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Non-canonical small noncoding RNAs in the plasma extracellular vesicles as novel biomarkers in gastric cancer

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Abstract

Identifying robust diagnostic biomarkers for gastric cancer (GC) remains a significant challenge. Emerging studies highlight extracellular vesicle (EV)-derived RNAs in cancer biology, but the diagnostic potential of circulating EV-derived small non-coding RNAs (sncRNAs) in GC is poorly understood. Using panoramic RNA display by overcoming RNA modification aborted sequencing (PANDORA-seq), we mapped non-canonical sncRNAs—specifically ribosomal RNA-derived small RNAs (rsRNAs) and transfer RNA-derived small RNAs (tsRNAs)—in plasma EVs. We identified a three-rs/tsRNA signature that discriminates GC patients from healthy individuals with high sensitivity (80.42%) and specificity (87.43%) (143 GC *vs* 167 controls). For early-stage GC (stage I), sensitivity and specificity were 81.97% and 81.44%, respectively. Furthermore, the three-rs/tsRNA signature was evaluated in two independent cohorts, resulting in AUC values of 0.97 and 0.91 for distinguishing GC from healthy controls. Functional analyses revealed that these rs/tsRNAs regulate the ErbB/Hippo pathways, suggesting them in the underlying pathogenesis and therapeutic potential. This study establishes a novel EV-derived sncRNA signature for early GC detection.

Keywords Gastric cancer, Biomarker, rsRNA, tsRNA, Extracellular vesicles

To the Editor,

Gastric cancer (GC) remains a leading cause of global cancer-related mortality [1]. Late-stage diagnosis often limits treatment efficacy and survival outcomes, emphasizing the need for biomarkers to detect GC earlier.

Circulating extracellular vesicles (EVs) carry stable tumor-associated RNAs in biofluids, particularly small non-coding RNAs (sncRNAs) that mediate key functions in cancer progression [2]. This study proposes developing an EV-based signature targeting novel sncRNAs for GC diagnosis.

Advanced sequencing has identified non-traditional sncRNAs, such as ribosomal RNA-derived (rsRNAs) and transfer RNA-derived small RNAs (tsRNAs) [3–5]. These RNAs evade standard detection due to heavy modifications but enriched in EVs, which shield them from degradation [6]. Notably, tsRNAs link to cancer

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progression [7–9], while rsRNAs dynamically respond to disease states [10], highlighting their promise as real-time biomarkers. Here, using panoramic RNA display by overcoming RNA modification aborted sequencing (PANDORA-seq) [6], we mapped plasma EV sncRNAs landscape in GC, identifying a novel rs/tsRNAs signature with early diagnostic potential.

Dysregulation of non-canonical sncRNAs in gastric cancer

The study design is schematized in Fig.S1. Plasma EVs (Fig.S2) from 30 GC patients and 30 healthy controls underwent PANDORA-seq, revealing altered sncRNA profiles in GC-derived EVs (Fig. 1A, B). The analysis detected 197 miRNAs, 519 piRNAs, 12,789 rsRNAs, 1,553 tsRNAs, and 787 snoRNAs. Scatter plots revealed expression differences between groups (Fig. 1C), with 43 sncRNAs significantly dysregulated (reads per million > 10, fold change > 10; Table S1). The top 10 upregulated (4 tsRNAs, 5 rsRNAs, and 1 piRNA) and downregulated sncRNAs are highlighted in Fig. 1D.

We first validated the top 10 upregulated sncRNAs in an independent training cohort (24 GC patients *vs* 24 controls), with RT-qPCR standard curves confirming assay sensitivity (Fig.S3). Six sncRNAs showed elevated levels in GC EVs (Fig. 1E).

