Global analysis of tRNA and translation factor expression reveals a dynamic landscape of translational regulation in human cancers

Zhao Zhang¹, Youqiong Ye¹, Jing Gong¹, Hang Ruan¹, Chun-Jie Liu², Yu Xiang¹, Chunyan Cai³, An-Yuan Guo², Jiqiang Ling⁴, Lixia Diao⁵, John N. Weinstein⁵ & Leng Han¹,⁶

The protein translational system, including transfer RNAs (tRNAs) and several categories of enzymes, plays a key role in regulating cell proliferation. Translation dysregulation also contributes to cancer development, though relatively little is known about the changes that occur to the translational system in cancer. Here, we present global analyses of tRNAs and three categories of enzymes involved in translational regulation in ~10,000 cancer patients across 31 cancer types from The Cancer Genome Atlas. By analyzing the expression levels of tRNAs at the gene, codon, and amino acid levels, we identified unequal alterations in tRNA expression, likely due to the uneven distribution of tRNAs decoding different codons. We find that overexpression of tRNAs recognizing codons with a low observed-over-expected ratio may overcome the translational bottleneck in tumorigenesis. We further observed overall overexpression and amplification of tRNA modification enzymes, aminoacyl-tRNA synthetases, and translation factors, which may play synergistic roles with overexpression of tRNAs to activate the translational systems across multiple cancer types.
Translational regulation is critical for biological functions and cellular processes. In the translational system, transfer RNAs (tRNAs) play essential roles by delivering amino acids to initiate or elongate a peptide chain on the ribosome, and they account for ~10% of total cellular RNAs by weight. The human genome includes approximately 600 annotated tRNA genes, which code for 62 codons and 21 amino acids. Activation of the oncogenic signaling pathways, including AKT-mTOR, RAS-MAPK, and MYC or loss of the tumor suppressor TP53 can regulate RNA polymerase III expression, thus leading to altered tRNA expression. In general, overexpression of tRNAs may enhance tumor progression by supplying the high demand codons of oncogenic pathways. Despite the essential functions of tRNAs in the cell, it is still challenging to perform high-throughput quantification of tRNAs, mainly due to the presence of post-transcriptional modifications and secondary structures. To address these challenges, several methods have been designed to quantify tRNA expression level, including tRNA microarrays, which can only achieve codon level resolution by recognizing the tRNA’s anticodon loop, and tRNA-sequencing methods, such as demethylase-tRNA-seq (DM-tRNA-seq), which has been applied in a few cell lines. Alternatively, it is also possible to quantify tRNA expression from miRNA-sequencing (miRNA-seq), which has been applied in small patient sample cohorts. These methods have previously not been applied in large numbers of cancer patient samples.

Multiple categories of enzymes are involved in translational regulation, including the tRNA modification enzymes, aminoacyl tRNA synthetases (ARSs), and translation factors. The first category, tRNA modification enzymes, maintains the stability and specificity of tRNA structure by chemically modifying tRNAs post-transcriptionally. Several modification enzymes, including those encoded by NSUN2, TRMT12, and TRMT2A have been reported to serve as oncogenes, and others, including RG9MTD2, KIAA1456, and TRDMT1, serve as tumor suppressors. However, there is still a lack of knowledge about the majority of tRNA modification enzymes in cancer. The second category, the ARSs, includes cytosolic ARSs (cy-ARSs) and mitochondrial ARSs (mt-ARSs). ARSs function to attach the appropriate amino acids to their respective unladen tRNAs, thereby initiating or elongating the peptide chain by recognizing the codon in the messenger RNA sequence. Cy-ARSs are involved in tumorigenesis through their interaction with aminoacyl tRNA synthetase-interacting multifunctional proteins to influence cancer cell proliferation and oncogenic transformation. Alterations in the expression of multiple cy-ARSs, such as CARS, IARS, and YARS, are involved in tumorigenesis by promoting oncogenic pathways, but it is unclear whether the mt-ARSs are altered and involved in tumorigenesis. The third category, translation factors, that mediate translational initiation and translational elongation, also play important roles in cancer. For example, EIF3H is up-regulated in prostate cancer, EIF4G is up-regulated in lung cancer, whereas EIF3F is down-regulated in melanoma and pancreatic cancer. Previous studies have generally described only one category of enzymes or even individual enzymes based on relatively small sample cohorts.

The UCSC Genome Atlas (TCGA) provides a uniquely comprehensive data resource, including ~10,000 human patients. In this study, we performed a comprehensive analysis elucidating a dynamic landscape of translational regulation, including tRNAs, tRNA modification enzymes, ARSs, and translation factors, across multiple cancer types in TCGA. Our results highlight a synergistic activation of the translational system in cancer.

Results

Expression landscape of tRNAs across 31 cancer types. We obtained tRNA annotations from the UCSC genome browser (http://genome.ucsc.edu/), including 604 tRNA transcripts, 62 codons, and 21 amino acids. We then mapped these reads obtained from miRNA-seq to tRNA annotation to infer the relative expression level of tRNAs. The tRNA expression data were merged to the codon level and amino acid level according to the anticodon and amino acid information (Supplementary Figure 1A). We first analyzed DM-tRNA-seq and miRNA-seq data from 293T cells to test our computational pipeline. Our analysis showed a high correlation at the tRNA level (Spearman’s correlation Rs = 0.73, p < 2 × 10−6), codon level (Rs = 0.61, p = 7.2 × 10−7), and amino acid level (Rs = 0.59, p = 4.1 × 10−3) (Supplementary Figure 1B) between both data types, indicating the reliability of our computational pipeline to infer the relative expression levels of tRNAs.

