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Systematic Profiling of Small Non-Coding RNA Landscapes in Human Serum and Plasma Extracellular Vesicle Subtypes

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ABSTRACT

Small non-coding RNAs (ncRNAs) function as bioactive molecules packaged inside extracellular vesicles (EVs), where they influence a broad spectrum of physiological and disease-related pathways. This investigation presents an in-depth expression atlas of seven distinct categories of small ncRNAs in EVs isolated from both serum and plasma across multiple conditions. Large EVs (lEVs) and small EVs (sEVs) alike contain considerable fractions of miRNAs (~28.2% in lEVs and ~20.8% in sEVs) and ribosomal RNAs (~24.0% in lEVs and ~19.1% in sEVs). Compared with sEVs, lEVs show markedly higher transfer RNA content (~38.8%), whereas sEVs are characterized by elevated Y RNA levels (~22.5%). In particular, Y RNA abundance rises substantially in sEVs obtained from older donors (age ≥60 years), an observation not replicated in lEVs. Serum-derived EVs display greater variety in their small ncRNA repertoire than plasma-derived counterparts. A substantial overlap (>50%) is evident among the 100 most abundant small ncRNAs shared between lEVs and sEVs. The miRNAs hsa-miR-16-5p, hsa-let-7a-5p, hsa-miR-142-3p, and hsa-miR-103-3p rank consistently within the ten most highly expressed small ncRNAs in both plasma- and serum-derived lEVs, as well as in peripheral blood mononuclear cells. In addition, serum-derived sEVs from patients with glioblastoma, breast cancer, prostate cancer, and gastric cancer are enriched in distinctive, highly abundant miRNAs, snoRNAs, small nuclear RNAs, and piRNAs. Collectively, these observations delineate the distinctive cargo signatures of small ncRNAs within lEVs and sEVs from serum and plasma under normal and pathological states, thereby supplying useful reference points for subsequent biomarker development and therapeutic exploration.

Keywords: Expression profiles, lEVs, Plasma, Serum, sEVs, Small ncRNAs

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Introduction

Extracellular vesicles (EVs) represent important vehicles for intercellular signaling, facilitating the delivery of proteins, lipids, and various RNA molecules to recipient cells [1, 2]. These vesicles are categorized primarily by size, whereby small EVs (sEVs) measure less than 200 nm in diameter and large EVs (lEVs) exceed 200 nm [3, 4]. An expanding body of research highlights the presence of abundant small non-coding RNAs (ncRNAs) within EVs, which exert pivotal control over gene expression networks [5, 6]. Among these, miRNAs transported by EVs have attracted particular attention for their involvement in both normal physiology and disease progression, positioning them as major functional elements of EV cargo [7, 8]. Beyond miRNAs, other small ncRNA families—including small nucleolar RNAs (snoRNAs), ribosomal RNAs (rRNAs), PIWI-interacting RNAs (piRNAs), small nuclear RNAs (snRNAs), transfer RNAs (tRNAs), and Y RNAs—encapsulated in EVs also display altered expression patterns in cancer development [9-11]. Despite these insights, a systematic and

exhaustive characterization of the full spectrum of small ncRNAs in lEVs and sEVs under heterogeneous conditions is still lacking.

Serum and plasma are the predominant biological fluids employed in EV studies [12]. EVs recovered from these matrices carry a heterogeneous collection of small ncRNAs that participate actively in transcriptional regulation and cell-to-cell communication [13, 14]. Existing literature has identified notable variations in vesicle subpopulations, lipid composition, and protein makeup between serum- and plasma-originating EVs [13, 15, 16]. Most contemporary investigations have concentrated on profiling either a single ncRNA class or selected individual transcripts within EVs sourced from particular biological contexts or disease states [17]. To date, however, no study has delivered a holistic assessment of all principal small ncRNA types present in EVs derived from serum and plasma.

In the present work, we constructed detailed expression profiles for seven small ncRNA classes (miRNAs, snoRNAs, piRNAs, snRNAs, rRNAs, tRNAs, and Y RNAs) in EVs obtained from plasma and serum of subjects representing a range of physiological and disease conditions. We first examined the overall small ncRNA composition in lEVs versus sEVs and assessed differences between serum- and plasma-derived EVs in both healthy and diseased settings. Next, we contrasted the small ncRNA repertoires of EVs with those detected in peripheral blood mononuclear cells (PBMCs). Lastly, we pinpointed small ncRNAs that are differentially or selectively expressed in tumor-associated EVs. Such broad-scale profiling offers strong potential for uncovering clinically relevant biomarkers.

Materials and Methods

Data collection and curation

Publicly accessible small RNA sequencing datasets originating from plasma- and serum-derived EVs were downloaded from the EVAtlas database [18] (http://guolab.wchscu.cn/EVAtlas/). Following data consolidation and rigorous quality filtering, 699 lEV and sEV samples from serum and plasma were retained for downstream analyses. These comprised 89 samples from healthy individuals (50 lEVs and 39 sEVs) sourced from serum (31) and plasma (58), along with 610 samples from patients across 10 different tumor types **(Figure 1a)**. To mitigate potential batch-related artifacts, an additional set of 28 high-quality PBMC small RNA-seq datasets was included, each demonstrating an average alignment rate above 50% and encompassing more than 25 samples.

