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ORIGINAL RESEARCH

Hsa-miRNA-16-5p Of Urinary Exosomes A Reliable Biosignature Effective For Prostate Cancer Screening

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Introduction: MicroRNAs (miRNAs) are small, non-coding RNA molecules critical for cellular function, growth, and development. Recent advances in remote diagnostic technologies have highlighted the potential of urinary miRNAs as non-invasive biomarkers for disease monitoring. This study introduces a simple, rapid, and cost-effective reagent for exosomal miRNA extraction, designed for urine-based exosome screening. We further identified hsa-miR-16-5p (miR-16) as a promising diagnostic biomarker for prostate cancer (PCa), with the goal of integrating this method with biosensors to enable rapid result acquisition and support early cancer detection and treatment planning.

Materials and Methods: The extraction reagent was formulated using polyethylene glycol (PEG), sodium dodecyl sulfate (SDS), and diethyl pyrocarbonate (DEPC), differing from commercial kits. miRNAs were extracted and validated through RT-qPCR, focusing on miR-16 and the reference control miR-21. Results were compared against commercial kits. A cohort of 39 clinical samples—28 PCa patients, 1 benign prostatic hyperplasia (BPH) case, and 10 healthy controls (Ctr)—was analyzed. Statistical evaluations included *T*-tests and receiver operating characteristic (ROC) analysis to assess the diagnostic value of miR-16.

Results: miR-16 expression significantly differed across PCa stages and between cancerous and non-cancerous individuals (Ctr vs Stage IV: $p < 0.05$; Stage II vs III: $p < 0.05$; Stage II vs IV: $p < 0.005$; Stage III vs IV: $p < 0.05$). ROC analysis confirmed miR-16's diagnostic potential, particularly in detecting mid-stage PCa.

Conclusion: The proposed extraction method matches commercial kits in performance but offers notable advantages: a simplified five-step process, reduced extraction time (1.08 hours), and low cost ($0.13/test). These findings support the use of urinary miR-16 as a biomarker for PCa staging and highlight the practical value of the newly developed extraction reagent.

Keywords: exosome extraction, urine miR-16, prostate cancer, diagnosis

Introduction

In modern clinical medicine, urine—alongside blood—is one of the most widely utilized non-invasive biological fluids for diagnostic purposes. Particularly in the context of remote medical monitoring and cellular microenvironment analysis, urine offers a convenient, relatively contamination-free, and reliable specimen. Compared to other biological fluids such as saliva, sweat, breast milk, and feces, urine provides distinct advantages in terms of collection ease and diagnostic consistency. Humans typically excrete approximately 1–2 liters of urine per day. A major advantage of using urine over other biological fluids is its ease of collection, allowing for frequent and large-volume sampling without the need for specialized equipment or technical expertise.[1–3] It is well established that many cancer-related miRNA biomarkers are encapsulated within urinary exosomes. The bilayer lipid membrane of these exosomes protects the miRNAs from degradation, thereby ensuring their stability and making them suitable for assessing tumor status.[4–12] Given their diagnostic potential, the isolation of urinary exosomes has become a crucial step in miRNA-based cancer biomarker

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research. However, traditional exosome extraction methods—such as ultracentrifugation and commercial precipitation kits—are often time-consuming, expensive, and require specialized equipment, limiting their application in routine clinical or point-of-care settings. To overcome these challenges, simplified and cost-effective extraction methods are increasingly being developed. These approaches aim to facilitate rapid isolation of exosomes and reliable recovery of miRNAs for downstream analysis, such as quantitative PCR or next-generation sequencing.

However, the pre-analytical handling of urine samples can be influenced by numerous variables, including differences in sample collection methods, patient preparation (eg, whether prostatic massage is performed prior to collection), storage duration, temperature, use of chemical preservatives, and centrifugation protocols. These factors could significantly affect the integrity and yield of urinary exosomes and their miRNA content. Therefore, maintaining consistency in pre-analytical procedures is essential to ensure the reliability of diagnostic results. To this end, our study emphasizes method validation and parameter optimization to identify both the largest and smallest variations that may impact the accuracy of exosomal miRNA analysis.