To comprehensively analyze the expression profiles of tRNAs from TCGA, we downloaded all miRNA-seq samples across 31 cancer types from the TCGA data portal (https://portal.gdc.cancer.gov/). After filtering out duplicated and low-quality samples, we retained 9931 cancer samples and 663 normal samples for analysis (Supplementary Figure 1C). The number of samples and detailed abbreviations for each cancer type are listed in Supplementary Figure 1D and Supplementary Table 1. We detected the expression of 490 distinct tRNA genes (trimmed mean of M values, TMM > 1) among the multiple cancer types. This figure accounts for 81.1% (490/604) of all annotated tRNA genes in the human genome. The average number of reads per detectable tRNA ranged from 2 to 9558, with the median as 141. The log2 expression values (log2 TMMs) of tRNA genes ranged from 0.13 to 14.36 with a median of 6.11 (5.86–6.40 for the different cancer types; Fig. 1a). The different cancer types showed strikingly similar overall average expression levels and patterns of tRNA expression (Fig. 1a). We were able to classify tRNAs into three groups by unsupervised clustering (Fig. 1b): 135 high-expression genes (cluster A), with a median expression of tRNA genes across cancer types ≥ 7.88; 200 medium-expression genes (cluster B), with median expression values between 4.99 and 7.88; and 155 low-expression genes (cluster C), with a median expression ≤ 4.99.

The primary function of tRNAs is to carry amino acids to the ribosome to initiate and elongate growing peptides. We analyzed tRNAs based on the amino acids they accepted. The number of tRNA genes detected for each amino acid ranged from two for selenocysteine (Sec) to 39 for leucine (Leu) (Supplementary Figure 2). The tRNA expression levels for each amino acid varied greatly. For example, all tRNA genes for histidine (His) were highly expressed, and more than 90% of tRNA genes for arginine (Arg), proline (Pro), aspartic acid (Asp), and the methionyl initiator of translation (tMet) were high or moderate expression. In contrast, more than 60% of tRNA genes for cysteine (Cys), asparagine (Asn), threonine (Thr), and methionine (Met) were low in expression. In particular, none of the tRNA genes for tryptophan (Trp), leucine (Leu), phenylalanine (Phe), Asn, or Sec were in the high-expression cluster (Fig. 1c). Taken together, our results reveal a diverse transcriptional landscape for different tRNAs at the tRNA level across multiple cancer types in more than 10,000 samples.

Alterations of tRNA gene expression across cancer types. To systematically understand the potential functions of tRNAs in tumorigenesis, we examined the differences in tRNA gene expression levels between paired tumor and normal samples. We identified a total of 474 differentially expressed tRNA genes (96.7% of the 490 detectable tRNA genes) across the 31 cancer types (Supplementary Data 1). There were 93 tRNAs with pervasive differential expression in at least eight cancer types (Fig. 2a). Among them, 66 tRNA genes were pervasively
up-regulated. The spectrum showed 18 tRNAArg, 16 tRNACys, and 7 tRNAVal with pervasive overexpression, as well as 25 tRNAs that carry 11 other amino acids. At the other end of the spectrum, 27 pervasively down-regulated tRNAs including six tRNAVal carrying nine amino acids. Of interest, tRNAVal showed up-regulation in nine cancer types and down-regulation in five cancer types, suggesting cancer-specific features of tRNAVal.

Interestingly, tRNAArg, tRNACys, and tRNAVal showed the highest percentage of altered expression across multiple cancer types (Fig. 2b), suggesting their functional roles in tumorigenesis. We observed 1518 (20.7%) up-regulated and 880 (12.0%) down-regulated tRNAs ([fold-change] ≥ 1.5, false discovery rate (FDR) < 0.05) across different cancer types (Supplementary Data 1). Nine cancer types, including bladder cancer (BLCA), uterine corpus endometrial carcinoma (UCEC), and breast cancer (BRCA), showed predominant up-regulation of tRNA expression. Six cancer types, including kidney chromophobe (KICH), cholangiocarcinoma (CHOL), and kidney renal clear cell carcinoma (KIRC), showed similar numbers of up-regulated and down-regulated tRNA genes (Fig. 2c). That observation suggests the overall overexpression of tRNAs at the tRNA level across multiple cancer types.

Alterations of tRNA gene expression at the codon level. tRNAs deliver amino acids to initiate and elongate peptide chains by recognizing specific codons. To further explore the possible functions of tRNAs in cancer, we merged tRNA genes to the codon level. Forty-five (72.5%) codons showed significantly differential expression ([fold-change] ≥ 1.5, FDR < 0.05) in at least one cancer type.
Eight cancer types (including BLCA, UCEC, and BRCA) showed predominantly up-regulated codons, whereas two cancer types (CHOL and liver hepatocellular carcinoma) showed predominantly down-regulated codons. Five cancer types (including LUSC, KICH, and KIRC) showed similar numbers of up- and down-regulated codons (Supplementary Figure 3A).

tRNAs for nine codons (represented here as tRNAamino acid (codon)) were pervasively up-regulated in at least five cancer types. Included were four Arg codons (tRNAArg(GTG), tRNAArg(AGA), tRNAArg(CGA)), tRNAArg(CGG)). Similarly, four tRNAs codons (tRNAThr(ACA), tRNAHis(CAC), tRNAVal(GAA), and tRNAArg(AGA)) were pervasively down-regulated (Fig. 3a).

