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Original Article

Circulating serum exosomes 5'Leader ArgTCG and 3'tRF SerGCT combined with tumor biomarkers as non-invasive diagnostic and monitoring indicators for colorectal cancer

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Abstract: Objectives: Colorectal cancer (CRC) has one of the highest incidence and mortality rates among cancers, particularly concerning the rates of diagnosis. Serum exosomes (Exos) are crucial mediators for the intercellular transmission of genetic information. These vesicles contain various tRNA-derived fragments (tRFs) that play a role in the noncoding regulation of tumor genes. Consequently, they are anticipated to become valuable non-invasive diagnostic and predictive biomarkers for CRC. Methods: At the Affiliated Taian City Central Hospital of Qingdao University, we isolated and extracted serum Exos from 201 healthy donors and 205 patients with CRC. To measure Exo physical morphology, we utilized qNano, transmission electron microscopy, and a particle size analyzer. Western blotting was conducted to confirm the expression of surface and nuclear proteins in the Exos. Gene chips were employed to screen for differentially expressed tRF RNAs. The quantitative PCR technology was deployed to ascertain 5'Leader-ArgTCG and 3'tRF-SerGCT differential expression. CRC diagnostic efficiency was assessed utilizing the area under the curve. Results: We compared 5'Leader-ArgTCG and 3'tRF-SerGCT expression in 205 patients with CRC and 201 healthy donors. Among them, 5'Leader-ArgTCG was significantly downregulated, while 3'tRF-SerGCT was significantly upregulated. The diagnostic efficiency of predicting 5'Leader-ArgTCG and 3'tRF-SerGCT in serum Exos using survival curves was 0.659 and 0.659, respectively. Meanwhile, the diagnostic efficiency of combining the two tumor markers was 0.954. Conclusion: Serum Exos 5'Leader-ArgTCG and 3'tRF-SerGCT were differentially expressed in patients with CRC. Combining these two tumor markers may serve as a predictive indicator for the non-invasive diagnosis of CRC, demonstrating notable statistical significance.

Keywords: Colorectal cancer, 5'Leader-ArgTCG, 3'tRF-SerGCT, non-invasive diagnostic predictors, biomarker

Introduction

As the economy improves and dietary habits change, colorectal cancer (CRC) has become increasingly prevalent, ranking as the third highest in occurrence and the second highest in mortality rate globally. Research indicates that colon health is closely linked to overall well-being [1]. CRC incidence rate is significantly higher in urban areas compared to rural areas, a difference closely tied to diet structure, lifestyle, and socio-economic status. Additionally, age and genetics play crucial roles in CRC risk [2-4]. Hereditary enteritis (familial non-polypic colorectal disease, familial adenomatous polyposis, and Lynch syndrome, among others) is significantly associated with CRC onset. Currently, early screening with colonoscopy is the most intuitive examination method; however, its disadvantages are high cost and low patient acceptance. There are multiple interfering factors in fecal occult blood tests, with many benign diseases having positive results, such as hemorrhoids and enteritis [5]. Exploring non-invasive diagnostic methods, early interventions, and treatment options for

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CRC can significantly reduce mortality rates among patients with cancer, thereby alleviating their suffering.

Sequencing technology advancements have demonstrated the significant function of non-coding RNAs (ncRNAs) in gene transcription and translation. Among them, tRNA-derived fragment (tRF) RNAs are a recently discovered small ncRNA class generated through tRNA cleavage. Conversely, tRNA-derived small RNAs (TDRs) are fragments of precursors or mature tRNA molecules with a length of 14-50 nucleotides. TDR is classified based on its biological location into tRNA half-segments and tRF-RNAs [6, 7]. The biogenesis of tRFs occurs through the breakdown of tRNA, and their synthesis is not by arbitrary splicing. The control of this process is governed by a highly conserved and meticulous site-specific cleavage mechanism [8]. This cleavage can be categorized into three types: 5'tRF, 3'tRF, and intermediate tRF, depending on where the cleavage occurs. The end of 5'tRF-1 aligns precisely with the downstream base of the RNaseZ cleavage site, while the end of 3'tRF-1 has the Pol III transcription termination motif. This evidence supports the notion that tRF-1 is a distinct and stable small RNA generated from the processing of precursor tRNA.

There are various forms of extracellular vesicles in fludids such as blood, milk, urine, and other various secretions in the human body. These vesicles can be extracted in large quantities. Numerous studies have demonstrated that cells can facilitate the transmission of genetic information via tRF RNAs carried by serum exosomes (Exos). However, further research is required to develop therapeutic effects through drug delivery [9, 10]. Exo genes are crucial in tumor metastasis, proliferation, and transmission. Additionally, Exos are extensively utilized in modern clinical diagnostics [11]. They enable real-time monitoring of disease progression and provide a reliable basis for timely tumor diagnosis and treatment [12].

The differential expression of 5'Leader-ArgTCG and 3'tRF-SerGCT in serum Exos has been used to diagnose CRC. The clinical and pathological correlation of patients with CRC provides a basis for non-invasive diagnosis and early intervention treatment, reducing the mortality rate of patients with CRC [13, 14]. Combining tRF RNAs from extracellular vesicles with tumor markers can address the limitations of traditional colonoscopy for CRC diagnosis, including high costs, significant trauma, lengthy procedures, and low patient acceptance. Non-invasive comprehensive screening can facilitate timely monitoring of cancer incidence and prompt intervention.