Exosomes were first discovered in the 20th century by biochemist Rose Johnstone during her studies on sheep reticulocytes, and she was the first to coin the term "exosomes" to describe these extracellular vesicles.[11,13] Exosomes are extracellular vesicles (EVs) ranging in size from approximately 30 to 120 nanometers.[4–9,11,14–17] They are composed of a double-layered phospholipid membrane and are predominantly found in various body fluids.[8] Exosomes are known to carry abundant and stable amounts of small molecules, including deoxyribonucleic acid (DNA), microRNA (miRNA), messenger RNA (mRNA), proteins, and transforming growth factor-β (TGF-β) etc. These molecules can function as intercellular signals, serving as a medium for transmission and playing a crucial role in regulating physiological processes.[3–8,14,16,18] Currently, exosomes are considered promising biomarkers for cancer diagnosis, as the gene regulatory mechanisms they carry are significantly and extensively influenced by miRNAs.[6,17,19–24] Exosomes, as carriers of miRNAs, are critical mediators of cancer progression, facilitating the modulation of gene expression within the tumor microenvironment and distant target cells. These miRNAs can regulate various processes such as cell proliferation, apoptosis, migration, and immune evasion, all of which contribute to tumor growth and metastasis. The stability of miRNAs within exosomes makes them ideal candidates for cancer diagnostics, as they reflect the molecular state of the tumor and can be easily detected in body fluids such as urine, blood, and saliva. As such, exosomal miRNAs hold great potential as biomarkers for early cancer detection, prognosis, and monitoring therapeutic responses.

Given these factors, studying the functions of small molecules in exosomes, particularly single-stranded microRNAs, becomes challenging once they lose the protection provided by the double-layered phospholipid membrane. Without this protection, miRNAs are prone to cleavage and structural changes, which can negatively impact the accuracy of analytical results. Therefore, efficient purification techniques are essential to minimize processing time and enhance miRNA extraction concentration, ensuring reliable and accurate results for subsequent testing.

Clinically, PCa diagnosis and screening primarily rely on prostate-specific antigen (PSA) blood test levels. However, there is no definitive threshold for normal or abnormal PSA levels in the blood. Typically, PSA levels at or below 4.0 ng/mL are considered normal. Nevertheless, some men with PSA levels below 4.0 ng/mL may still have PCa, while many men with PSA levels between 4 and 10 ng/mL may not have the disease. This uncertainty in screening results often necessitates more advanced and costly diagnostic tools, such as magnetic resonance imaging (MRI), or invasive procedures like biopsy. These approaches not only increase patient anxiety but also contribute to the inefficient use of medical resources.

Our previous study demonstrated that the expression of miR-21 in urine is significantly higher in PCa patients compared to healthy individuals ($p < 0.001$), and this expression can even differentiate PCa from benign prostatic hyperplasia (BPH) ($p < 0.04$). Furthermore, the diagnostic sensitivity of miR-21 in urine was shown to be superior to conventional PSA blood testing.[25] These findings are consistent with numerous reports indicating that the overexpression of miR-21 is associated with the initiation and progression of various cancers.[26–31] Specifically, it has been shown to have a significant impact on PCa, lung cancer, pancreatic cancer, liver cancer, and colorectal cancer.[26–28,30,32] In contrast to the role of miR-21 in tumorigenesis, downregulated miRNAs, such as hsa-miRNA-16-5p (miR-16), can also promote excessive tumor cell proliferation by targeting key tumor suppressor genes. MiR-16 has been shown to target several important tumor suppressors, including PTEN, p53, and Bcl-2. By downregulating these genes, miR-16 disrupts normal

cell cycle control, inhibits apoptosis, and enhances cell survival, all of which contribute to tumor growth and progression. Given its role in regulating multiple pathways critical to tumorigenesis, miR-16 holds promise as both a diagnostic biomarker and a potential therapeutic target. Modulating miR-16 expression or its downstream targets could offer new strategies for controlling tumor growth and improving cancer treatment outcomes.[32–37] In this study, we not only investigated the role of exosomal miR-16 in PCa but also aimed to develop a rapid, user-friendly exosomal miRNA extraction kit to facilitate efficient biomarker analysis. We demonstrated that precise amounts of exosomal miRNA can be obtained through a streamlined five-step process, while also reducing associated costs. Additionally, we evaluated the diagnostic potential of miR-16 expression in urine samples from clinical PCa patients, as well as its utility in tumor monitoring. It is anticipated that this method could be effectively integrated into clinical telemedicine applications in the future.