To understand the effects of codon usage frequency on their expression, we used the observed-over-expected ratio (O/E ratio) to represent the codon usage frequency. Interestingly, those codons that tend to be overexpressed in cancer samples showed significantly lower O/E ratio than those codons that tend to be down-regulated (Fig. 3a, Student’s t test p = 0.043; Wilcoxon’s test p = 0.017), suggesting that over-expression of codons with low O/E ratio may overcome the bottleneck in tumor development.

We next asked whether tRNA expression at the codon level has prognostic value, and found that the expression of several codons was correlated with patient survival times across different cancer types (Supplementary Figure 3A, Cox’s model). For example, overexpression of multiple codons, including tRNAArg(GTG) (two-sided log-rank test, FDR = 7.3 x 10^{-5}) and tRNAArg(AGA) (FDR = 2.2 x 10^{-6}), were associated with worse survival in KIRC (Fig. 3b). In contrast, down-regulation of several codons, including tRNAThr(ACA) (FDR = 6.4 x 10^{-3}) and tRNAPro(CCA) (FDR = 5.8 x 10^{-6}), were associated with worse survival (Fig. 3b). These results suggest the possibility of tRNA expression levels to serve as a prognostic marker.
level and observed diverse patterns across different cancer types. tRNA\text{Arg} was pervasively up-regulated in eight cancer types, and tRNA\text{Asn} was up-regulated in five cancer types (Fig. 4a and Supplementary Data 3). Those findings are consistent with their up-regulation at the tRNA and codon levels. In contrast, tRNA\text{His} was down-regulated in at least five cancer types. tRNA\text{Ser}, tRNA\text{Thr}, tRNA\text{Pro}, and tRNA\text{Leu} showed no significant alterations of expression (Fig. 4a). Interestingly, tRNA\text{Val} showed significant up-
regulation in three cancer types and down-regulation in four cancer types, further confirming that tRNAVal may have divergent functions across different cancer types.

We analyzed the correlations between alterations at the amino acid expression level and the observed-over-expected ratio (O/E ratio) across cancer types. Interestingly, we observed a significantly negative correlation between expression alterations and the amino acid O/E ratio in multiple cancer types (Fig. 4b), such as in kidney renal papillary cell carcinoma (KIRP) ($R_s = -0.55, p = 0.01$, Supplementary Figure 4). Other studies have demonstrated that tRNAArg is up-regulated in cancer cells\[17,18\], and that overexpression of tRNAArg enhances the
ability of cancer cells to invade other tissues and metastasize. It apparently does so by increasing the codon-dependent translation and translation of genes with high Arg codon content. For example, tRNATyr was up-regulated in breast cancer, and the oncogene TERT protein was also up-regulated in breast cancer samples, while TERT has significantly higher Arg usage frequency (O/E = 1.98) compared to the genomic Arg usage frequency (O/E = 0.57). These results suggest that overexpression of a rare amino acid could facilitate the overexpression of the genes with high amino acid usage frequency, thus overcoming the bottleneck in tumor development.

In general, alterations at the tRNA level (defined as tRNA^amino acid (anticodon) will lead to alterations at the codon level (defined as tRNA^amino acid (codon)) and at the amino acid level (defined as tRNA^amino acid). For example, our results showed consistent up-regulation of tRNA^Arg at the tRNA level, codon level, and amino acid level, suggesting that they may function as oncogenes (Fig. 4c). We observed consistent down-regulation for tRNA^His and tRNA^Glu, suggesting that they might act as tumor suppressor genes. Despite connections among tRNAs, codons, and amino acids, the tRNA expression alterations at the three levels also appeared inconsistently with each other. That inconsistency is probably due to the uneven distribution of tRNAs and codons. Each amino acid has one (e.g., Trp, Met) to five (e.g., Arg, Leu, Ser) detectable codons (Supplementary Figure 5A), and each codon has 1 (e.g., tRNA^Cys(ACG)) to 31 (e.g., tRNA^Glu(GGA)) detectable tRNA genes (Supplementary Figure 5B). We observed expression alteration at the tRNA level but not in the codon or amino acid level for two possible reasons. First, alteration of tRNAs in opposite directions may lead to unaltered expression at the codon level or amino acid level. For example, tRNA^Tyr-GTA-5-3 and tRNA^Tyr-GTA-9-1 were up- regulated, whereas tRNA^Tyr-GTA-6-1 was down-regulated in BRCA, leading to no significant difference for tRNA^Tyr(TAC) and tRNA^Tyr (Fig. 4c and Supplementary Data 1–3). Second, the alteration of only a few tRNAs may not be sufficient to imply significant alterations at the codon or amino acid level. For example, in KIRC, we observed 3/16 (18.8%) up-regulated tRNA^Ala (AAG) but no alterations in the expression of tRNA^Ala (GCT) or tRNA^Ala (Fig. 4c and Supplementary Data 1–3). Through this comprehensive analysis, we revealed unequal alterations at multiple levels, which is largely due to the uneven distribution of tRNAs and codons.

