tRNA-derived fragments tRF^{GlnCTG} induced by arterial injury promote vascular smooth muscle cell proliferation

Xiao-Ling Zhu,1,6 Tao Li,2,6 Yu Cao,1,6 Qing-Ping Yao,3 Xing Liu,4 Ying Li,1 Yang-Yang Guan,1 Ji-Jun Deng,1 Rui Jiang,5 and Jun Jiang1,2

1Department of Thyroid Surgery, The Affiliated Hospital of Southwest Medical University, Luzhou, China; 2Collaborative Innovation Center for Prevention and Treatment of Cardiovascular Disease of Sichuan Province, Southwest Medical University, Luzhou, China; 3Institute of Mechanobiology & Medical Engineering, School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai, China; 4Department of Cardiology, The Affiliated Hospital of Southwest Medical University, Luzhou, China; 5Department of Urology, The Affiliated Hospital of Southwest Medical University, Luzhou, China; 6Department of Urology, The Affiliated Hospital of Southwest Medical University, Luzhou, China

INTRODUCTION
Transfer RNAs (tRNAs) are molecules that assemble amino acids into proteins. In recent years, studies have found that when cells are under environmental stress, such as starvation, oxidative stress, or hypoxia, endogenous tRNAs are prone to be cleaved into small fragments at the specific sites.1 The half molecules formed by the cleavage on the tRNA anticodon loop are called tRNA halves (tiRNAs). The small fragments of tRNA are called tRNA-derived fragments (tRFs). tRFs are approximately 16~18 nt in length and are derived from mature or precursor tRNA. According to their corresponding position on the parent tRNA, they can be further classified into tRF-5, which corresponds to the 5’ end of mature tRNA and is produced by cleaving the tRNA on the D-loop; tRF-3, which corresponds to the 3’ end of mature tRNA, is produced by cutting the tRNA on the T-loop, and contains the common carotid artery (CCA) sequence; tRF-1, which is derived from the 3’ tail of the precursor tRNA and includes a poly U sequence at the 3’ end; and i-tRF, which is derived from the internal region of mature tRNA (Figure S1A). Unlike intact tRNAs, tRFs/tiRNAs perform another function: if their nucleic acid sequences are complementary to messenger RNAs (mRNAs), then they interfere with the production of proteins. tRFs/tiRNAs play essential roles in biological regulation and are related to diverse diseases. tRNA or tRFs are found to be involved in degenerative neural diseases,2–4 acquired metabolic disorders,5,6 stress injuries,7,8 and cancers.9,10 Their action mechanisms include gene silencing,11 affecting protein translation,12 competitive binding to essential proteins,9,13 and so on.

Intimal hyperplasia is a fundamental pathological change in cardiovascular diseases, such as hypertension and atherosclerosis. It also occurs after vascular surgeries, such as arterial bypass, balloon dilatation and stenting, and arteriovenous fistula. Neointima formation is a crucial cause of vascular stenosis, leading to the failure of revascularization surgery or aggravation of the original disease. There are a variety of cells involved in the formation of neointima. After the endothelium injury, platelets accumulate on the sites where endothelial cells (ECs) fall off, triggering inflammation. Then vascular smooth muscle cells (VSMCs) or adventitial fibroblasts (AFs) accumulate under the intima, hyperproliferating and synthesizing excessive extracellular matrix. In the end, ECs regenerate and cover the intima, reshaping the protective barrier of the intima. In the process of neointima formation, the migration and proliferation of VSMCs play pivotal roles.
We hypothesized that as a stress condition, the vascular injury might regulate the production of tiRNA or tRF, which have specific biological effects. In this study, we replicated the rat carotid artery intimal hyperplasia model by balloon injury (Figure 1A) and performed a high-throughput deep RNA sequencing. tiRNA and tRFs were identified and analyzed. One tRF, the tRNAGlnCTG-derived fragment (tRFGlnCTG), was found to be upregulated in the injured arteries. RNA fluorescent in situ hybridization (RNA-FISH) demonstrated that tRFGlnCTG was expressed in the cytoplasm of cells from the tunica media and the neointima. The synthesized tRFGlnCTG mimetics promoted VSMC proliferation and migration. In contrast, the inhibitors of tRFGlnCTG suppressed the migration and proliferation of VSMCs. Bioinformatics analysis suggested that tRFGlnCTG may modulate genes involved in cell growth and death. We identified its target genes as FAS cell surface death receptor (FAS). Our study provides new insights for further understanding of intimal hyperplasia mechanism and searching for gene-therapy targets.

