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Basic Study

5'tiRNA-Pro-TGG, a novel tRNA halve, promotes oncogenesis in sessile serrated lesions and serrated pathway of colorectal cancer

5'tiRNA-Pro-TGG promotes oncogenesis in SSL

Xin-Yuan Wang, Yujie Zhou, Hai-Ying Chen, Jin-Nan Chen, Shan-Shan Chen, Hui-Min Chen, Xiao-Bo Li

Abstract

BACKGROUND

Transfer ribonucleic acid (tRNA)-derived small RNAs (tsRNAs) are small fragments that form when tRNAs severe. tRNA halves (tiRNAs), a subcategory of tsRNA, are involved in the oncogenic processes of many tumors. However, their specific role in sessile serrated lesions (SSLs), a precancerous lesion often observed in the colon, has not yet been elucidated.

AIM

To identify SSL-related tiRNAs and their potential role in the development of SSLs and serrated pathway of colorectal cancer (CRC).

METHODS

Small-RNA sequencing was conducted in paired SSLs and their adjacent normal control (NC) tissues. The expression levels of five SSL-related tiRNAs were validated by qPCR. Cell counting kit and wound healing assays were performed to detect cell proliferation and migration. The target genes and sites of tiRNA-1:33-Pro-TGG-1 (5′tiRNA-Pro-TGG) were predicted by TargetScan and miRanda algorithms. Metabolism-associated and immune-related pathways were analyzed by single-sample gene set enrichment analysis. Functional analyses were performed to establish the roles of 5′tiRNA-Pro-TGG based on the target genes.

RESULTS

In total, we found 52 upregulated tsRNAs and 28 downregulated tsRNAs in SSLs compared to NC. The expression levels of tiRNA-1:33-Gly-CCC-2, tiRNA-1:33-Pro-TGG-1, and tiRNA-1:34-Thr-TGT-4-M2 5′tiRNAs were higher in SSLs than those in NC, while that of 5′tiRNA-Pro-TGG was associated with the size of SSLs. It was demonstrated that 5′tiRNA-Pro-TGG promoted cell proliferation and migration of RKO cell *in vitro*. Then, heparanase 2 (*HPSE2*) was identified as a potential target gene of 5′tiRNA-Pro-TGG. Its lower expression was associated with a worse prognosis in CRC. Further, lower expression of *HPSE2* was observed in SSLs compared to normal controls or adenomas and in *BRAF*-mutant CRC compared to *BRAF*-wild CRC. Bioinformatics analyses revealed that its low expression was associated with a low interferon γ response and also with many metabolic pathways such as riboflavin, retinol, and cytochrome p450 drug metabolism pathways.

CONCLUSION

tiRNAs may profoundly impact the development of SSLs. 5′tiRNA-Pro-TGG potentially promotes the progression of serrated pathway CRC through metabolic and immune pathways by interacting with *HPSE2* and regulating its expression in SSLs and *BRAF*-mutant CRC. In the future, it may be possible to use tiRNAs as novel biomarkers for early diagnosis of SSLs and as a potential therapeutic target in serrated pathway of CRC.

Key Words: Noncoding RNA; TRNA halves; Sessile serrated lesions; Colon cancer; Serrated pathway

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Core Tip: Our study identified the tsRNA expression profile of SSLs for the first time and found that tiRNA-1:33-Pro-TGG-1, which was associated with polyp size, were highly expressed in SSLs and promoted oncogenesis in colorectal cancer cell. Furthermore, tiRNA-1:33-Pro-TGG-1 potentially promotes the progression of serrated pathway CRC through metabolic and immune pathways by interacting with HPSE2 in SSLs and BRAFmut CRC. In the future, tiRNA-1:33-Pro-TGG-1 may serve as a potential target for the early diagnosis of SSLs and treatment of CRC that arises from the serrated pathway.