**Dynamic landscape of enzymes involved in translation.** Numerous categories of enzymes, including tRNA modification enzymes, ARSs, and translation factors, are involved in translational regulation. To gain mechanistic insights into how these enzymes are altered in cancer, we examined the gene expression landscape and copy number variations (CNVs) of these enzymes. For tRNA modification enzymes, we observed a total of 97 up-regulated enzymes compared to a total of 30 down-regulated enzymes, suggesting the overall up-regulation of these enzymes across different cancer types (two-sided χ² test, p = 0.02, Fig. 5a, left panel; Supplementary Data 4). We found that 20/29 (69.0%) tRNA modification enzymes showed up-regulation in at least one cancer type (Fig. 5b). For example, METTL1 has been reported to promote lung cancer, while we observed significant overexpression of METTL1 in nine cancer types, suggesting it as the master oncogenic event. In addition, we identified several novel oncogenic enzymes with up-regulation in multiple cancer types, including PUS1, a tRNA pseudouridylate synthase, and TRMT1 and TRMT6, the tRNA methyltransferases. These enzymes play essential roles to maintain tRNA structure through modifying certain nucleotide residues. In contrast, several enzymes, including KIAA1456, RG9MTD2, and TRDMT1, showed down-regulation and can potentially act as tumor suppressors. Consistent with the overall overexpression pattern, we also observed overall amplification of CNV for tRNA modification enzymes, including those encoded by TRMT12, NSUN2, and TRMT6 (Supplementary Figure 6A). Taken together, the overall overexpression of tRNA modification enzymes may stabilize tRNAs to facilitate overexpression of tRNAs.

In analyzing 37 ARSs, including 20 cy-ARSs and 17 mt-ARSs, across different cancer types, we observed a total of 128 up-regulated and 9 down-regulated enzymes (two-sided χ² test, p = 1.7 × 10⁻⁹, Fig. 5a, middle panel). Specifically, we observed overall up-regulation across different cancer types, with 31 out of 37 ARSs (83.8%), exemplified by GARS and VARS, up-regulated in at least one cancer type (Fig. 5c). Among these, mt-ARSs, which have been neglected by previous studies, also showed abundant up-regulation across different cancer types. Consistent with the overall overexpression pattern, we also observed overall copy number amplifications for ARSs, including those encoded by TARS2, TARS, and DARS2 (Supplementary Figure 6B). In particular, GARS consistently showed overexpression and copy number amplification in colon adenocarcinoma (COAD), KICH, KIRP, lung adenocarcinoma, and rectum adenocarcinoma (READ). Interestingly, the isoenzymes in the cytoplasm and mitochondria may show very distinct expression patterns. For example, VARS is up-regulated in 10 cancer types, whereas VARS2 is only up-regulated in two cancer types. More interestingly, FARS2 is down-regulated in KICH, while the two paralog ARSs, FARS and FARS8, are up-regulated in six and four cancer types, respectively. The inconsistent between cy-ARSs and mt-ARSs may involve in metabolic pathway in tumor development. Further studies on ARSs, especially mt-ARSs are necessary to understand their roles in tumorigenesis. Taken together, the overexpression of ARSs may facilitate the accelerated charging process of tRNAs.

We further investigated the translation factors across cancer types and observed an overall overexpression and amplification pattern that 342 enzymes are up-regulated versus 201 down-regulated enzymes (two-sided χ² test, p = 2.1 × 10⁻⁵, Fig. 5a, right panel). We observed up-regulation of several enzymes, including EIF4EBP1 and EIF3B, and down-regulation, including EIF4E3 and EIF1AY (Fig. 5d). Interestingly, EEF1A2 has been reported to be overexpressed in multiple cancer types, including breast cancer and liver cancer, while our analyses showed that EEF1A2 is up-regulated in six cancer types, but strikingly down-regulated in...
four cancer types, namely COAD, esophageal carcinoma, head and neck squamous cell carcinoma, and stomach adenocarcinoma, suggesting a potential controversial function of EEF1A2 in these cancer types. Consistent with the overall overexpression pattern, we also observed overall copy number amplifications for translation factors, including EIF3H, EEF1D, and EIF3E (Supplementary Figure 7). Taken together, the overall overexpression of translation factors may promote the translational process. In summary, we showed overall overexpression and copy number amplification for tRNA modification enzymes,
Fig. 5 Expression alteration of tRNA modification enzymes, ARSs, and translation factors across cancer types. a Two-sided χ² test for up-regulated and down-regulated enzymes for tRNA modification enzymes (left), aminoacyl tRNA synthetases (middle), and translation factors (right). b Differentially expressed tRNA modification enzymes between paired tumor and normal samples. Magenta denotes up-regulation; blue denotes down-regulation. X-axis represents 16 cancer types with > 5 tumor-normal paired RNA-seq samples. Squares denote enzymes with expression alterations. c Differentially expressed aminoacyl tRNA synthetases (ARSs) between paired tumor and normal samples. Left panel, cytosolic ARSs (cy-ARSs); right panel, mitochondrial (mt-ARSs). The color bars in the middle panel summarize the comparisons between paralog ARSs. Squares denote enzymes with expression alterations. d Differentially expressed translation factors between paired tumor and normal samples. Squares denote enzymes with expression alterations.