Material and Methods

The Processing of Clinical Urine Sample for the Exosome miR-16 Detection

A total of 90 clinical urine samples were initially collected from the Department of Urology at Chang Gung Memorial Hospital (CGMH), Linkou, Taiwan. All procedures involving human participants were conducted in accordance with the Declaration of Helsinki and were approved by the Institutional Review Board of the CGMH Human Subjects Research Ethics Committee (IRB No. 202300229B0). Written informed consent was obtained from all participants prior to sample collection. The primary objective of this study was to identify specific biomarkers for PCa; therefore, strict exclusion criteria were applied, such as patients diagnosed with other types of cancer or presenting with serious comorbidities were excluded from the study. As a result, thirty-nine cases met the inclusion criteria and were included in the final analysis. Among the 39 participants, ten were healthy individuals with no cancer diagnosis (control group), one was diagnosed with BPH, and twenty-eight were PCa patients at various clinical stages. Specifically, the PCa group included one patient at stage I, eight patients at stage II, sixteen patients at stage III, and three patients at stage IV. To evaluate the stability of urinary exosomal miRNAs, urine samples were randomly collected without any prior processing or preparatory procedures. Following collection, the samples were immediately stored at –80°C and analysed within two weeks to ensure sample integrity.

Collecting Urinary Exosome Components Included miRNAs From Urine Through Homemade Formula

A 10 mL urine sample was centrifuged (Hettich, Germany) at 580× g for 15 minutes, the supernatant was collected to remove most impurities, and 2 mL of 2.82M polyethylene glycol (PEG) (Sigma-Aldrich®, Darmstadt, Germany) was added and left to stand at 4°C for 15 min. Centrifuge twice at 145× g for 30 and 5 minutes each time and discarded the supernatant to ensure minimal waste remains at the bottom of the tube. Finally, add 95 μL 0.1% diethyl pyrocarbonate (DEPC) (Sigma-Aldrich®, St. Louis, USA) solution and 5 μL 0.1% sodium dodecyl sulfate (SDS) (Sigma-Aldrich®, Darmstadt, Germany) solution to resuspend the exosomes.

Isolation and Quantification of microRNAs by Commercial Kit for Comparison with Homemade Formula

Firstly, total RNA, including all sizes of exosomal miRNAs, was extracted from urine samples using Norgen's Urine MicroRNA Purification Kit (#29000, Norgen Biotek, Thorold, ON, Canada). The extraction protocol was slightly modified based on our previous method,[38] isolating only small RNAs with a molecular size of less than 200 nucleotides. One milliliter of urine per sample was lysed and centrifuged at 7155× g for 5 minutes. The samples were then washed twice and centrifuged at 15339 × g for 5 minutes to remove contaminants. The purified miRNAs were eluted in 50 μL of elution buffer and stored at −80°C until further use. The final RNA yield varied depending on the specimen, with an average concentration of approximately 30 ng per 10 μL.

Urine samples from ten healthy subjects, used as controls, were further processed using commercial miRNA extraction kits for comparison. These included the miRNA Kit (Norgen Biotek, #29600, Thorold, Ontario, Canada)

and the ExoQuick-TC Kit (System Biosciences, #EXOTC50A-1, Palo Alto, California, USA). After measuring the total RNA concentrations, the relative fold changes in miRNA levels were analyzed using qRT-PCR to estimate the relative concentrations of the target miRNAs.

Quantification of miR-16 Using Traditional Real-Time Polymerase Chain Reaction (RT-qPCR)

A 5 μL RNA sample with a concentration of 9 ng/μL was added to 7 μL of reverse transcription reaction mixture, which contained 0.15 μL of 100 mM dNTPs, 1 μL of 50 U/μL MultiScribe™ Reverse Transcriptase, 1.5 μL of 10× reverse transcription buffer, 0.19 μL of 20 U/μL RNase inhibitor, and 4.16 μL of nuclease-free water; In addition, 3 μL of 5× RT primer was added to the reaction mixture. Reverse transcription was performed using a thermal cycler (Applied Biosystems, #293641474) under the following conditions: 16 °C for 30 minutes, 42 °C for 30 minutes, 85 °C for 5 minutes, followed by a hold at 4 °C. The primers designed used for reactions as below: miR-16: 5′-TAG CAG CAC GTA AAT ATT GGC G-3′, miR-21: 5′- TAG CTT ATC AGA CTG ATG TTG A-3′. For the probes designed: miR-16: 5′-UAG CAG CAC GUA AAU AUU GGC G-3′ and miR-21: 5′-UAG CUU AUC AGA CUG AUG UUG A-3′. The resulting cDNA was stored at –20 °C until further use. To measure miRNA expression levels, 0.67 μL of cDNA was added to a 9.34 μL PCR reaction mixture consisting of 0.5 μL of 20× TaqMan™ Small RNA Assay, 5 μL of TaqMan™ Fast Advanced Master Mix, and 3.84 μL of nuclease-free water. Quantification was carried out using the QuantStudio™ 3 Real-Time PCR System (Thermo Scientific™, #A2813). Expression of miR-21 was concurrently measured in each sample as a positive control. To normalize miRNA expression levels, we used a mixture of samples from 10 healthy subjects as a baseline for comparison. Each individual was initially tested separately, and outliers were excluded to ensure consistency, resulting in the final selection of 10 representative samples.