RESULTS

The expression profiles of tiRNAs and tRFs were markedly different between the healthy and injured rat CCAs

On day 14 after the balloon injury, the rat CCAs were harvested. Histological sections showed the neointima formed in the carotid artery with a marked increase in the intimal/medial area ratio (Figure 1B). The intimal hyperplasia model was successfully made. Small RNA fragments consistent with pre-tRNA and mature-tRNA sequences were screened from the sequencing results for differential expression analysis between two groups. The expression profiles of tiRNAs and tRFs in the healthy and injured CCAs were markedly different. A total of 1,131 tiRNAs and tRFs were identified, of which 283 were only expressed in normal CCAs, and 87 were exclusively expressed in injured CCAs (Figure 1C). Fragments with expression difference >2.0-fold between the two groups and p values <0.05 were defined as the differentially expressed tiRNAs or tRFs (Figures 1D and 1E). The tiRNAs and tRFs expressed in arteries were mainly tiRNA-5, tRF-5, and i-tRF. Other types, such as tRF-3, tRF-1, and tiRNA-3, just occupied a minimum proportion. The proportion of tRF-5 was higher in the injured arteries than in the healthy arteries (Figure 2A). The source tRNA of tiRNAs and tRFs was also different in the healthy arteries and injured arteries (Figure 2B). There were 14 statistically differentially expressed tiRNAs and tRFs between the healthy and injured arteries (Figure 2C). Nine of them were upregulated, and the other five were downregulated in the injured arteries. In the upregulated tRFs, tRFGlnCTG had a high expression abundance (with high tag
counts per million of total aligned tRNA reads (TPM) values) in carotid arteries.

tRF^{GlnCTG} was highly expressed in the neointima and also in platelet-derived growth factor (PDGF)-BB- or transforming growth factor (TGF)-β1-induced VSMCs

The results of deep sequencing showed that the expression of tRF^{GlnCTG} was increased in injured arteries (Figure 3A). According to its sequence, tRF^{GlnCTG} was derived from the internal area of tRNA^{GlnCTG} (Figure 3B), so-called i-tRFs. We utilized quantitative real-time PCR to validate its expression in healthy and injured arteries. Its increased expression in injured arteries was confirmed (Figure 3C).

RNA-FISH demonstrated that tRF^{GlnCTG} were localized in the cytoplasm of VSMCs from the tunica media and in the cytoplasm of cells from the neointima. In the neointima, the expression of tRF^{GlnCTG} was markedly elevated (Figure 3D).

VSMCs from rat thoracic aorta were stimulated with recombinant proteins of PDGF-BB (rPDGF-BB) and TGF-β1 (rTGF-β1) in vitro. Both rPDGF-BB and rTGF-β1 resulted in increased expression of tRF^{GlnCTG} (Figure 3E).

**tRF^{GlnCTG} promoted the proliferation and migration of VSMCs**

We investigated the biological effects of tRF^{GlnCTG} by transfecting the synthetic RNA mimetics or inhibitors into rat VSMCs (Figure 4A).

The Cell Counting Kit-8 (CCK-8) assay showed that the mimetics increased, and the inhibitors decreased the cell viability (Figure 4B).

For the 5-ethyl-2'-deoxyuridine (EdU) incorporation assay, 50 nM of synthetic tRF^{GlnCTG} mimetics or inhibitors were transfected into VSMCs by Lipofectamine 3000 for 24 h. Then EdU was added to the culture medium. After another 24 h, the number of proliferating cells was counted under a fluorescence microscope, and the cell proliferation rate was calculated. The proliferation of VSMCs transfected with tRF^{GlnCTG} mimetics was increased. On the contrary, tRF^{GlnCTG} inhibitors reduced the number of proliferating cells (Figure 4C).

tRF^{GlnCTG} enhanced the migration of VSMCs

The pretreatment of VSMCs was identical to the cell proliferation assay. Cell motility was assessed by the Transwell and scratch wound-closure assay. The transfection of tRF^{GlnCTG} mimetics significantly increased the migration (Figure 4D) and the wound-closure velocity of VSMCs (Figure 4E). On the other hand, the inhibitors of tRF^{GlnCTG} alleviate cell migration.

**Target gene prediction of tRF^{GlnCTG}**

In the R environment, TargetScan and miRanda were used to predict the possible target genes of tRF^{GlnCTG}. We performed functional
enrichment and pathway prediction of the assumed genes by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (Figure 5A). The genes were functionally clustered, and genes related to cell growth, proliferation, death, and movement were paid attention. By querying the results of the GEO (GEO: GSE48279), we found that some of the assumed genes were downregulated in intimal hyperplasia and were previously reported to be involved in vascular biology. These target genes may be involved in the regulation of vascular cell death and movement.