INTRODUCTION

Colorectal cancer (CRC) typically develops from colorectal polyps. there are two main categories: conventional adenomas (ADs) and serrated lesions (SLs)[1]. SLs, precancerous lesions often observed in the colon, are again of four subtypes: 1) sessile serrated lesions with or without dysplasia (SSLs-D and SSLs, respectively); 2) traditional serrated adenomas (TSAs); 3) hyperplastic polyps (HPs); and 4) unclassified serrated adenomas[2].

The association between common colorectal polyps and the development of CRC has been well studied. In recent years, a large number of studies have also confirmed the malignant potential of SLs, in particular, that of SSLs[3]. Unlike ADs, which tend to develop into microsatellite-stability (MSS) or microsatellite-instability–low (MSI-L) CRC and carry a mutation in the Kirsten Rat Sarcoma Viral Oncogene Homolog (*KRAS*) gene, SSLs tend to develop into microsatellite-instability–high (MSI-H) CRC and carry a mutation in the v-raf murine sarcoma viral oncogene homolog B (*BRAF*) gene[4,5]. At present, we do not know much about the exact mechanisms through which SSLs develop and how they progress to CRC. In addition, endoscopic detection of SSLs is very difficult because of the flattened morphology, surface coverage with an overlying mucus cap, and greyish hue of the lesion[6]. Several studies have shown that patients with SSLs are at an increased risk of developing both concurrent and heterochronic advanced adenomas[7–9]. As the effective intervention in CRC depends on early detection and diagnosis, there is an urgent need to find effective early diagnostic markers for SSLs, which will help in the prevention and accurate treatment of CRC.

Recent research has revealed the essential role of non-coding RNAs, including long non-coding (lnc) RNAs, transfer (t) RNAs, and micro (mi) RNAs, in the development and progression of various diseases[10–12]. tRNAs play an important role in the translation of proteins by transporting amino acids to the ribosome[13]. However, under conditions of nutritional, physicochemical, and oxidative stress, cells selectively reduce protein synthesis to conserve energy. Under these situations, tRNAs may be enzymatically cleaved to form tRNA-derived small RNAs (tsRNAs)[14,15]. tsRNAs can be classified into two categories based on their length and enzymatic cleavage sites: tRNA halves (tiRNAs) and tRNA-related fragments (tRFs). tiRNAs comprise 31–40 nucleotides (nt). They form when mature tRNAs are cleaved in the anticodon loop region comprising 5′tiRNAs and 3′tiRNAs. tRFs are 14–30 nt in length and form from mature or precursor tRNA. tRFs have four isoforms: tRF-1, tRF-2, tRF-3, and tRF-5.

It was reported in the last decade that tsRNAs are involved in the regulation of several physiological processes. For example, 5′tiRNA-GLY can promote the proliferative and invasive capabilities of papillary thyroid cancer cells by binding to RBM17[16]. Other studies have shown that tRF-Val can attach directly to binding protein EEF1A1 to promote proliferation and inhibit apoptosis of gastric cancer cells[17]. It was also reported that tRF-Gly promotes migration of hepatocellular carcinoma cells by binding to NDFIP2 in liver cancer[18]. Thus, although the role of tsRNAs in diseases remains an interesting research topic, dysregulation of their expression may present possible biomarkers for many diseases, such as CRC and breast cancer[19–21]. However, the expression and function of tsRNAs and small tRNA-derived fragments in colorectal polyps, particularly those of SSLs, have not yet been explored.

This study aims to identify the expression profiles of tsRNAs in SSLs and paired normal control (NC) tissues using small-RNA sequencing and their potential role in the development of SSLs and serrated pathway of CRC.

