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Original Article

A panel of serum-derived exosomal miRNAs as markers of cardiovascular risk assessed by carotid intima-media thickness in patients with type 2 diabetes

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Abstract: Objectives: Patients with type 2 diabetes mellitus (T2DM) exhibit accelerated atherosclerosis progression and an increased risk of cardiovascular disease (CVD). Early CVD diagnosis and timely intervention are critical to mitigate complications and mortality in this population. Methods: Serum exosomes were isolated from 12 T2DM patients with or without carotid atherosclerosis (CAS). miRNA profiling was performed using microarray analysis. A total of 187 T2DM patients were divided into the test and validation cohorts. Plasma-derived exosomal miRNAs were quantified using quantitative PCR (qPCR), and their diagnostic potential was assessed using receiver operating characteristic curve analysis and area under the curve (AUC) calculations. Results: Microarray analysis identified 23 differentially expressed miRNAs (DEMIs), including 19 upregulated and 4 downregulated miRNAs. Four of these (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, and hsa-miR-122-5p) were significantly elevated in patients with CAS and showed positive correlation with carotid intima-media thickness. The results also showed that these miRNAs demonstrated diagnostic efficacy for CAS detection in patients with T2DM, and were stratified by disease severity. Conclusions: The identified miRNA panel represents a promising diagnostic biomarker for CAS in patients with T2DM, providing a foundation for the development of targeted therapies to address diabetic cardiovascular complications.

Keywords: Exosomes, miRNA, carotid intima-media thickness, atherosclerosis, diabetes

Introduction

Diabetes mellitus (DM) is a complex, multifactorial metabolic disorder characterized by persistent hyperglycemia. The confluence of population aging and accelerated urbanization has precipitated a global surge in the prevalence of DM, particularly in developing nations [1]. Type 2 diabetes mellitus (T2DM), which constitutes over 90% of diabetes cases, predisposes patients to cardiovascular disease (CVD), a cluster of vascular pathologies that has emerged as the predominant complication in this population [2-4]. Mechanistically, T2DM accelerates atherogenesis and promotes premature CVD development [5]. Epidemiologic data reveal a 2-4-fold higher CVD incidence in patients with T2DM than in their non-diabetic counterparts [6]. Therefore, early diagnosis and prompt intervention for CVD in T2DM patients are essential to mitigate complications, improve prognosis, and alleviate healthcare burdens. The identification of new predictive biomarkers for CVD in T2DM patients remains imperative.

Exosomes, with a diameter of 40-160 nm, are secreted by virtually all types of human cells [7-9] and are widely present in various body fluids [10, 11], including saliva, breast milk, cerebrospinal fluid, urine, and blood [12]. These vesicles transport diverse biomolecules, including DNAs, RNAs, proteins, lipids, and other metabolites [13]. These encapsulated biomolecules reflect cellular origins and mediate intercellular signaling [14, 15]. The inherent stability and pathophysiological relevance have positioned exosomes as frontier targets for diagnostic applications [16, 17].

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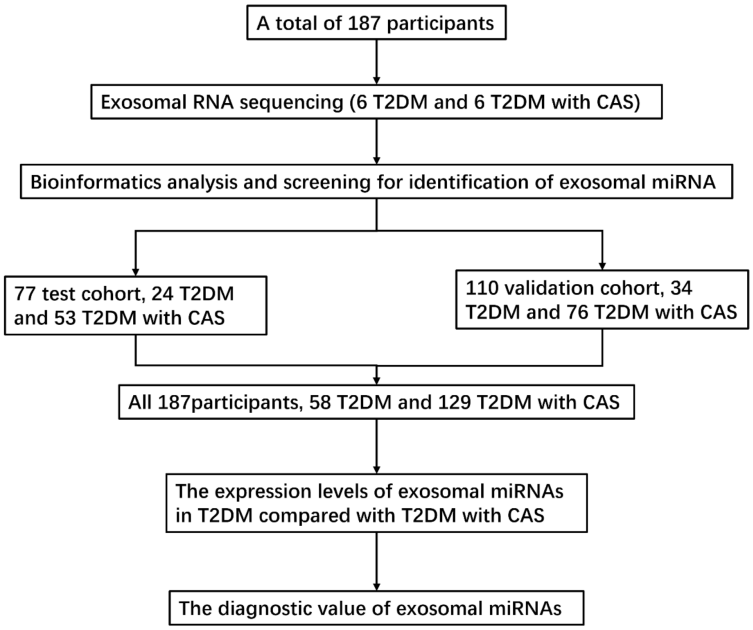


Figure 1. A flow chart of the inclusion process for patients with T2DM. A total of 187 patients meeting the inclusion criteria were enrolled in the study. In the discovery cohort, 12 T2DM patients (6 with CAS and 6 without CAS) underwent comprehensive miRNA microarray profiling. Subsequently, 58 T2DM patients and 129 T2DM + CAS patients were divided into test and validation cohorts for further validation of the miRNA microarray results. CAS: carotid atherosclerosis; T2DM: type 2 diabetes mellitus.

MicroRNAs (miRNAs) are a class of short single-stranded non-coding RNAs of 21-25 nucleotides that modulate various biological processes, including autophagy, proliferation, metabolism, and differentiation [18]. Substantial evidence implicates miRNA dysregulation in atherosclerotic pathogenesis [19]. miRNAs circulating in the serum or plasma can be used as promising non-invasive biomarkers for early detection and monitoring of the progression of asymptomatic atherosclerotic disease [20]. In animals, miRNAs are packaged into exosomes and account for a majority of exosomal RNA. Exosomal miRNAs have received increasing attention because of their noninvasiveness, accessibility, and stability [21, 22]. Although exosomal miRNA signatures have diagnostic potential in the cardiovascular and diabetic contexts [23-25], their utility in diabetic vascular complications remains underexplored.