Materials and methods

Sample preparation and clinical pathological analysis

Between March 2021 and April 2022, we collected 205 tissue samples from patients with CRC and 201 samples from healthy donors at The Affiliated Taian City Central Hospital of Qingdao University. All studies involving human participants were reviewed and approved by the Affiliated Taian City Central Hospital Committee of Qingdao University (approval number: 20230510). Blood samples were collected pre-treatment, and samples were obtained from the laboratory on the same day after testing. The healthy donor samples were selected from individuals undergoing medical assessments. The supernatant was collected after centrifuging approximately 3 mL of serum at 1,000 g for 10 min at 4°C. We recorded detailed data on the patients with CRC, including gender, age, type of cancer, lifestyle habits, and tumor metastasis.

Extracted extracellular vesicles

The extraction of extracellular vesicles was conducted using ultracentrifugation. First, the collected serum samples were thawed on ice. The plasma was then centrifuged at 10,000 *g* for 30 min at 4°C, aiming at eliminating large vesicles. Subsequently, the extracellular vesicles were centrifuged at 100,000 g for 2 h at 4°C (Type 50.4 TiRotor; Beckman Coulter). This was followed by PBS washing, and the precipitate from the outer cut was collected through ultracentrifugation at 100,000 *g*.

Performance evaluation of extracellular vesicles

The volume, size, and distribution of extracellular vesicles were determined using a particle size analyzer, the qNano (New Zealand), along with statistical analysis. Exo physical morphol-

ogy was determined by fixing the Exos on a dedicated copper mesh, which was then observed using a transmission microscope (Transmission Electron Microscope; FEI Tecnai T20e, FEI Company, USA). Subsequently, Western blotting was employed to identify extracellular vesicle proteins on a PVDF membrane (Millipore, Billerica, MA, USA). High expression of proteins TSG101 (Cell signaling technology, 72312T, 1:1000), CD9 (Cell signaling technology, 13174T, 1:1000), and CD87 (Abacom, ab307895, 1:800) was detected on the Exo surface (Proteintech, United States) [15].

RNA extraction and reverse transcription

After resuspending the extracted exosomes in 1 mL of PBS, we thoroughly lysed them by adding 1 mL of triazole. RNA was then precipitated with isopropanol and rinsed with 75% iced ethanol before extraction. The purified RNA was reverse transcribed into cDNA in preparation for the subsequent qPCR step (TaKaRa Bio, Nojihigashi, Kusatsu, Japan). To minimize RNA degradation during the experiment, all operations were conducted on ice.

Small RNA microarray profiling

Total RNA isolation was performed utilizing Trizol (Invitrogen), with RNA quantity measured by a NanoDrop ND-1000 spectrophotometer and RNA integrity assessed using a Bioanalyzer 2100 or denaturing gel electrophoresis. For small RNA microarray profiling, 100 ng of total RNA was first subjected to dephosphorylation with 3 units of T4 polynucleotide kinase at 37°C for 40 min. This process was aimed at removing both (P) and (cP) chemical groups from the 3' end of RNAs, resulting in the formation of a 3-OH end. This reaction was halted by heating at 70°C for 5 min and instantly cooling to 0°C. To denature the RNAs, 7 μL of DMSO was added and heated to 100°C for 3 min and chilled instantly to 0°C. The process of RNA end labeling was carried out by combining ligase buffer, BSA, a final concentration of 50 mM pCp-Cy3, and 15 units of T4 RNA ligase in a 28 μL reaction. The reaction was then incubated at 16°C overnight. The slides were subjected to scanning using an Agilent G2505C microarray scanner. Identifying differentially expressed small RNAs between two comparison groups was conducted through filtration at the pre-determined fold change and statistical significance (*P*-value) thresholds. Finally, we generated hierarchical clustering, as well as scatter and volcano plots, using R software.

qPCR and statistical analysis

Reverse transcription was followed by a qPCR assay to detect differentially expressed tRF RNAs in healthy donors and patients with CRC. The internal reference was U6, and statistical analyses were conducted based on ΔCT values, with three independent replications for each data set ($P < 0.001$). GraphPad Prism (version 6.0; GraphPad Software) was initially utilized to determine if the data followed a normal distribution. Depending on the results, either a t-test or a Mann-Whitney unpaired test was applied. The diagnostic efficacy of the candidate predictors was calculated by the area under the curve (AUC). A *P*-value < 0.05 was considered statistically significant, analyzed using the Statistical Package for the Social Sciences (version 22.0) (Ehningen, Germany). One - way analysis of variance (ANOVA) was employed to assess whether there were statistically significant differences in the mean values among three or more independent samples.

Results

Serum Exos identification

The results revealed that the shape of extracellular vesicles was predominantly spherical, with varying volume sizes of 80-140 nm and main diameter sizes of 100-120 nm (**Figure 1A**, **1B**). Western blotting results revealed high CD9/63 and TSG101 protein expression, which significantly differed from the expression of cell surface proteins. These findings confirm that we successfully extracted extracellular vesicles (**Figure 1C**).

Serum Exos tRF RNAs expression by high-throughput sequencing

Based on GeneChip, 24 differentially expressed tRF RNAs were screened (**Table 1**). Among these, 12 were significantly upregulated and 12 were downregulated (**Figure 2**). Therefore,

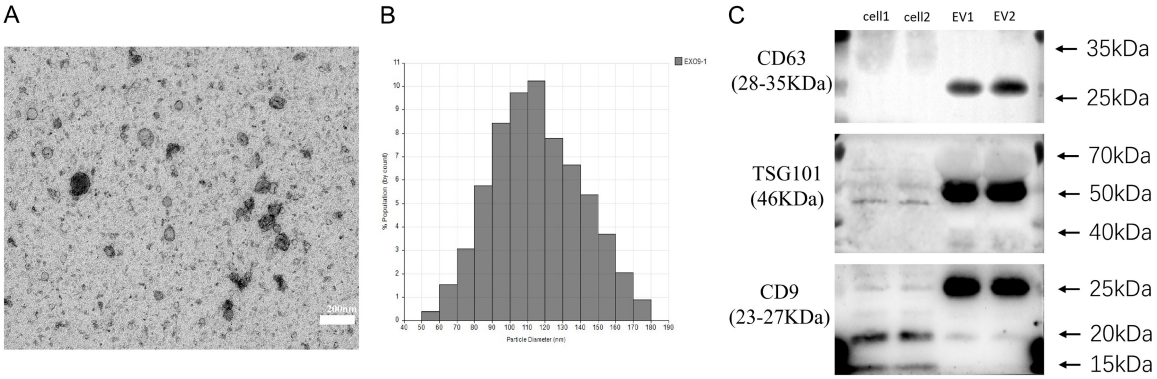

Figure 1. Validating the performance of serum exosomes. A. Identification of the physical morphology of exosomes by transmission electron microscopy (TEM). B. Measurement of exosomal size distribution using particle size analyzer by qNano. C. Western blot validation of serum exosomes surface protein markers.