MATERIALS AND METHODS

Clinical samples

Twenty paired SSL tissues and adjacent normal tissues belonging to the same patient were collected from Renji Hospital, School of Medicine, Shanghai JiaoTong University (China). The inclusion criteria were as follows: age, ≥18 years; adequate bowel preparation and cecum reach; and received a colonoscopy with an assured diagnosis of SSL by experienced endoscopists. Two pathologists confirmed the diagnosis using biopsy specimens according to the 2019 WHO 5th classification. Our study was approved by the Ethics Committee of Renji Hospital (KY2021-004).

Data collection

RNA sequencing (RNA-Seq) data of SSLs, conventional adenomas, and the corresponding control tissue were obtained from GSE76987 from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds/). The gene expression data of colorectal adenocarcinoma (COAD) were downloaded from TCGA (https://portal.gdc.cancer.gov/) and cBioPortal for Cancer Genomics (https://www.cbioportal.org/).

RNA extraction

Total RNA was extracted from fresh tissues stored in RNA using TRIzol (Invitrogen, CA, USA). The purity and concentration of the total RNA samples were determined with NanoDrop ND-1000 (Thermo Fisher Scientific, DE, USA).

Sequence processing of tRF and tiRNA

RNA samples were extracted from four paired SSL tissues and adjacent normal tissues. The purity and concentration of the total RNA samples were determined before conducting small-RNA sequencing, as mentioned before. Next, a commercial RNA pretreatment kit (rtStar™ tRF and tiRNA Pretreatment Kit, AS-FS-005, Arraystar Inc., MD, USA) for tRF and tiRNA-seq library preparation was used, which was then used to remove some RNA modifications that interfered with small RNA-seq library construction, including 3′-aminoacyl (charged) deacylation to 3′-OH for 3′adaptor ligation, 3′-cP (2′,3′-cyclic phosphate) removal to 3′-OH for 3′adaptor ligation, 5′-OH (hydroxyl group) phosphorylation to 5′-P for 5′-adaptor ligation, m1A and m^3C demethylation for reverse transcription, cDNA synthesis, and library PCR amplification. The prepared RNA of each sample was ligated to 3′ and 5′ small-RNA adapters. Then, cDNA was synthesized and amplified using proprietary reverse transcription (RT) primers and amplification primers (Illumina). Subsequently, ~134–160 bp PCR-amplified fragments were extracted and purified using the PAGE gel. The concentration and quality of the libraries were assessed *via* absorbance spectrometry on Agilent BioAnalyzer 2100 (Agilent Technologies Inc., CA, USA). The libraries were denatured and diluted to a loading volume of 1.3 mL and a loading concentration of 1.8 pM. Then, they were loaded onto a reagent cartridge and forwarded to sequencing run on the Illumina NextSeq 500 system using NextSeq 500/550 V2 kit (FC-404- 2005, Illumina), according to the manufacturer's instructions. Raw sequencing read data that passed the Illumina chastity filter were used for subsequent analysis. Trimmed reads (with 5′,3′-adaptor bases removed) were aligned to mature-tRNA and pre-tRNA reference sequences. Statistical analysis of the alignment results was applied to retain the valid sequences for subsequent tRF and tiRNA expression profiling analysis.

Sequencing data analysis

Sequencing quality was examined using the FastQC software (v0.11.7) and trimmed reads were aligned allowing for only one mismatch to mature-tRNA sequences. The reads that do not map are aligned allowing for only one mismatch to precursor tRNA sequences using the Bowtie software (v1.2.2, http://bowtie-bio.sourceforge.net/index.shtml). The abundance of tRF and tiRNA was evaluated using their sequencing counts and is normalized as counts per million (CPM) of the total aligned reads. The differentially expressed tRFs and tiRNAs were screened based on the count value with R package edgeR. The R packages (R 4.1.2), including FactoMineR, factoextra, ggvenn, pheatmap, and ggplot2, were used for principal component analysis (PCA), Venn plots, Hierarchical clustering heatmap analysis, and Volcano plots.

