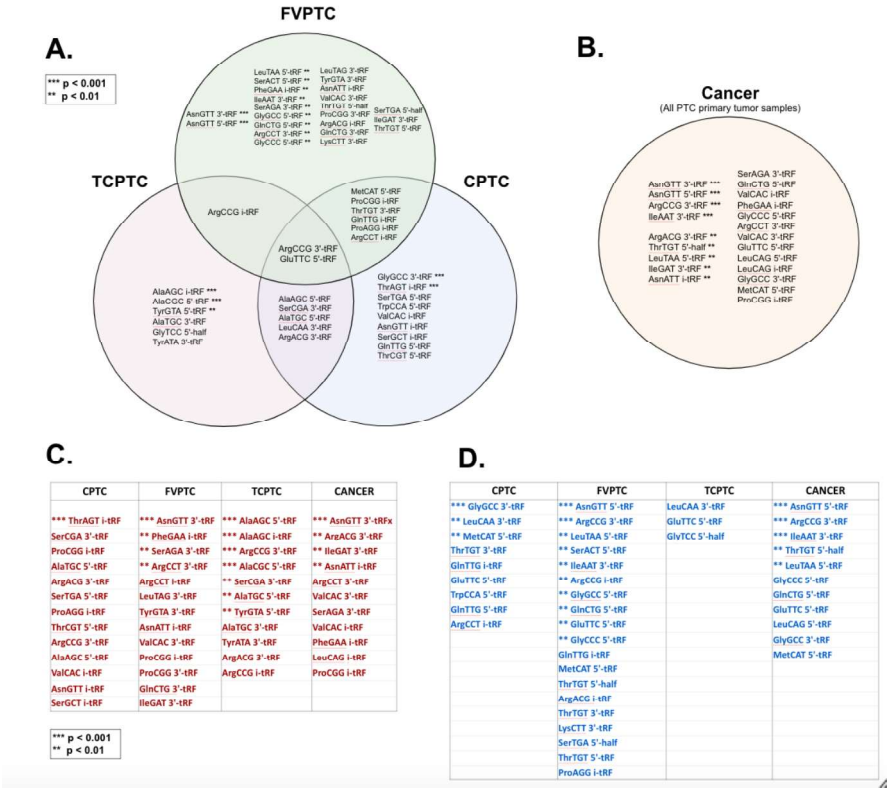


Figure 1. Volcano plots depicting the negative logarithm of Kruskal-Wallis p-values plotted against the $\log_2 FC$ of tRF abundance between primary papillary thyroid carcinoma (PTC) primary tumor samples (n=511) and normal samples (n=59). The red line denotes the negative logarithm of p-value = 0.05, which was our benchmark for significance. (**A) depicts the results of** an inclusive cohort of PTC samples, with 22 significantly dysregulated tRFs. (**B)** depicts the results of only classical PTC **primary tumor samples (n=311) with** 22 significantly dysregulated tRFs. (**C) depicts the results of follicular variant PTC samples (n=87)** with 32 significantly dysregulated tRFs. (**D) depicts the results of tall cell PTC samples (n=30) with** 14 significantly dysregulated tRFs.



CPTC	FVPTC	TCPTC	CANCER
*** ThrAGT i-tRF	*** AsnGTT 3'-tRF	*** AlaAGC 5'-tRF	*** AsnGTT 3'-tRFx
SerCGA 3'-tRF	** PheGAA i-tRF	*** AlaAGC i-tRF	** ArzACG 3'-tRF
ProCGG i-tRF	** SerAGA 3'-tRF	*** ArgCCG 3'-tRF	** IleGAT 3'-tRF
AlaTGC 5'-tRF	** ArgCCT 3'-tRF	*** AlaCGC 5'-tRF	** AsnATT i-tRF
ArgACG 3'-tRF	ArgCCT i-tRF	** SerCGA 3'-tRF	ArgCCT 3'-tRF
SerTGA 5'-tRF	LeuTAG 3'-tRF	** AlaTGC 5'-tRF	ValCAC 3'-tRF
ProAGG i-tRF	TyrGTA 3'-tRF	** TyrGTA 5'-tRF	SerAGA 3'-tRF
ThrCGT 5'-tRF	AsnATT i-tRF	AlaTGC 3'-tRF	ValCAC i-tRF
ArgCCG 3'-tRF	ValCAC 3'-tRF	TyrATA 3'-tRF	PheGAA i-tRF
AlaAGC 5'-tRF	ProCGG i-tRF	ArgACG 3'-tRF	LeuCAG i-tRF
ValCAC i-tRF	ProCGG 3'-tRF	ArgCCG i-tRF	ProCGG i-tRF
AsnGTT i-tRF	GlnCTG 3'-tRF		
SerGCT i-tRF	IleGAT 3'-tRF		