Table 1. tRF RNA expression profiling (12 up-regulated and 12 down-regulated)

tRFRNA	Fold change	Description	tRFRNA	Fold change	Description
5’tRF-LysTTT	4.7759	Up	5’tiRNA-CysGCA	0.4690	Down
3’tRF-SerGCT	3.4686	Up	i-tRF-AspGTC	0.4755	Down
5’tiRNA-mtGlnTTG	1.9435	Up	3’tRF-ThrTGT	0.1893	Down
i-tRF-ArgCCT	1.6096	Up	5’Leader-ArgTCG	0.2455	Down
5’tRF-GlnTTG	1.8371	Up	3’tRF-MetCAT	0.4999	Down
5’Leader-IleAAT	2.2334	Up	3’tRF-GlyGCC	0.3603	Down
5’tRF-HisGTG	1.5121	Up	3’tRF-LeuCAG	0.4856	Down
5’tRF-mtLeuTAA	1.6629	Up	3’tiRNA-ValTAC	0.4314	Down
5’tRF-LeuTAA	1.7855	Up	i-tRF-MetCAT	0.4511	Down
5’Leader-LysTTT	2.1421	Up	5’tiRNA-LeuCAA	0.3831	Down
i-tRF-GluTTC	1.5337	Up	3’tiRNA-LeuTAA	0.3714	Down
i-tRF-GluCTC	1.9288	Up	3’tRF-IleAAT	0.2791	Down

we analyzed 3 groups of healthy donors, 3 groups of non-metastatic patients with CRC, and 3 groups of metastatic patients with CRC, and created a clustering plot based on the differentially expressed tRF RNAs. Additionally, we validated the expression of these RNAs in the serum of 205 CRC cases through quantitative PCR.

Clinicopathologic link between serum Exos 5’Leader-ArgTCG and 3’tRF-SerGCT for the diagnosis of CRC analysis

The real-time quantitative PCR results revealed that 5’Leader-ArgTCG and 3’tRF-SerGCT were differentially expressed, with 5’Leader-ArgTCG being significantly downregulated and 3’tRF-SerGCT being significantly upregulated in the serum of a patient with CRC (**Figure 3A**, **3B**).

Analysis of clinicopathologic grading in patients with 5’Leader-ArgTCG and 3’tRF-SerGCT

To assess the correlation between patients with CRC survival and stage grading, it is essential to diagnose CRC occurrence immediately. This plays a significant role in enhancing patient survival. Our results indicated no statistically significant association between 5’Leader-ArgTCG and T-stage grading of CRC primary foci (**Figure 4A**). Tumor presence and lymph node metastases were evident, with most tumors being identified at an advanced stage. The findings demonstrated a statistically significant disparity in lymph node metastasis between N0 and N1 stages (P = 0.038*, **Figure 4B**) without significant difference between N2 and N3 stages. In tumor staging, there was statistical significance (P < 0.05*) between stages I/II/III, while no significance

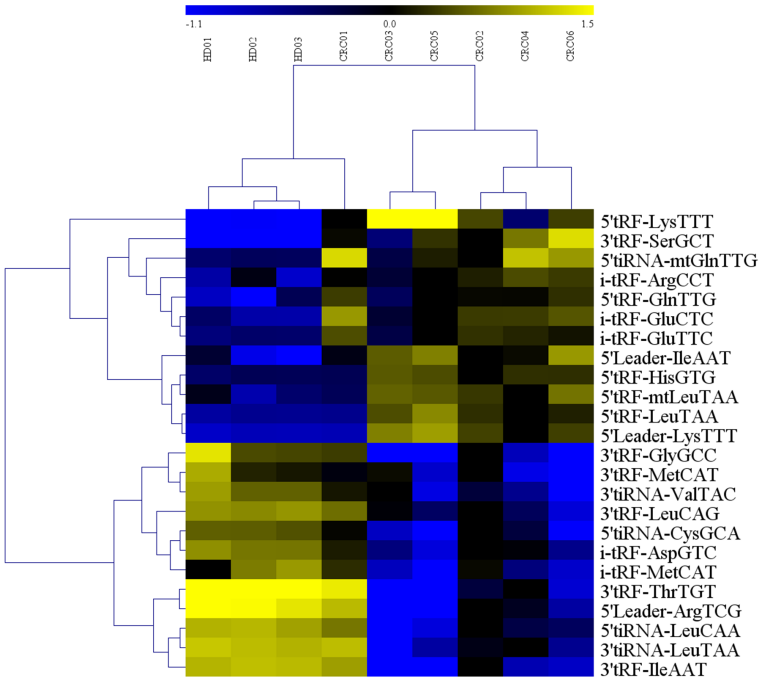

Figure 2. Exosomes tRF RNAs profile of the CRC patients. Cluster analysis of differentially expressed serum exosomes tRF RNAs between 6 CRC patients and 3 healthy donors.