The biological processes and signaling pathways of the potential target genes of tRF<sub>Gln<i>CTG</i></sub> were analyzed with Ingenuity Pathway Analysis (IPA) software (QIAGEN Bioinformatics, Hilden, Germany, https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis). In the forecasted protein-protein interact networks, ATF6 (activating transcription factor 6), FAS, IL-10 (interleukin 10), MAPK1 (mitogen-activated protein kinase 1), and SPRY2 (Sprouty homolog 2) were located at the hubs of the network (Figure 5B).

FAS was the target gene of tRF<sub>Gln<i>CTG</i></sub> in VSMCs
Bioinformatic analysis forecasted the potential binding sites of tRF<sub>Gln<i>CTG</i></sub> with AFT6, FAS, IL-10, MAPK1, and SPRY2 (Figure 6A). That tRF<sub>Gln<i>CTG</i></sub> mimetics inhibited the expression of FAS mRNA (Figure 7B).

FAS overexpression (FAS-OE) abolished the effect of tRF<sub>Gln<i>CTG</i></sub>
To validate the causal link between tRF<sub>Gln<i>CTG</i></sub> and FAS, a recombinant lentiviral vector carrying the rat FAS gene was transfected to rat VSMCs, the control group treated with an empty vector. Western blotting confirmed the overexpression of FAS in VSMCs (Figure 7C). Then, the proliferation of FAS-OE VSMCs was investigated under tRF<sub>Gln<i>CTG</i></sub> overexpression. FAS-OE VSMCs showed decreased proliferation compared with the control group (Figure 7D). Thus, the results indicated that the overexpression of FAS in VSMCs eliminated the upregulation of proliferation by tRF<sub>Gln<i>CTG</i></sub>.

DISCUSSION
Studies have shown that in many species, under certain stressful conditions, such as starvation or hypoxia, endogenous tRNAs or their precursors are prone to be cleaved to produce specific small RNA fragments. Such RNA fragments are a class of gene-expression regulators, and their mechanisms of action are diverse. Some inhibit mRNA translation by microRNAs (miRNAs) manners. Some serve as reverse transcription primers for the retroviral RNA genome. Some are involved in the assembly of precursor rRNA
cleavage complexes. Others inhibit protein translation. tRFs/tiRNAs play crucial roles in diseases. For example, CU1276, a tRF from human mature B cells, suppresses proliferation and regulates the molecular response to DNA damage, and it is absent in germinal center-derived lymphomas. tRFs originated from tRNA^Glu, tRNA^Asp, tRNA^Gly, and tRNA^Tyr suppress breast cancer metastasis.
by competitively binding to YBX1, the stabilizer of pro-oncogenic transcripts.9

Vascular injury induces excessive proliferation of the intima, leading to stenosis or even occlusion of blood vessels. We speculate that intimal injury, as a stress condition, may cause changes in tiRNA and tRF expression. The small RNA sequencing to the healthy and injured rat CCA demonstrated that the types and expression levels of tRNA-derived small fragments markedly changed in intimal hyperplasia. A total of 14 differentially expressed tiRNAs and tRFs were identified. Among them, we investigated the regulation of tRF\textsuperscript{GlnCTG} on the biological behavior of VSMCs. Synthetic tRF\textsuperscript{GlnCTG} mimetics promoted the proliferation and migration of VSMCs in vitro. On the contrary, inhibitors of tRF\textsuperscript{GlnCTG} weaken VSMC proliferation and migration. Previous studies have also found that tRFs can negatively regulate cell death or apoptosis, promoting cell proliferation.13,20,21 The change of VSMC biological behavior is pivotal in the process of neointima formation. Therefore, tRF\textsuperscript{GlnCTG} may be essential for the diagnosis and treatment of intimal hyperplasia.