*** p < 0.001
** p < 0.01

CPTC	FVPTC	TCPTC	CANCER
*** GlyGCC 3'-tRF	*** AsnGTT 5'-tRF	LeuCAA 3'-tRF	*** AsnGTT 5'-tRF
** LeuCAA 3'-tRF	*** ArgCCG 3'-tRF	GluTTC 5'-tRF	*** ArgCCG 3'-tRF
** MetCAT 5'-tRF	** LeuTAA 5'-tRF	GlyTCC 5'-half	*** IleAAT 3'-tRF
ThrTGT 3'-tRF	** SerACT 5'-tRF		** ThrTGT 5'-half
GlnTTG i-tRF	** IleAAT 3'-tRF		** LeuTAA 5'-tRF
GluTTC 5'-tRF	** ArgCCG i-tRF		GlyCCC 5'-tRF
TrpCCA 5'-tRF	** GlyGCC 5'-tRF		GlnCTG 5'-tRF
GlnTTG 5'-tRF	** GlnCTG 5'-tRF		GluTTC 5'-tRF
ArgCCT i-tRF	** GluTTC 5'-tRF		LeuCAG 5'-tRF
	** GlyCCC 5'-tRF		GlyGCC 3'-tRF
	GlnTTG i-tRF		MetCAT 5'-tRF
	MetCAT 5'-tRF		
	ThrTGT 5'-half		
	ArgACG i-tRF		
	ThrTGT 3'-tRF		
	LysCTT 3'-tRF		
	SerTGA 5'-half		
	ThrTGT 5'-tRF		
	ProAGG i-tRF		

Figure 2. Differentially expressed tRFs in primary tumor samples of papillary thyroid carcinoma (PTC). **(A)** Dysregulated tRFs ($p < 0.05$) are organized in a Venn diagram by subtypes to highlight common dysregulated tRFs. Samples are separated into cohorts by PTC subtype: follicular variant PTC (FVPTC), classical PTC (CPTC), and tall cell PTC (TCPTC). **(B)** Dysregulated tRFs in the cancer cohort, which includes all PTC primary tumor samples in the MINTbase v2.0 database, irrespective of subtype. **(C)** Upregulated tRFs ($p < 0.05$, $|\log FC| \geq 1$) and **(D)** downregulated tRFs ($p < 0.05$, $|\log FC| < 1$) in the CPTC, FVPTC, TCPTC, and cancer cohorts. Three asterisks are used to denote $p < 0.001$, and two asterisks are used to denote $p < 0.01$.

2b) Differentially Expressed Oncogenes and Tumor Suppressor Genes

Next, significantly upregulated oncogenes (OG) and downregulated tumor suppressor genes (TSG) were identified in PTC primary tumor samples (n=509) against normal tissue samples (n=58) by analyzing gene expression data from The Cancer Genome Atlas. Genes of interest were compiled using a list of 108 OGs and 185 TSGs from the Catalogue of Somatic Mutations in Cancer (COSMIC), one of the largest available repositories for somatic mutational data in cancer.

Identified in the PTC cohort (n=509) were 9 upregulated OGs and 14 significantly downregulated TSGs ($p < 0.05$). Among FVPTC primary tumor samples (n=86) were 10 significantly upregulated OGs and 12 significantly downregulated TSGs. CPTC primary tumor samples (n=311) yielded the greatest number of significantly upregulated OGs and downregulated TSGs overall, with 9 and 15, respectively. Conversely, TCPTC primary tumor samples (n=30) had the least number of dysregulated genes, with 4 significantly upregulated OGs and 7 downregulated TSGs. Notably, oncogenes CYSLTR2, MET, FOXA1, TNC, ERBB3, MACC1, JAK3, TMSB4X, and CDH17 were dysregulated in common across all cohorts. Similarly, many of the same tumor suppressor genes were dysregulated; in at least 3 cohorts, we identified SFRP4, WNK2, USP44, PTPRT, FBLN2, AXIN2, FAT4; across all cohorts, tumor suppressor genes LRP1B, SOX21, CNTNAP2, PHOX2B were significantly downregulated.