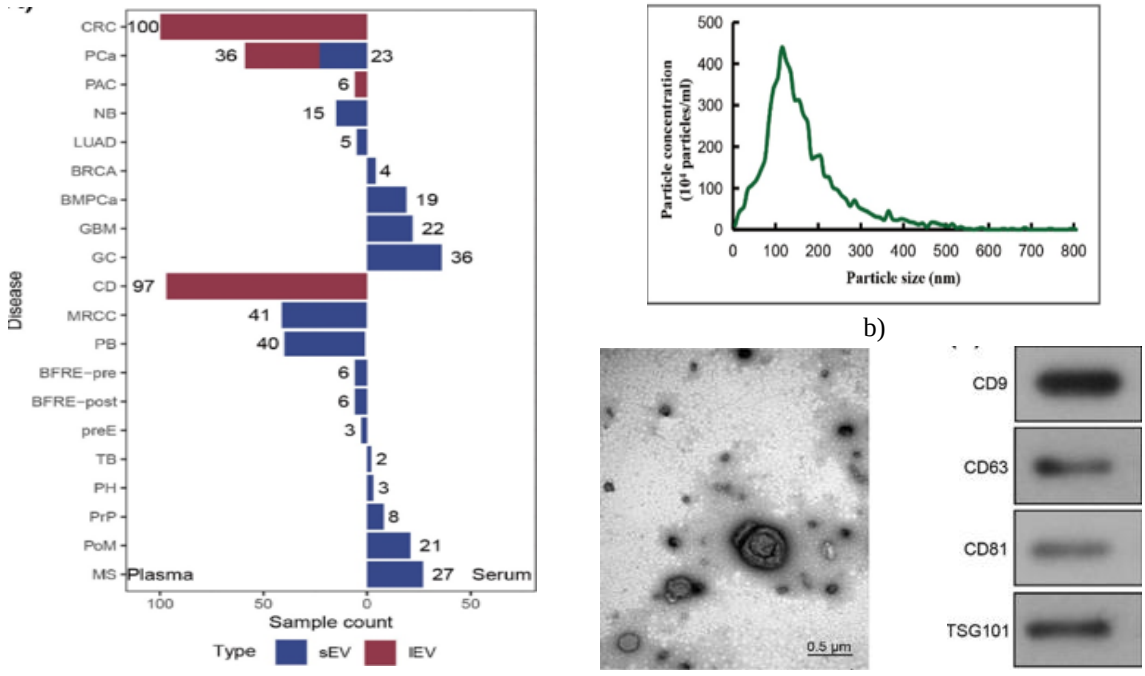


Figure 1. Overview of EV samples retrieved from public repositories together with key properties of the EV samples analyzed in this study.

(a) Breakdown of serum- and plasma-derived EV samples according to tumor and non-tumor origin. (b) Nanoparticle tracking analysis illustrating particle size distribution of the EV preparations. (c) Representative transmission electron microscopy images of EV samples. Scale bars, 0.5 μm. (d) Western blot confirmation of EV marker proteins (CD9, CD63, CD81, TSG101) in the examined EV samples. EV, extracellular vesicle.

Preparation and sequencing of PBMC, serum and plasma-derived EV samples

Beyond the incorporation of publicly sourced datasets, PBMCs and EVs were freshly isolated from peripheral blood of six healthy volunteers to conduct small RNA sequencing. This newly generated dataset was referred to as the A950 project. Ethical approval for the A950 project was granted by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (approval number 2017-S086). Blood was drawn into EDTA-containing tubes for PBMC isolation. Following initial centrifugation at 500 × g for 5 min, red blood cells were eliminated using PharmLyse reagent (BD Biosciences) and the mononuclear cells were subsequently washed. To prepare lEVs, serum and plasma fractions were centrifuged at 4000 × g for 15 min, after which the resulting supernatant was diluted in PBS and ultracentrifuged at 4°C and 16,000 × g for 60 min to recover the lEV pellet. Total RNA was then extracted from both PBMC and EV preparations for small RNA sequencing.

For the six healthy donor specimens, RNA extraction from PBMC and EV materials was performed with the MiPure Cell/Tissue miRNA Kit (Vazyme). Small RNA concentration was measured using the QubitTM microRNA Assay Kit (Invitrogen). Library construction utilized 3 ng of total RNA per sample as starting material. Sequencing libraries were prepared according to the instructions of the QIAseq miRNA Library Kit (Qiagen, Frederick, MD), incorporating index sequences for sample identification. Unique molecular index-containing reverse transcription primers were included to enable precise miRNA quantification during cDNA generation and PCR amplification. Library quality control was conducted on the Agilent Bioanalyzer 2100 instrument and via qPCR. Final libraries were sequenced on the Illumina NovaSeq 6000 platform to produce paired-end reads at EchoBiotech Co. Ltd., Beijing, P. R. China. The raw sequencing data generated in this study are accessible through the China National Center for Bioinformation (CNCB: https://ngdc.cncb.ac.cn/) under the accession PRJCA032563.

Small RNA-seq data quantification and statistical analyses

Prior to determining the expression levels of the seven small ncRNA categories (miRNA, snoRNA, piRNA, snRNA, rRNA, tRNA, and Y RNA) across datasets, any samples sharing multiple SRR identifiers and likely representing technical replicates were combined. Quality control and quantification of individual small ncRNA species were subsequently carried out using the in-house developed tool EVAtool [19]. Analyses proceeded with default settings, and resulting read counts were normalized to reads per million (RPM) for all downstream evaluations. Statistical processing was executed in the R environment with the assistance of the "tidyr" and "dplyr" packages.

Identification of differentially expressed small ncRNAs

The 610 small RNA-seq EV samples (excluding the 89 healthy controls) were assigned to tumor, non-tumor disease, or matched control categories. Tumor types included colorectal cancer (CRC), breast cancer (BRCA), glioblastoma (GBM), gastric cancer (GC), lung adenocarcinoma (LUAD), neuroblastoma (NB), pancreatic cancer (PAC), bone-metastatic prostate cancer (BMPCa), prostate cancer (PCa), and metastatic renal cell carcinoma (MRCC). Non-tumor disease categories comprised post-blood flow-restricted resistance exercise (BFRE-post), pre-blood flow-restricted resistance exercise (BFRE-pre), postmenopausal (PoM), premenopausal (PrP), cognitive decline (CD), multiple sclerosis (MS), pre-eclampsia (preE), premature birth (PB), pulmonary hypertension (PH), and tuberculosis (TB).