Diagnostic potential of the rs/tsRNA signature in gastric cancer

Validation in a larger cohort (119 GC *vs* 143 controls) confirmed elevated S2/S7/S10 in GC (Fig. 2A). ROC analysis across two cohorts (143 GC *vs* 167 controls) demonstrated diagnostic performance: S2 (AUC 0.86; sensitivity 79.72%, specificity 82.63%), S7 (AUC 0.73; sensitivity 54.55%, specificity 88.62%), S10 (AUC 0.86; sensitivity 71.13%, specificity 86.23%). Combined signature improved accuracy (AUC 0.91; sensitivity 80.42%, specificity 87.43%; Fig. 2B), even in stage I GC (AUC 0.88; sensitivity 81.97%, specificity 81.44%; Fig.S4).

Three random forest models were developed to evaluate clinical potential. The three-sRNA model (AUC 0.95, S2 as key feature) demonstrated higher accuracy than clinical model (AUC 0.70, CA19-9 as the main feature). Their combination achieved an AUC of 0.95 (Fig. 2C, D). The integrated model showed 83.9% sensitivity across all samples, higher than the sRNAs (77.6%) and clinical (43.4%) models (Fig. 2E).

In the testing stage, the three-rs/tsRNA signature showed high diagnostic performance in two test cohorts (Cohort 1: 47 GC *vs* 47 controls; Cohort 2: 40 GC *vs* 43 controls), with individual AUCs of 0.75–0.90 (Cohort 1) and 0.85–0.86 (Cohort 2) (Fig. 2F, Fig.S5). Combined signature achieved higher accuracy (Cohort 1: AUC 0.97; sensitivity 93.6%, specificity 91.5%; Cohort 2: AUC 0.91; sensitivity 85%, specificity 95.4%; Fig. 2G). Postoperative analysis revealed decreased rs/tsRNA levels in 20 GC patients, suggesting potential for monitoring residual tumors and recurrence (Fig.S6).

Functional characterization of the rs/tsRNA signature

To explore the potential functions of three rs/tsRNAs, we performed bioinformatics analysis. RNAhybrid and miRanda (consensus targets) identified critical targets of three rs/tsRNAs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses revealed enrichment in cancer pathways (ErbB/Hippo signaling, gastric cancer progression, etc.) (Fig.S7). Luciferase assays confirmed that S2 bound ErbB (CDKN1A/PAK3) and Hippo (NF2/TEAD3/LATS1) components; S7 targeted SMAD4/BMP5 (Hippo); S10 interacted with NRG4/PRKCA (ErbB) and LIMD1/PPP2R1B/WTIP (Hippo) (Fig. 2H, Fig.S8). Corresponding protein suppression was confirmed by Western blot in GC cells (MKN-45/AGS; Fig. 2I, Fig.S9). These findings revealed that the three rs/tsRNAs regulate ErbB/Hippo pathways, linking them to GC pathogenesis and therapeutic potential.

Conclusions

This study is the first to establish a modification-agnostic profiling of plasma EV sncRNAs in GC through PANDORA-seq, moving beyond traditional miRNA/tsRNA focus. The novel rs/tsRNAs signature shows promise as a blood-based biomarker for early-stage GC detection, improving diagnosis and clinical outcomes. The findings

(See figure on next page.)

Fig. 1 Identification of differentially expressed plasma EV non-canonical sncRNAs in gastric cancer. **A** Read summaries and length distributions of different small RNA categories under PANDORA-seq. **B** Heatmap displaying the expression profile of plasma EV small RNAs in GC group and healthy controls. **C** Scatter plot illustrating the changes in expression levels of miRNAs, piRNAs, rsRNAs, tsRNAs, and snoRNAs. **D** Ranked differential expression of top 10 upregulated (red) and downregulated (blue) sncRNAs (selection criteria: reads per million > 10, fold-change > 10). Parent RNA origins indicated by iconography (rRNA for rsRNAs, tRNA for tsRNAs). **E.** RT-qPCR validation of candidate sncRNAs in training cohort (24 GC patients *vs* 24 healthy controls). Data are shown as the mean ± SEMs. Significance determined by unpaired two-tailed Student's t-test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$