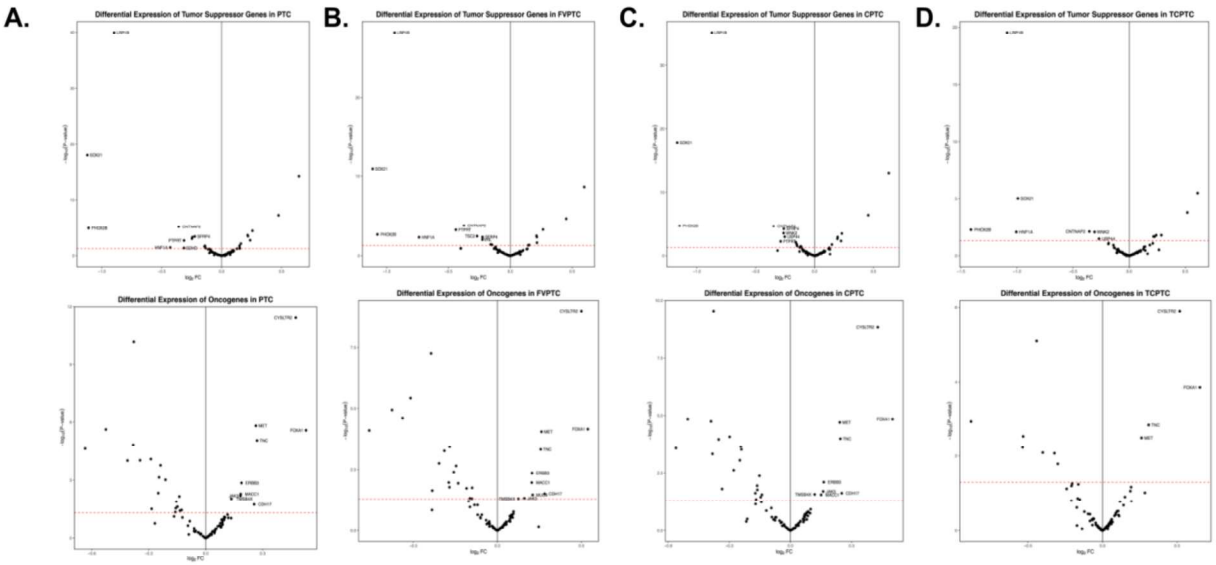


Figure 3. Volcano plots depicting the negative logarithm of Kruskal Wallis p-values plotted against the $\log_2 FC$ of genetic expression (CPM) between primary papillary thyroid carcinoma (PTC) tumor samples (n=509) and normal samples (n=58). The red line denotes the negative logarithm of p-value = 0.05, which was our benchmark for significance. **(A)** depicts the results for an inclusive cohort of all PTC samples (n=509), **(B) d**epicts the results for follicular variant PTC primary tumor samples (n=86), **(C)** depicts the results for classical PTC primary tumor samples (n=311), and (D) depicts the results for tall cell PTC primary tumor samples (n=30).

2c) tRFs Anticorrelated with the Expression of Oncogenes and Tumor Suppressor Genes

The abundance of significantly dysregulated tRFs was then analyzed against the expression of significantly dysregulated oncogenes and tumor suppressor genes with Spearman's rank correlation coefficient. Pairings were selected for significant anticorrelated expression ($r < 0$, $p < 0.05$), where upregulated tRFs were correlated with downregulated oncogenes and downregulated tRFs were correlated with upregulated tumor suppressor genes. Across all PTC samples (n=509) 5 significant anticorrelated pairs were identified from pairing 22 dysregulated tRFs, 9 dysregulated oncogenes, and 14 dysregulated tumor suppressor genes. Among CPTC samples (n=311), 8 anticorrelated tRF and tumor suppressor gene pairs were identified from 22 tRFs, 9 oncogenes, and 15 tumor suppressor genes. Among FVPTC samples (n=86), 3 anticorrelated tRF-Oncogene pairs and 1 tRF-Tumor Suppressor Gene pair were identified. There were no significant anticorrelated gene pairs among TCPTC samples (n=30).