In the context of primary prevention, increased carotid intima-media thickness (CIMT) measured using ultrasonography is considered a subclinical marker of atherosclerosis and an important predictor of cardiovascular events [26]. However, conventional methods have limitations in terms of the precision and profundity of scanning [27]. More practical approaches are required to identify individuals with carotid atherosclerosis (CAS), especially at the subclinical stage. In the present study, we explored the diagnostic accuracy and role of circulating exosomal miRNAs as emerging biomarkers of subclinical CAS. We hypothesized that serum-derived exosomal miRNAs can be used to detect subclinical CAS.

Materials and methods

Study population

In this two-phase investigation, we adopted a discovery-validation design and the experiments were conducted at the First Affiliated Hospital of Soochow University. In the discovery cohort, 12 T2DM patients (6 with CAS and 6 without CAS) underwent comprehensive miRNA microarray profiling. Subsequently, 58 T2DM patients and 129 with T2DM + CAS were enrolled (June 2022-June 2024) for further validation of the miRNA microarray results. The study design is illustrated in **Figure 1**. CAS was defined as a CIMT value of > 0.9 mm. Patients with confirmed T2DM (as per the ADA criteria) aged 18-80 years were enrolled in the study. The exclusion criteria were as follows: (1) neuropsychiatric disorders impairing protocol compliance and (2) incomplete clinical records. This study was approved by the First Affiliated Hospital of Soochow University (approval no. 2015124) and strictly complied with the Declaration of Helsinki (2008). Written informed consent was obtained from all the participants before sample collection.

Clinical data collection

Demographic parameters (age, sex, and body mass index (BMI), lifestyle factors (smoking and alcohol use), and comorbidities (hypertension duration) were systematically recorded. Fasting blood glucose (FBG), serum creatinine (SCr), total cholesterol (TC), triglyceride (TG),

low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were routinely tested using an automatic biochemical analyzer (7600, HITACHI Company, Japan). Glycosylated hemoglobin A1C (HbA1c) levels were assessed by high-performance liquid chromatography (HLC-723G8, TOSOH Company, Japan).

Ultrasound evaluation of CIMT

The CIMT of the bilateral common carotid arteries (CCA) was measured. It was performed using a standardized protocol with a Philips iE33 ultrasound system (San Jose, CA, USA) equipped with a 12 L-RS linear transducer (6-13 MHz). Imaging was performed at 10-MHz frequency, capturing 1 cm arterial segments extending ≥ 0.5 cm proximal to the carotid bifurcation. The mean value of triplicate measurements from both CCAs was calculated, with CIMT > 0.9 mm indicating pathological thickening. Focal lesions exceeding 1.5 mm were classified as atherosclerotic plaques per established criteria [28, 29]. All scans were performed in duplicate by certified sonographers who were blinded to the participants' clinical data.

Isolation and identification of exosomes

Fasting venous blood (12-hour fast) was collected from the participants and processed through gradient centrifugation (2,000 ×g, 20 min, 4°C) to obtain cell-free serum. Exosomes were isolated using an isolation kit (EZBioscience) with protocol optimization. Briefly, serum (1 mL) was mixed with 30% volume of the precipitation reagent and incubated at 4°C for 12 h. After centrifuging the mixture at 15,000 ×g for 30 minutes at 4°C, the supernatants were aspirated out. Next, 100 μL of phosphate-buffered saline (PBS) was used to resuspend the pellet that contained the exosomes at the tube's bottom.

A multistep procedure was used to confirm the presence of the serum-isolated exosomes. Transmission electron microscopy (TEM; H-7650; Hitachi, Ltd., Tokyo, Japan) was used to examine the morphology. The diameter and quantity of the vesicles were determined using ZetaView PMX 110 (Particle Metrix, Meerbusch) and analyzed using nanoparticle tracking analysis (NTA) software (ZetaView 8.02.28). Finally, specific exosomal markers (CD9, CD63, and TSG101) and the negative marker, calnexin, were determined by western blotting.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from exosomes using TRIzol Reagent (Invitrogen). cDNA was synthesized according to the manufacturer's protocol using a Bulge-Loop miRNA qRT-PCR Starter Kit (Sangon Biotech). Subsequent qRT-PCR amplification utilized the SYBR Green One™ miRNA qPCR Detection Kit (BioTeke) on an ABI 7500 system with SYBR Green Mastermix (Toyobo). U6 served as an endogenous reference. miRNA expression levels were normalized to U6 using the 2-ΔΔCq method [30].

Western blotting

Following a previously established methodology [9], total protein was extracted from the exosomal isolates using an EZBioscience extraction kit. Protein samples (15 μg per lane) were loaded and separated on 10% SDS-PAGE gels (FD341-100, Fudebio), transferred to polyvinylidene difluoride membranes (Millipore Sigma), and blocked with 5% non-fat milk for 2 h at room temperature. Membranes were incubated overnight at 4°C with primary antibodies: anti-CD9 and CD63 (1:1000, Santa Cruz Biotechnology); anti-TSG101 and Calnexin (1:1500, Proteintech). After incubation with secondary antibodies (1:2500, Sangon) for 1.5 h, protein bands were visualized using the Zen-Bio ECL reagent (Chengdu, China).