Quantitative real-time reverse-transcription PCR

Total RNA collected from 16 paired SSLs and adjacent normal tissues was extracted using TRIzol (Invitrogen, CA, USA), as stated previously. tiRNAs were reverse-transcribed into cDNA using a Bulge-Loop miRNA qRT-PCR Starter Kit (Ribobio, Guangzhou, China). Subsequently, qPCR was performed with SYBR Premix Ex Taq (Takara) , as instructed by the manufacturer. The expression levels of tiRNAs were normalized to that of U6. The primers of qPCR were as follows: *HPSE2*-F: 5′-ATGGCCGGGCAGTAAATGG-3′; *HPSE2*-R: 5′-GCTGGCTCTGGAATAAATCCG -3′; *ACTB*-F: 5′-CACCATTGGCAATGAGCGGTTC-3′, and *ACTB*-R: 5′-AGGTCTTTGCGGATGTCCACGT-3′. Other primers involved in reverse transcription and qPCR were purchased from RiboBio (China).

Cell culture

The RKO cell line was purchased from the Typical Culture Preservation Commission Cell Bank, Chinese Academy of Sciences (Shanghai, China). The cell was cultured in the RPMI 1640 medium with 10% fetal bovine serum (Gibco, USA) at 37 °Cwith 5% CO_2.

Cell transfection

The RKO cell was seeded in plates (Corning Life Sciences, USA) the day before transfection. The 5′tiRNA-Pro-TGG mimic and inhibitor (50 nM), both modified with 2′-

O-methyl, were purchased from GenePharma Technology (Shanghai, China) and transfected using DharmaFECT 1 siRNA transfection reagent (Thermo Fisher Scientific Dharmacon Inc., USA). The corresponding scramble sequences were used as negative controls. The RNA oligonucleotide sequences were as follows: 5′tiRNA-Pro-TGG mimic: 5′-GGCUCGUUGGUCUAGUGGUAUGAUUCUCGCUUU-3′; 5′tiRNA-Pro-TGG inhibitor: 5′-AAAGCGAGAAUCAUACCACUAGACCAACGAGCC-3′; mimic scramble control: 5′-ACGUUUGACCUGUGUCGAGUUUUCUGUUUGGCG-3′; and inhibitor scramble control: 5′-GGGAAAGCGAAUAAAUCCAAACACCCAAUCCGC-3′.

Cell counting kit-8 (CCK-8) assay

Cell proliferation was measured by CCK-8 (Dojindo, Japan). The RKO cell was seeded in 96-well plates at a density of 2×10^3 cells per well. After transfection for 48 h, 10 μL of CCK-8 solution and 100 μL of the RPMI 1640 medium per well were added to the wells after discarding the previous medium; the OD values (450 nm) were measured after 2 h. All assays were conducted three times.

Wound-healing assay

The cells were inoculated in a 6-well plate. When 90% confluence was reached, a sterile 200-μL pipette tip was used to create vertical wounds. Finally, the wells were photographed under a microscope (Olympus, Japan) at ×200 magnification. The pictures were analyzed by ImageJ. All assays were conducted three times.

Single-sample gene set enrichment analysis (ssGSEA)

The ssGSEA analysis was used to investigate the expression levels of immune- and metabolism-related pathways by GSVA R package. Next, 41 metabolism pathway gene sets and 29 immune pathway gene sets were obtained from Molecular Signatures Database (MSigDB; https://www.gsea-msigdb.org/).

Gene ontology (GO) and Kyoto encyclopedia of genes and genome (KEGG) enrichment analyses of target genes

To investigate the potential biological function of dysregulated tiRNAs in SSLs, the target gene predictions were conducted by TargetScan (http://www.targetscan.org/vert 72/) and Miranda (http://www.microrna.org/microrna/) with a context score < -0.1. KEGG pathway and GO analyses to the target gene sets were performed by using the clusterProfiler R package.