Discussion

tRNAs can play important roles in cancer by accelerating the translational regulation and by supplying molecules that are in high demand for tumor metabolism14,15. Here, we developed a computational pipeline to infer the relative tRNA expression levels from miRNA-seq data from ~10,000 patient samples from TCGA across 31 cancer types. This is the first time to our knowledge that tRNAs have been analyzed in large-scale cancer samples, and provides a unique opportunity as the same samples have been comprehensively characterized by TCGA at the DNA, RNA, protein, pathological, and clinical levels. Our analysis achieved the highest resolution to date of tRNA expression to individual tRNAs, and showed overexpression of tRNAs across multiple cancer types. We further demonstrated the unequal expression of tRNAs at the tRNA, codon, and amino acid levels. First, tRNA alterations in opposite directions may compensate for alterations of tRNAs at the tRNA, codon, and amino acid levels. Furthermore, the O/E ratios of amino acids tend to be negatively correlated with alterations in expression between tumor and normal samples. These observations suggest that overexpression of tRNAs from codons and amino acids with low O/E ratios may overcome a bottleneck posed to tumor development by the process of increased protein translation. Furthermore, overexpression of tRNAArg will promote breast cancer metastasis17, while we observed overexpression of tRNAArg in multiple cancer types. Our work laid the groundwork for an integrated functional interpretation to illuminate the functional roles of tRNAs.

Finally, we identified a series of enzymes with alterations across multiple cancer types, including unprecedented tRNA modification enzymes, such as those encoded by PUS1, TRMT1, and TRMT6. Particularly, we revealed the global gene overexpression of mt-ARSs, and individual mt-ARS exhibit divergent alterations with their cytosolic paralogs. More interestingly, we observed global overexpression and amplification of tRNAs, tRNA modification enzymes, ARSs, and translation factors. Thus, overexpression of tRNA modification enzymes stabilize tRNAs in order to increase the expression level of tRNAs. Overexpression of tRNAs and ARSs may accelerate the process of aminoacyl tRNA synthesis. Overexpression of translation factors may accelerate the translational initiation and translational elongation. Taken together, overexpression of tRNAs and enzymes involved in translational regulation highlights the synergistic activation of protein translation in cancer.

Methods

Quantitation of tRNA expression across different cancers. Supplementary Figure 1 shows a schematic of the overall analysis pipeline. The TCGA data portal was accessed to download miRNA-seq data 16,591 samples (https://portal.gdc.}

Fig. 6 Synergistic activation of translation system in cancer. Overall overexpression of tRNAs, tRNA modification enzymes, ARSs, and translation factors across different cancer types, suggests the synergistic activation of translation system in cancer. Magenta and blue arrows denote number of up-regulated and down-regulated tRNA/enzyme, respectively.

ARs, and translation factors across multiple cancer types, which may play synergistic roles with the overall overexpression of tRNAs (Fig. 6).
We considered FDR < 0.05 as statistically significant.

In brief, we calculated the reads counts (\(N_k\)) for each gene and the total number of reads (\(N\)) for each sample. TMM introduced M value as

\[
M_k = \log_2 \left( \frac{N_k}{\sum_{k'} w_{kk'} N_{k'}} \right).
\]

then was estimated as

\[
\text{log}(\text{TMM}) = \sum_{k} w_{kk} \log(\text{reads}) \cdot \frac{N_k}{\sum_{k'} w_{kk'} N_{k'}}.
\]

TMM is implemented as the TMM module in the edgeR R Biocductor.

\(\text{tRNAs with an average TMM} > 1\) across samples in each cancer type were defined as detectable tRNAs (Supplementary Figure 1). We downloaded the mRNA expression data for DM-IRNA-seq in cell line from gene expression omnibus (GEO, GSE7259). We measured distance by Euclidean distance based on mRNA expression, and then classified tRNAs using unweighted pair-group method with arithmetic mean methods.

Analysis of tRNA expression at tRNA, codon, and amino acid levels. We used paired Student's t test to perform differentially expressed analyses in those cancer types with \(\geq 5\) paired tumor and normal samples, which normal samples are extracted from the adjacent tissues (Supplementary Table 1). For each cancer type, we estimated the \(p\) value of each tRNA between tumor and normal samples and then adjusted the \(p\) value by FDR (Benjamini–Hochberg procedure). We identified differentially expressed tRNAs with \([\text{fold-change}] \geq 1.5\) and FDR < 0.05 considered to be significantly up-regulated or down-regulated. The detailed information for accepted amino acid accepted by each codon can be viewed at http://www.cbs.dtu.dk/courses/27619/codon.html. We then merged tRNA expression at codon and amino acid levels based on strict Watson-Crick match considering \([\text{fold-change}] \geq 1.5\) and FDR < 0.05 to be significant. By downloading the original data, the interested reader can reproduce the calculations for other degrees of stringency. Cancer types with more up-regulated or down-regulated tRNAs, codons, and amino acids \((\text{fold-change} \geq 1.5)\) were considered as pre-dominantly up-regulated or down-regulated cancer types, respectively. The O/E ratio was estimated by observed value/expected value (http://www.tiem.utk.edu/~gross/bioed/webmodules/aminoacid.html). The observed value is the frequency of an amino acid or codon in the human genome. The expected frequency of a particular codon can be calculated by multiplying the frequencies of each DNA base comprising the codon. The expected frequency of the amino acid can then be calculated by adding the frequencies of each codon that codes for the amino acid.