Statistical analysis

Statistical analyses were performed using SPSS 23.0 and GraphPad Prism 8.0. Normally distributed data are presented as mean ± SD and analyzed by two-tailed unpaired t-test, while non-normally distributed data are expressed as median (interquartile range) with the Mann-Whitney U test. Categorical variables were described as frequencies (%) and compared using χ^2 test or Fisher's exact test. Spearman's correlation coefficient (r) was calculated to evaluate the association between differentially expressed miRNAs (DEMIs) and clinical characteristics. The diagnostic performance of indi-

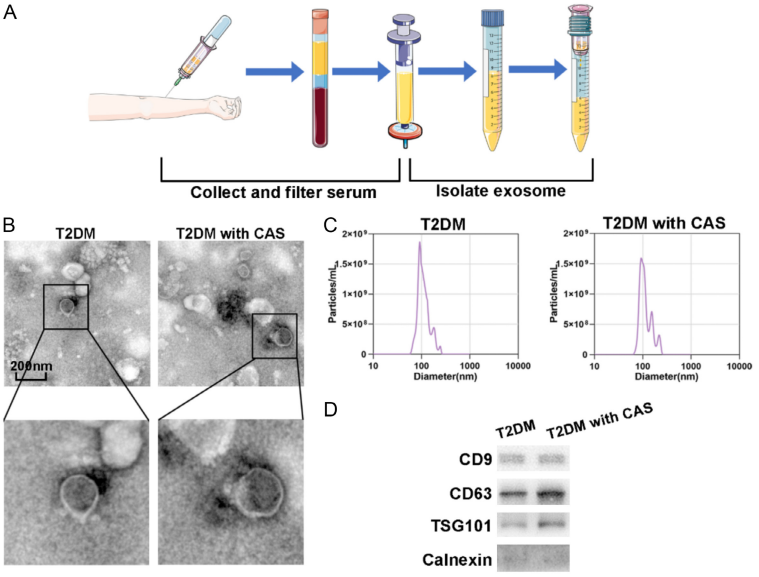


Figure 2. Isolation and identification of exosomes. A. Serum exosomes were successfully isolated from T2DM patients with and without CAS. B. TEM revealed characteristic cup-shaped vesicles with diameters approximating 100 nm. C. NTA demonstrated a size distribution between 40-200 nm, aligning with established exosomal size parameters. D. Western blotting analysis confirmed positive expression of exosomal markers (CD9, TSG101, CD63) and absence of the negative marker calnexin. NTA: nanoparticle tracking analysis; T2DM: type 2 diabetes mellitus; TEM: transmission electron microscopy.

vidual miRNAs and their combinations was assessed using receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) quantification. Statistical significance was defined as $P < 0.05$.

Results

Clinical characteristics of patients

A total of 187 participants were enrolled in our study, comprising 58 T2DM patients (T2DM group) and 129 patients with T2DM and CAS (CAS group) (Table S1). The T2DM group had a median age of 44 years (interquartile range: 37-53.5 years), whereas the CAS group had a significantly older median age of 55 years (interquartile range: 50-61 years). The male: female ratios were 40:18 in the T2DM group and 88:41 in the CAS group, respectively.

Isolation and identification of exosomes

Serum exosomes were successfully isolated from T2DM patients with and without CAS (**Figure 2A**). Comprehensive characterization using TEM, NTA, and western blotting confirmed the isolation of exosomes. TEM revealed characteristic cup-shaped vesicles with diameters of approximately 100 nm (**Figure 2B**). NTA demonstrated a size distribution between 40-200 nm, which aligned with established exosomal size parameters [24] (**Figure 2C**). Western blot analysis confirmed the positive expression of exosomal markers (CD9, TSG101, and CD63) and the absence of the negative marker calnexin (**Figure 2D**).

Profile of exosomal miRNA expression in the serum of patients with T2DM and CAS

Microarray analysis of 12 serum exosome samples (six T2DM vs. six T2DM with CAS) identified 24 DEMIs using thresholds of |fold change| > 2 and an FDR-adjusted *p*-value < 0.05. Nineteen miRNAs were upregulated and four were downregulated in the CAS group (**Figure 3A**). This revealed distinct exosomal miRNA profiles in T2DM patients with and without CAS.

Validation of exosomal miRNA profile identified by microarray

Ten miRNAs with the highest statistical significance (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, hsa-miR-122-5p, hsa-miR-27a, hsa-miR-375, hsa-miR-942-5p, hsa-miR-548d-3p, hsa-miR-371b-5p, and hsa-miR-101) were selected for further analysis. To further confirm these 10 potential biomarkers, we purified serum exosomes and extracted miRNAs from T2DM patients with (58 patients) and those without CAS (129 patients). To increase the reliability of the results, samples were divided into training and validation sets. In the training cohort (24 T2DM vs. 53 T2DM with CAS), five miRNAs were significantly upregulated, and has-miR-27a was downregulated in the CAS group (**Figure 3B**). Correlation analysis with CIMT stratification revealed that four miRNAs (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, and hsa-miR-122-5p)