Statistical analysis

Mean and standard deviations (mean ± SD) were used to analyze all quantitative variables. Two-tailed Student's *t*-tests and Wilcoxon rank test were performed. A *P*-value <0.05 was considered statistically significant. Spearman correlation analysis was used to determine the relationship between 5'tiRNAs and polyp size. Pearson correlation analysis was used to determine the relationship between tiRNA-1:33-Pro-TGG-1 and *HPSE2*. Kaplan–Meier survival analysis was performed to evaluate the association between the *HPSE2*-expression level and the overall survival of CRC patients. All analyses were performed by GraphPad Prism 9.3.1 (GraphPad Software, USA).

RESULTS

Expression profiles of tRFs and tiRNAs in paired SSL and NC groups

To identify the expression profiles of tRFs and tiRNAs in patients with SSLs, four pairs of SSLs and the corresponding NC tissues from different patients were collected for small-RNA sequencing analysis. Variations in all tRFs and tiRNAs expressed in SSL and NC groups are shown using a heatmap (Figure 1A). The expression levels of tRFs and tiRNAs in SSLs were different from those in NC groups, as determined by PCA (Figure 1B). We used a Venn diagram to show the tRFs and tiRNAs that were both generally and specifically expressed between the SSL and NC groups (Figure 1C). As seen in Figure 1C, 54 types of tRFs and tiRNAs were exclusively found in SSLs, while 123 types were found only in NCs. Differences in tRFs and tiRNAs found in SSLs and NCs were determined under the following conditions: ≥1.5 fold change and $P < 0.05$. Under these conditions, we identified 52 upregulated and 28 downregulated tRFs and tiRNAs and have shown them in a hierarchical cluster heatmap (Figure 1D).

Distribution of tRF and tiRNA subtypes in SSL and NC groups

The expression of tRF-1 and 5′tiRNA increased, while that of tRF-5c and tRF-3b decreased in SSLs compared with that of NCs (Figure 2A and B). tRNAs with the same anticodon translate the same amino acid. Hence, we herein separately determined the number of different tRFs and tiRNAs with the same anticodon (Figure 2C and D).

Validation for discrepant expression levels of 5′tiRNAs

5′tiRNAs play an important role in the development of many diseases, including CRC. Because our analysis showed a significant increase in tiRNA-5 in SSLs compared to that in NCs, we further verified the expression of 5′tiRNA in SSLs. Our previous screening criteria showed that six 5′tiRNAs (five upregulated and one downregulated) with different expression levels emerged between SSLs and NCs. tiRNA-1:33-Gly-CCC-2, tiRNA-1:33-Pro-TGG-1, tiRNA-1:34-Thr-TGT-4-M2, tiRNA-1:34-Lys-CTT-1-M2, and tiRNA-1:32-chrM.Val-TAC were upregulated in SSLs with 43.99-, 25.50-, 24.00-, 12.61-, and 8.73-fold change, respectively ($P < 0.05$), while tiRNA-1:33-Gly-CCC-3 was downregulated with a 36.95-fold change in SSLs compared to that in NCs ($P < 0.05$, Figure 3A and B).

Since we previously reported increased levels of 5′tiRNA expression in SSLs (Figure 2A and B), we herein focus on the abovementioned five upregulated 5′tiRNAs in SSLs. To further validate our sequencing data, we collected 16 pairs of SSLs and the corresponding NCs to confirm the expressions of the five upregulated 5′tiRNAs using RT-PCR (Figure 3C–G). The size of all collected lesions ranged from 4 to 15 mm, with an average of 6.31±3.07 mm. tiRNA-1:33-Gly-CCC-2, tiRNA-1:33-Pro-TGG-1, and tiRNA-1:34-Thr-TGT-4-M2 were significantly upregulated in SSLs compared to those in the paired NC (P = 0.0059, 0.0309, and 0.0008, respectively). The expression of tiRNA-1:34-Lys-CTT-1-M2 and tiRNA-1:32-chrM.Val-TAC did not show any statistically significant differences between SSLs and NCs (P = 0.0641 and 0.9838, respectively).

