Original Article

TiRNA-Gly-GCC-002 is associated with progression in patients with hepatocellular carcinoma

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Background: The transfer RNA (tRNA)-derived fragments, generated by the cleavage of mature and pre-tRNAs, play a vital role in the tumorigenesis and progression of hepatocellular carcinoma (HCC). However, the relationship between tRNA-derived fragments and the prognosis of patients with HCC has not been thoroughly studied. This study aims to discuss the relationship between tiRNA-Gly-GCC-002 and the prognosis of HCC patients and its role in guiding HCC treatment.

Methods: In this study, the differently expressed tRNA-derived fragments were screened out from the tumor tissues and paracancerous tissues. These tRNA-derived fragments were validated in the tissues and serum samples of patients with HCC by quantitative real-time polymerase chain reaction (qRT-PCR). The target genes of the tRNA-derived fragments were predicted with the microRNA target prediction database (miRDB), which was proceeded with gene set enrichment analysis (GSEA). After that, we analyzed the prognostic effect of the tRNA-derived fragment in relapse-free survival (RFS). Based on univariate and multivariate Cox regression analysis, independent prognostic factors for RFS were obtained. In addition, a column chart was constructed based on clinical pathological features and tiRNAGly-GCC-002.

Results: The tiRNA-Gly-GCC-002 was ultimately served as the candidate gene. Function analysis indicated that tiRNA-Gly-GCC-002 was primarily involved in adenyl nucleotide binding, cell cycle, cell cycle process and chromosome organization. We found that patients with high expression level of tiRNA-Gly-GCC-002 had worse prognosis than low expression level. The univariable and multivariable Cox regression analyses showed that tiRNAGly-GCC-002 was an important prognostic factor. Furthermore, the nomogram by combining tiRNA-Gly-GCC-002 expression level (P=0.03) and serum gamma-glutamyl transferase (GGT) level (P=0.001) was established to predict the prognosis of patients with HCC [concordance index (C-index): 0.789].

Conclusions: In summary, the tiRNA-Gly-GCC-002 can predict the outcome of patients with HCC, which may play a vital role in directing the treatment of HCC.

Keywords: tiRNA-Gly-GCC-002; hepatocellular carcinoma (HCC); predict; prognosis; nomogram

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Introduction

Hepatocellular carcinoma (HCC), the sixth most common cancer worldwide, is a highly aggressive and fatal malignancy (1). The etiology and progression of HCC are attributed to various factors, including alcohol consumption, hepatitis B virus (HBV) infection, and hepatitis C virus (HCV) infection (2). Currently, liver transplantation, local ablation, and surgical resection are effective treatments for early-stage HCC. In 2019, 42,030 individuals were diagnosed with HCC, and 31,780 succumbed to the disease in the United States (3). It was reported that the mortality to occurrence rate ratio was 95%, which showed an extremely bad prognosis (4). In recent years, with the extensive employment of the sequencing technology, a good deal of studies indicated that molecular pathological subtyping and mutations of tumor driving genes distinctly influence the outcomes of patients with HCC (5-7). However, the potential molecular circadian mechanism of the tumor growth and progression still remains unclear (8). Therefore, identifying novel perspectives for research is essential for more accurately predicting HCC prognosis.

For the past few years, researchers have demonstrated that dysregulated gene could affect the prognosis of patients with HCC. For example, six genes (*BAG6*, *PGBD3*, *RNF5*, *UTP11*, *PGM5P3-AS1*, and *KCND2*) were identified to be associated with prognosis of HCC (9). However, due to the lack of sufficient validation, these gene signatures have not been extensively applied in the clinical medicine, making prognosis still being poor (10). Transfer RNA (tRNA) plays a critical role in tumor genesis and progression. Specific tRNAs can drive the expression of oncogenes, thereby influencing patient outcomes (11). The upregulated expressive level of some tRNAs (methionine tRNA) could contribute to the tumorigenesis (12). Recently, a new kind of small non-coding RNAs (sncRNAs) has been identified, which is generated by the characteristic cleavage of mature and pre-tRNAs and called tRNA-derived fragments (13,14). According to the diverse cleavage sites of the mature and pre-tRNAs, tRNA-derived fragments can be divided into two groups, including tRNA-derived small RNA fragments (tRFs) and tRNA halves (tiRNAs) (15). For the past few years, it has been indicated that tRNA-derived fragments are involved in the cell biology functions regulation by impacting cell metabolism, proliferation, apoptosis and differentiation (16). The relationship between tumor and tRNA-derived fragments has gradually come into researchers' vision and some prominent development has been produced (17). Some studies have shown that particular tRNA-derived fragments could promote the expression of oncogene (18,19). The upregulation of expression levels of tRNA-derived fragments may contribute to the development of tumors; and tumor cells can affect the progress of tumors through regulating the tRNA-derived fragments expression level (20,21). Furthermore, the tRNA-derived fragments have also been proved as the crucial biomarker for the tumor (22). However, the association between HCC and tRNA-derived fragments expression maladjustment is currently poorly understood. So far, the association between the prognosis of HCC and tRNA-derived fragments has rarely been reported.

In this research, we first examined the expression profile of tRNA-derived fragments in tumor tissues and paracancerous tissues. The differentially expressed tRNA-derived fragments were further validated and the tiRNA-Gly-GCC-002 was selected as the candidate gene. Additionally, we predicted the target gene of tiRNA-Gly-GCC-002 and conducted a functional enrichment analysis. We also assessed the prognostic potential of this tRNA-derived fragment and established a prognostic model by combining it with clinical information for HCC. We hope that this tRNA-derived-fragments-based prognosis model could be applied to instruct individualized therapy for HCC. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-644/rc).