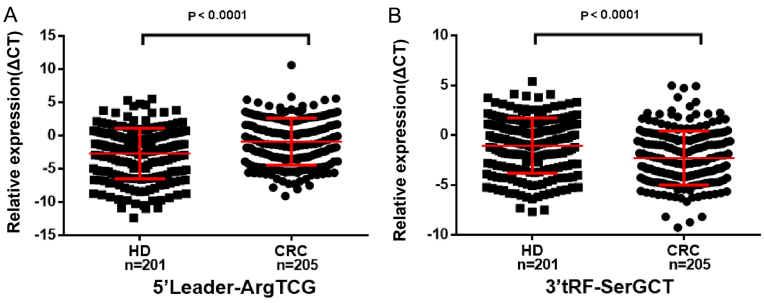

Figure 3. Serum exosomes 5’Leader-ArgTCG and 3’tRF-SerGCT are potential diagnosis biomarkers of colorectal cancer. The expression levels of serum exosomes 5’Leader-ArgTCG and 3’tRF-SerGCT in (A) CRC and (B) healthy donors (****P < 0.0001).

was observed between stages I and IV (**Figure 4C**).

The statistical analysis of clinical staging data in patients with CRC, focusing on the primary tumor in the T-stage (**Figure 4D**), indicated that 3’tRF-SerGCT was statistically non-significant. However, the comparison of N0 and N1 lymph node metastasis displayed a statistically significant difference (P = 0.0235*). No significant differences were found between N0 and N2/N3 (**Figure 4E**). The tumor staging experienced a significant difference between stages I and II (P = 0.0037**) and I and III (P = 0.0146*), while no significant difference was observed between stages I and IV (**Figure 4F**). Analysis of data from 205 patients with CRC (**Table 2**) revealed that 5’Leader-ArgTCG expression differed significantly between genders, with male patients exhibiting a higher prevalence of this expression. This difference was demonstrated to be statistically significant. The clinicopathological data revealed that 5’Leader-ArgTCG and 3’tRF-SerGCT were statistically non-significant concerning age, pathology type, life habits, and tumor metastasis.

5’Leader-ArgTCG and 3’tRF-SerGCT as predictors for CRC diagnosis

5’Leader-ArgTCG and 3’tRF-SerGCT were identified as diagnostic indicators for CRC through qPCR. The predictive capability of these markers was assessed using AUC. CRC diagnostic rate using 5’Leader-ArgTCG and 3’tRF-SerGCT as predictors was found to be 0.659 (**Figure 5A**, **5B**). Additionally, 5’Leader-ArgTCG and 3’tRF-SerGCT combined diagnosis was more effective, reaching 0.830 (95% confidence interval [CI]: 0.789-0.869, sensitivity: 0.896, specificity: 0.620, cutoff value: 0.516, **Figure 5C**). The combined diagnostic efficiency was high, which is noteworthy for CRC diagnosis.

5’Leader-ArgTCG and 3’tRF-SerGCT combined tumor markers improve CRC diagnosis

Tumor-associated antigens are primarily utilized in clinical practice for the purpose of predicting CRC. One of these markers, carcinoembryonic antigen (CEA), is found in high levels in the blood of patients with CRC. However, CEA

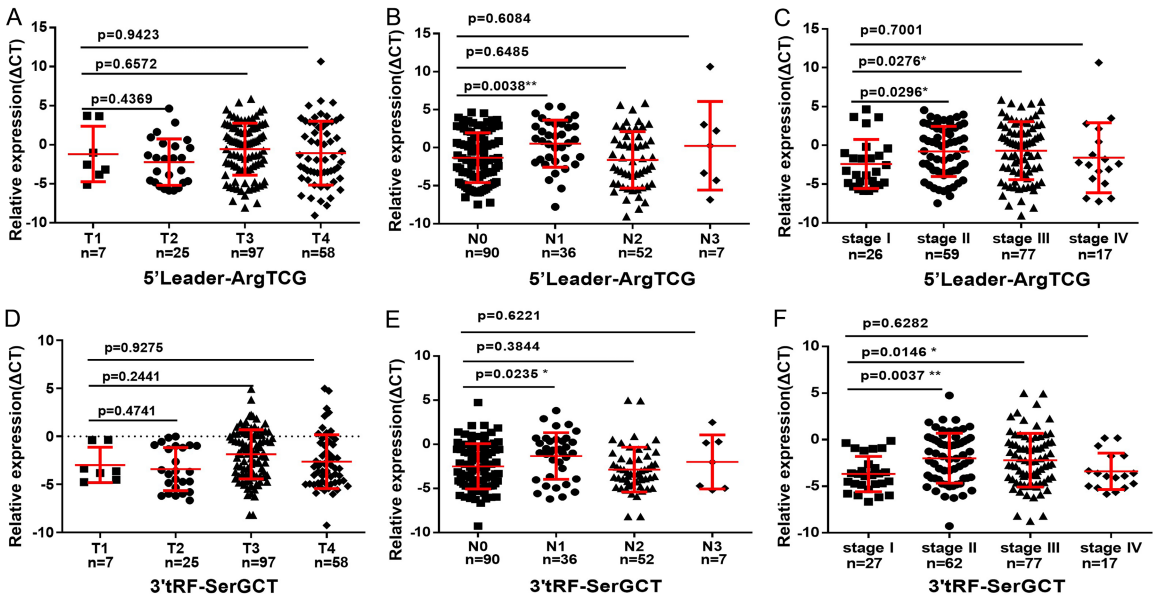

Figure 4. Analysis between the serum exosomal tRF RNAs and tumor stage; tumor metastasis. Expression levels of 5'Leader-ArgTCG (A) and (B) in T and N stages patients. (C) Serum exosomal 5'Leader-ArgTCG expression in early stage (I+II) and advanced stage (III+IV) patients. (D, E) Statistical levels of 3'tRF-SerGCT in T and N stages patients (F) Serum exosomal 3'tRF-SerGCT expression in stage (**P < 0.01, *P < 0.05, ns, not significant).