Overall survival times for patient samples were obtained from TCGA's data portal (https://tcga-data.nci.nih.gov/tcga). We used the univariate Cox model to test relationship between overall survival time and tRNA expression. We also used two-sided log-rank model to test the difference of survival time between two groups, for example, high tRNA expression group and low tRNA expression group. We considered FDR < 0.05 as statistically significant.

Analysis of enzymes involved in translational regulation. tRNA modification enzymes were collected from the Modomics database (http://modomics.genesilico.pl)\(^{64}\) and from the literature\(^{63,65}\). ARS and translation factors were collected from previous studies\(^{89,90,91}\).

Gene expression and CNV data were downloaded from TCGA's data portal (https://tcga-data.nci.nih.gov/tcga). Genes were considered to be differentially expressed if the \([\text{fold-change}] \geq 1.5\) and FDR < 0.05. TCGA CNV scores for each gene were downloaded from a previous study\(^{70}\). CNV score > \(\log(3)\) or < \(\log(1)\) were defined as gain or loss, respectively\(^{70}\).

Code availability. Custom scripts are available upon request.

Data availability

All datasets of the current study are freely available in Synapse (https://www.synapse.org, syn3867000).

Received: 19 July 2018 Accepted: 27 November 2018
Published online: 21 December 2018

References
1. Ewing, B. & Green, P. Analysis of expressed sequence tags indicates 35,000 human genes. Nat. Genet. 25, 232–234 (2000).
2. van’t Veer, L. J. et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 415, 530–536 (2002).
3. Byron, S. A., Van Keuren-Jensen, K. R., Engelthaler, D. M., Carpten, J. D. & Craig, D. W. Translating RNA sequence into clinical diagnostics: opportunities and challenges. Nat. Rev. Genet. 17, 257–271 (2016).
4. Veleculescu, V. E., Zhang, L., Vogelestein, B. & Kinzler, K. W. Serial analysis of gene expression. Science (80-.). 270, 484–487 (1995).
5. Dever, T. E. & Green, R. The elongation, termination, and recycling phases of translation in eukaryotes. Cold Spring Harb. Perspect. Biol. https://doi.org/10.1101/cshperspect.a013706, 1–16 (2012).
6. Kirchner, S. & Ignatova, Z. Emerging roles of tRNA in adaptive translation, signalling dynamics and disease. Nat. Rev. Genet. 16, 98–112 (2015).
7. Lander, E. S. et al. Initial sequencing and analysis of the human genome. Nature 409, 860–921 (2001).
8. Lowe, T. M. & Eddy, S. R. TRNasc: SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25, 955–964 (1996).
9. Bild, A. H. et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature 439, 353–357 (2006).
10. Felton-Edkins, Z. A. et al. The mitogen-activated protein (MAP) kinase ERK induces tRNA synthesis by phosphorylating TFIIB. EMBO J. 22, 2422–2432 (2003).
11. White, R. J. Direct activation of RNA polymerase III transcription by c-Myc. Nature 421, 1698–1701 (2003).
12. Kantidakis, T., Ramsbottom, B. A., Birch, L., Dowding, S. N. & White, R. J. Correction for Kantidakis et al., mTOR associates with TFIIIC, is found at tRNA and 35 RNA genes, and targets their repressor Maf1. Proc. Natl. Acad. Sci. USA. 109, 11,465–11,467 (2012).
13. Wei, Y., Tsang, C. K. & Zheng, X. F. S. Mechanisms of regulation of RNA polymerase III-dependent transcription by TORC1. EMBO J. 28, 2220–2230 (2009).
14. Grewal, S. S. Why should cancer biologists care about tRNAs? TRNA synthesis, mRNA translation and the control of growth. Biochem. Biophys. Acta 1849, 898–907 (2014).
15. Tuitt, M. L. & Ruggero, D. New frontiers in translational control of the cancer genome. Nat. Rev. Cancer 16, 288–304 (2016).
16. Zheng, G. et al. Efficient and quantitative high-throughput tRNA sequencing. Nat. Methods 12, 835–837 (2015).
17. Goodarzi, H. et al. Modulated expression of specific tRNAs drives gene expression and cancer progression. Cell 165, 1416–1427 (2016).
18. Pavon-Eternod, M. et al. tRNA over-expression in breast cancer and functional consequences. Nucleic Acids Res. 37, 7268–7280 (2009).
19. Parnell, J. L. & Hueyns, M. A. Clustering of codons with rare cognate tRNAs in human genes suggests an extra level of expression regulation. PLoS Genet. 5, e1000121 (2009).
20. Evans, M. E., Clark, W. C., Zheng, G. & Pan, T. Determination of tRNA aminoclaylation levels by high-throughput sequencing. Nucleic Acids Res. https://doi.org/10.1093/nar/gkx514, 1–8 (2017).
21. Dai, Q., Zheng, G., Schwartz, M. H., Clark, W. C. & Pan, T. Selective enzymatic demethylation of N2,N2-dimethylguanosine in RNA and its application in high-throughput tRNA sequencing. Angew. Chem. Int. Ed. 56, 5017–5020 (2017).
22. Guo, Y. et al. A micro-tRNA expression signature for human NAFLD progression. J. Gastroenterol. 51, 1022–1030 (2016).
23. Pundhir, S. & Gorodkin, J. Differential and coherent processing patterns from small RNAs. Sci. Rep. 5, 12,062 (2015).
24. Pang, Y. L. J., Abe, R., Levine, S. S. & Dedon, P. C. Diverse cell stresses induce unique patterns of tRNA up- and down-regulation: tRNA-seq for quantifying changes in tRNA copy number. Nucleic Acids Res. 42, e170 (2014).
25. Krishnan, P. et al. Genome-wide profiling of transfer RNAs and their role as novel prognostic markers for breast cancer. Nat. Publ. Gr. https://doi.org/10.1038/srep32843, 1–12 (2016).
26. Beck, D., Ayers, S. & Wen, J. Integrative analysis of next generation sequencing for small non-coding RNAs and transcriptional regulation in myelodysplastic syndromes. BMC Med. Genom. 4, 19 (2011).
27. Danielson, K. M., Rubio, R., Abderazzaq, F., Das, S. & Wang, Y. E. High throughput sequencing of extracellular RNA from human plasma. PLoS ONE 12, 1–18 (2017).
28. Guo, Y. et al. Transfer RNA detection by small RNA deep sequencing and disease association with myelodysplastic syndromes. BMC Genom. 16, 727 (2015).
29. Zhong, J. et al. Transfer RNAs mediate the rapid adaptation of Escherichia coli to oxidative stress. PLoS Genet. 11, 1–24 (2015).
30. Phizicky, E. M. & Alfonzo, J. D. Do all modifications benefit all tRNAs? FEBS Lett. 584, 265–271 (2010).
31. Torres, A. G., Batlle, E. & Ribas de Pouplana, L. Role of tRNA modifications in human diseases. *Trends Mol. Med.* 20, 306–314 (2014).