tRNAs are one of the main sources of natural small noncoding RNAs. By bioinformatic tools, we predicted possible target genes of tRF\textsuperscript{GlnCTG}, ATF6,22–24 FAS,25–29 IL-10,30–32 MAPK1,33,34 and SPRY235–38 have been reported to regulate vascular cells’ growth. We confirmed that tRF\textsuperscript{GlnCTG} decreased the level of FAS. Furthermore, the overexpression of FAS reduced the proliferation of VSMCs upregulated by tRF\textsuperscript{GlnCTG}. Studies have shown that the decrease in FAS is one of the critical reasons for intimal hyperplasia. FAS binds to the FAS ligand and forms a death complex, reducing inflammatory cells’ infiltration and the proliferation of smooth muscle cells.25,26,27 Intimal hyperplasia can be alleviated by local overexpression of the FAS ligand in the arteries injured by the balloon.25 ECs are relatively resistant to the FAS mechanism. Overexpression of the FAS ligand in transplanted ECs attenuates intimal hyperplasia.28 The combination of the overexpressing FAS ligand and nitric oxide suppresses smooth muscle growth without affecting endothelium repairment.29 Our study found that vascular injury induces increased expression of tRF\textsuperscript{GlnCTG}, which promotes neointimal formation by inhibiting FAS (Figure 7E). The tRF\textsuperscript{GlnCTG}-FAS pathway may be one of the mechanisms leading to intimal hyperplasia after arterial injury. The utilization of tRF\textsuperscript{GlnCTG} inhibitors might be a feasible strategy to alleviate neointima formation.

Our understanding of the correlation between tRFs/tiRNAs and vascular biology is still in the preliminary stage. Although we have predicted the possible targets for tRF\textsuperscript{GlnCTG}, the relationship between tRF\textsuperscript{GlnCTG} and neointima formation requires more experiments to determine. Besides, studies of other differentially expressed tRFs and tiRNAs, especially those that are downregulated in intimal hyperplasia, have not been conducted. We hope that shortly, knowledge of the modulation mechanisms of tiRNA and tRFs on vascular biology will be developed and enriched and will provide more tools for the diagnosis and treatment of cardiovascular diseases.
Figure 6. FAS was the target gene of tRF<sup>GlnCTG</sup> in VSMCs

(A) ATF6, FAS, IL-10, MAPK1, and SPRY2 have been reported to regulate the growth of vascular cells. The possible bindings sites between them and tRF<sup>GlnCTG</sup> and their functions in vascular biology are listed in the table. (B) Rat VSMCs were transfected with tRF<sup>GlnCTG</sup> mimetics or inhibitors, and the levels of ATF6, FAS, IL-10, MAPK1, and SPRY2 were detected. tRF<sup>GlnCTG</sup> mimetics decreased the level of FAS, and the inhibitors increase it (n = 7). Data are expressed as means ± SEM. *p < 0.05, **p < 0.01.
MATERIALS AND METHODS

**Intima hyperplasia model of rat CCA**

The animal protocol conformed to the Animal Management Rules of China (documentation 55, 2201, Ministry of Health, China) and the recommendations in the 8th edition of the Guide for the Care and Use of Laboratory Animals of the NIH (NIH revised 2011). All experiments were approved by the Southwest Medical University Laboratory Animal Care and Use Committee. 8-week-old healthy male Sprague-Dawley rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (300 mg/kg), as described in the previous study.39 A 2F Fogarty catheter (Edward Lifesciences, Irvine, CA, USA) was inserted into the left CCA of the rat, dragged, and rotated. The right CCA of the rat was dissected, but not invaded, as a sham control. The rats were sacrificed under general anesthesia 14 days after surgery. The bilateral CCAs were harvested, sectioned, and subjected to a small RNA sequencing.

**Small RNA sequencing**

Total RNA of arteries was extracted by the TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) method. First, the samples were pretreated to remove the modifications, including 3'-aminoacyl, 2',3'-cyclic phosphate, 5'-OH, m1A, and m3C. With the use of RT primers and amplification primers, cDNA was synthesized and amplified. Second, 135—160 bp PCR-amplified fragments (corresponding to a small RNA size of 15—40 nt) were extracted and purified from the polyacrylamide gel electrophoresis (PAGE) gel. Finally, the completed libraries were sequenced by a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). The diluted library was sequenced on a NextSeq 500 system (Illumina, San Diego, CA, USA).

**Quantitative real-time PCR**

RNA modifications, including terminal and methylation, were removed using the rtStar tRF&tiRNA Pretreatment Kit (Arraystar, Rockville, MD, USA), according to the manufacturer’s instructions. Total RNA of each sample was sequentially ligated to 3' and 5' small RNA adaptors, and each adaptor was 60 nt. Therefore, the length of
the amplified cDNA is about 134–160 bp (120 nt + 15–40 nt) (Figure S1B). cDNA was synthesized and amplified using Illumina’s proprietary RT primers and amplification primers. With the use of the rTF First-Strand cDNA Synthesis Kit (Arraystar), cDNA was synthesized and amplified. Stem-loop primers were added into the RT mixtures, and the reaction mixtures were incubated at 95°C for 10 min, followed by 40 cycles of 95°C for 10 s and 60°C for 60 s in a ViiA 7 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). U6 was used as an internal control. The 2^−ΔΔCt methods were used for the analysis of the gene expression.