ArgCCG 3'-tRF, which was previously found to be significantly dysregulated in all cohorts, was found to be significantly anticorrelated with several oncogenes within the inclusive PTC cohort, namely CYSLTR2, ERBB3, JAK3, MACC1, and MET. Several tRFs of AsnGTT (AsnGTT 5'-tRF, AsnGTT i-tRF, and AsnGTT 3'-tRF) were significantly paired to genes in the follicular variant PTC, classical PTC, and PTC cohorts respectively. Notably, ProCGG i-tRF-PHOX2B was the only tRF-gene pair found to be significant in more than one cohort (PTC and classical PTC); all other tRF-gene pairs were found to be unique to their own cohort. Within the PTC cohort, AsnGTT 3'-tRF was found to be significantly anticorrelated with multiple tumor suppressor genes while ArgCCG 3'-tRF was anticorrelated with multiple oncogenes. In the classical PTC cohort, there were two tRFs (ArgACG 3'-tRF and AsnGTT i-tRF) found paired with multiple tumor suppressor genes. In addition, there were no oncogenes or tumor suppressor genes found to be anticorrelated in all three cohorts.

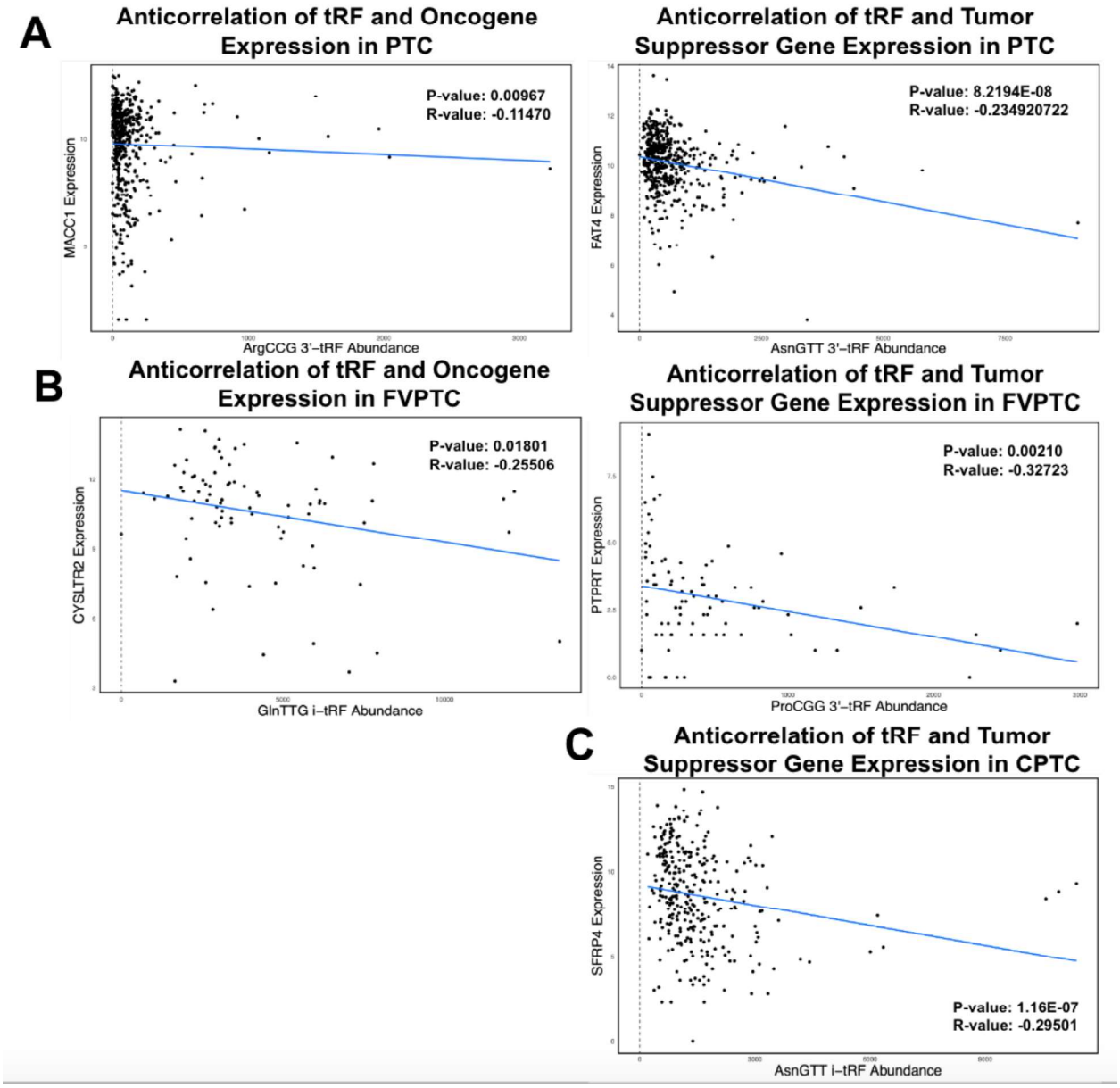


Figure 4: Scatterplots showing the relationship between tRF Abundance and gene expression. **(A)** Shows selected scatterplots with the largest r-value per set. Left-hand scatterplots depict tRF-oncogene pairs while right-hand scatterplots depict tRF-tumor suppressor gene pairs. Depicts pairs from papillary thyroid carcinoma (PTC) inclusive cohort. **(B)** The second row shows pairs from follicular variant PTC cohort. **(C)** Third row shows tRF-tumor suppressor gene pair from classical PTC cohort. No scatterplots were produced for the tall cell PTC cohort as no significant anticorrelated tRF-gene pairs were identified.

2d) Measuring Binding Affinity of Anticorrelated tRFs, Oncogenes, and Tumor Suppressor Genes