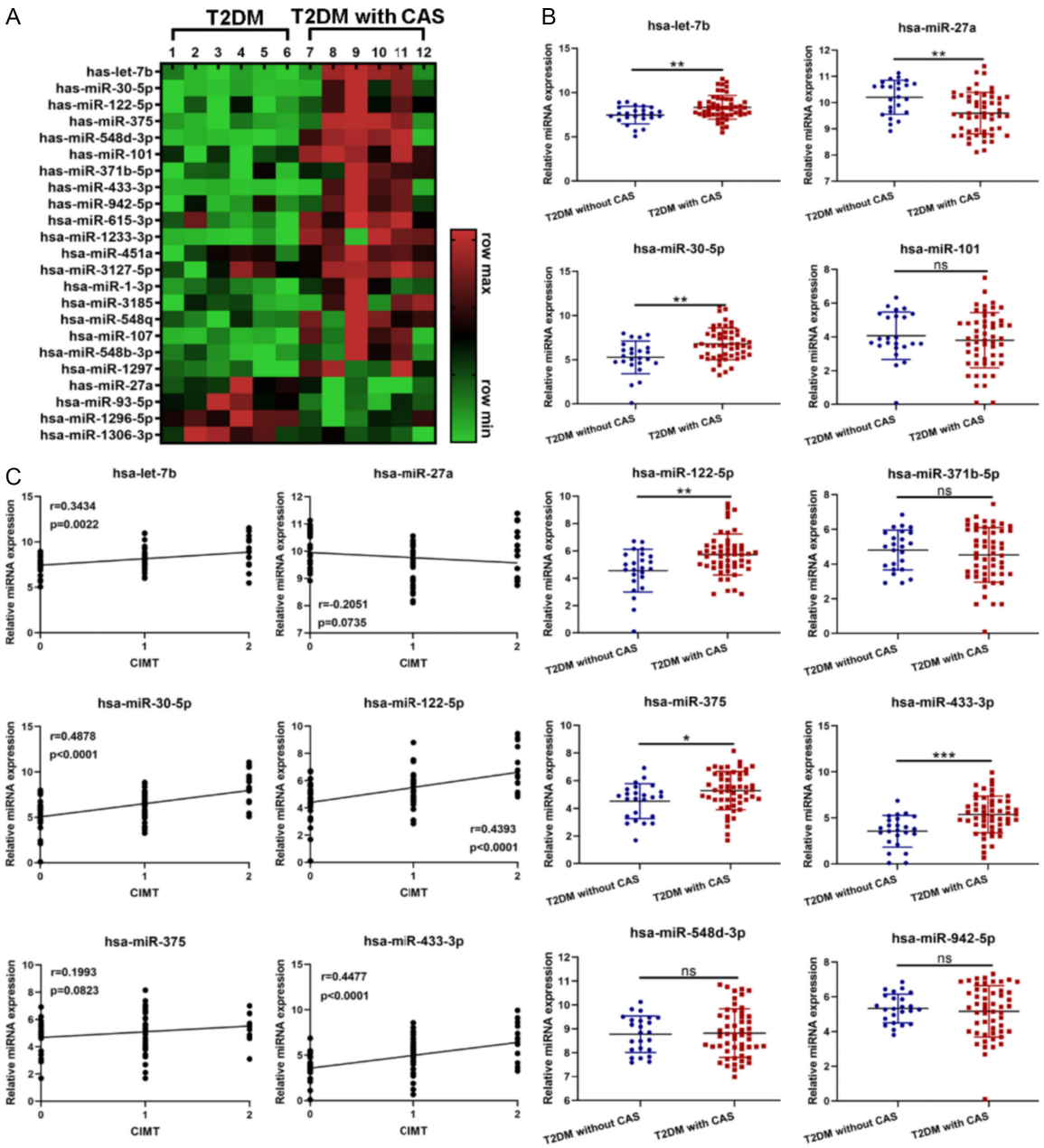
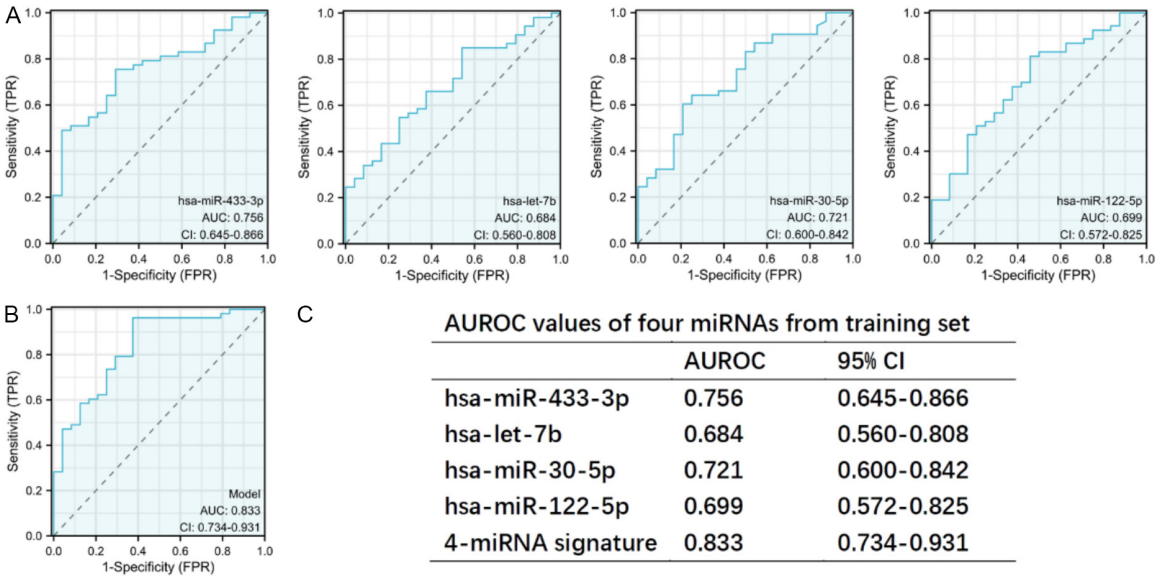


Figure 3. Validation of exosomal miRNA profile identified by microarray. A. The heat map showed that microarray analysis of 12 serum exosome samples (6 T2DM vs 6 T2DM with CAS) identified 23 DEMIs. B. Ten miRNAs with highest statistical significance were selected for validation in the training set. C. Correlation analysis between expression of miRNAs with CIMT severity was performed. CAS: carotid atherosclerosis; CIMT: carotid intima-media thickness; DEMIs: differentially expressed miRNAs; T2DM: type 2 diabetes mellitus.

positively correlated with CIMT severity (**Figure 3C**).