**Rat thoracic aorta VSMC culture**
As mentioned in the previous study,^40^ the rat thoracic aorta was dissected under the aseptic circumstance. First, the tunica adventitia was removed. Subsequently, the ECs on the intima were digested using 0.1% type I collagenase. Finally, the tunica media were cut into small pieces and immersed in DMEM containing 15 mM HEPES, 100 U/mL penicillin, 100 mg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA), and 10% newborn bovine serum (Gibco). VSMCs were identified by immunocytochemistry using the anti-alpha-smooth muscle actin (α-SMA) antibody (Cell Signaling Technology, Danvers, MA, USA). Passages 4–7 VSMCs were used for subsequent experiments.

**PDGF-BB and TGF-β1 stimulation on VSMCs**
VSMCs were cultivated on 6-well plates at a density of 2 × 10^5 cells with DMEM containing 10% neonatal bovine serum. 25 ng/mL rPDGF-BB (R&D Systems, MN, USA) or 1 ng/mL rTGF-β1 (R&D Systems) was added into the culture medium. The expression of tRFGlnCTG in VSCMs was detected after 24 h by qPCR.

**Transfection of RNA mimetics or inhibitors**
The exogenous tRF mimetics and inhibitors were synthesized (GenePharma, Shanghai, China.). VSMCs were seeded at a density of 2 × 10^5 cells/mL and cultured in 6-well plates for 24 h. RNA mimetics or inhibitors were transfected into VSMCs at a final concentration of 50 nM using Lipofectamine 3000 (Thermo Fisher Scientific), according to the manufacturer’s protocol.

**Cell viability and proliferation assay**
VSMCs were transfected with tRF mimetics or inhibitors and then seeded at 5 × 10^4 cells/mL in a 96-well plate for 48 h. 10 μL of CCK-8 (Dojindo, Kumanoto, Japan) solution was added to the VSMCs, and the cells were incubated at 37 °C. The absorbance was measured at 450 nm using an iMark microplate reader (Bio-Rad, Hercules, CA, USA).

VSMCs were cultured in a 96-well plate for 8 h. 50 μM EdU was added into the culture medium for 2 h. Then the staining solution was added for 30 min in the dark. The proliferation cells were observed using a fluorescence microscope (Olympus BX-51).

**Cell migration assay**
After transfected with tRF mimetics or inhibitors, VSMCs were seeded at a density of 2 × 10^5 cells/mL in 24-well plates and cultivated 48 h to the confluence. A 200 μL pipette tip was used to scratch the cell layer to form a wound. The cells were photographed under a BH-2 optical microscope (Olympus, Tokyo, Japan) at 0 h and 24 h after wounding.

A Costar Transwell chamber (pore size of 8 μm; Corning Life Sciences, Tewksbury, MA, USA) containing serum-free DMEM was used to culture VSMCs. The chamber was immersed in the DMEM with 20% fetal bovine serum for 8 h. The un migrated cells on the inner side of the chamber were removed using a cotton bar. The migrated cells on the chamber’s outer side were dyed with crystal violet and counted using an optical microscope.

**Bioinformatical analysis**
In the R environment, TargetScan and miRanda databases are applied to predict the potential target genes and sites of tRF\(^{\text{GlnCTG}}\). The Database for Annotation, Visualization and Integrated Discovery (DAVID) version (v.)6.8 was used to perform the functional clustering of target genes. KEGG pathway was used to analyze the signaling pathways in which target genes may participate. RNAhybrid 2.2 ([https://bibiserv.bibi.helmholtz-muenchen.de/rnahybrid/submission.html](https://bibiserv.bibi.helmholtz-muenchen.de/rnahybrid/submission.html)) was used to analyze the potential binding sites and their MFE between tRNA\(^{\text{GlnCTG}}\) and the target gene.

**Western blotting**
The rat CCAs or cultured cells’ total protein were abstracted using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). Proteins were isolated by PAGE and transferred to a nitrocellulose membrane (GE Healthcare Life Sciences, PA, USA). The target proteins were identified with ATF6, FAS, IL-10, MAPK1, and SPRY2 antibodies (Absin, Shanghai, China).

**Luciferase reporter assay**
1.0 × 10^4 HEK293T cells were cultured in a 96-well plate. tRF\(^{\text{GlnCTG}}\) mimetics or nontarget control were diluted in 5 μL of Opti-MEM medium (Beyotime, China). FAS 3’ UTR dual reporter vector (or mutation vector) and 0.25 μL of Lipo6000 transfection reagent (Beyotime, China) were diluted in 5 μL of Opti-MEM medium. The two solutions were mixed gently and added to the cells. The transfection concentration of mimetics was 50 nM. The plasmid concentration was 50 ng/well. After 48 h of transfection, the medium was removed, and luciferase reagent (Promega, Fitchburg, WI, USA) was added. The fluorescence value was measured with a GloMax 96 spectrophotometer (Promega).