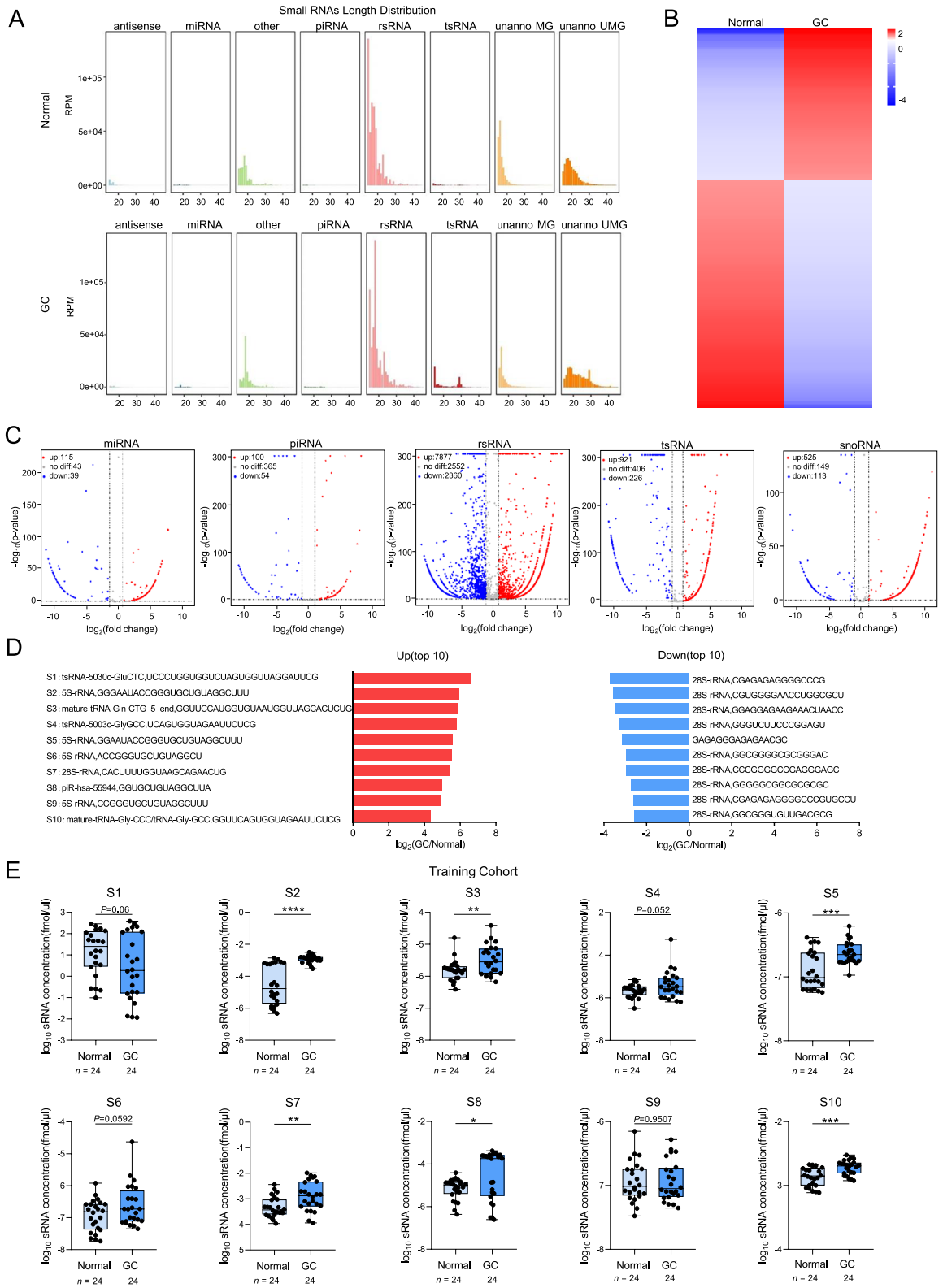