Methods

Patients and database

We randomly selected 70 HCC patients who underwent surgical resection at The First Affiliated Hospital of Wenzhou Medical University and Peking University Shenzhen Hospital. The clinicopathological information of the 70 HCC patients is shown in the *Table 1*. Collected clinical data included sex, age, HBV status, cirrhosis, alpha-fetoprotein (AFP) level, alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, gamma-glutamyl transferase (GGT) level, tumor stage, tumor grade, and microvascular invasion (MVI). All patients were followed up regularly. Follow-up was in the form of patient outpatient visits and telephone calls. The survival data recorded were relapse-free survival (RFS) and this research was in agreement with the Committee for Ethical Review of Research. The date from the first surgery until

Table 1 Clinicopathological characteristics of 70 liver cancer patients

Parameters	N
Sex	
Male	62
Female	8
Age (years)	
≤60	36
>60	34
HBV	
Yes	50
No	20
Cirrhosis	
Yes	46
No	24
AFP (ng/L)	
≤25	37
>25	30
ALT (U/L)	
≤40	38
>40	32
AST (U/L)	
≤40	34
>40	36
GGT (U/L)	
≤50	24
>50	39
Grade	
I	26
II	26
III	16
Stage	
T1	36
T2	14
T3	14
T4	1
MVI	
Yes	51
No	19

A few patients' clinical pathological data do not include AFP (ng/L), GGT (U/L), grade, and stage. Therefore, the total number of corresponding items is less than 70 cases. HBV, hepatitis B virus; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; MVI, microvascular invasion.

first recurrence or death was defined as RFS. In addition, the gene expression profiles of 424 HCC samples were downloaded from The Cancer Genome Atlas (TCGA) database (23). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by The First Affiliated Hospital of Wenzhou Medical University (No. 2020023) and Peking University Shenzhen Hospital (No. 2023132), and individual consent for this retrospective analysis was waived.

The selection of the candidate gene

Total RNA from tumor tissues and paracancerous tissues of 6 patients were extracted and quantified through the NanoDrop ND-1000 instrument. Then, we preprocessed the RNA samples to remove certain RNA modifications, which could interfere with the establishment of the small RNA-seq library. Total RNA after preprocess of each sample was applied to construct tRF & tiRNA-seq library. The library construction procedure was as follows: (I) 3'-adapter ligation; (II) 5'-adapter ligation; (III) complementary DNA (cDNA) synthesis; (IV) polymerase chain reaction (PCR) amplification; (V) filtration of the 134–160 bp PCR amplified fragments (corresponding to the 14–40 nt small RNAs). Agilent 2100 Bioanalyzer was used to quantify the completed libraries. The DNA fragments in the libraries were denatured into single-stranded DNA molecules using 0.1 M NaOH and diluted to a loading concentration of 1.8 pM. Based on the manufacturer's instructions, the diluted libraries were loaded onto reagent cartridge and the sequencing run was carry out on NextSeq system using NextSeq 500/550 V2 kit. Sequencing was performed by running 50 cycles. According to the sequencing result, we filtered the differentially expressive tRNA-derived fragments between tumor tissues and paracancerous tissues ($P<0.05$). Then, we further selected genes from the above differentially expressive tRNA-derived fragments [$|\log_2$ fold change (FC)$|$ >1]. Based on the specific primers of these tRNA-derived fragments, the obtained tRNA-derived fragments were validated in 6 pairs of tumor tissues and paracancerous tissues through quantitative real-time polymerase chain reaction (qRT-PCR). The up-regulated tRNA-derived fragments in the tumor tissues were selected. The up-regulated tRNA-derived fragments were further validated using qRT-PCR in the serum samples of 10 hepatitis samples and 10 cancer samples, which was included above 6 patients. The final candidate gene was obtained by the

P value <0.05. The expression level of this candidate gene was measured in the serum samples of the other 60 samples by qRT-PCR.

qRT-PCR

According to the manufacturer's protocol, total RNA was extracted using TRIzol (Invitrogen, California, USA). The tRNA-derived fragments are heavily modified by RNA modifications. The rtStar tRF & tiRNA Pretreatment Kit (Arraystar, California, USA) was used to remove these modifications to avoid interference with small RNA cDNA library construction. According to the manufacturer's instructions of the rtStar™ First-Strand cDNA Synthesis Kit (Arraystar), cDNA was synthesized. Based on the 2x PCR master mix (Arraystar), the qRT-PCR was proceeded with the ViiA 7 Real-time PCR System. The Primer 5.0 was used to design the primers and the sequences of primers are shown in the Table S1. The tRNA-derived fragments expression levels were calculated through the ΔΔCt way.

Functional enrichment analysis

The target genes of the tRNA-derived fragments (tRF-Ala-AGC-060, tiRNA-Gly-GCC-002, tRF-Ala-AGC-010, tRF-Leu-AAG-001 and tRF-Gly-GCC-011) were predicted by the microRNA target prediction database (miRDB). In order to explore the potential function of the tRNA-derived fragments, Gene Ontology (GO), analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of tRNA-derived fragments target genes were proceeded by the 'clusterProfiler' package. Gene set enrichment analysis (GSEA) of tiRNA-Gly-GCC-002 was also performed using Molecular Signatures Database (MSigDB C2 and C5 databases) (24).