To determine the binding capabilities that the pairs of tRFs and OG/TSGs with significantly anticorrelated expression **(Section 2c)**, the binding affinity of each significant pair was analyzed through RNA22 v2. We performed this analysis only on significant tRF and OG/TSG pairs with false discovery rates below 25%. RNA22 analysis identified pairs with significant binding affinities and the specific binding sites between each pair. Of the pairs identified within the PTC cohort, 5 tRF-TSG pairs were found to have significant binding affinities. Of the classical PTC cohort, 4 tRF-TSG pairs were found to exhibit significant binding affinity. Lastly, all 3 tRF-OG pairs and 1 tRF-TSG pairs in the follicular variant PTC cohort were found to have significant binding affinities. The TCPTC cohort was not included in this analysis since no significant anticorrelated pairs of tRFs and genes were identified (**Section 2c**). Of all three cohorts, only the follicular variant cohort exhibited tRF-oncogene pairs with significant binding affinities. Of the specific tRFs found in multiple anticorrelated gene pairs (**Section 2c)**, tRFs AsnGTT 3'-tRF and AsnGTT i-tRF were found to have significant binding affinities with tumor suppressor genes within cohorts PTC and classical PTC respectively.

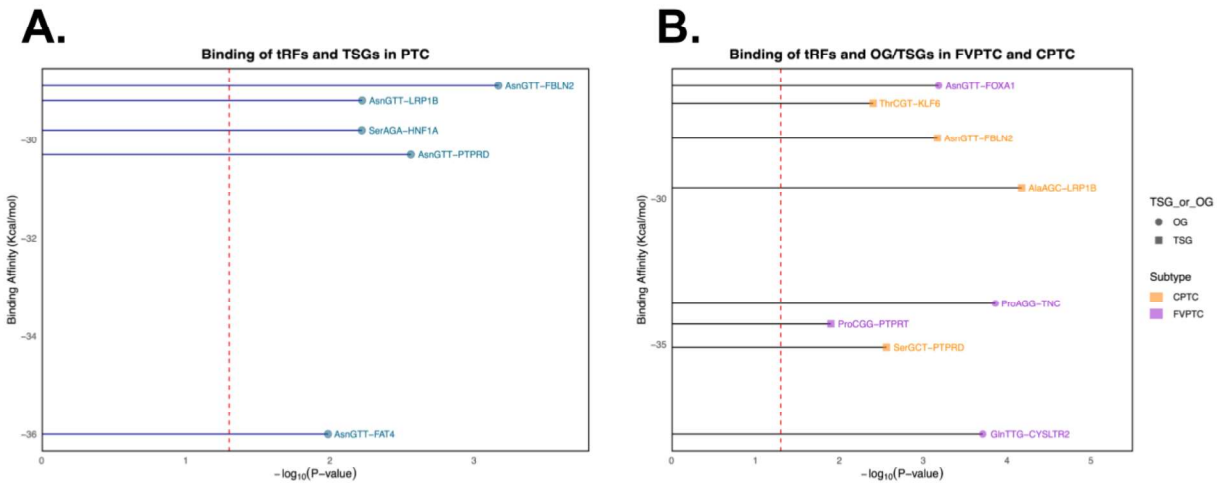


Figure 5. Lollipop plots show the binding affinities in relation to the $-\log_{10}(p\ value)$of each significantly dysregulated tRF-gene pairs. Red dotted line marks p-value = 0.05. **(A)** Plot shows the tRF-TSG pairs found within inclusive PTC cohort (n=511). **(B)** Plot shows tRF-gene pairs within classical PTC (n=311) and follicular variant PTC cohorts (n=87). Points marked with a

square represent tumor suppressor genes while circles represent oncogenes. Purple plots represent follicular variant PTC and orange represents classical PTC.