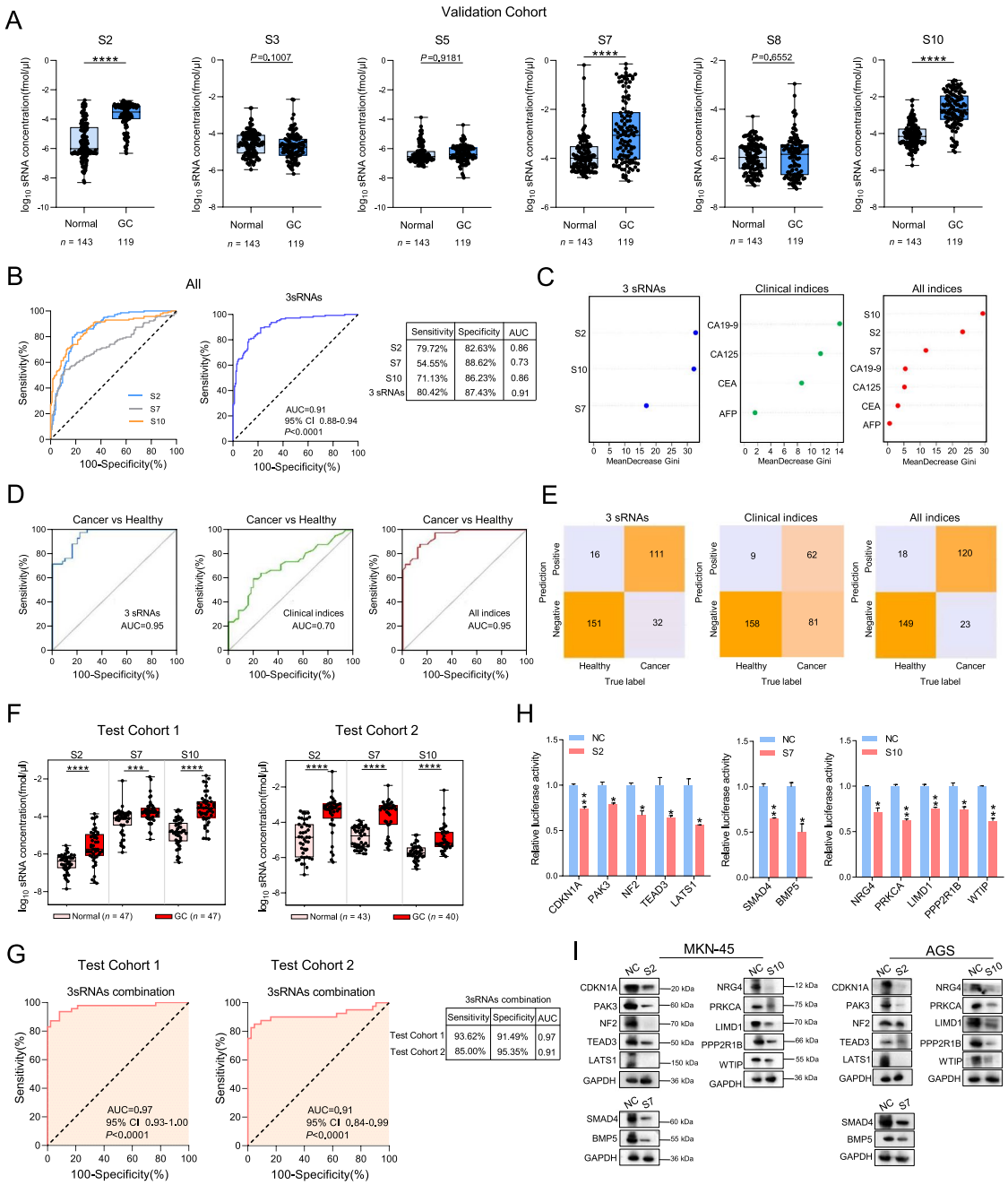