Prognostic role of candidate gene and statistical analysis

The expression levels of the tiRNA-Gly-GCC-002 were regarded as the risk score of patients with HCC. The optimal cutoff value of the risk score was obtained through the R package called 'survminer'. According to this optimal cutoff value, all HCC patients were ultimately divided into the high-risk group or low-risk group. Kaplan-Meier (KM) analysis was performed using the 'survival' package in R software. In order to confirm whether the tiRNA-Gly-GCC-002 was an independent prognosis biomarker of HCC patients, the univariable and multivariable Cox regression analyses of tiRNA-Gly-GCC-002 expression levels and clinicopathological information were proceeded. Receiver operating characteristic (ROC) analysis was also used to evaluate the sensitivity and specificity of tiRNA-Gly-GCC-002 in prognostic prediction using the 'survivalROC' package in R. At the same time, the area under the curve (AUC) was gained via the above ROC curve (25). The image of these results of the tiRNA-Gly-GCC-002 prognostic effect was acquired using the 'ggplot2' package in R. In addition, based on the factors of the P values <0.05 in multivariate Cox regression analysis, we constructed a nomogram to predict the survival probability of patients with HCC, which contained the tiRNA-Gly-GCC-002 expression level and clinicopathologic factors. The nomogram prediction performance was demonstrated with concordance index (C-index) by R package called 'rms'. We applied the R version 3.6.0 software.

Results

Differential expression of tRNA-derived fragments in tumor tissues and paracancerous tissues

Of the 70 cases, we quantitatively assessed the expression levels of tRNA-derived fragments in 6 pairs of tumor and paracancerous tissues to identify differentially expressed tRNA-derived fragments. All significantly differential expressed tRNA-derived fragments are shown in the *Figure 1A*, containing 55 upregulated tRNA-derived fragments and 95 downregulated tRNA-derived fragments. According to the $|\log_2 FC| > 1$ and genetic stability, 9 tRNA-derived fragments were selected (*Figure 1B*). After that, we verified the expression levels of these tRNA-derived fragments in the tissues of 6 patients using PCR. The result revealed that 5 tRNA-derived fragments (tRF-Ala-AGC-060, tiRNA-Gly-GCC-002, tRF-Ala-AGC-010, tRF-Leu-AAG-001 and tRF-Gly-GCC-011) were significantly higher in the tumor tissues versus paracancerous tissues (*Figure 1C*). Then, 5 tRNA-derived fragments were further validated in the blood sample, including 10 cancer samples and 10 hepatitis samples and the tiRNA-Gly-GCC-002 had the higher expression in the HCC (*Figure 1D*). Therefore, the tiRNA-Gly-GCC-002 acted as the final candidate gene.

Function analysis of the tRNA-derived fragments

To evaluate the main functions of the tRNA-derived fragments, GO, KEGG, and GSEA analysis were