Four serum-exosome-derived miRNAs acted as biomarkers for the diagnosis of CAS

The ROC curve analysis demonstrated the diagnostic potential of four miRNAs: hsa-miR-433-3p (AUC=0.756), hsa-let-7b (AUC=0.684), hsa-miR-30-5p (AUC=0.721), and hsa-miR-122-5p (AUC=0.699) (**Figure 4A**). The combined model achieved a superior diagnostic performance (AUC=0.833) (**Figure 4B** and **4C**). Validation in an independent cohort (34 T2DM vs. 76 T2DM with CAS) confirmed the elevated expression of these miRNAs in patients with CAS (**Figure 5A**), with maintained diagnostic accuracy (combined AUC=0.816) (**Figure 5B** and **5C**).

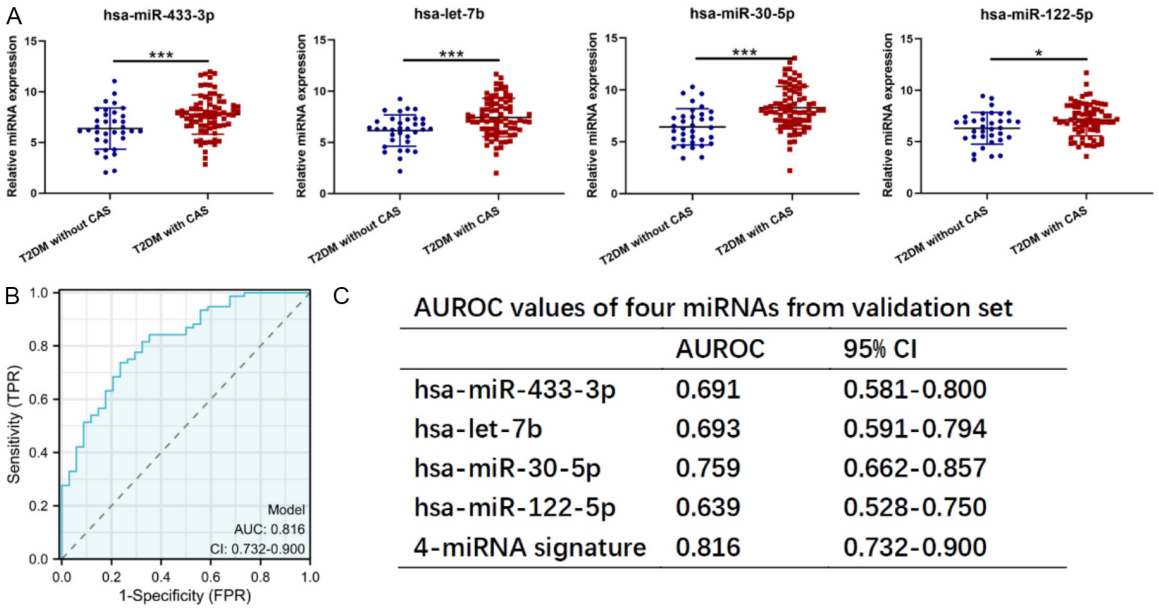


C

AUROC values of four miRNAs from training set

	AUROC	95% CI
hsa-miR-433-3p	0.756	0.645-0.866
hsa-let-7b	0.684	0.560-0.808
hsa-miR-30-5p	0.721	0.600-0.842
hsa-miR-122-5p	0.699	0.572-0.825
4-miRNA signature	0.833	0.734-0.931

Figure 4. Diagnostic potential of the four selected miRNAs was detected in the training set. A. ROC analysis demonstrated diagnostic potential for the four miRNAs (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, and hsa-miR-122-5p). B. Diagnostic performance of the combined model was detected. C. The detailed diagnostic performance was listed. ROC: receiver operating characteristic.