Table 2. Characteristics of colorectal cancer patients for differentially expressed exosomal 5'Leader-ArgTCG and 3'tRF-SerGCT

Characteristic		5'Leader-ArgTCG			3'tRF-SerGCT		
		No. cases	Median with interquartile range	*P*-value	No. cases	Median with interquartile range	*P*-value
Age (year)	≤ 62	76	-0.8097 (-8.048-10.65)	0.8801	76	-2.601 (-8.731-4.750)	0.7013
	> 62	129	-1.005 (-9.054-5.873)		129	-2.576 (-9.252-4.984)	
Gender	Male	126	-1.477 (-9.054–5.873)	0.0289*	126	-1.477 (-9.054-5.873)	0.1207
	Female	79	0.6431 (-6.835-10.65)		79	-2.246 (-8.731-4.946)	
Smoking	Smoker	44	-1.523 (-8.048-3.694)	0.0885	42	-2.166 (-8.173-2.157)	0.8142
history	non-smoker	161	-0.1025 (-9.054-10.65)		159	-2.582 (-9.252-4.984)	
Drinking	Drinker	43	-1.342 (-8.048-3.694)	0.1321	41	-1.342 (3.694–8.048)	0.3092
history	non-drinker	162	-0.3762 (-9.054-10.65)		160	-2.462 (4.984–8.731)	
Lymph node metastasis	Yes	95	-0.0255 (-9.054-10.65)	0.2051	95	-2.579 (-8.175-4.984)	0.4699
	No	90	-1.643 (-7.477-4.644)		90	-2.745 (-9.252-4.750)	
	unknow	0			0		
Distant metastasis	Yes	17	-1.793 (-7.241-10.65)	0.6024	17	-3.593 (-5.632-0.1797)	0.4396
	No	167	-1.211 (-9.054-5.873)		167	-2.582 (-9.252-4.984)	

CRC, colorectal cancer; Red font annotation and statistical significance, *$P < 0.05$.

lacks certain specificity to accurately predict CRC and cannot identify the specific organ where the cancer originated. Consequently, it is important to combine CEA with other tumor indicators for a reliable clinical diagnosis. CA724 is a laboratory marker used to detect gastric cancer and other digestive tract cancers. It is primarily found in gastrointestinal disorders and is highly sensitive in diagnosing various cancers, including gastric cancer, biliary system tumors, CRC, and pancreatic cancer. The diagnostic efficiency of 5'Leader-ArgTCG and 3'tRF-SerGCT, when combined with tumor marker assays, exhibited significant improvement for CRC identification. The diagnostic efficiency of 5'Leader-ArgTCG and CEA was 0.837 (sensitivity: 0.737, specificity: 0.823, cutoff value: 0.56, **Figure 6A**), while the combination

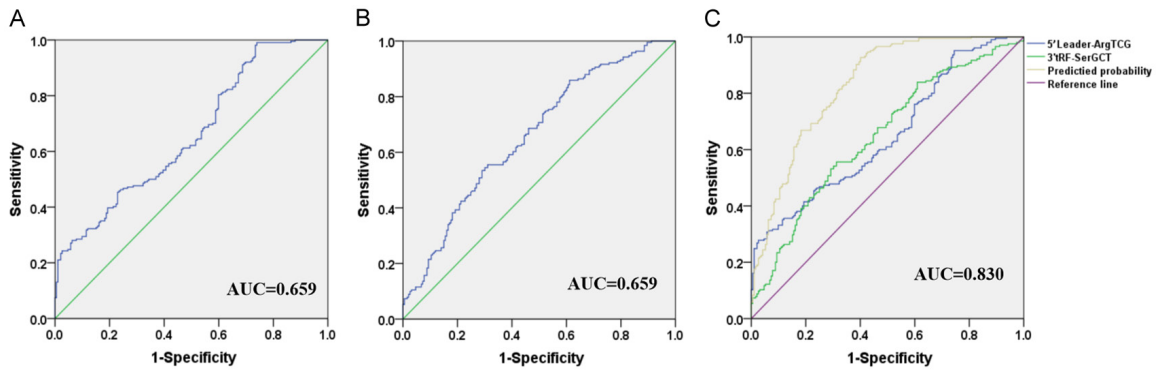

Figure 5. Diagnostic role of serum exosomal 5'Leader-ArgTCG and 3'tRF-SerGCT expression levels in CRC patients patients. The AUCs of 5'Leader-ArgTCG (A), 3'tRF-SerGCT (B), and both (C) in CRC patients relative to healthy donors. AUC (area under the curve, 0-1, The larger the value, the higher the diagnostic efficiency).

with CA724 yielded an efficiency of 0.723 (**Figure 6B**). In contrast, 5'Leader-ArgTCG in combination with two tumor markers achieved a diagnostic efficiency of 0.860 (**Figure 6C**). The 3'tRF-SerGCT combined with CEA and CA724 were tested for their AUCs, respectively, revealing a 3'tRF-SerGCT and CEA diagnostic efficiency of 0.836 (95% CI: 0.811-0.887, sensitivity: 0.614, specificity: 0.932, cutoff value: 0.546, **Figure 6D**). The combination of 3'tRF-SerGCT and CA724 resulted in a diagnostic efficiency of 0.702 (**Figure 6E**), while the combination of 3'tRF-SerGCT, CEA, and CA724 achieved a diagnostic rate of 0.854 (95% CI: 0.804-0.886, **Figure 6F**). 5'Leader-ArgTCG and 3'tRF-SerGCT together demonstrated a combined CEA diagnostic rate of 0.918 (**Figure 6G**) and a combined CA724 diagnostic rate of 0.905 (**Figure 6H**). Ultimately, the combination of 5'Leader-ArgTCG and 3'tRF-SerGCT, along with two tumor markers, achieved a diagnostic rate of 0.954 (95% CI: 0.936-0.973, sensitivity: 0.828, specificity: 0.933, cutoff value: 0.771, **Figure 6I**).