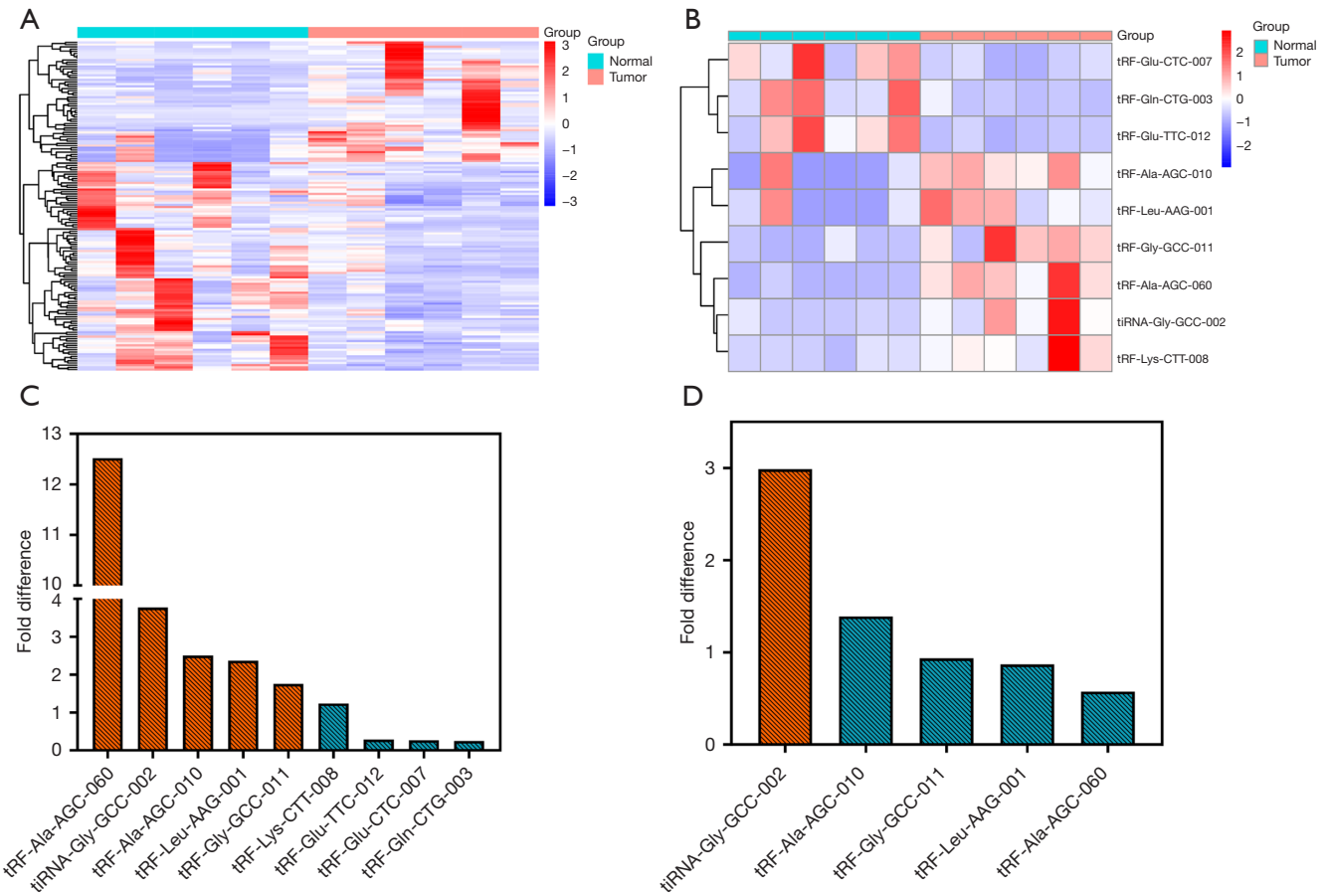

Figure 1 The differential expressed tRNA-derived fragments. (A) Heatmap of all differential expressed tRNA-derived fragments of tumor tissues and paracancerous tissues. (B) Heatmap of nine differential expressed tRNA-derived fragments of tumor tissues and paracancerous tissues. (C) The fold difference of 9 tRNA-derived fragments in tumor tissues and paracancerous tissues. The tRNA-derived fragments with highly expressed in cancer were marked orange. (D) The fold difference of 5 tRNA-derived fragments in cancer and hepatitis blood sample. Orange indicates that the difference was significant. tRNA, transfer RNA.

performed. According to the miRDB database, we predicted the target genes of the above 5 tRNA-derived fragments and 1,384 target genes were obtained. The GO enrichment analysis indicated that the genes were significantly enriched in the synapse organization, regulation of cell morphogenesis, presynapse and proximal promoter sequence-specific DNA binding (Figure S1A). The KEGG enrichment analysis indicated that these genes were mainly enriched in the Herpes simplex virus 1 infection (Figure S1B). Additionally, GSEA analysis of 236 target genes of tiRNA-Gly-GCC-002 based on the TCGA database revealed that adenyl nucleotide binding, cell cycle, cell cycle process and chromosome organization were enriched in the patients with HCC (*Figure 2*).

tiRNA-Gly-GCC-002-based expression level predicting RFS

In order to explore whether the tiRNA-Gly-GCC-002 could predict the prognosis of the patients with HCC, we examined the expression level of tiRNA-Gly-GCC-002, which served as the risk score. Using the survminer package, 1.24 was identified as the optimal cutoff value. Consequently, patients were divided into high-risk and low-risk groups based on this cutoff (*Figure 3A*). *Figure 3B* shows the survival time and survival status of patients. We found that the patients in the low-risk group had worse outcomes than the high-risk group [P=0.002; hazard ratio (HR) =2.7; 95% confidence interval (CI): 1.1, 6.6] (*Figure 3C*). In addition, based on clinical information, patients were divided into different groups to validate the prognostic