Discussion

Differential expression analysis (**Section 2a**) revealed that tRFs are dysregulated in PTC across all subtypes, but that the identity of the dysregulated tRFs varies by subtype. Because a salient issue in diagnosing PTC is the lack of rigorous, standardized practices in distinguishing between subtypes [5], subtype-specific dysregulated tRFs may be utilized in a novel approach to differentiating between PTC subtypes. Although two tRFs, ArgCCG 3'-tRF and GluTTC 5'-tRF, were found to be dysregulated in every cohort, only GluTTC 5'-tRF was consistently downregulated; ArgCCG3'-tRF, on the other hand, was upregulated in CPTC and TCPTC but downregulated in FVPTC and PTC (inclusive cohort). Beyond that, most tRFs were characteristic of a PTC subtype. Namely, AsnGTT 5'-tRF and AsnGTT 3'-tRF were only found to be dysregulated in FVPTC, and GlyGCC 3'-tRF and ThrAGT i-tRF were only found to be dysregulated in CPTC. Similarly, TCPTC was the only cohort found to exhibit dysregulation of AlaAGC i-tRF and AlaCGC 5'-tRF. These characteristic tRFs may serve as potential biomarkers in distinguishing between PTC subtype in clinical settings, however, the viability of tRFs to be detected and quantified with high sensitivity in common practice is uncertain.

Next, anticorrelated expression of dysregulated genes with dysregulated tRFs was analyzed. Apart from the correlation between ProCGG i-tRF and PHOX2B (found in both classical PTC and the all-inclusive PTC cohort), each cohort was found to have a unique set of significant tRF-gene pairs. The exclusive presence of AsnGTT 3'-tRF and ArgCCG 3'-tRF within PTC and ArgACG 3'-tRF and AsnGTT i-tRF within CPTC with multiple different gene pairs suggests that tRFs exhibit subtype-specific pathological influence in papillary thyroid carcinoma.

Accordingly, the potential for tRFs to form heteroduplexes with oncogenes and tumor suppressor genes was assessed using RNA22 binding affinity predictions. Thirteen of the 27 signficantly anticorrelated tRF-gene pairs exhibited sufficient complementarity for binding in PTC, including in the FVPTC and CPTC cohorts ($p < 0.05$). Of the tRF-gene pairs with significant binding affinities, no pairing was present in more than one cohort. Moreover, of the 13 tRF-gene pairs with significant binding affinities, 10 were anticorrelated with tumor suppressor genes specifically. Because there were more significant results between tRFs and TSGs than there were between tRFs and OGs in both anticorrelation

analysis **(section 2c)** and binding affinity analysis **(section 2d), it is plausible** that tRFs exhibit greater post-transcriptional influence on tumor suppressor genes than they do on oncogenes.

Data on defined base position cutoffs between tRFs belonging to the same parent tRNA (e.g. 5'-tRFs, i-tRFs, 3'-tRFs) were not available, so binding affinity analysis with RNA22 was conducted with whole tRNA sequences. This limits confidence in the binding affinity results; For instance, while binding affinity between AsnGTT and FBLN2 was found to be significant, it is unclear whether complementary binding would occur with AsnGTT 3'-tRF or AsnGTT i-tRF (both tRFs were significantly anticorrelated within different cohorts). As a result, both tRF-gene pairs were included in the results and figures of **(section 2D).**

To the best of our knowledge , this study is among the first to investigate the presence and function of tRFs in PTC and is the first to analyze the tRF landscape with respect to PTC subtype. However, it is important to note that this study only categorizes the 3 most common subtypes of PTC (CPTC, FVPTC, and TCPTC) and that many other PTC subtypes that were not included (oncocytic, columnar cell, diffuse sclerosing and solid forms). Therefore, additional information on these less common subtypes may provide more insight into tRF-subtype relationships. In addition, the limited sample size for TCPTC (n=30) is likely what caused inconclusive results in anticorrelation analysis between tRF and gene expression. This precluded binding affinity analysis from being performed on TCPTC samples. Future studies may look to include all subtypes and increase sample size to allow a more holistic interpretation. Lastly, because the analysis in this study is computational in nature, further *in vitro* validation is necessary to identify dysregulated tRFs and determine whether tRF and mRNA binding occurs within cells.