Statistical Evaluation of the Clinical Relevance of miR-16

In this study, qRT-PCR was performed on each sample, included one BPH case, and 28 PCa cases with different clinical statuses then further compared to the non-cancer group (The control group consisted of pooled urine samples from ten healthy subjects who were selected from the original twelve subjects. To prevent bias in the data, two subjects were excluded from the analysis due to outliers of unknown reasons). IBM SPSS Statistics 23 software (IBM Corporation) was used to perform independent sample t-tests, paired sample t-tests, and Receiver Operating Characteristic (ROC) curve analysis. The ROC analysis was specifically conducted to evaluate the effectiveness of miR-16 expression in distinguishing between different stages of PCa, thereby providing a quantitative measure of the diagnostic performance. A p-value of less than 0.05 was considered statistically significant.

Results

The Homemade Extraction Formula Rapidly Produced the Highest Quality and the Greatest Total Yield of Purified miRNAs Compared to Other Methods Tested

Regarding our homemade extraction formula, PEG, known for its highly hydrophilic properties, was combined with SDS, a detergent effective at disrupting phospholipid bilayer membranes. This mixture was then dissolved in DEPC-treated, RNase-free water to minimize the risk of RNA degradation. The final formulation was used for the cryogenic treatment of urinary exosomes to enhance miRNA recovery. For comparison, two commercially available kits—the miRNA Kit (Norgen Biotek, #29600) and the Exo Kit (System Biosciences, #EXOTC50A-1)—were also employed to extract total RNA from urine samples collected from 10 healthy subjects. Following extraction, total RNA concentrations and relative fold changes in miRNA levels were assessed using qRT-PCR to estimate miRNA concentrations. As illustrated in Figure 1A, 7 out of 10 subjects exhibited higher RNA concentrations when extracted using the homemade kit, compared to the commercial alternatives, indicating the potential efficacy of the homemade formulation. In addition, to further assess miRNA expression levels, the relative fold changes measured by qRT-PCR were compared among the homemade kit and the two commercially available kits. The results indicated no significant difference in miRNA recovery between the homemade kit and the Exo Kit, while the miRNA Kit produced lower concentrations, as it need