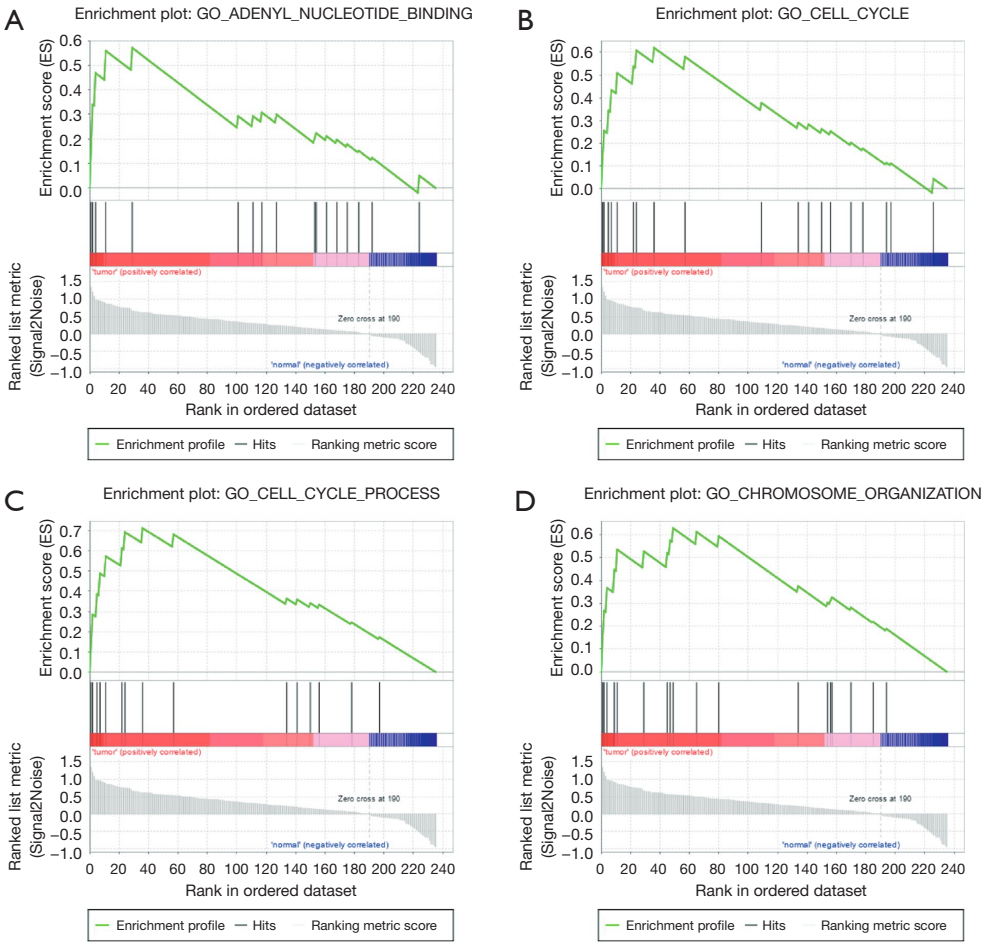

Figure 2 GSEA of the target genes of ti-RNA-Gly-GCC-002 was based on TCGA database. (A) GSEA-enriched gene set GO_ADENYL_NUCLEOTIDE_BINDING. (B) GSEA-enriched gene set GO_CELL_CYCLE. (C) GSEA-enriched gene set GO_CELL_CYCLE_PROCESS. (D) GSEA-enriched gene set GO_CHROMOSOME_ORGANIZATION. GO, Gene Ontology; GSEA, gene set enrichment analysis; TCGA, The Cancer Genome Atlas.

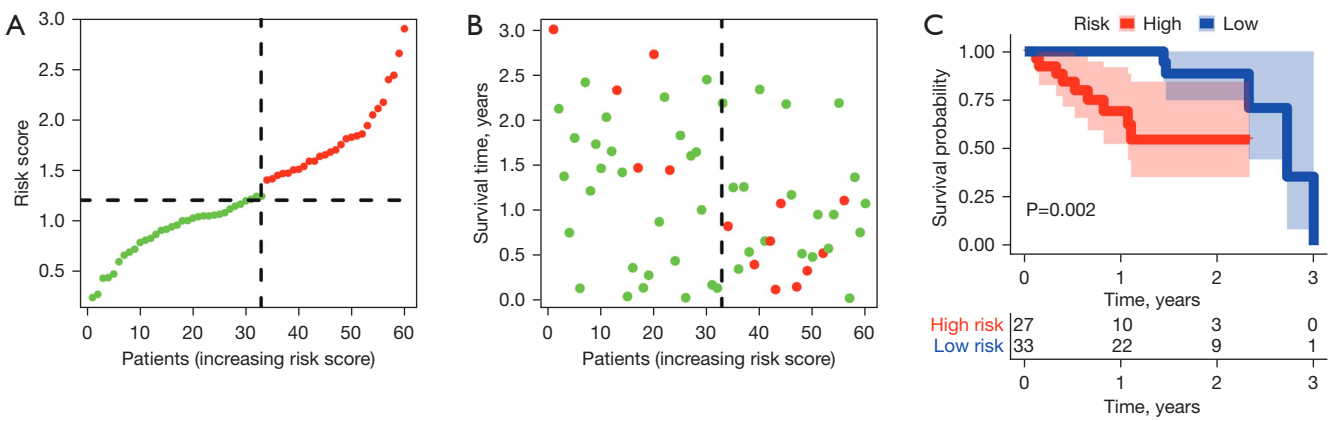

Figure 3 Survival analysis of the HCC patients. (A) Risk scores distribution of the patients. The red points represented patients with high-risk scores. The green points represented patients with low-risk scores. (B) Relapse time and status of the patients. The red points represented patients with relapse. The green points represented patients with dead status. (C) Kaplan-Meier survival curve of patients. The red represented patients in the high-risk group. The blue represented patients in the low-risk group. HCC, hepatocellular carcinoma.

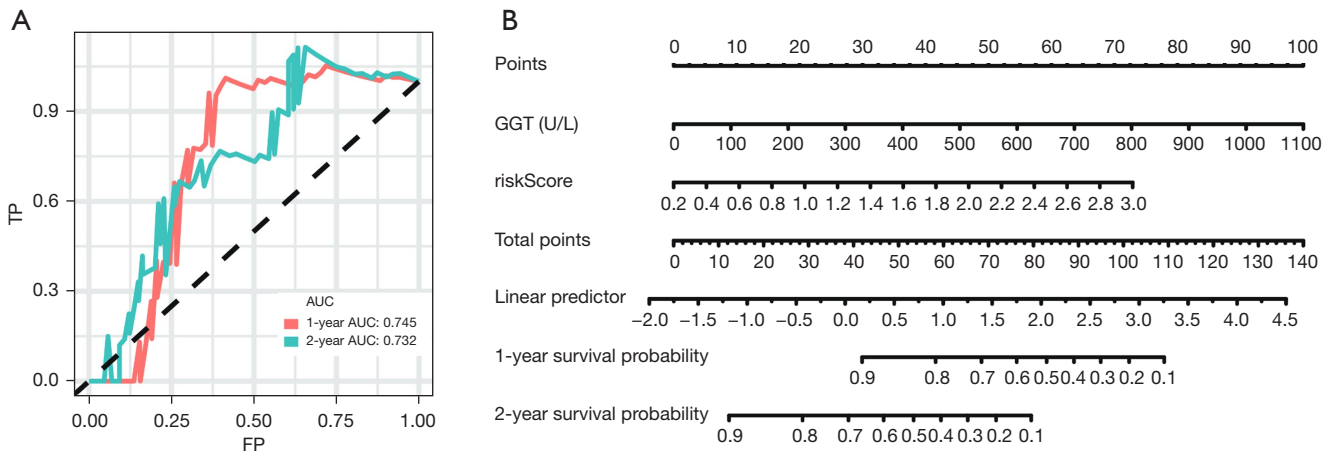

Figure 4 The ROC curves and nomogram of tiRNA-Gly-GCC-002. (A) The ROC curves of the tiRNA-Gly-GCC-002. (B) A nomogram for predicting the RFS in HCC. FP, false positives; TP, true positives; AUC, area under the curve; GGT, gamma-glutamyl transferase; ROC, receiver operating characteristic; RFS, relapse-free survival; HCC, hepatocellular carcinoma.