C

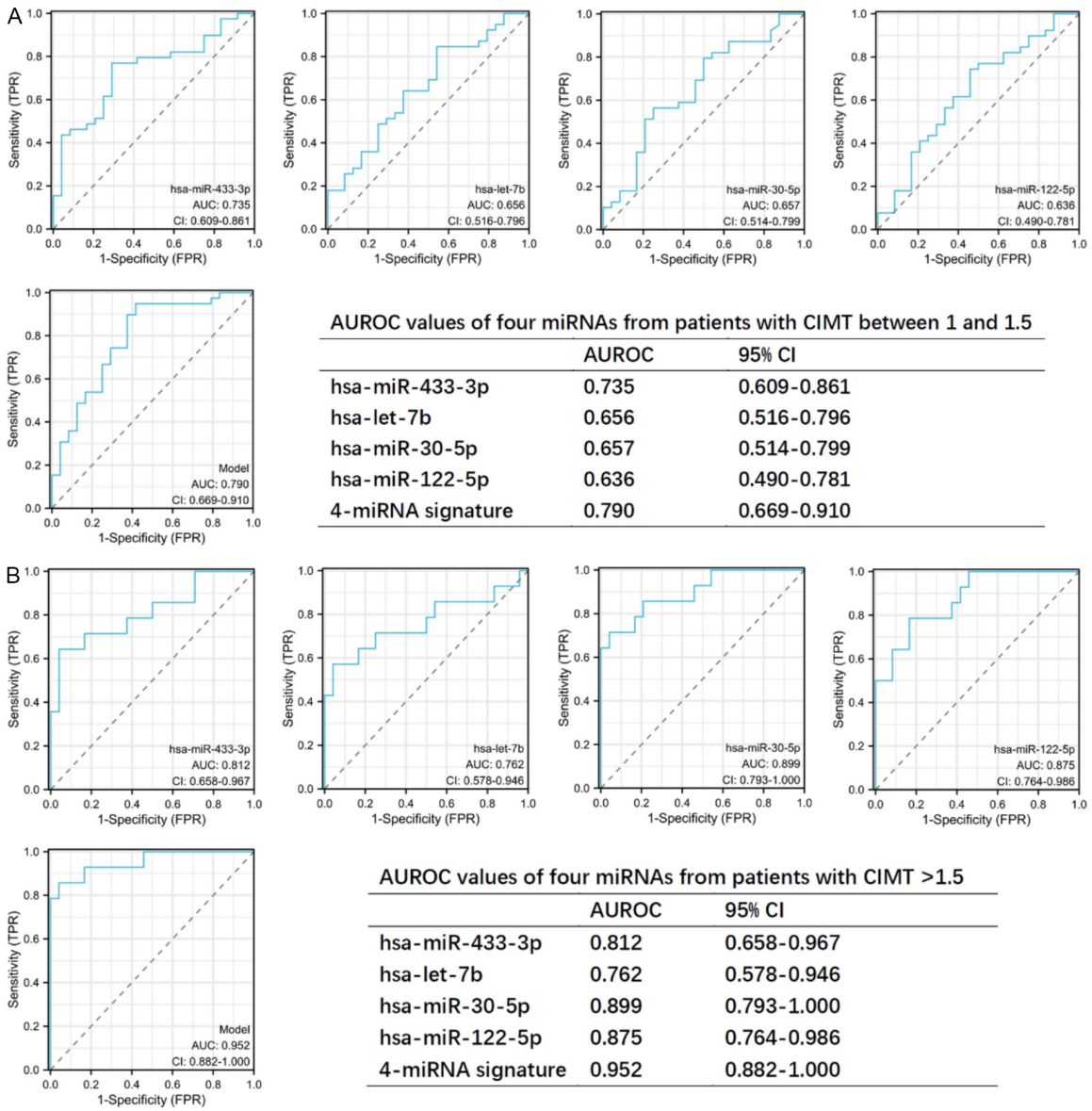
AUROC values of four miRNAs from validation set

	AUROC	95% CI
hsa-miR-433-3p	0.691	0.581-0.800
hsa-let-7b	0.693	0.591-0.794
hsa-miR-30-5p	0.759	0.662-0.857
hsa-miR-122-5p	0.639	0.528-0.750
4-miRNA signature	0.816	0.732-0.900

Figure 5. Diagnostic potential of the four selected miRNAs was detected in the validation set. A. Expression of the four miRNAs was confirmed in the validation set. B. ROC analysis demonstrated diagnostic potential for the four miRNAs. C. The detailed diagnostic performance was listed. ROC: receiver operating characteristic.

Diagnostic potential of the 4 DEMIs for the severity of CAS

We also investigated the application of these four serum exosome-derived miRNAs in the early diagnosis of CAS. Patients with CAS were divided into two groups: CIMT between 1 and 1.5, and CIMT > 1.5. ROC curve analysis showed that all four serum exosome-derived miRNAs could be used to diagnose CAS at an early stage (CIMT between 1 and 1.5), and the combined miRNA panel could effectively detect early stage CAS (CIMT 1-1.5 mm, AUC=0.79) (**Figure 6A**). Interestingly, it showed enhanced



AUROC values of four miRNAs from patients with CIMT between 1 and 1.5

	AUROC	95% CI
hsa-miR-433-3p	0.735	0.609-0.861
hsa-let-7b	0.656	0.516-0.796
hsa-miR-30-5p	0.657	0.514-0.799
hsa-miR-122-5p	0.636	0.490-0.781
4-miRNA signature	0.790	0.669-0.910

AUROC values of four miRNAs from patients with CIMT >1.5

	AUROC	95% CI
hsa-miR-433-3p	0.812	0.658-0.967
hsa-let-7b	0.762	0.578-0.946
hsa-miR-30-5p	0.899	0.793-1.000
hsa-miR-122-5p	0.875	0.764-0.986
4-miRNA signature	0.952	0.882-1.000

Figure 6. Diagnostic potential of the 4 DEMIs for the severity of CAS. Patients with CAS were divided into two groups: CIMT between 1 and 1.5, and CIMT > 1.5. A. ROC mode showed that all four serum-exosome-derived miRNAs could diagnose CAS at its early stage (CIMT between 1 and 1.5). B. ROC mode showed that all four serum-exosome-derived miRNAs exhibited enhanced diagnostic capacity for advanced lesions (CIMT > 1.5 mm). CAS: carotid atherosclerosis; CIMT: carotid intima-media thickness; DEMIs: differentially expressed miRNAs; ROC: receiver operating characteristic; T2DM: type 2 diabetes mellitus.

diagnostic capacity for advanced lesions (CIMT > 1.5 mm). The AUC of the combined model was 0.952 (**Figure 6B**).