Discussion

The morbidity and mortality rates of CRC are increasing annually, making it the second-highest incidence of cancer globally [15, 16]. Early detection and diagnosis of CRC are very necessary. Colonoscopy is currently the most intuitive diagnostic modality, which is generally difficult to carry out as a routine procedure. Therefore, there is a critical need for simple and non-invasive diagnostic methods. We have chosen to investigate non-invasive tRF RNAs and tumor markers as potential screening methods for CRC. This approach offers several advantages, including minimizing the discomfort associated with colonoscopy [17] and improving patient compliance by accurately predicting the omission of small colorectal lesions through blood tests.

The study has several limitations regarding the patient specimens collected. Notably, the data did not include the early-stage patient number. Additionally, there was an insufficient number of patients with early-stage cancer, and all the collected patient cases were in an advanced stage. Colorectal diseases are primarily chronic conditions that often receive little attention from patients, resulting in few individuals undergoing routine physical examinations. Consequently, when collecting data and specimens, it is essential to broaden the approach by focusing on screening samples from early-stage patients [11]. The value of 5'Leader-ArgTCG and 3'tRF-SerGCT as an early diagnosis of CRC remains unclear. Currently, there are fewer studies on tRF RNAs for CRC diagnosis, which represents a novel aspect of this research [18, 19]. In future studies, we aim to concentrate on evaluating the diagnostic efficacy of tRF-RNA specimens in early CRC to enhance the early detection rate of the disease.

The study indicated that 5'Leader-ArgTCG expression was significantly suppressed in patients with CRC *in vivo*, while 3'tRF-SerGCT was significantly overexpressed in the patients' serum Exos. A review of the existing lit-

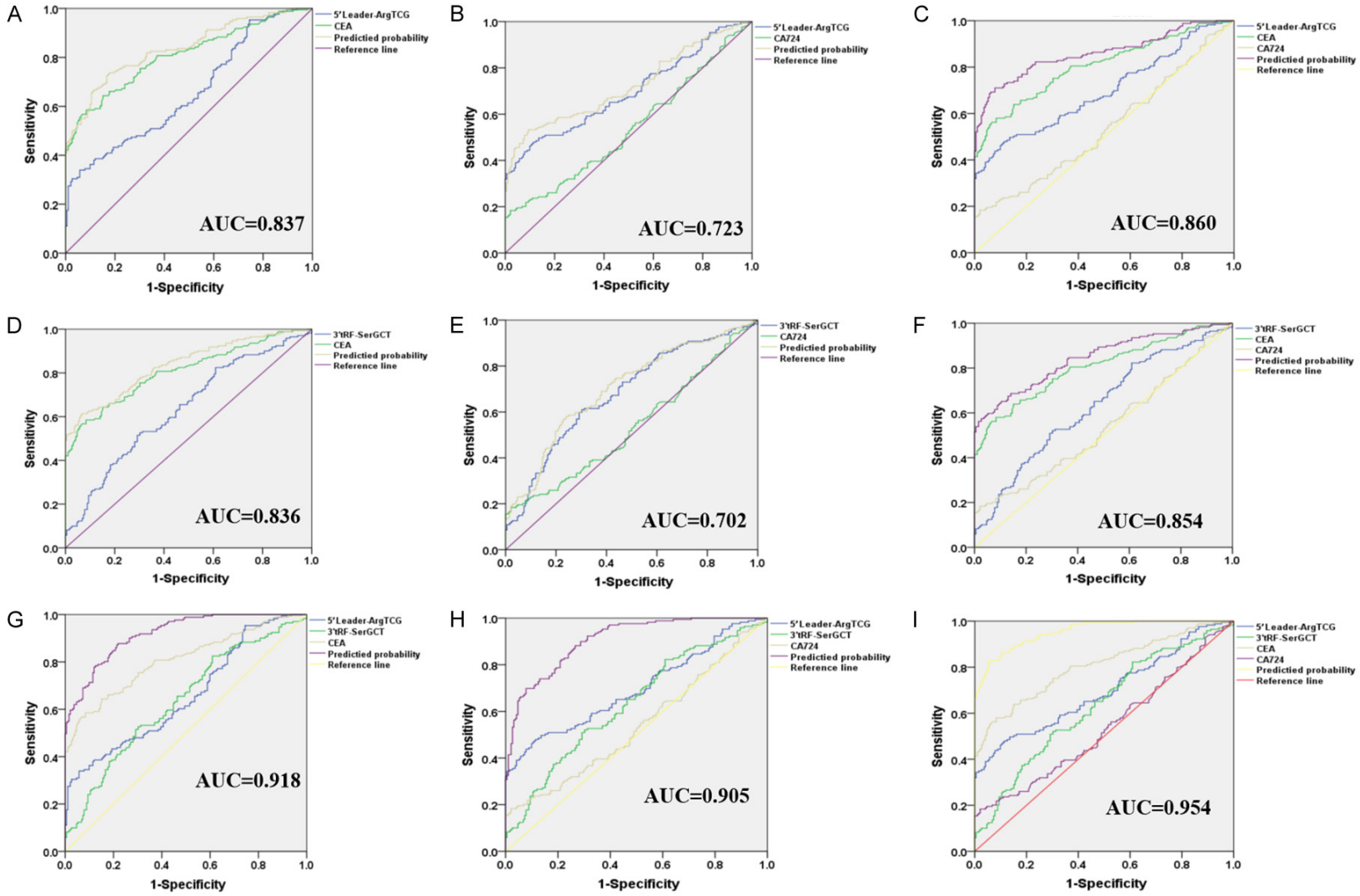

Figure 6. Improved diagnostic capacity of serum exosomes 5'Leader-ArgTCG and 3'tRF-SerGCT combined with established tumor markers in CRC patients. The AUCs of 5'Leader-ArgTCG combined with CEA (A), CA724 (B), and both (C). The AUCs of 3'tRF-SerGCT combined with CEA, CA724 (D, E), and both three (F). The AUCs of 5'Leader-ArgTCG and 3'tRF-SerGCT combined with CEA (G), CA724 (H), and both four (I). AUC (area under the curve, 0-1).