role of tiRNA-Gly-GCC-002, including male group (Figure S2A), young age group (≤60 years) (Figure S2B), old age group (>60 years) (Figure S2C), high level group of ALT (>40 U/L) (Figure S2D), low level group of ALT (≤40 U/L) (Figure S2E), high level group of AST (>40 U/L) (Figure S2F), low level group of AST (≤40 U/L) (Figure S2G), high level group of GGT (>50 U/L) (Figure S2H), low level group of GGT (≤50 U/L) (Figure S2I), high level group of AFP (>25 ng/L) (Figure S2J), low level group of AFP (≤25 ng/L) (Figure S2K), stage T1 and T2 group (Figure S3A), stage T3 and T4 group (Figure S3B), grade I and II group (Figure S3C), grade III group (Figure S3D), group without MVI (Figure S3E), group with MVI (Figure S3F), group without HBV (Figure S3G), group with HBV (Figure S3H), group without cirrhosis (Figure S3I) and group with cirrhosis (Figure S3J). These results revealed that patients with high expression levels of tiRNA-Gly-GCC-002 had an obviously shorter RFS.

The prognostic effect and applications of tiRNA-Gly-GCC-002

To assess the prognostic effect of the tiRNA-Gly-GCC-002-based risk score, we performed the ROC analysis, which demonstrated that the AUC value for 1-, 2-year survival time were 0.745 and 0.732, respectively (*Figure 4A*). This result indicated that the tiRNA-Gly-GCC-002 could be used to predict the prognosis of HCC patients. Based on the data including both tiRNA-Gly-GCC-002 expression level and patient clinic information, the univariable and multivariable Cox regression analyses were performed to select the factors associated with prognosis for RFS of HCC patients. The univariate Cox regression analysis indicated that the factors of tiRNA-Gly-GCC-002-based prognostic score (P=0.03) and serum GGT level (P=0.001) were significant risk factors, which were associated with RFS of patients with HCC. Subsequently, the multivariate Cox regression analysis also showed that the factors of tiRNA-Gly-GCC-002-based prognostic score (P=0.03) and serum GGT level (P=0.001) were associated with RFS of patients with HCC, which acted as the independent predictors of RFS (*Table 2*). According to the result of the multivariable Cox regression analysis, the tiRNA-Gly-GCC-002 expression levels and serum GGT levels of 60 patients with liver cancer were selected as independent predictors of overall survival to establish the nomogram model. The combined use of tiRNA-Gly-GCC-002 and serum GGT better predicted the 1- and 2-year survival probabilities of HCC patients. The C-index for the prognosis prediction of the nomogram model was 0.789, which also demonstrated that the tiRNA-Gly-GCC-002 served as a crucial and momentous prognosis factor of patients with HCC. This nomogram allows for the development of personalized follow-up strategies or treatment options based on the predicted risk of recurrence, improving long-term outcomes (*Figure 4B*).

Discussion

Around 780,000 new cases of HCC are diagnosed

Table 2 Univariate and multivariate Cox PH regression in survival analysis

Parameters	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Sex	1.042 (0.133, 8.169)	0.97	–	–
Age	0.983 (0.940, 1.027)	0.44	–	–
HBV	2.891 (0.633, 13.20)	0.17	–	–
Cirrhosis	2.369 (0.639, 8.790)	0.20	–	–
AFP	1.000 (1.000, 1.000)	0.98	–	–
ALT	1.007 (0.998, 1.016)	0.12	–	–
AST	1.009 (1.000, 1.019)	0.056	–	–
GGT	1.004 (1.002, 1.007)	0.001	1.004 (1.002, 1.007)	0.001
Grade	1.086 (0.519, 2.272)	0.83	–	–
Stage	1.247 (0.586, 2.651)	0.57	–	–
MVI	1.787 (0.792, 4.032)	0.16	–	–
Prognostic score	2.692 (1.102, 6.579)	0.03	3.304 (1.138, 9.597)	0.03

PH, proportional hazard; HR, hazard ratio; CI, confidence interval; HBV, hepatitis B virus; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; MVI, microvascular invasion.

worldwide each year (26). The deep and wide researches have been carried out in the fields of genomic alteration and prognosis of HCC patients (27). Over the past few years, there are vast biomarkers which have been confirmed to have prognostic function and are applied extensively in the various prognosis assessment (28). As we all know, the prognostic genes can evaluate and forecast the outcomes of patients with HCC from diverse perspectives. In the current study, the tiRNA-Gly-GCC-002 was ultimately selected and the prognostic effect in the RFS of this tRNA was also detected in the cohort of HCC patients. Based on the tiRNA-Gly-GCC-002 expression level, we studied HCC from a new perspective.

tRFs and tiRNAs are related with the cell proliferation, invasive metastasis, apoptosis, genes methylation, protein translation regulation, RNA interference, which demonstrate that the tRNA-derived fragments have immense potential and value in the progression of cancer (29). The recognition of all differently expressed tRNA-derived fragments is crucial for the comprehension of their potential functions. Several researches have demonstrated that certain tRNA-derived fragments accelerate the tumor progression (30). However, several researches collided with the above points that some tRNA-derived fragments could be able to suppress the tumor development and progression (31). The components of cells involved in tumor occurrence and development are complex, and thus, the potential mechanisms of tRNA-derived fragments in cancer cells remain to be further studied.