Differential expression analysis via edgeR [20] omitted diseases possessing fewer than three matched controls (specifically BRCA, BMPCa, MRCC, CD, MS, and TB). The control for PoM samples was defined as PrP, and BFRE-post samples were compared against BFRE-pre controls. For all other tumor and non-tumor groups, appropriately matched healthy samples served as controls. Small ncRNAs demonstrating an FDR ≤ 0.05 together with $|\log2FC| \geq 1$ were regarded as significantly upregulated or downregulated.

Detection of specifically expressed small ncRNAs in sources and diseases

Prior to specific expression analysis, all 699 samples were integrated and batch effects were adjusted using the ComBat function available in the SVA Bioconductor package [21]. The corrected dataset was then partitioned into three distinct subsets: (1) plasma-sEV samples from PCa, MRCC, LUAD, NB cases and their matched healthy controls; (2) plasma-lEV samples from CRC, PAC, PCa cases and matched healthy controls; and (3) serum-sEV samples from GBM, GC, BMPCa, BRCA cases and matched healthy controls. Identification of small ncRNAs specifically expressed within each of these three categories was achieved using SEGtool [22] under rigorous settings (exp_cutoff = 1 and detect_mod = 3).

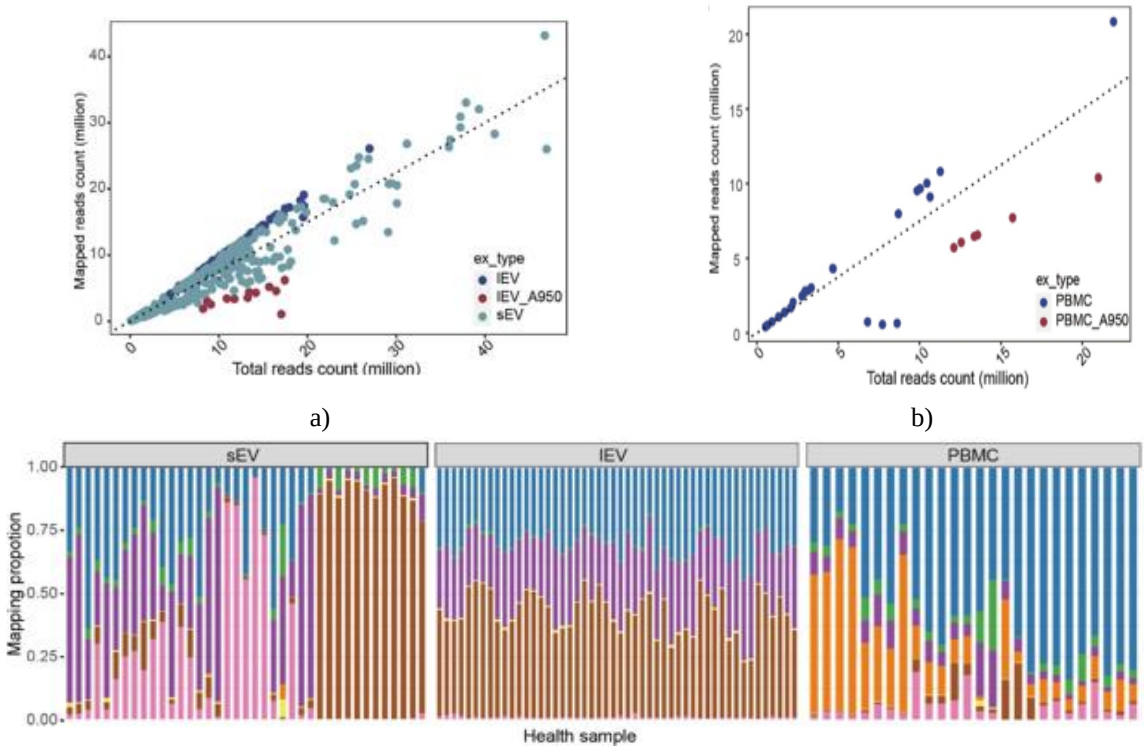
Results and Discussion

Distinct small ncRNA profiles in lEVs and sEVs

In order to characterize the small ncRNA content of extracellular vesicles (EVs), this study drew upon sequencing data from 699 publicly available EV samples representing 10 tumor types **(Figure 1a)**, alongside 12 EV samples freshly isolated from six healthy donors and subjected to small RNA sequencing within the A950 project. Confirmation of EV integrity was achieved through assessment of particle size, morphology, and surface marker proteins **(Figures 1b-1d)**. Sequencing of these EV samples generated an average of 9.96 million raw reads per sample, of which 20%–98.5% successfully aligned to reference sequences for the seven major classes of small ncRNAs **(Figure 2a)**.

Corresponding PBMC samples (28 sourced from public repositories and 6 generated under the A950 project) produced over 14.7 million raw reads per sample, attaining an approximate mapping rate of 51.0% **(Figure 2b)**. A comprehensive read distribution analysis was subsequently conducted on both EV and PBMC datasets from public sources to delineate small ncRNA composition. In samples from healthy individuals, sEVs showed primary alignment to Y RNA (~22.5%), miRNA (~20.8%), and rRNA (~19.1%) **(Figure 2c)**. By comparison, lEVs were markedly enriched in tRNAs (~38.8%), miRNAs (~28.2%), and rRNAs (~24.0%). PBMCs, however, exhibited a different pattern, featuring elevated snoRNA content (17.2%) together with miRNAs (48.8%) and rRNAs (5.97%).