**Overexpression of FAS**
Lentiviral vector overexpressing recombinant FAS (rFAS-OE-LV) encoding FAS (GenBank: NM_139194.2) was obtained by cloning the coding region of FAS from rat VSMC cDNA into lentiviral vector GV492 (GeneChem, Shanghai, China). VSMCs were infected with
FAS-OE-LV particles, and the efficacy of transfection was investigated by western blotting.

**Statistical analysis**

If there is no specific remark in all of the experiments, then the replicates used in each experimental group are 5 (n = 5). The statistical analysis was processed with Prism 7 (GraphPad, San Diego, CA, USA). Two-tailed Student’s t test was performed for pairwise comparison, and a one-way ANOVA was performed for multiple comparisons. Bonferroni corrected p < 0.05 was considered statistically significant. The results were expressed as the mean ± standard error of the mean (SEM).

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2020.12.010.

**ACKNOWLEDGMENTS**

This study was supported by the National Natural Science Foundation of China (grant numbers 82070288 and 31900813); Science & Technology Department of Sichuan Province (grant numbers 202ZYF2108 and 2019YJ0409); Office of Science and Technology and Intellectual Property of Luzhou (grant number: 2019LZXNYD129); Talent Development Project of The Affiliated Center for Prevention and Treatment of Cardiovascular Disease Hospital of Southwest Medical University; and Collaborative Innovation Center for Prevention and Treatment of Cardiovascular Disease of Sichuan Province, Southwest Medical University (grant number xtcx2019-18).

**AUTHOR CONTRIBUTIONS**

J.J. designed the research and wrote and reviewed the manuscript. J.J. and R.J. analyzed the data. X.-L.Z., T.L., Y.C., Q.-P.Y., X.L., Y.L., Y.-Y.G., and J.-J.D. performed the research.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

**REFERENCES**

1. Emara, M.M., Ivanov, P., Hickman, T., Davra, N., Tisdale, S., Kedersha, N., Hu, G.F., and Anderson, P. (2010). Angiogenin-induced tRNA-derived stress-induced RNAs promote stress-induced stress granule assembly. J. Biol. Chem. 285, 10959–10968.

2. Hanada, T., Weitzer, S., Mair, B., Bernreuther, C., Wainger, B.J., Ichida, J., Hanada, R., Orthofer, M., Cronin, S.J., Komnenovic, V., et al. (2013). CLP1 links tRNA metabolism to progressive motor neuron loss. Nature 495, 474–480.

3. Greenway, M.I., Anderson, P.M., Russ, C., Ennis, S., Cashman, S., Donaghy, C., Patterson, V., Swingler, R., Kieran, D., Prehn, J., et al. (2006). ANG mutations segregate with familial and ‘sporadic’ amyotrophic lateral sclerosis. Nat. Genet. 38, 411–413.

4. van Es, M.A., Schelhaas, H.J., van Vught, P.W., Ticozzi, N., Andersen, P.M., Groen, E.J., Schulte, C., Blau, H.M., Koppers, M., Dijkstra, F.P., et al. (2011). Angiogenin variants in Parkinson disease and amyotrophic lateral sclerosis. Ann. Neurol. 70, 964–973.

5. Chen, Q., Yan, M., Cao, Z., Li, X., Zhang, Y., Shi, J., Feng, G.H., Peng, H., Zhang, X., Zhang, Y., et al. (2016). Sperm tRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science 351, 397–400.

6. Sharma, U., Conine, C.C., Shea, J.M., Boskovic, A., Derr, A.G., Bing, X.Y., Belloannner, C., Kućukural, A., Serra, R.W., Sun, F., et al. (2016). Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. Science 351, 391–396.

7. Mishima, E., Inoue, C., Saigusa, D., Inoue, R., Ito, K., Suzuki, Y., Jinno, D., Tsukui, Y., Akamatsu, Y., Araki, M., et al. (2014). Conformational change in transfer RNA is an early indicator of acute cellular damage. J. Am. Soc. Nephrol. 25, 2316–2326.

8. Wang, Q., Lee, L., Ren, J., Ajay, S.S., Lee, Y.S., and Bao, X. (2013). Identification and functional characterization of tRNA-derived RNA fragments (tRFs) in respiratory syncytial virus infection. Mol. Ther. 21, 368–379.