Association of tiRNA-1:33-Pro-TGG-1 with lesion size and promotion of oncogenesis in CRC cells

We further analyzed the correlation between the lesion size and the expression levels of the three 5′tiRNAs that had been validated as significantly highly expressed in SSLs. It was tiRNA-1:33-Pro-TGG-1 (Figure 4A-B), not tiRNA-1:33-Gly-CCC-2 or tiRNA-1:34-Thr-TGT-4-M2 (Figure 4C-D), that positively correlated with lesion size. Therefore, we focused on tiRNA-1:33-Pro-TGG-1, also known as 5′tiRNA-Pro-TGG. It comprises 33 nucleotides and is a type of 5′tiRNA that originated in tRNA-Pro-TGG-1 (Figure 4E). The inhibition of 5′tiRNA-Pro-TGG reduces the proliferation (Figure 4F) and migratory capacity of cancer cells in RKO, a colon cancer cell line carrying the *BRAF* V600E mutation, while its overexpression enhanced the migratory capacity of the cancer cells (Figure 4G and H).

Involvement of potential target gene *HPSE2* in the serrated pathway

To further investigate the potential functions of upregulated 5′tiRNAs in the progression of SSLs, we identified potential target genes that might bind to 5′tiRNA-Pro-TGG using TargetScan and miRanda algorithms and could predict 502 target genes. When context plus score < -0.5 and structure score > 300, the filtering parameters of the TargetScan and miRanda algorithms were predicted to be 33 and 10 target genes, respectively, with the intersection at *HPSE2* (Figure 5A). Two possible binding sites were predicted within the 3′UTR of *HPSE2* for the seed regions of 5′tiRNA-Pro-TGG (Figure 5B). *HPSE2* encodes heparinase II. A mutation in *HPSE2* is responsible for the urofacial syndrome and has been progressively identified as a tumor suppressor. We examined the expression levels of 5′tiRNA-Pro-TGG and *HPSE2* and found a significant negative correlation between their expression levels (Figure 5C). We also found that the expression level of *HPSE2* in SSLs was significantly lower than that in the right uninvolved colon (UR), right control colon (CR) tissue, and common adenoma (AD) ($P < 0.05$; Figure 5D). The qPCR results also confirmed that the expression level of *HPSE2* in SSLs was lower than that in NC ($P < 0.05$; Figure 5E). Not coincidentally, the *HPSE2* expression level was lower in CRC lesions carrying *BRAF* mutations than those with *BRAF* wild-type CRC (Figure 5F). An analysis of survival outcomes in CRC patients demonstrated that the lower level of *HPSE2* was associated with poorer prognosis (Figure 5G).

HPSE2-associated immune and metabolic profiles in CRC

To explore the function of *HPSE2* in the development of serrated pathway of CRC, we grouped CRC patients into *HPSE2* high (*HPSE2*-high) and low (*HPSE2*-low) expression groups. We then scored both groups for the immune cell type (Figure 6A), immune cell function (Figure 6B), and metabolic pathways (Figure 6C) using the ssGSEA algorithm. In the *HPSE2*-low group, a part of the immune cell scores were lower, including that for tumor infiltration lymphocyte (TIL), dendritic cells (DCs), T helper cells, B cells, and mast cells. The scores for response to interferon γ (IFNγ) and T-cell co-stimulation were lower in the *HPSE2*-low group than they were in the *HPSE2*-high group. Notably, many metabolic pathways scored lower in the *HPSE2*-low-expression group, implying the downregulation of these pathways, including that of riboflavin, retinol, and cytochrome P450 drug metabolism pathways. However, the metabolism of glyoxylate, dicarboxylate, and pyrimidine upregulated in the *HPSE2*-low group.