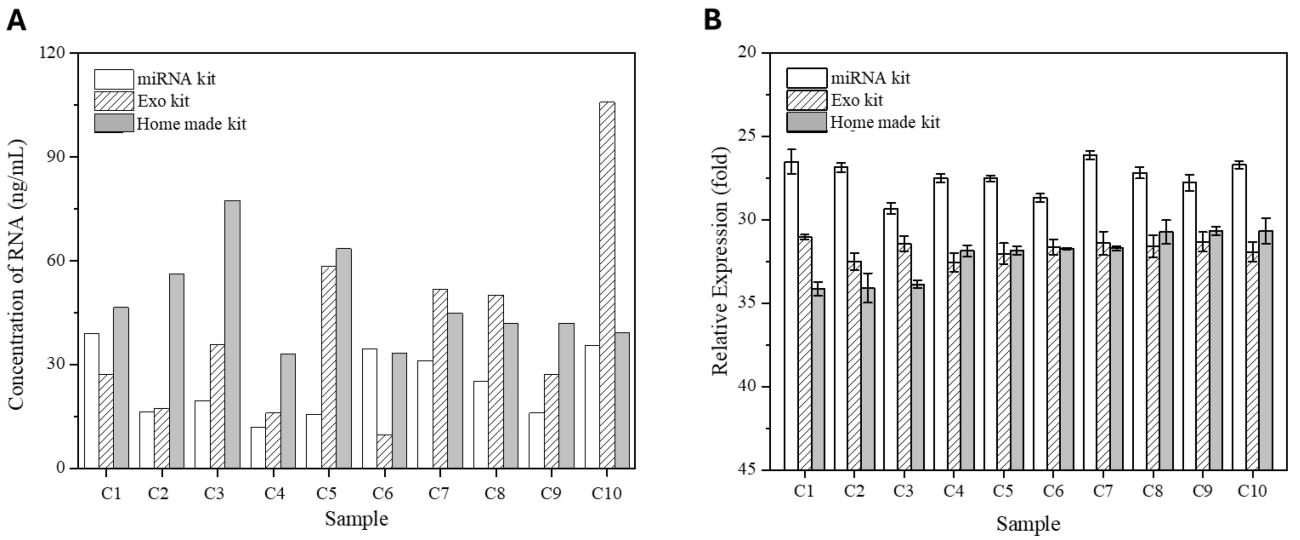

Figure 1 Comparison of extraction efficiency using the miRNA kit, Exo kit, and homemade kit. (**A**) Total miRNA concentrations measured by NanoDrop showed that 7 out of 10 samples yielded higher levels when extracted with the homemade kit. (**B**) qRT-PCR analysis of miR-16 expression revealed similar trends between the Exo kit and homemade kit across samples, whereas the miRNA kit consistently produced lower expression levels.

higher amplification folds during qRT-PCR (Figure 1B). Nevertheless, the correlation trends in miRNA expression levels were consistent across all methods in all the samples, indicating similar detection reliability. In summary, the findings indicate that the homemade kit demonstrates comparability to the commercially available Exo Kit in the extraction of urinary exosomal miRNAs. Furthermore, it presents the additional benefits of substantially reduced costs and expedited processing durations.

Efficient Assessment of miR-16 Expression Levels in Urinary Exosomes to Differentiate Between Non-Cancer Individuals and Prostate Cancer Patients

To ensure the reliability of the control group, urine samples from ten non-cancer subjects were individually tested. After verifying that they all exhibited similar values. These samples were then combined to form a pooled control group, minimizing individual variation and reducing potential bias in the analysis. The control group was then compared with cases of BPH and PCa at different stages. Based on the Gleason grading system for PCa, a significant decrease in miR-16 expression was observed in the advanced stages IV of PCa ($p < 0.041$) when compared to healthy subjects. Further, Although the results from earlier stages (I~III) of PCa were statistically close to significance, low miR-16 expression was still observed in many individual cases. In addition, cross-comparison among the different stages of PCa revealed significant differences. For example, comparisons between stage II and stage III yielded a p-value of < 0.02; stage II versus stage IV showed a p-value of < 0.001; and stage III versus stage IV resulted in a p-value of < 0.031. (Figure 2A) (Table 1).

Significant Alterations in Urinary Exosomal miR-16 Expression Post-Surgery

To evaluate whether the presence of tumor tissue influences miR-16 expression levels in urine, paired samples were collected from each PCa patient both preoperatively and one month postoperatively. The results revealed that preoperative miR-16 expression levels varied substantially with tumor stage. In contrast, one month after surgery, miR-16 expression levels across all PCa patients converged within a relatively consistent range. (Figure 2B). Meanwhile, miR-21 expression was employed as a positive control in this study. Consistent with expectations, its expression exhibited a downward trend one-month post-surgery (Figure 2C). The results clearly demonstrated that, irrespective of tumor stage, postoperative patients showed statistically significant differences in miRNAs expression levels compared to their preoperative counterparts. Both miR-16 and miR-21 displayed highly significant changes, with p-values across stages I to IV all less than 0.001 analysed by *T*-test.

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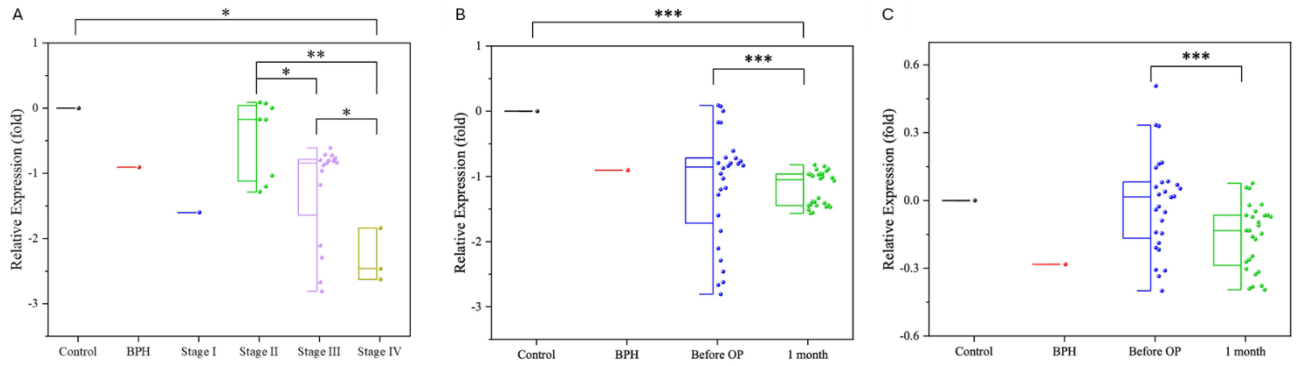