Analysis of A950-project lEVs confirmed miRNA, rRNA, and tRNA as the dominant small ncRNA species, while PBMCs from both A950 and public cohorts were dominated by miRNA, rRNA, and snoRNA **(Figure 2d)**. These profiles aligned closely with broader public dataset observations. Notably, sEVs from healthy elderly donors (≥60 years) displayed pronounced Y RNA enrichment, a feature absent in matched lEV samples **(Figure 2e)**.



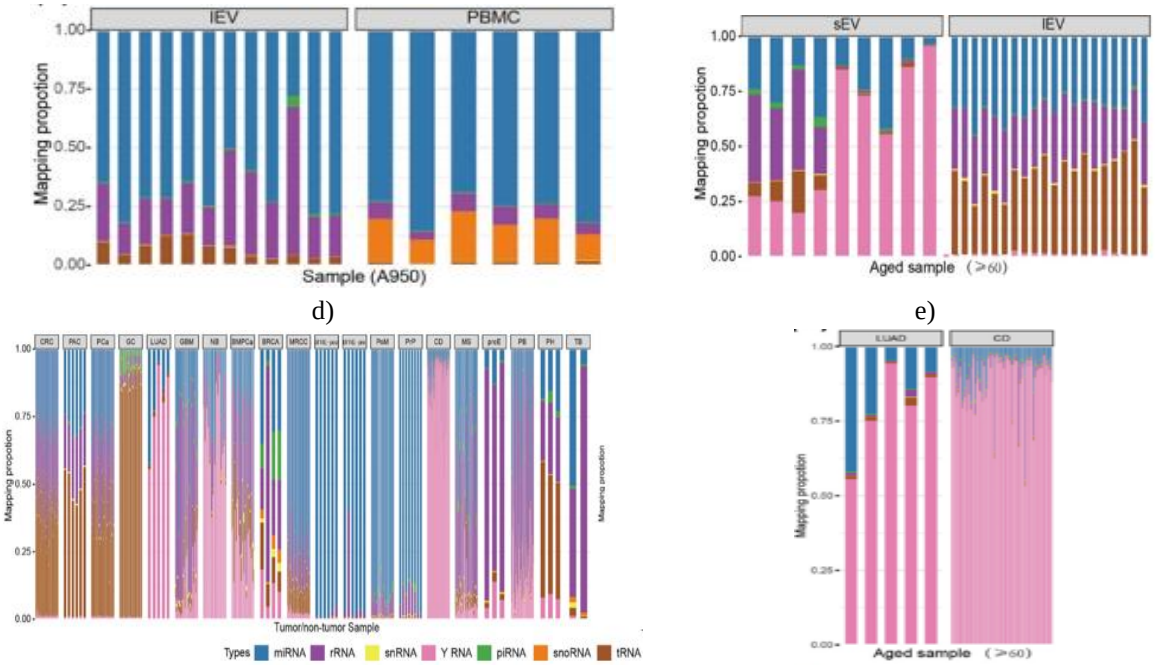


Figure 2. Read distribution patterns of small ncRNAs in sEVs, lEVs, and PBMCs derived from healthy, tumor-bearing, and non-tumor samples. (a–b) Mapped read counts in EV (a) and PBMC (b) samples originating from public databases and the A950 project. (c) Relative abundance of the seven small ncRNA classes in sEVs, lEVs, and PBMCs from public databases, and (D) in lEVs and PBMCs from the A950 project. (e) Relative abundance of the seven small ncRNA classes in sEVs and lEVs from aged samples in public databases. (f) Relative abundance of the seven small ncRNA classes in EVs from tumor versus non-tumor samples. (g) Relative abundance of the seven small ncRNA classes in sEVs and lEVs from aged patients with cognitive decline (CD) and lung adenocarcinoma (≥60 years). EV, extracellular vesicle; ncRNAs, non-coding RNA; PBMC, peripheral blood mononuclear cell.

Further examination of EV small ncRNA read distributions across diverse biological conditions, including tumor and non-tumor states, revealed several defining patterns **(Figure 2f)**: (1) pronounced tRNA enrichment in EVs isolated from CRC, PAC, PCa, GC, and PH; (2) widespread miRNA dominance across nearly all EV populations, surpassing 80% of mapped reads in samples from MRCC, BFRE-post, BFRE-pre, PoM, and PrP; and (3) elevated Y RNA representation in EVs from CD, LUAD, NB, and BMPCa (approximately 84.7%, 71.2%, 51.7%, and 21.1%, respectively). Mirroring the pattern seen in healthy elderly donors, EVs obtained from aged CD and LUAD patients (≥60 years) likewise exhibited clear Y RNA enrichment **(Figure 2g)**.

Diversity of small ncRNAs in plasma- and serum-derived EVs

The abundance of small ncRNAs was assessed in EVs isolated from plasma and serum in both healthy donors and cancer patients. Upon exclusion of rRNA sequences, serum-derived EVs contained substantially more small ncRNAs than plasma-derived EVs **(Figures 3a and 3b)**. Among healthy sEVs, serum samples displayed significantly higher counts of snRNAs and tRNAs compared with plasma **(Figure 3a)**. In healthy lEVs, miRNA abundance was likewise significantly elevated in serum relative to plasma **(Figure 3b)**.