Fig. 1 (See legend on previous page.)



	Sensitivity	Specificity	AUC
S2	79.72%	82.63%	0.86
S7	54.55%	88.62%	0.73
S10	71.13%	86.23%	0.86
3 sRNAs	80.42%	87.43%	0.91

3sRNAs combination	Sensitivity	Specificity	AUC
Test Cohort 1	93.62%	91.49%	0.97
Test Cohort 2	85.00%	95.35%	0.91

Fig. 2 Multi-cohorts validation and functional characterization of plasma EV-derived rs/tsRNA signature in gastric cancer. **A** qRT-PCR analysis of six candidate sncRNAs in validation cohort (119 GC patients *vs* 143 healthy controls). Data are shown as the mean ± SEMs. Significance determined by unpaired two-tailed Student's t-test. ****$P < 0.0001$. **B** ROC analyses of the three-rs/tsRNA signature across training and validation cohorts (143 GC patients *vs* 167 healthy controls). **C** Feature importance ranking by mean decrease Gini index of three rs/tsRNAs(S2/S7/S10), four clinical protein indices (CA19-9, CA125, CEA, AFP), and their combination, as determined by random forest analysis. **D** ROC analysis of the performance of three rs/tsRNAs, four clinical protein indices, and all index combinations in the validation set of machine learning model. **E** Confusion matrix showing the classification accuracy of three rs/tsRNAs, four clinical protein indices, and their combined use in distinguishing GC patients from healthy controls through machine learning. **F** Validation in two independent test cohorts (Test Cohort 1: 47 GC *vs* 47 controls; Test Cohort 2: 40 GC *vs* 43 controls). Data are shown as the mean ± SEMs. Statistical significance was assessed using unpaired two-tailed t tests, ***$P < 0.001$; ****$P < 0.0001$.**G** ROC curves demonstrating diagnostic values of three rs/tsRNAs in two test cohorts. **H** Functional validation of rs/tsRNA-target interactions via dual-luciferase reporter assay. Data are shown as the mean ± SEMs. Statistical significance was assessed using unpaired two-tailed t tests, *$P < 0.05$; **$P < 0.01$; **I** Western blot verification of ErbB/Hippo pathway modulation in GC cells. MKN-45 and AGS cells transfected with sncRNA mimics and scramble negative control RNA, respectively. GAPDH served as internal control. Quantification represents mean ± SEM from three independent experiments

suggest its utility as a complementary tool in GC screening, particularly for risk stratification and prioritizing biopsies in high-risk groups (Fig.S10). Nonetheless, its clinical applicability requires prospective validation in large-scale populations.

Abbreviations

AUC	Area under the curve
EV	Extracellular vesicle
GC	Gastric cancer
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
PANDORA-seq	Panoramic RNA display by overcoming RNA modification aborted sequencing
rsRNA	Ribosomal RNA-derived small RNA
sncRNA	Small noncoding RNA
tsRNA	Transfer RNA-derived small RNA

Supplementary Information

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Additional file1 (PDF 551 KB)

Additional file2 (PDF 539 KB)

Additional file3 (PDF 416 KB)

Author contributions

Methodology and data validation: P.Y., Z.L., X.C., Y.H., X.W., Y.L.; Formal analysis: P.Y., C.M.; Guidance to the study: F.J., T.M.; Methodology and writing—original draft: P.Y., F.J.; Collection of samples and clinical information: P.Y., S.Z.; Bioinformatics analysis: C.M.; Chief designer of the whole experiment: F.J., T.M.. All authors have reviewed and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during the study are included in this published article and its supplementary information. The PANDORA-seq data have been uploaded to GEO(GSE291630).

Declarations

Ethics approval and consent to participate

The study was authorized by the Medical Ethics Committee of Nanjing Drum Tower Hospital (Approval No: 2021–244-01) and all patients had provided informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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