Materials and Methods

4.1 Data Acquisition

All tRF expression values were extracted from MINTbase v2.0, and can be found using the query "thyroid." 511 primary tumor samples and 59 normal samples were obtained by downloading a zip file with patient tRF expression profiles (https://cm.jefferson.edu/tcga-mintmap-profiles/; accessed 17 May 2023). Patient samples were analyzed in 4 cohorts: all PTC samples irrespective of subtype (n=511), classical PTC (n=311), follicular variant PTC (n=87), tall cell PTC (n=30). Sample IDs correspond to samples hosted by The Cancer Genome Atlas (TCGA); cross-referencing between TCGA and MINTbase v2.0 allowed for distinction of PTC subtype. Likewise, gene

expression data was obtained from TCGA and matched by patient ID; 107 OGs and 185 TSGs were selected from the COSMIC database. The software STAR (2.7.11B) was used to map the sequencing files to oncogene and tumor suppressor gene sequences, yielding normalized read counts of each gene. Default parameters were chosen.

4.2 Differential Expression Analysis

Differential expression analysis was conducted through edgeR library to identify tRFs that significantly differentially expressed within the following cohorts: (1) all-inclusive PTC samples, (2) classical PTC samples, (3) follicular variant PTC samples, and (4) tall cell PTC samples against adjacent normal samples. Significance was identified and filtered by $p\text{-}value < 0.05$. Similarly, significantly differentially expressed oncogenes and tumor suppressor genes were identified by cohort through differential expression analysis through edgeR library. Adjacent normal samples were used as a control and significance was filtered by $p\text{-}value < 0.05$.

4.3 Anti-correlation of tRF abundance and Oncogene and Tumor Suppressor Gene Expression

Spearman's rank correlation coefficient was run in R to measure the strength and direction of correlation between dysregulated tRF abundance and dysregulated oncogene/tumor suppressor gene expression for all cohorts: (1) all-inclusive PTC samples, (2) classical PTC samples, (3) follicular variant PTC samples, and (4) tall cell PTC samples. Upregulated tRFs were run against downregulated tumor suppressor genes, while downregulated tRFs were run against upregulated oncogenes.

4.4 Binding Affinity

The software RNA22v2 was used to compute the binding affinities of tRF-gene pairs (default parameters). tRF sequences and gene sequences were input and assessed for regions of sufficient complementarity. Computed statistical significance was used to compare the observed rate of base pairing with a spurious rate of base pairing

4.5 Plots

All figures were generated with the R package ggplot2.

4.6 Star Methods

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Weg M. Ongkeko (rongkeko@health.ucsd.edu).

Data and Code Availability

This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table. This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Author Contribution

Conceptualization, W.M.O.; Methodology, W.M.O., S.M., and M.U.; Formal Analysis, A.N.D. and M.K.N.; Investigation, A.N.D., M.K.N., S.M., and M.U.; Resources, W.M.O.; Writing—Original Draft Preparation, A.N.D. and M.K.N.; Writing—Review and Editing, A.N.D., M.K.N., S.M., M.U., and W.M.O.; Visualization, A.N.D. and M.K.N.; Supervision, W.M.O.; Project Administration, W.M.O.; Funding Acquisition, W.M.O. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability

The datasets used in this study are available at (https://cm.jefferson.edu/tcga-mintmap-profiles/; accessed 17 May 2023) and (https://portal.gdc.cancer.gov/projects/TCGA-THCA; accessed 17 May 2023).

Acknowledgments

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

Conclusion

The role of transfer-RNA derived fragments (tRFs) within papillary thyroid carcinoma (PTC) has yet to be widely studied. Specific tRFs were found to be dysregulated in PTC primary tumor samples and exhibit anticorrelated expression with dysregulated oncogenes and tumor suppressor genes. Predictive software revealed that anticorrelated

tRF-gene pairs have sufficient complementarity to bind, potentially forming an RNA-induced silencing complex. This study begins to analyze tRF differentiation between the three most common PTC subtypes (classical, follicular variant, and tall cell), but further research is required for a more comprehensive understanding of the role of tRFs in PTC.