In this study, we screened out the differently expressed tRNA-derived fragments between HCC tissues and paracancerous tissues. Clinical information of 6 patients is shown in Table S2. This information of the patient samples shows differences, which may have some impact on the quantification of tRNA-derived fragments, but the impact on the selection of tRNA-derived fragments is limited. In order to further confirm the reliability of our result, these differently expressed tRNA-derived fragments were validated in the tissues and serum samples of patients with HCC. Ultimately, the tiRNA-Gly-GCC-002 was obtained, which had significantly higher expression level in the HCC patients than that in the hepatitis patients. The potential molecular biological functions of tRNA-derived fragments were also explored. Moreover, we assessed the prognostic performance of the tiRNA-Gly-GCC-002. Based on the univariable and multivariable Cox regression analyses, independent prediction factors of RFS were obtained. In addition, according to the clinicopathological characteristic and tiRNA-Gly-GCC-002, the nomogram was constructed.

Recently, a research demonstrated the role of specific tRF-GG or tRF-Gly-GCC as the inhibitor of genes related with the endogenous reverse transcription factors murine endogenous retrovirus-L (MERVL) to regulate the production of the noncoding RNAs and the histone levels (32). The correlation between the prognosis and tRNA expression level of lung adenocarcinoma patients was explored and the studies showed that tRNA-Tyr-ATA, mt-tRNA-Ser-GCT and tRNA-Lys-CTT-1 were related with cancer-specific survival (33). In addition, compared with hepatitis patients, the expression levels of 5'-tRNA-Lys-TTT halves, 5'-tRNA-Glu-CTC and 5'-tRNA-Arg-CCT were lower in the clear cell renal cell carcinoma patients indicating its potential effect as the tumor inhibitor (34). Compared with the benign prostatic hyperplasia tissue, 3'-tRNA-Asp-GUC-half and 5'-tRNA-Asp-GUC-half were increased in the prostate cancer tissues and both the tRNA-derived fragments can be acted as potential biomarkers to predict and monitor the disease progression (35). The tiRNA-5034-GluTTC-2, which was significantly related with the tumor size, was reduced in plasmas and tissues of patients with gastric cancer. The overall survival of the patients with higher expression levels of tiRNA-5034-GluTTC-2 was longer than the patients with lower expression (36). Furthermore, Wang *et al.* revealed that tiRNA-Phe-GAA-003, tRF-Gly-CCC-001 and tRF-Arg-CCT-017 could act as the new diagnostic biomarkers and tiRNA-Phe-GAA-003 and tRF-Arg-CCT-017 could predict the prognosis of the breast cancer patients (37). The above researches indicated that the tRNA-derived fragments might be acted as the promising biomarkers to predict the prognosis of tumor patients.

Moreover, GSEA analysis indicated that the target genes of tiRNA-Gly-GCC-002 were enriched in the adenyl nucleotide binding, cell cycle, cell cycle process and chromosome organization pathways. Cell cycle and cell cycle process pathway are well-known vital factors, which can regulate the growth and reproduction of the cancer cells. A growing number of studies have indicated that some genes could regulate the cell cycle to change the occurrence and development of tumors, so as to achieve the purpose of targeted treatment (38,39). The above result showed that the tiRNA-Gly-GCC-002 might play an important role in HCC progression.

We acknowledge that there are still many shortcomings in this study. One of the most vital limitations of the current study is that the functions of the tiRNA-Gly-GCC-002 were not verified with the experiments. In the second place, additional data sets are required to verify the reliability and veracity of the results. In addition, the number of HCC patients selected for tRNA-derived fragment analysis in our research process is relatively small, and the overall patient selection is also limited. In the end, our study was conducted in China, where the prevalence of HBV is high. Therefore, there may be geographical limitations that restrict its application in other countries.