Importantly, the aggregate count of the six small ncRNA categories proved significantly higher in serum-derived sEVs than in serum-derived lEVs, whereas plasma-derived sEVs and lEVs showed no such disparity **(Figure 3c)**. PBMCs exhibited a markedly greater total of these six small ncRNA classes than plasma-derived lEVs, plasma-derived sEVs, or serum-derived lEVs **(Figure 3c)**. Apart from Y RNA and piRNA, all other small ncRNA types occurred in significantly higher numbers in PBMCs than in either plasma- or serum-derived lEVs **(Figure 3d)**. Taken together, serum-derived EVs, encompassing both sEVs and lEVs, demonstrate superior small ncRNA diversity relative to plasma-derived EVs.

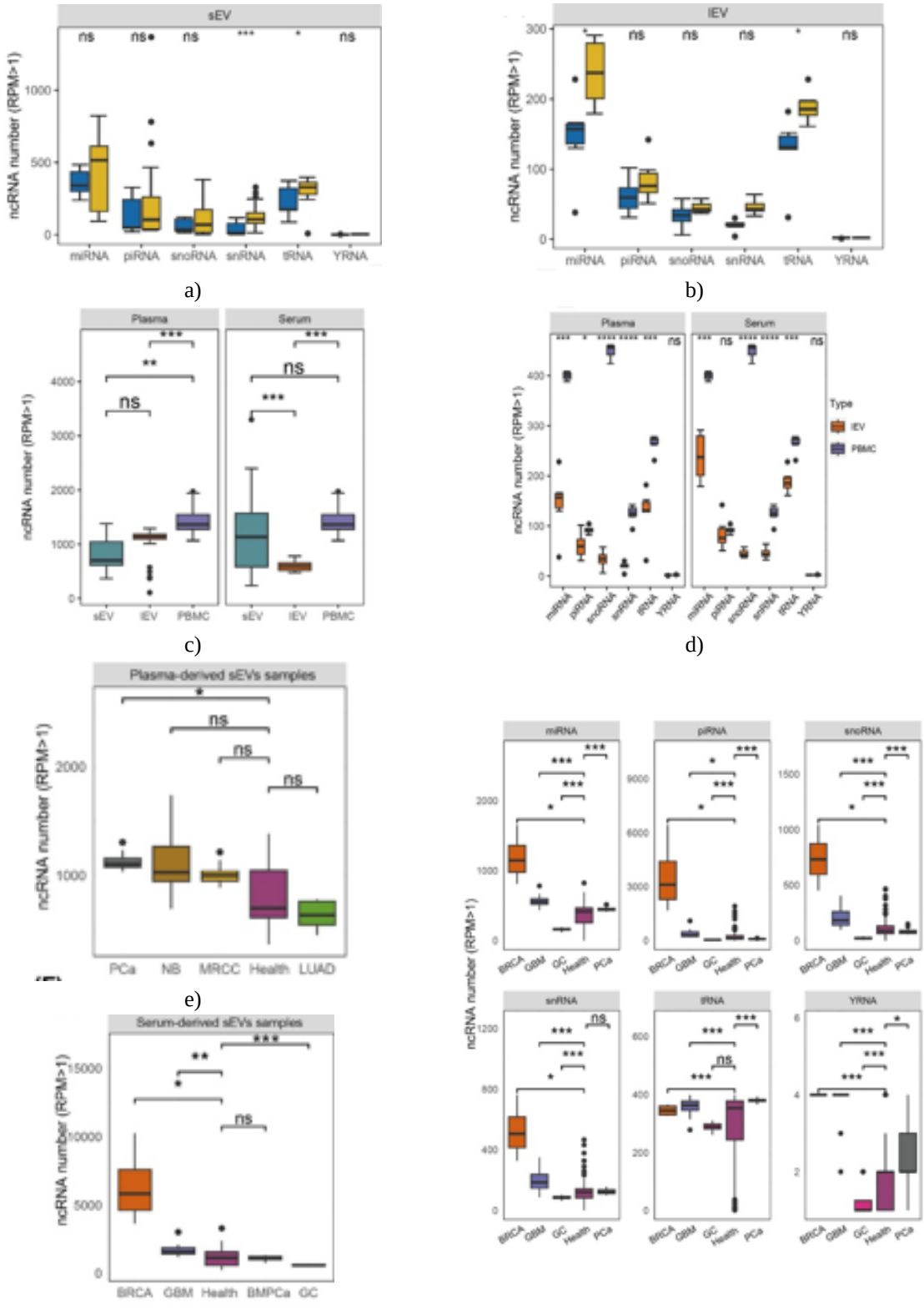


Figure 3. Abundance profiles of small ncRNAs detected in sEVs, lEVs, and PBMCs. (a–b) Quantitative distribution of six small ncRNA classes in sEVs (a) and lEVs (b) isolated from serum versus plasma. (c) Quantitative distribution of small ncRNAs across sEVs, lEVs, and PBMCs. (d) Quantitative distribution of the six small ncRNA classes in plasma- and serum-derived lEVs and PBMCs from the A950 project. Total small ncRNA counts in plasma- (e) or serum- (f) derived sEVs from tumor patients and healthy controls. (g) Quantitative distribution of individual small ncRNA classes in sEVs from tumor and healthy samples. Error bars indicate upper and lower limits; statistical comparisons were performed using the t-test. EV,

extracellular vesicle; ncRNAs, non-coding RNA; ns, not significant; PBMC, peripheral blood mononuclear cell. : p-value <0.05, : p-value <0.01, : p-value <0.001, : p-value <0.0001.

Further evaluation targeted the six small ncRNA classes in plasma- or serum-derived EVs from patients with PCa, GBM, BRCA, and GC. Plasma-derived sEVs from PCa cases contained higher small ncRNA counts than those from healthy individuals **(Figure 3e)**. In serum-derived sEVs, GBM and BRCA samples showed significantly increased small ncRNA numbers compared with healthy controls, while GC samples displayed lower numbers **(Figure 3f)**. Most small ncRNA classes were also significantly more abundant in GBM and BRCA sEVs than in healthy sEVs, except for tRNAs in the BRCA cohort **(Figure 3g)**.