erature revealed that there have been no prior reports on the expression of 5'Leader-ArgTCG and 3'tRF-SerGCT in patients with CRC. Our research is the first to demonstrate that 5'Leader-ArgTCG is differentially expressed in the Exos of patients with CRC, suggesting its potential use as a predictor for relevant interventions [20, 21]. The diagnostic efficiency of single tRF RNAs was high, with an AUC value of 0.659. Moreover, the diagnostic power of combined tumor markers was significantly enhanced. Glycoprotein CEA is a widely used blood-based molecular marker for CRC and serves as a valuable tool for patient monitoring [22-24]. From a prognostic point of view, detecting CEA expression can aid in determining the occurrence of CRC, particularly in metastatic patients with increased expression. However, one notable limitation is its lack of specificity [25, 26]. Glycoprotein antigen (CA724) is another test marker that detects various digestive tract cancers [27]. While it is a non-specific tumor marker, it demonstrates high sensitivity for biliary system tumors, CRC, pancreatic cancer, and others [28-30]. When combining tumor markers for diagnosis, our findings revealed a diagnostic efficacy of 0.954 (95% CI: 0.902-0.955), supporting the diagnosis of non-invasive CRC.

5'Leader-ArgTCG and 3'tRF-SerGCT were statistically significant in lymph node metastasis in patients with CRC. This suggests a potential connection to tumor metastasis, indicating that these factors could serve as predictive intervention targets. Additionally, 5'Leader-ArgTCG and 3'tRF-SerGCT were significantly correlated with the tumor stage of patients with CRC. Notably, stages I/II/III were statistically significant, while stages I/IV were statistically non-significant. The relatively small number of patients in stage IV indicates a need for further investigation to understand the specific reasons behind this observation.

The limitations of this study include the collection of serum samples from patients with CRC and the limited data from early-stage patients. Factors such as overall CRC progression, multiple adenomas, low detection rates during disease development, and poor patient compliance all contribute to the increasing incidence of major cancers [31-34]. Early diagnosis and ongoing monitoring of cancer are also shortcomings of this study. In the future, we aim to gather enhanced case information on patients with CRC and investigate the relationship between additional tRF RNAs and CRC to improve early diagnosis. Simultaneously, we will explore the connection between these factors and tumor metastasis to enhance the survival rate of patients with CRC through timely intervention and treatment.

Conclusion

Both 5'Leader-ArgTCG and 3'tRF-SerGCT are recently developed diagnostic indexes with a strong combined diagnostic effect, demonstrating high efficiency in predicting CRC development. These indices are expected to serve as novel predictive variables for clinical applications.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Thanikachalam K and Khan G. Colorectal cancer and nutrition. Nutrients 2019; 11: 164.
[2] Li N, Lu B, Luo C, Cai J, Lu M, Zhang Y, Chen H and Dai M. Incidence, mortality, survival, risk factor and screening of colorectal cancer: a comparison among China, Europe, and northern America. Cancer Lett 2021; 522: 255-268.
[3] Avelar-Barragan J, DeDecker L, Lu ZN, Coppedge B, Karnes WE and Whiteson KL. Distinct colon mucosa microbiomes associated with tu-