Correlations between expression levels of the 4 DEMIs and biochemical parameters in patients with T2DM and CAS

Correlations between the expression levels of the four DEMIs and biochemical parameters are shown in Table S2. The results indicated that the expression levels of hsa-miR-433-3p, hsa-let-7b, and hsa-miR-30-5p positively correlated with HbA1c levels. Hsa-miR-122-5p was positively correlated with LDL-C levels and negatively correlated with HDL-C levels. No significant associations were found between FBG, SCr, TC, and TG levels, suggesting that these exosomal miRNAs have specific clinical relevance beyond CAS diagnosis.

Discussion

In the present study, serum exosomes from 12 patients with T2DM, with or without CAS, were analyzed using a human miRNA microarray. A total of 23 DEMIs were identified, and 10 miRNAs were selected for further investigation. We observed elevated circulating levels of five miRNAs (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, hsa-miR-375, and hsa-miR-122-5p) in the CAS group; however, only hsa-miR-27a was downregulated. Four DEMIs (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, and hsa-miR-122-5p) were significantly correlated with CIMT and exhibited diagnostic potential for CAS. Our findings also suggest that the above 4 DEMIs have diagnostic potential for determining the severity of CAS.

Atherosclerosis, the pathological foundation of cardiovascular and cerebrovascular diseases, has shown an increasing prevalence in recent decades [31]. Its association with cardiovascular events has attracted substantial scientific interest [32, 33], emphasizing the critical need for early detection and intervention. Increasing evidence supports the diagnostic value of circulating miRNAs in various diseases [34-36]. Among these, miRNAs in cytoplasmic exosomes have attracted our attention. In this context, serum from 12 T2DM patients with or without CAS were collected and exosomes were isolated. DEMIs were screened using a miRNA microarray, and their expression levels were validated across the training and validation cohorts.

Emerging studies have highlighted specific miRNAs involved in AS pathogenesis and diagnosis [37-39]. For instance, circulating miR-18a-5p shows elevated expression in patients with AS, regardless of the medication status, correlating positively with CIMT values [40]. Another study proposed a 10-miRNA panel for CVD risk assessment in patients [41]. SHUFANG et al. focused on patients with coronary heart disease (CHD) and hyperglycemia [24]. They identified serum exosomal hsa-let-7b-5p as a biomarker of coronary stenosis severity in patients with diabetic CHD. Consistent with these findings, our study detected 23 DEMIs in T2DM patients, of which 4 DEMIs (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, and hsa-miR-122-5p) demonstrated significant associations with CIMT progression and CAS diagnosis.

Among the four miRNAs associated with CIMT, miR-122-5p has been extensively linked to cardiovascular pathology. Previous studies have reported its correlation with cardiac troponin levels in athletes [42], association with secondary cardiovascular events [43-46], and its role in plaque instability [47] and myocardial infarction [48]. Notably, miR-122-5p inhibition attenuates LPS-induced myocardial injury [49]. Our findings align with these reports, showing a significant upregulation of miR-122-5p in the CAS group and its diagnostic value. Similarly, exosomal let-7b-5p, previously implicated in the severity of coronary stenosis [24], and miR-433-3p, which is associated with vascular calcification [50-52], reinforce their clinical relevance. However, the role of miR-30-5p in the cardiovascular context remains poorly understood.

Beyond the clinical predictive value elucidated in this study, miRNAs are well-established as versatile regulators involved in a myriad of physiological and pathological processes, including tumor progression, inflammatory responses, and the development of atherosclerosis [38, 53]. The canonical mechanism of action of miRNAs involves binding to the 3'-untranslated region (3'-UTR) of target messenger RNAs (mRNAs), leading to post-transcriptional repression via mRNA degradation or translational inhibition. Furthermore, miRNAs can participate in complex regulatory networks, such as competing endogenous RNA (ceRNA) crosstalk, to fine-tune gene expression. In the context of cardiovascular diseases, numerous miRNAs have been implicated in the modulation of key atherosclerotic pathways such as those governing vascular inflammation, endothelial dysfunction, lipid metabolism, and smooth muscle cell proliferation [54].

However, among the four-miRNA panel (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, and hsa-miR-122-5p) identified in our study, the direct mechanistic roles and specific target genes within the pathophysiology of carotid atherosclerosis in T2DM remain largely unexplored. Although some members, such as miR-122-5p and let-7b [24, 43], have been associated with general cardiovascular events or coronary stenosis, their precise functions in the diabetic vasculature are not fully defined. The lack of extensive prior mechanistic data for this specific panel underscores the novelty of our

clinical findings and highlights a significant knowledge gap that warrants further investigation.

This study had several limitations. The cross-sectional design precludes causal inference and requires validation in prospective longitudinal cohorts. The single-center design and lack of healthy controls limit the generalizability and ability to distinguish between diabetes-and atherosclerosis-specific effects, necessitating external validation in diverse populations. Furthermore, the underlying mechanistic pathways of the identified miRNAs remain unexplored, and our study did not include qualitative plaque characterization. Finally, clinical translation is currently constrained by reliance on qPCR-based methodology, highlighting the need for standardized point-of-care testing platforms. Future research should focus on translating this miRNA panel into integrated, rapid point-of-care testing platforms and leveraging emerging microfluidic and biosensing technologies to facilitate its clinical utility in risk stratification. Despite these limitations, our findings provide a foundation for future studies.