9. Goodarzi, H., Liu, X., Nguyen, H.C., Zhang, S., Fish, L., and Tavazoie, S.F. (2015). Endogenous tRNA-Derived Fragments Suppress Breast Cancer Progression via YBX1 Displacement. Cell 161, 790–802.

10. Maute, R.L., Schneider, C., Sumazin, P., Holmes, A., Calilano, A., Basso, K., and Dalla-Favera, R. (2013). tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. Proc. Natl. Acad. Sci. USA 110, 1404–1409.

11. Venkatesh, T., Suresh, P.S., and Tutsuimi, R. (2016). tRFs: miRNAs in disguise. Gene 579, 133–138.

12. Sobala, A., and Hutvagner, G. (2013). Small RNAs derived from the 5’ end of tRNA can inhibit protein translation in human cells. RNA Biol. 10, 553–563.

13. Saikia, M., Jobava, R., Parisien, M., Putnam, A., Krokowski, D., Gao, X.H., Guan, B.J., Yuan, Y., Jankowsky, E., Feng, Z., et al. (2014). Angiogenin-cleaved tRNA halves interact with cytochrome c, protecting cells from apoptosis during osmotic stress. Mol. Cell. Biol. 34, 2450–2463.

14. Kosozoma, A., and Griffiths-Jones, S. (2014). miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 42, D68–D73.

15. Schopman, N.C., Heynen, S., Haasnoot, J., and Berkhour, B. (2010). A miRNA-tRNA mix-up: tRNA origin of proposed miRNA. RNA Biol. 7, 573–576.

16. Morris, S., and Leis, J. (1999). Changes in Rous sarcoma virus RNA secondary structure near the primer binding site upon tRNATrp primer annealing. J. Virol. 73, 6307–6318.

17. Ruggero, K., Gullanti, A., Corradin, A., Sharma, V.K., De Bellis, G., Corti, G., Grassi, A., Zanovello, P., Bronte, V., Cinamin, V., and D’Agostino, D.M. (2014). Small non-coding RNAs in cells transformed by human T-cell leukemia virus type 1: a role for a tRNA fragment as a primer for reverse transcription. J. Virol. 88, 3612–3622.

18. Couvillion, M.T., Bounova, G., Purdom, E., Speed, T.P., and Collins, K. (2012). A Tryptophan Piwi bound to mature tRNA 3’ tRNA fragment as a primer for reverse transcriptase. J. Virol. 86, 3961–3966.

19. Ivanov, P., Emara, M.M., Villen, J., Gygi, S.P., and Anderson, P. (2011). Angiogenin-induced tRNA fragments inhibit translation initiation. Mol. Cell. Biol. 41, 633–643.

20. Jiang, X., and Wang, X. (2004). Cytochrome C-mediated apoptosis. Annu. Rev. Biochem. 73, 87–106.

21. Mei, Y., Yong, J., Liu, H., Shi, Y., Meinkoth, J., Dreyfuss, G., and Yang, X. (2010). tRNA binds to cytochrome c and inhibits caspase activation. Mol. Cell 37, 668–678.

22. Wang, X., Karamariti, E., Simpson, R., Wang, W., and Xu, Q. (2015). Dickkopf Homolog 3 Induces Stem Cell Differentiation into Smooth Muscle Lineage via ATF6 Signalling. J. Biol. Chem. 290, 19844–19852.

23. Karamariti, E., Zhai, C., Yu, B., Qiao, L., Wang, Z., Potter, C.M.F., Wong, M.M., Simpson, R.M.L., Zhang, X., Wang, X., et al. (2018). DKK3 (Dickkopf 3) Alters Atherosclerotic Plaque Phenotype Involving Vascular Progenitor and Fibroblast Differentiation Into Smooth Muscle Cells. Arterioscler. Thromb. Vasc. Biol. 38, 425–437.

24. Karali, E., Bellou, S., Stellas, D., Klinakis, A., Murphy, C., and Fotsis, T. (2014). VEGF Signals through ATF6 and PERK to promote endothelial cell survival and angiogenesis in the absence of ER stress. Mol. Cell 54, 559–572.

25. Luo, Z., Sata, M., Nguyen, T., Kaplan, J.M., Akita, G.Y., and Walsh, K. (1999). Adenovirus-mediated delivery of fas ligand inhibits interimal hyperplasia after balloon injury in immunologically primed animals. Circulation 99, 1776–1779.