Heterogeneity of small ncRNA expression in EVs and peripheral blood mononuclear cells

To delineate small ncRNA expression features in lEVs, sEVs, and PBMCs from healthy subjects, the most abundant small ncRNAs (top 100) were compared. Plasma-derived lEVs and sEVs shared 51 of these top 100 small ncRNAs **(Figure 4a)**. Relative to PBMCs, plasma-derived lEVs and sEVs shared 25 and 53 of the top 100 small ncRNAs, respectively. Plasma- and serum-derived sEVs together shared 76 of the top 100 small ncRNAs **(Figure 4b)**.

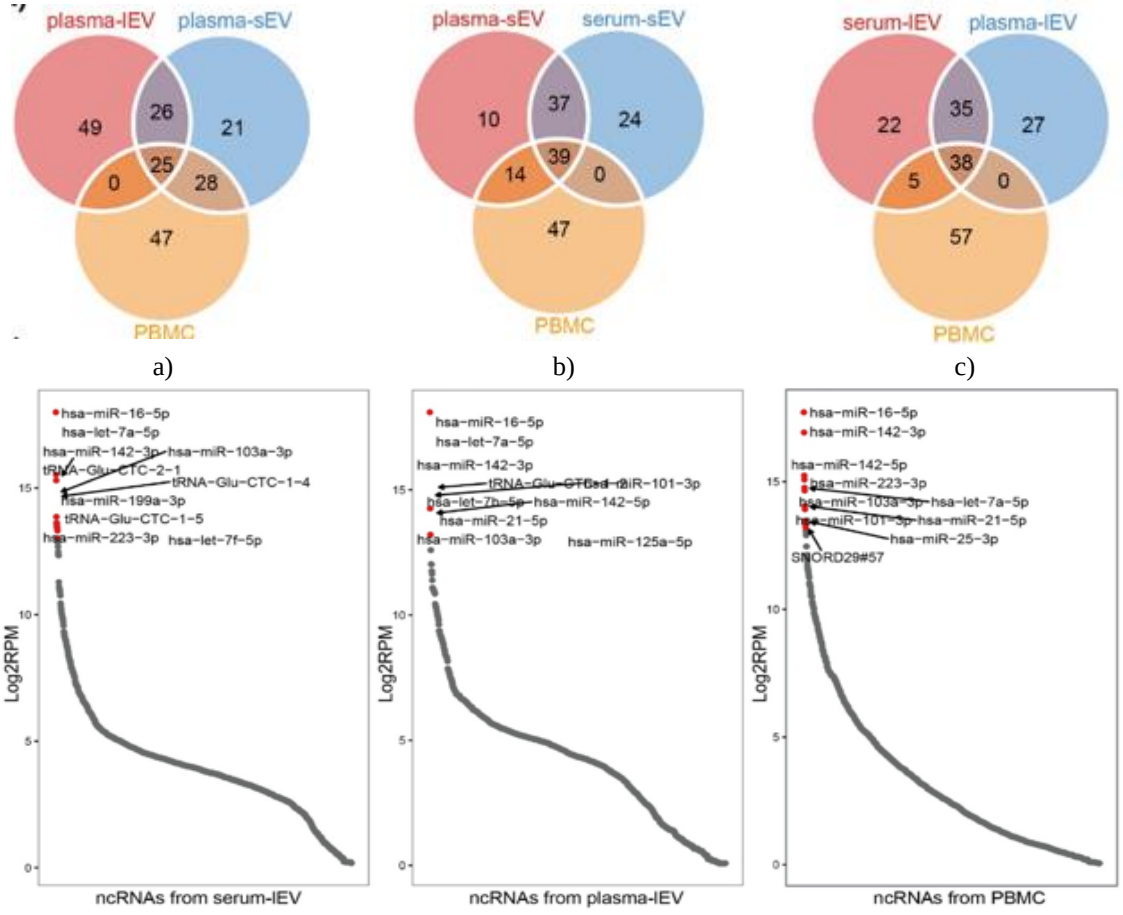


Figure 4. Small ncRNA expression patterns in plasma-derived EVs, serum-derived EVs, and PBMCs. (a) Overlap of the top 100 small ncRNAs in plasma-derived lEVs, sEVs, and PBMCs from public databases. (b) Overlap of the top 100 small ncRNAs in plasma-derived sEVs, serum-derived sEVs, and PBMCs from public databases. (c) Overlap of the top 100 small ncRNAs in serum- and plasma-derived lEVs and PBMCs from the A950 project. (d) Expression distribution patterns of small ncRNAs in serum- and plasma-derived lEVs and PBMCs from the A950 project. EV, extracellular vesicle; ncRNAs, non-coding RNA; PBMC, peripheral blood mononuclear cell.

In the A950 project, serum- and plasma-derived lEVs shared 73 of the top 100 small ncRNAs **(Figure 4c)**. Plasma- and serum-derived lEVs shared 38 and 43 of the top 100 small ncRNAs with PBMCs, respectively **(Figure 4c)**. Overall, highly expressed small ncRNAs displayed considerable overlap across lEVs, sEVs, and PBMCs. Detailed comparison of expression distributions in serum- and plasma-derived lEVs versus PBMCs identified hsa-miR-16-5p, hsa-let-7a-5p, hsa-miR-142-3p, and hsa-miR-103-3p among the ten most highly expressed small ncRNAs common to these sample types **(Figure 4d)**. These observations illustrate both shared and unique small ncRNA expression signatures across lEVs, sEVs, and PBMCs, with notable convergence in their most abundant transcripts.