Conclusions

In conclusion, four serum exosomal miRNAs (hsa-miR-433-3p, hsa-let-7b, hsa-miR-30-5p, and hsa-miR-122-5p) were significantly associated with the incidence and severity of CAS in T2DM patients, demonstrating their diagnostic potential. Future studies should elucidate the pathophysiological mechanisms of atherosclerosis to identify novel therapeutic targets for managing diabetic cardiovascular complications.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

AUC, area under the curve; BMI, body mass index; CAS, carotid atherosclerosis; CCA, common carotid artery; CHD, coronary heart disease; CIMT, carotid intima-media thickness; CVD, cardiovascular disease; DEMIs, differentially expressed miRNAs; DM, diabetes mellitus; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; miRNA, microRNA; NTA, nanoparticle tracking analysis; PBS, phosphate buffered saline; qPCR, quantitative PCR; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; ROC, receiver operating characteristic; SCr, serum creatinine; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TEM, transmission electron microscopy; TG, triglyceride; WB, western blotting.

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Table S1. Clinical characteristics of individuals in test cohort, validation cohort, and total participants

Characteristic	Test cohort (n=77)		Validation cohort (n=110)		Total participants (n=187)	
	T2DM (n=24)	T2DM with CAS (n=53)	T2DM (n=34)	T2DM with CAS (n=76)	T2DM (n=58)	T2DM with CAS (n=129)
Gender, Female, n (%)	7 (29.2)	17 (32.1)	11 (32.4)	24 (31.6)	18 (31.0)	41 (31.8)
Age, years	45 (37.0-55.0)	54 (48-59.0)	43 (38.75-52.75)	55 (50-62.75)	44 (37-53.5)	55 (50-61)
BMI, Kg/m^2	24.70±0.47	24.79±0.34	24.43±0.44	23.69±0.32	24.56±0.32	24.15±0.24
Smoking, n (%)	5 (20.8)	14 (26.4)	7 (20.6)	18 (23.7)	12 (20.7)	32 (24.8)
Drinking, n (%)	3 (12.5)	9 (16.9)	4 (11.8)	12 (15.8)	7 (12.1)	21 (16.3)
Hypertension, n (%)	4 (16.7)	23 (43.4)	5 (14.7)	34 (44.7)	9 (15.5)	57 (44.2)
SBP, mmHg	124 (117-131)	124 (119.5-128)	121.5 (116-131.25)	120.5 (117-133.5)	122 (116-131)	122 (118-130)
DBP, mmHg	77 (72-91)	75 (69.5-79)	75.5 (69-85)	78 (71-85.75)	76 (70-87.5)	75 (71-84)
FBG, mM	7.9 (6.7-8.6)	8.93 (7.23-10.28)	7.9 (7.28-9.3)	9.2 (7.63-10.1)	7.9 (7.15-9.05)	9.13 (7.57-10.17)
HbA1C (%)	9.0 (8.6-10.4)	9.5 (8.55-10.3)	9.1 (8.5-10.03)	9.5 (8.8-10.4)	9.1 (8.5-10.05)	9.5 (8.7-10.4)
SCr, mg/dL	56.8 (54.5-63.6)	58 (53.55-62.3)	57.05 (46.15-61.35)	59.8 (54.0-63.25)	56.8 (47.6-61.4)	59.3 (53.7-62.75)
TC, mM	4.68 (4.12-5.84)	4.75 (4.22-5.23)	5.02 (4.32-5.25)	5 (4.70-5.35)	4.93 (4.2-5.26)	4.95 (4.54-5.33)
TG, mM	1.48 (1.10-2.30)	1.80 (1.47-2.27)	1.59 (1.35-2.12)	1.53 (1.37-1.90)	1.54 (1.24-2.14)	1.63 (1.40-2.01)
LDL-C, mM	2.99 (2.31-3.26)	3.38 (3.02-4.07)	3.10 (2.76-3.58)	3.33 (2.88-3.61)	3.06 (2.58-3.27)	3.35 (2.92-3.69)
HDL-C, mM	0.98 (0.81-1.11)	1.05 (0.84-1.20)	1.01 (0.84-1.17)	1.04 (0.78-1.18)	1.00 (0.82-1.13)	1.05 (0.82-1.19)

BMI: body mass index; CAS: carotid atherosclerosis; DBP: diastolic blood pressure; FBG: fasting blood glucose; HbA1c: hemoglobin A1c; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; SCr: serum creatinine; T2DM: type 2 diabetes mellitus; TC: total cholesterol; TG: triglyceride.

Table S2. Pearson correlation analysis of the correlations between expression levels of the selected 4 miRNAs and biochemical parameters in total participants

miRNA	Correlation coefficient, rho-values						
	FBG	HbA1C	SCr	TC	TG	LDL-C	HDL-C
hsa-miR-433-3p	0.067	0.504[c]	-0.178	-0.083	-0.009	-0.082	-0.146
hsa-let-7b	0.014	0.297[b]	-0.099	-0.205	0.023	0.131	0.142
hsa-miR-30-5p	-0.008	0.284[a]	-0.05	0.074	-0.085	0.063	-0.044
hsa-miR-122-5p	0.126	0.007	-0.159	0.095	0.126	0.251[a]	-0.264[a]

FBG: fasting blood glucose; HbA1c: hemoglobin A1c; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SCr: serum creatinine; TC: total cholesterol; TG: triglyceride. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.