We next performed KEGG and GO enrichment analyses using the potential target genes of 5'tiRNA-Pro-TGG. KEGG enrichment analysis revealed that these target genes could be involved in pathways such as the biosynthesis of cofactors, antifolate resistance, and choline metabolism in cancer (Figure 6D). GO enrichment analysis demonstrated the possible target genes involved in cell-to-cell adhesion and regulation of the secretory pathway and exocytosis (Figure 6E).

DISCUSSION

Formation of SSLs and the process by which they progress to CRC is known as the serrated neoplastic pathway. However, the mechanisms and processes involved in this pathway are still not fully understood. SSLs that progress to CRC have a prevalence of 10–15% in the general population. The main pathology of this type of cancer involves a structurally distorted serrated crypt, with a *BRAF* mutation (*BRAF*mut), MSI-H, and

CpG island methylator phenotype-high (CIMP-H)[22]. SSLs presenting with *BRAF*mut often develop into CRC with a worse prognosis[23]. Therefore, early diagnosis of SSLs can help in reducing the incidence of *BRAF*mut CRC. In addition, understanding the pathogenesis of SSLs can help identify new targets for intervention in the early and precise treatment of *BRAF*mut CRC.

Studies conducted in the last decade reported that tsRNAs can play an important role as a biomarker in colon cancer[24], and that 5′-tiRNA-Pro-TGG levels can be used as an independent prognostic marker in CRC for predicting its recurrence[20]. Another study showed that in CRC, higher levels of tRF-phe-GAA-031 and tRF-VAL-TCA-002 expression were associated with reduced survival. Hence, they could also be used as prognostic predictors of CRC[19]. Therefore, the present study investigated the expression levels of tsRNAs, specifically that of 5′tiRNAs, in SSLs and their potential biological roles. We sequenced small RNAs from SSLs and their corresponding NCs, and found that among the 80 dysregulated tsRNAs, 5′tiRNAs, 3′tiRNAs, and tRFs-1 were more highly expressed in the SSLs, which suggests that tiRNAs may play an important role in these lesions.

Further analysis confirmed tiRNA-1:33-Gly-CCC-2, tiRNA-1:33-Pro-TGG-1, and tiRNA-1:34-Thr-TGT-4-M2 to be significantly upregulated in SSLs with a 2.92-, 3.69-, and 2.37-fold change, respectively. The expression level of 5′tiRNA-Pro-TGG was positively correlated with the size of SSLs. In addition, 5′tiRNA-Pro-TGG promoted carcinogenic processes in the colon cancer cell. We further screened *HPSE2* as the potential target gene. Interestingly, we found that *HPSE2* appeared to be specifically hypo-expressed in SSLs, as well as in *BRAF*-mutant CRC, and its low expression predicted lower survival. 5′tiRNA-Pro-TGG is associated with poor prognosis in CRC[20]. *HPSE2*, a novel tumor-suppressor gene, has been reported to have reduced expression levels and poor prognosis in colon and breast cancers[25,26]. Our study identifies for the first time the specific low expression of *HPSE2* in SSLs and *BRAF*mut CRC and reveals that it may play an essential role in the serrated pathway but not in other colorectal carcinogenesis pathways. To the best of our knowledge, this study is the first to report the high expression of 5′tiRNA-Pro-TGG in SSLs and its potential regulatory relationship with *HPSE2*.

Analysis of immune cells and their functions suggested that *HPSE2* is involved in regulating the functions of various immune cells in CRC, including TIL, particularly in response to IFNγ. IFNγ promotes antigen presentation and tumor killing[27]. Our finding that patients with low *HPSE2* had lower IFNγ-response scores suggested that it might be involved in regulating the tumor immune escape. Metabolic analysis revealed that *HPSE2* could downregulate various metabolic pathways, such as riboflavin and retinol metabolism. Riboflavin may reduce the risk of CRC in women[28]. In addition, a negative association between the plasma retinol concentration and the risk of proximal colon cancer has also been reported[29]. Recently, a lack of retinoic acid synthesis was found to promote the accumulation of myeloid-derived suppressor cells (MDSC) in CRC, thereby mediating the immune escape, while exogenous retinoic acid supplementation in an *in vitro* model attenuated the polymorphonuclear MDSC production[30].