32. Frye, M. & Watt, F. M. The RNA methyltransferase Mts2 (NSun2) mediates Myc-induced proliferation and is upregulated in tumors. *Curr. Biol.* 16, 971–981 (2006).

33. Vachon, C. M. et al. Strong evidence of a genetic determinant for mammographic density, a major risk factor for breast cancer. *Cancer Res.* 67, 8412–8418 (2007).

34. Mantripragada, K. et al. Telomerase activity is a biomarker for high grade malignant peripheral nerve sheath tumors in neurofibromatosis type 1 individuals. *Genes Chromosomes Cancer* 47, 238–246 (2008).

35. Bartlett, J. M. et al. Mammotest as a tool to stratify breast cancer patients at risk of recurrence during endocrine therapy. *Breast Cancer Res.* 12, R47 (2010).

36. Bogley, U. et al. Human tRNA methyltransferase Nde1 protein prevents tumour growth by regulating LIN9 and HIF1-a. *EMBO J.* 35, 366–383 (2016).

37. Berg, M. et al. Distinct high resolution genome profiles of early onset and late onset colorectal cancer integrated with gene expression data identify candidate susceptibility loci. *Mol. Cancer* 9, 100 (2010).

38. Schäfer, M., Hagemann, S., Hanna, K. & Lyko, F. Azacytidine inhibits RNA methylation at DNMT2 target sites in human cancer cell lines. *Cancer Res.* 69, 8127–8132 (2009).

39. Kim, S., You, S. & Hwang, D. Aminoacyl-tRNA synthetases and tumorigenesis: more than housekeeping. *Nat. Rev. Cancer* 11, 708–718 (2011).

40. Beltran, A. S., Graves, L. M. & Blancafort, P. Novel role of engrailed 1 as a prosurvival transcription factor in basal-like breast cancer and engineering of interference peptides block its oncogenic function. *Oncogene* 33, 1–11 (2013).

41. Park, S. G., Schimmel, P. & Kim, S. Aminoacyl-tRNA synthetases and their connections to disease. *Proc. Natl Acad. Sci. USA* 105, 11043–11049 (2008).

42. Ray, P. S. & Fox, P. L. A post-transcriptional pathway represses monocyte VEGF-A expression and angiogenic activity. *EMBO J.* 26, 3360–3372 (2007).

43. Hershey, J. W. B. & Merrick, W. C. The pathway and mechanism of initiation of protein synthesis. *Transl. Control Gene Exp.* [https://doi.org/10.1101/087969618.39.33] (2000).

44. Saramäki, O. et al. Amplification of EIF3S3 gene is associated with advanced stage in prostate cancer. *Am. J. Pathol.* 159, 2089–2094 (2001).

45. Furey, M. & Watt, F. M. The RNA methyltransferase Misu (NSun2) mediates Myc-induced proliferation and is upregulated in tumors. *Curr. Biol.* 16, 971–981 (2006).