26. Mano, T., Luo, Z., Suhara, T., Smith, R.C., Eser, S., and Walsh, K. (2000). Expression of wild-type and noncleavable Fas ligand by tetracycline-regulated adenoviral vectors to limit interimal hyperplasia in vascular lesions. Hum. Gene Ther. 11, 1625–1635.
27. Jiang, C., Yang, Y.F., and Cheng, S.H. (2004). Fas ligand gene therapy for vascular intimal hyperplasia. Curr. Gene Ther. 4, 33–39.

28. Sata, M., Luo, Z., and Walsh, K. (2001). Fas ligand overexpression on allograft endothelium inhibits inflammatory cell infiltration and transplant-associated intimal hyperplasia. J. Immunol. 166, 6964–6971.

29. Kural, M.H., Wang, J., Gui, L., Yuan, Y., Li, G., Leiby, K.L., Quijano, E., Tellides, G., Saltzman, W.M., and Niklason, L.E. (2019). Fas ligand and nitric oxide combination to control smooth muscle growth while sparing endothelium. Biomaterials 212, 28–38.

30. Mazighi, M., Pellè, A., Gonzalez, W., Mtairag, E.M., Philippe, M., Hénin, D., Michel, J.-B., and Feldman, L.J. (2004). IL-10 inhibits vascular smooth muscle cell activation in vitro and in vivo. Am. J. Physiol. Heart Circ. Physiol. 287, H866–H871.

31. Verma, S.K., Garikipati, V.N., Krishnamurthy, P., Khan, M., Thorne, T., Qin, G., Losordo, D.W., and Kishore, R. (2016). IL-10 Accelerates Re-Endothelialization and Inhibits Post-Injury Intimal Hyperplasia following Carotid Artery Denudation. PLoS ONE 11, e0147615.

32. Feldman, L.J., Aguirre, L., Ziol, M., Brideau, J.P., Nevo, N., Michel, J.B., and Steg, P.G. (2000). Interleukin-10 inhibits intimal hyperplasia after angioplasty or stent implantation in hypercholesterolemic rabbits. Circulation 101, 908–916.

33. Pintucci, G., Saunders, P.C., Gulkarov, I., Sharony, R., Kadian-Dodov, D.L., Bohmann, K., Raumann, F.G., Galloway, A.C., and Mignatti, P. (2006). Anti-proliferative and anti-inflammatory effects of topical MAPK inhibition in arterialized vein grafts. FASEB J. 20, 398–400.

34. Evans, B.C., Hocking, K.M., Osgood, M.J., Vokresensky, I., Dmowka, J., Kilchrist, K.V., Brophy, C.M., and DuVall, C.L. (2015). MK2 inhibitory peptide delivered in nanopolyplexes prevents vascular graft intimal hyperplasia. Sci. Transl. Med. 7, 291ra95.

35. Taniguchi, K., Sasaki, K., Watari, K., Yasukawa, H., Imairumi, T., Ayada, T., Okamoto, F., Ishizaki, T., Kato, R., Kohno, R., et al. (2009). Suppression of Sproutys has a therapeutic effect for a mouse model of ischemia by enhancing angiogenesis. PLoS ONE 4, e5467.

36. Wietecha, M.S., Chen, L., Ranzer, M.J., Anderson, K., Ying, C., Patel, T.B., and DiPietro, L.A. (2011). Sprouty2 downregulates angiogenesis during mouse skin wound healing. Am. J. Physiol. Heart Circ. Physiol. 300, H459–H467.

37. Byasheva, D., Veliceasa, D., Topczewski, J., Topczewska, J.M., Mzgirev, I., Vinokour, E., Reddi, A.L., Licht, J.D., Revskoy, S.Y., and Volpert, O.V. (2012). miR-27b controls venous specification and tip cell fate. Blood 119, 2679–2687.

38. Zhang, C., Chaturvedi, D., Jaggar, L., Magnuson, D., Lee, J.M., and Patel, T.B. (2005). Regulation of vascular smooth muscle cell proliferation and migration by human sprouty 2. Arterioscler. Thromb. Vasc. Biol. 25, 533–538.

39. Xu, J.Y., Chang, N.B., Rong, Z.H., Li, T., Xiao, L., Yao, Q.P., Jiang, R., and Jiang, J. (2019). circDiaph3 regulates rat vascular smooth muscle cell differentiation, proliferation, and migration. FASEB J. 33, 2659–2668.

40. Xu, J.Y., Chang, N.B., Li, T., Jiang, R., Sun, X.L., He, Y.Z., and Jiang, J. (2017). Endothelial Cells Inhibit the Angiotensin II Induced Phenotypic Modulation of Rat Vascular Adventitial Fibroblasts. J. Cell. Biochem. 118, 1921–1927.