bular adenomas and serrated polyps. NPJ Biofilms Microbiomes 2022; 8: 69.
[4] Li J, Ma X, Chakravarti D, Shalapour S and DePinho RA. Genetic and biological hallmarks of colorectal cancer. Genes Dev 2021; 35: 787-820.
[5] Feng RM, Zong YN, Cao SM and Xu RH. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? Cancer Commun (Lond) 2019; 39: 22.
[6] Kumar P, Mudunuri SB, Anaya J and Dutta A. tRFdb: a database for transfer RNA fragments. Nucleic Acids Res 2015; 43: D141-145.
[7] Lee YS, Shibata Y, Malhotra A and Dutta A. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes Dev 2009; 23: 2639-2649.
[8] Losh JS, King AK, Bakelar J, Taylor L, Loomis J, Rosenzweig JA, Johnson SJ and van Hoof A. Interaction between the RNA-dependent ATPase and poly(A) polymerase subunits of the TRAMP complex is mediated by short peptides and important for snoRNA processing. Nucleic Acids Res 2015; 43: 1848-1858.
[9] Batrakova EV and Kim MS. Using exosomes, naturally-equipped nanocarriers, for drug delivery. J Control Release 2015; 219: 396-405.
[10] Kimiz-Gebologlu I and Oncel SS. Exosomes: large-scale production, isolation, drug loading efficiency, and biodistribution and uptake. J Control Release 2022; 347: 533-543.
[11] Zhou B, Xu K, Zheng X, Chen T, Wang J, Song Y, Shao Y and Zheng S. Application of exosomes as liquid biopsy in clinical diagnosis. Signal Transduct Target Ther 2020; 5: 144.
[12] Bastos N, Ruivo CF, da Silva S and Melo SA. Exosomes in cancer: use them or target them? Semin Cell Dev Biol 2018; 78: 13-21.
[13] Lu S, Wei X, Tao L, Dong D, Hu W, Zhang Q, Tao Y, Yu C, Sun D and Cheng H. A novel tRNA-derived fragment tRF-3022b modulates cell apoptosis and M2 macrophage polarization via binding to cytokines in colorectal cancer. J Hematol Oncol 2022; 15: 176.
[14] Wu Y, Yang X, Jiang G, Zhang H, Ge L, Chen F, Li J, Liu H and Wang H. 5'-tRF-GlyGCC: a tRNA-derived small RNA as a novel biomarker for colorectal cancer diagnosis. Genome Med 2021; 13: 20.
[15] Shin AE, Giancotti FG and Rustgi AK. Metastatic colorectal cancer: mechanisms and emerging therapeutics. Trends Pharmacol Sci 2023; 44: 222-236.
[16] Aljama S, Lago EP, Zafra O, Sierra J, Simón D, Santos C, Pascual JR and Garcia-Romero N. Dichotomous colorectal cancer behaviour. Crit Rev Oncol Hematol 2023; 189: 104067.
[17] Zygulska AL and Pierzchalski P. Novel diagnostic biomarkers in colorectal cancer. Int J Mol Sci 2022; 23: 852.
[18] Han Y, Peng Y, Liu S, Wang X, Cai C, Guo C, Chen Y, Gao L, Huang Q, He M, Shen E, Long J, Yu J, Shen H and Zeng S. tRF3008A suppresses the progression and metastasis of colorectal cancer by destabilizing FOXK1 in an AGO-dependent manner. J Exp Clin Cancer Res 2022; 41: 32.
[19] Li H, Wu C, Aramayo R, Sachs MS and Harlow ML. Synaptic vesicles contain small ribonucleic acids (sRNAs) including transfer RNA fragments (trfRNA) and microRNAs (miRNA). Sci Rep 2015; 5: 14918.
[20] Christodoulou S, Katsaraki K, Vassiliu P, Danias N, Michalopoulos N, Tzikos G, Sideris DC and Arkadopoulos N. High Intratumoral i-tRF-Gly(GCC) expression predicts short-term relapse and poor overall survival of colorectal cancer patients, independent of the TNM stage. Biomedicines 2023; 11: 1945.
[21] Chen H, Xu Z, Cai H, Peng Y, Yang L and Wang Z. Identifying differentially expressed tRNA-derived small fragments as a biomarker for the progression and metastasis of colorectal cancer. Dis Markers 2022; 2022: 2646173.
[22] Gao Y, Wang J, Zhou Y, Sheng S, Qian SY and Huo X. Evaluation of serum CEA, CA19-9, CA72-4, CA125 and ferritin as diagnostic markers and factors of clinical parameters for colorectal cancer. Sci Rep 2018; 8: 2732.
[23] Su YT, Chen JW, Chang SC, Jiang JK and Huang SC. The clinical experience of the prognosis in opposite CEA and image change after therapy in stage IV colorectal cancer. Sci Rep 2022; 12: 20075.
[24] Mahmoud NN. Colorectal cancer: preoperative evaluation and staging. Surg Oncol Clin N Am 2022; 31: 127-141.
[25] Khaled YS, Khot MI, Aiyappa-Maudsley R, Maisey T, Pramanik A, Tiernan J, Lintern N, Al-Enezi E, Shamsuddin SH, Tomlinson D, Coletta L, Millner PA, Hughes TA and Jayne DG. Photoactive imaging and therapy for colorectal cancer using a CEA-Affimer conjugated Foslip nanoparticle. Nanoscale 2024; 16: 7185-7199.
[26] Aakif M, Balfe P, Elfaedy O, Awan FN, Pretorius F, Silvio L, Castinera C and Mustafa H. Study on colorectal cancer presentation, treatment and follow-up. Int J Colorectal Dis 2016; 31: 1361-1363.
[27] Orăseanu A, Brisc MC, Maghiar OA, Popa H, Brisc CM, Solea SF, Maghiar TA and Brisc C. Landscape of innovative methods for early diagnosis of gastric cancer: a systematic review. Diagnostics (Basel) 2023; 13: 3608.

[28] Li XC, Wang S, Yang XX, Li TJ, Gu JX, Zhao L, Bao YR and Meng XS. Patrinia villosa treat colorectal cancer by activating PI3K/Akt signaling pathway. J Ethnopharmacol 2023; 309: 116264.
[29] Tong Y, Zhu Y, Zhao Y, Jiang C, Wang W, Shan Z, Sun F, Liu D and Zhang J. CA724 predicts tumor regression grade in locally advanced gastric cancer patients with neoadjuvant chemotherapy. J Cancer 2021; 12: 6465-6472.
[30] Xu Y, Zhang P, Zhang K and Huang C. The application of CA72-4 in the diagnosis, prognosis, and treatment of gastric cancer. Biochim Biophys Acta Rev Cancer 2021; 1876: 188634.
[31] Hisabe T, Hirai F and Matsui T. Development and progression of colorectal cancer based on follow-up analysis. Dig Endosc 2014; 26 Suppl 2: 73-77.
[32] Dharwadkar P, Greenan G, Singal AG and Murphy CC. Is colorectal cancer in patients younger than 50 years of age the same disease as in older patients? Clin Gastroenterol Hepatol 2021; 19: 192-194, e3.
[33] Patel SG, Karlitz JJ, Yen T, Lieu CH and Boland CR. The rising tide of early-onset colorectal cancer: a comprehensive review of epidemiology, clinical features, biology, risk factors, prevention, and early detection. Lancet Gastroenterol Hepatol 2022; 7: 262-274.
[34] Saus E, Iraola-Guzmán S, Willis JR, Brunet-Vega A and Gabaldón T. Microbiome and colorectal cancer: roles in carcinogenesis and clinical potential. Mol Aspects Med 2019; 69: 93-106.