46. Shi, J. et al. Decreased expression of eukaryotic initiation factor 3f deregulates cancer-relevant splicing networks. *Clin. Transl. Control Gene Expr.* [https://doi.org/10.1101/2018.11.20.19.2612] (2018).

47. Doldan, A. et al. Loss of the eukaryotic initiation factor 3f in melanoma. *Cancer Cell* 28, 2094–2105 (2015).

48. Maza, E. In papyro comparison of TMM (edgeR), RLE (DESeq2), and MRN normalization methods for a simple two-conditions-without-replicates RNA-seq experimental design. *Front. Genet.* 7, 164 (2016).

49. D’Haeseleer, P. How does gene expression clustering work? *Nat. Biotechnol.* 28, 1499–1501 (2010).

50. Ibba, M. & Söll, D. Aminoacyl-tRNAs: setting the limits of the genetic code. *Trends Mol. Med.* 11, 366–383 (2005).

51. Gong, J. et al. A pan-cancer analysis of the expression and clinical relevance of cancer susceptibility loci. *Nat. Genet.* 47, 1113–1120 (2013).

52. Rnas, S. et al. LTR-retrotranspon control by RNA-derived small RNAs. *Cell* 170, 61–71.e11 (2017).

53. Iba, M. & Soll, D. Aminoacyl-tRNAs: setting the limits of the genetic code. *Genes Dev.* 18, 731–738 (2004).

54. Dever, T. E. & Green, R. The elongation, termination, and recycling phases of translation in eukaryotes. *Cold Spring Harb. Perspect. Biol.* 4, 1–16 (2012).

55. Ducrest, A. L., Szutorisz, H., Lingner, J. & Nabholz, M. Regulation of the human telomerase reverse transcriptase gene. *Oncogene* 21, 541–552 (2002).

56. Wikman, H. et al. CDK4 is a probable target gene in a novel amplicon at 12q13.3-q14.1 in lung cancer. *Genes Chromosomes Cancer* 42, 193–199 (2005).

57. Kulkarni, G. et al. Expression of protein elongation factor eEF1A2 predicts favorable outcome in breast cancer. *Breast Cancer Res. Treat.* 102, 31–41 (2007).

58. Pellegrino, R. et al. EEF1A2 inactivates p53 by way of PI3K/AKT/mTOR-dependent stabilization of MDM4 in hepatocellular carcinoma. *Hepatology* 59, 1886–1899 (2014).

59. Li, H. et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079 (2009).

60. Robinson, M. & Oshlack, A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol.* 11, R25 (2010).

61. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139–140 (2009).

62. Maza, E. In papporo comparison of TMM (edgeR), RLE (DESeq2), and MRN normalization methods for a simple two-conditions-without-replicates RNA-seq experimental design. *Front. Genet.* 7, 164 (2016).

63. D’Haeseleer, P. How does gene expression clustering work? *Nat. Biotechnol.* 28, 1499–1501 (2010).

64. Machnicka, M. A. et al. MODOMICS: a database of RNA modification pathways—2013 update. *Nucleic Acids Res.* 41, 262–267 (2013).

65. Calvi, J. M. & Eisenberg, E. Do all modifications benefit all tRNAs? *Eric. Biophys. Chem.* 257, 2432–2437 (2010).

66. Silvera, D., Formenti, S. C. & Schneider, R. J. Translational control in cancer etiology. *Nat. Rev. Cancer* 10, 254–266 (2010).

67. Tuller, T. The effect of dysregulation of tRNA genes and translation efficiency mutations in cancer and neurodegeneration. *Front. Genet.* 3, 1–3 (2012).

68. Biffó, S., Manfrini, N., Ricciardi, S., Gebauer-Hernandez, F. & Abdel-Wahab, O. Crosstalks between translation and metabolism in cancer. *Curr. Opin. Genet. Dev.* 48, 75–81 (2018).

69. Langerhans, E. et al. The pathogenic role of tissue-resident immune cells in psoriasis. *Trends Immunol.* 117, 2385–2388 (2007).

70. Sebestyén, E. et al. Large-scale analysis of genome and transcriptome alterations in multiple tumors unveils novel cancer-relevant splicing networks. *Genome Res.* 26, 732–744 (2016).

Acknowledgements

We gratefully acknowledge contributions from TCGA Research Network. This work was supported by Cancer Prevention and Research Institute of Texas (RP150085 to L.H.); UTHealth Innovation for Cancer Prevention Research Training Program Post-doctoral Fellowship (Cancer Prevention and Research Institute of Texas, RP160015); National Institute of Health (NIGMS R01GM115431 to J.L.); Cancer Prevention and Research Institute of Texas, RP160015; National Institute of Health (NIGMS R01GM115431 to J.L.); China Scholarship Council (201606160508 to C.-J.L.); National Natural Science Foundation of China (31771458 to A.G.). We thank LeeAnn Chastain for editorial assistance.

Author contributions

I.H. conceived and supervised the project. Z.Z., Y.Y., J.G., C.-J.L., H.R., A.-Y.G., Y.X., C.C., L.D., J.L., and L.H. performed the research. Z.Z., L.D., J.N.W., and L.H. wrote the manuscript with input from all other authors.

Additional information

Supplementary information accompanies this paper at https://doi.org/10.1038/s42003-018-0239-8.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.