Our study has some limitations. Firstly, more *in vitro* and *in vivo* experiments are needed to validate the function of the tiRNAs discussed. Secondly, because of the low prevalence of SSLs and their frequent neglect by endoscopists, it became difficult to collect a large number of samples. In addition, the expression level of 5′tiRNA-Pro-TGG and its association with recurrence and prognosis in SSL patients require further studies in large samples.

CONCLUSION

Our study is the first to identify the tsRNA expression profile of SSLs. It also reported that tiRNA-1:33-Gly-CCC-2, tiRNA-1:33-Pro-TGG-1, and tiRNA-1:34-Thr-TGT-4-M2 were highly expressed in SSLs. tiRNA-1:33-Pro-TGG-1 potentially promotes the serrated pathway for CRC progression through metabolic and immune pathways by interacting with *HPSE2* in SSLs and *BRAF*mut CRC. Our results showed that tiRNA-1:33-Pro-TGG-1 may serve as a potential target for early diagnosis of SSLs and treatment of CRC arising from the serrated pathway.

ARTICLE HIGHLIGHTS

Research background

tRNA halves (tiRNAs), a subcategory of tRNA-derived small RNAs (tsRNA), are involved in the oncogenic processes of many tumors, yet their specific role in sessile serrated lesions (SSLs) has not yet been elucidated.

Research motivation

To identify SSL-related tiRNAs and their potential role in the development of SSLs and serrated pathway of colorectal cancer (CRC).

Research objectives

Endoscopic detection of SSLs is very difficult and we do not know much about the exact mechanisms through which SSLs develop and how they progress to CRC in present.

Research methods

Small-RNA sequencing was conducted in paired SSLs and their adjacent normal control (NC) tissues. The expression levels of five SSL-related tiRNAs were validated by qPCR. Cell counting kit and wound healing assays were performed to detect cell proliferation and migration. The target genes and sites of tiRNA-1:33-Pro-TGG-1 (5′tiRNA-Pro-TGG) were predicted by TargetScan and miRanda algorithms. Metabolism-associated and immune-related pathways were analyzed by single-sample gene set enrichment analysis. Functional analyses were performed to establish the roles of 5′tiRNA-Pro-TGG based on the target genes.

Research results

The expression levels of tiRNA-1:33-Gly-CCC-2, tiRNA-1:33-Pro-TGG-1, and tiRNA-1:34-Thr-TGT-4-M2 5′tiRNAs were higher in SSLs than those in NC, while that of 5′tiRNA-Pro-TGG was associated with the size of SSLs. It was demonstrated that 5′tiRNA-Pro-TGG promoted cell proliferation and migration of RKO cell *in vitro*. Then, heparanase 2 (*HPSE2*) was identified as a potential target gene of 5′tiRNA-Pro-TGG. Its lower expression was associated with a worse prognosis in CRC. Further, lower expression of *HPSE2* was observed in SSLs compared to NC or adenomas and in *BRAF*-mutant CRC compared to *BRAF*-wild CRC. Bioinformatics analyses revealed that its low expression was associated with a low interferon γ response and also with many metabolic pathways such as riboflavin, retinol, and cytochrome p450 drug metabolism pathways.

Research conclusions

5′tiRNA-Pro-TGG potentially promotes the progression of serrated pathway CRC through metabolic and immune pathways by interacting with *HPSE2* and regulating its expression in SSLs and *BRAF*-mutant CRC.

Research perspectives

In the future, it may be possible to use 5′tiRNA-Pro-TGG as novel biomarkers for early diagnosis of SSLs and as a potential therapeutic target in serrated pathway of CRC.

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