Conclusions

In summary, the tiRNA-Gly-GCC-002 was selected to predict the outcomes of patients with HCC, which may play a vital role in directing the treatment of HCC. In addition, the nomogram incorporating tiRNA-Gly-GCC-002 expression level and serum GGT level was established, which could instruct individualized therapy for HCC. Further studies are required to explain the latent molecule mechanisms of the tiRNA-Gly-GCC-002 in HCC.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-644/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-644/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-644/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-644/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by The First Affiliated Hospital of Wenzhou Medical University (No. 2020023) and Peking University Shenzhen Hospital (No. 2023132), and individual consent for this retrospective analysis was waived.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;385:117-71.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7-34.
4. Petrick JL, Braunlin M, Laversanne M, et al. International trends in liver cancer incidence, overall and by histologic subtype, 1978-2007. Int J Cancer 2016;139:1534-45.
5. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 2009;10:57-63.
6. Chen E, Yi J, Jiang J, et al. Identification and validation of a fatty acid metabolism-related lncRNA signature as a predictor for prognosis and immunotherapy in patients with liver cancer. BMC Cancer 2022;22:1037.
7. Yang F, Deng K, Zheng H, et al. Progress of targeted and immunotherapy for hepatocellular carcinoma and the application of next-generation sequencing. Ann Hepatol 2022;27:100677.
8. DiStefano JK, Davis B. Diagnostic and Prognostic Potential of AKR1B10 in Human Hepatocellular Carcinoma. Cancers (Basel) 2019;11:486.
9. Zhang Y, Wang Y, Liu H, et al. Six genes as potential diagnosis and prognosis biomarkers for hepatocellular carcinoma through data mining. J Cell Physiol 2019;234:9787-92.
10. Zhu CQ, Tsao MS. Prognostic markers in lung cancer: is it ready for prime time? Transl Lung Cancer Res 2014;3:149-58.
11. Gingold H, Tehler D, Christoffersen NR, et al. A dual program for translation regulation in cellular proliferation and differentiation. Cell 2014;158:1281-92.
12. Pavon-Eternod M, Gomes S, Rosner MR, et al. Overexpression of initiator methionine tRNA leads to global reprogramming of tRNA expression and increased proliferation in human epithelial cells. RNA 2013;19:461-6.
13. Speer J, Gehrke CW, Kuo KC, et al. tRNA breakdown products as markers for cancer. Cancer 1979;44:2120-3.
14. Anderson P, Ivanov P. tRNA fragments in human health and disease. FEBS Lett 2014;588:4297-304.
15. Sun C, Fu Z, Wang S, et al. Roles of tRNA-derived fragments in human cancers. Cancer Lett 2018;414:16-25.
16. Lee YS, Shibata Y, Malhotra A, et al. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes Dev 2009;23:2639-49.
17. Mleczko AM, Celichowski P, Bąkowska-Żywicka K. Ex-translational function of tRNAs and their fragments in cancer. Acta Biochim Pol 2014;61:211-6.
18. Yang W, Gao K, Qian Y, et al. A novel tRNA-derived fragment AS-tDR-007333 promotes the malignancy of NSCLC via the HSPB1/MED29 and ELK4/MED29 axes. J Hematol Oncol 2022;15:53.
19. Zhao R, Yang Z, Zhao B, et al. A novel tyrosine tRNA-derived fragment, tRF(Tyr), induces oncogenesis and lactate accumulation in LSCC by interacting with LDHA. Cell Mol Biol Lett 2023;28:49.
20. Zeng T, Hua Y, Sun C, et al. Relationship between tRNA-derived fragments and human cancers. International Journal of Cancer 2020;147:3007-18.
21. Zhu L, Ge J, Li T, et al. tRNA-derived fragments and tRNA halves: The new players in cancers. Cancer Lett 2019;452:31-7.
22. Martens-Uzunova ES, Olvedy M, Jenster G. Beyond microRNA--novel RNAs derived from small non-coding RNA and their implication in cancer. Cancer Lett 2013;340:201-11.
23. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015;19:A68-77.

24. Liberzon A. A description of the Molecular Signatures Database (MSigDB) Web site. Methods Mol Biol 2014;1150:153-60.
25. Kamarudin AN, Cox T, Kolamunnage-Dona R. Time-dependent ROC curve analysis in medical research: current methods and applications. BMC Med Res Methodol 2017;17:53.
26. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359-86.
27. Rebouissou S, Nault JC. Advances in molecular classification and precision oncology in hepatocellular carcinoma. J Hepatol 2020;72:215-29.
28. Liu GM, Zeng HD, Zhang CY, et al. Identification of a six-gene signature predicting overall survival for hepatocellular carcinoma. Cancer Cell Int 2019;19:138.
29. Li M, Radvanyi L, Yin B, et al. Downregulation of Human Endogenous Retrovirus Type K (HERV-K) Viral env RNA in Pancreatic Cancer Cells Decreases Cell Proliferation and Tumor Growth. Clin Cancer Res 2017;23:5892-911.
30. Olvedy M, Scaravilli M, Hoogstrate Y, et al. A comprehensive repertoire of tRNA-derived fragments in prostate cancer. Oncotarget 2016;7:24766-77.
31. Goodarzi H, Liu X, Nguyen HC, et al. Endogenous tRNA-Derived Fragments Suppress Breast Cancer Progression via YBX1 Displacement. Cell 2015;161:790-802.
32. Boskovic A, Bing XY, Kaymak E, et al. Control of noncoding RNA production and histone levels by a 5' tRNA fragment. Genes Dev 2020;34:118-31.
33. Kuang M, Zheng D, Tao X, et al. tRNA-based prognostic score in predicting survival outcomes of lung adenocarcinomas. Int J Cancer 2019;145:1982-90.
34. Zhao C, Tolkach Y, Schmidt D, et al. 5'-tRNA Halves are Dysregulated in Clear Cell Renal Cell Carcinoma. J Urol 2018;199:378-83.
35. Zhao C, Tolkach Y, Schmidt D, et al. tRNA-halves are prognostic biomarkers for patients with prostate cancer. Urol Oncol 2018;36:503.e1-7.
36. Zhu L, Li T, Shen Y, et al. Using tRNA halves as novel biomarkers for the diagnosis of gastric cancer. Cancer Biomark 2019;25:169-76.
37. Wang J, Ma G, Ge H, et al. Circulating tRNA-derived small RNAs (tsRNAs) signature for the diagnosis and prognosis of breast cancer. NPJ Breast Cancer 2021;7:4.
38. Liu J, Mei J, Li S, et al. Establishment of a novel cell cycle-related prognostic signature predicting prognosis in patients with endometrial cancer. Cancer Cell Int 2020;20:329.
39. Liao Y, Wang F, Zhang Y, et al. Silencing SHMT2 inhibits the progression of tongue squamous cell carcinoma through cell cycle regulation. Cancer Cell Int 2021;21:220.

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