Differentially and specifically expressed small ncRNAs in tumor-derived EVs

To uncover potential EV-based biomarkers for malignancies, differential expression and tumor-specific analyses were carried out on small ncRNAs by contrasting tumor specimens with their corresponding healthy controls. The analysis uncovered 529, 89, 86, 52, 28, 12, and 6 significantly differentially expressed small ncRNAs in EVs derived from GBM, GC, PCa, NB, CRC, PAC, and LUAD, respectively, relative to matched non-cancer controls **(Table 1)**. Among these, HY1 stood out as the only significantly altered Y RNA, exhibiting upregulation in EVs from PCa tumors compared to healthy donors (log2FC = 1.06, p value = $5.96 \times 10{-}6$) **(Table 1)**.

Following integration and categorization of the differentially expressed small ncRNAs by class, clear tumor-type-specific patterns became apparent, most prominently in the miRNA profiles **(Figure 5a)**. Prior investigations have associated hsa-miR-200a-3p, hsa-miR-885-5p, and hsa-miR-208a-3p with disease progression or cell proliferation in NB, GBM, and lung cancer, respectively [23-25]. In non-tumor samples, EVs from PH, PB, preE, PoM, and BFRE-post conditions contained 48, 10, 6, 2, and 1 significantly differentially expressed small ncRNAs, respectively **(Table 1)**.

Table 1. Summary statistics for differentially expressed small ncRNAs in tumor and non-tumor samples.

Small ncRNA	CRC	GBM	GC	LUAD	NB	PCa	PAC	PreE	PB	PH	BFRE-post	PoM
miRNA	7	137	56	3	11	22	2	1	1	4	0	2
piRNA	11	206	0	0	12	25	5	1	4	4	1	0
snoRNA	4	68	14	0	7	23	2	2	0	4	0	0
snRNA	2	88	3	1	4	6	1	0	0	1	0	0
tRNA	4	30	16	2	18	9	2	2	5	35	0	0
YRNA	0	0	0	0	0	1	0	0	0	0	0	0
Total	28	529	89	6	52	86	12	6	10	48	1	2

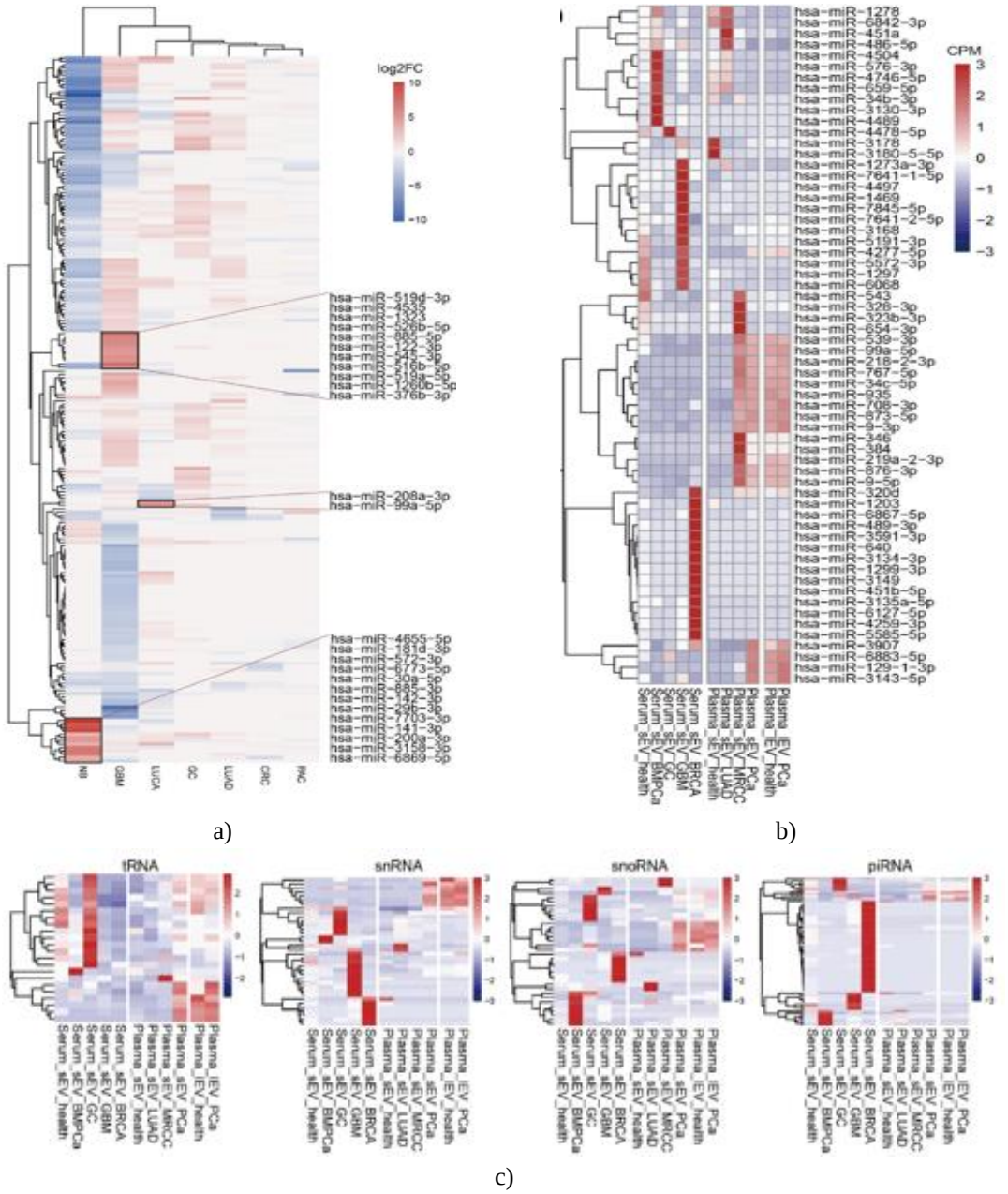


Figure 5. Comprehensive overview of differentially and specifically expressed small ncRNAs in tumor-originating EVs.

(a) Heatmap of differentially expressed miRNAs in tumor-derived EVs, with color intensity corresponding to scaled expression (red indicating highest levels and blue the lowest). (b) Heatmap of specifically expressed miRNAs in serum- and plasma-derived sEVs and plasma-derived lEVs across healthy, tumor, and non-tumor groups. (c) Specific expression of four small ncRNA classes under varying biological conditions. EV, extracellular vesicle; ncRNAs, non-coding RNA.

Attention was then directed toward small ncRNAs showing exclusive or preferential expression in tumors. Results indicated that serum- and plasma-derived sEVs from individual tumor types possessed distinct suites of highly expressed small ncRNAs compared with healthy reference samples. Focusing on miRNAs, unique sets of highly expressed species were identified in serum- and plasma-derived sEVs depending on the tumor context **(Figure 5b)**, notably including the miR-34 family, which has been implicated in PCa pathogenesis [26]. Serum-derived sEVs isolated from BMPCa, GC, GBM, and BRCA cases featured characteristic enrichments of highly expressed small ncRNAs across miRNAs, snoRNAs, snRNAs, and piRNAs. Conversely, plasma-derived sEVs from LUAD, MRCC, and PCa patients displayed relatively balanced representation among the five ncRNA categories without

pronounced specificity **(Figure 5c)**. Overall, these data emphasize the richness of tumor-specific small ncRNAs in serum-derived sEVs and support their utility in future biomarker development for oncology.

The study of extracellular vesicles (EVs) has advanced considerably over the last decade [27], with the detailed characterization of small ncRNA cargo in blood-derived EVs representing a key development in elucidating EV functions in intercellular signaling and identifying RNA-based disease biomarkers [28, 29]. Prior investigations have largely examined small ncRNA expression profiles in EVs from particular disease states or isolated sources, such as sEVs in the context of PCa [30]. The present work offers a broad examination of small ncRNAs in serum- and plasma-derived EVs, revealing their complex and varied profiles across physiological and pathological states. This investigation uncovered marked heterogeneity in both the abundance and expression patterns of the seven major small ncRNA classes within serum- and plasma-derived EVs. Emerging evidence indicates that many small ncRNAs, particularly miRNAs, encapsulated in blood-derived EVs hold promise as tumor biomarkers [31]. Although most research has emphasized sEVs, attention has recently shifted toward lEVs due to their distinctive molecular cargo and biological activities [32]. The current findings demonstrate clear distinctions between lEVs and sEVs in small ncRNA composition and relative abundance. lEVs were predominantly enriched in tRNAs, while sEVs showed prominent representation of Y RNA alongside miRNAs and rRNAs. Additionally, sEVs from aged individuals exhibited a notable elevation in Y RNA content, a feature absent in lEVs. Considering that Y RNA homologs in Caenorhabditis elegans have been linked to aging processes, it is plausible that Y RNAs in human EVs contribute to age-related regulatory mechanisms [33]. miRNAs remain the most thoroughly studied small ncRNA species. The present data confirm that miRNAs accounted for over 20% of reads in nearly all sEV and lEV samples, supporting their central involvement in EV-mediated molecular transfer and their value as potential cancer biomarkers [31].

The observed variability in small ncRNA expression across lEVs, sEVs, and PBMCs highlights the intricate regulatory networks governing these molecules and their multifaceted contributions to cellular communication. Notably, hsa-miR-208a-3p was upregulated in LUAD samples relative to other malignancies. Earlier work has established that this miRNA modulates proliferation and chemoresistance in lung cancer cells, pointing to its possible utility in diagnostic and prognostic applications [24]. Beyond the elevated Y RNA levels in aged sEVs, a significant increase in HY1 expression was detected in PCa tumor-derived EVs compared with healthy controls. Such observations reinforce the potential involvement of Y RNAs in oncogenesis. Small ncRNAs transported by EVs—including miRNAs, snoRNAs, tRNAs, snRNAs, and piRNAs—displayed distinctive expression signatures, particularly within serum-derived sEVs from diverse tumor types. Many of these molecules, such as miRNAs and piRNAs, are actively investigated as disease biomarkers [34]. The tumor-specific expression analysis identified discrete groups of small ncRNAs uniquely enriched in serum-derived sEVs from healthy versus cancerous samples, underscoring their relevance for liquid biopsy strategies [35, 36].

Although this study delivers an extensive overview of small ncRNA profiles in EVs based on available datasets, larger cohorts processed under uniform standardized protocols will be essential to minimize false positives in expression and compositional analyses [37, 38]. Minor variations arising from differences in EV isolation procedures or RNA sequencing platforms may also influence observed small ncRNA levels. Subsequent studies should prioritize validation of these candidate small ncRNA biomarkers in independent, larger patient populations and investigate their mechanistic contributions to disease development and therapeutic responses [39, 40].

Conclusion

In summary, the detailed characterization of small ncRNAs in serum- and plasma-derived EVs presented here constitutes a valuable reference for deciphering molecular mechanisms underlying multiple diseases. These insights lay the groundwork for innovative diagnostic tools and targeted therapies, with the potential to enhance clinical outcomes for patients.

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Conflict of Interest: None

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Ethics Statement: None

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