Figure 2 Relative expression of miR-16 measured by RT-qPCR. (**A**) *T*-test analysis of miR-16 expression in non-cancer individuals, patients with benign prostatic hyperplasia (BPH), and prostate cancer (PCa) patients. (**B**) Relative expression trends of miR-16 and (**C**) miR-21 were assessed in non-cancerous, BPH, and PCa patients before surgery and one-month post-operation. * Indicated $p < 0.05$; ** indicated $p < 0.005$; *** indicates $p < 0.001$.

Receiver Operating Characteristic Curve (ROC) Analysis of miR-16 and miR-21 for Stage-Specific Discrimination in PCa

To ensure that candidate clinical biomarkers reliably reflect PCa tumor burden and disease progression, miR-16 and miR-21 were analyzed using ROC curve analysis, a standard method for validating diagnostic markers. Notably, ROC curve analysis comparing both miRNAs expression with tumor stages and PSA levels revealed a strong correlation between miR-16 expression and PCa stages II and III (p= 0.001 and 0.004), consistent with the diagnostic relevance of PSA at these stages (p< 0.0001). These findings highlight the potential of miR-16 for disease stratification. Moreover, pairwise comparisons among the different stages showed statistically significant differences across all groups ($p < 0.05$), exception no significant association was observed for stage I and stage IV patients, likely due to small sample sizes in both groups —particularly in the advanced stage—thereby limiting the statistical power of the analysis. Furthermore, similar results were observed in the analysis of miR-21. ROC curve analysis demonstrated high diagnostic accuracy for PCa stages II and III, with p-values < 0.000 and AUC values of 1 and 0.969, respectively, indicating strong discrimination between these stages and non-cancer controls. Additionally, pairwise comparisons among the different stages revealed statistically significant differences between stage II and stage IV ($p = 0.014$), as well as between stage III and stage IV ($p = 0.034$) (Table 1 and Figure 3).

Discussion

The primary objective of cancer screening tests is to detect malignancies at an early stage to enable timely intervention, prevent disease progression, reduce morbidity, and ultimately lower mortality. In PCa screening, elevated serum PSA

Table 1 Statistical Analysis of Hsa-miRNA-16 Expression Levels Among Healthy Subjects, Prostate Cancer Patients, and Different Cancer Stages

Comparison of Groups	*T*- Test	ROC								
		miR-16			miR-21			PSA#		
	p-value	AUC*	p-value	95% CI$	AUC	p-value	95% CI	AUC	p-value	95% CI
Control vs Stage II	0.492	0.975	**0.001**	0.913–1.000	1.000	**0.000**	1.000–1.000	1.000	**0.000**	0.000–0.000
Control vs Stage III	0.135	0.844	**0.004**	0.697–0.997	0.969	**0.000**	0.910–1.000	1.000	**0.000**	0.000–0.000
Control vs Stage IV	**0.041**	0.733	0.237	0.461–1.000	0.133	0.063	0.000–0.336	1.000	**0.011**	1.000–1.000
Stage II vs Stage III	**0.020**	0.914	**0.001**	0.752–1.000	0.500	1.000	0.241–0.759	0.813	**0.014**	0.636–0.989
Stage II vs Stage IV	**0.001**	1.000	**0.014**	1.000–1.000	1.000	**0.014**	1.000–1.000	0.958	**0.025**	0.839–1.000
Stage III vs Stage IV	**0.031**	0.875	**0.044**	0.713–1.000	0.896	**0.034**	0.744–1.000	0.479	0.911	0.115–0.843

Notes: Bold indicated significance; * Represented area under the curve of receiver operating characteristic curve (ROC) analysis; # Represented prostate specific antigen; $ Represented confidence interval.

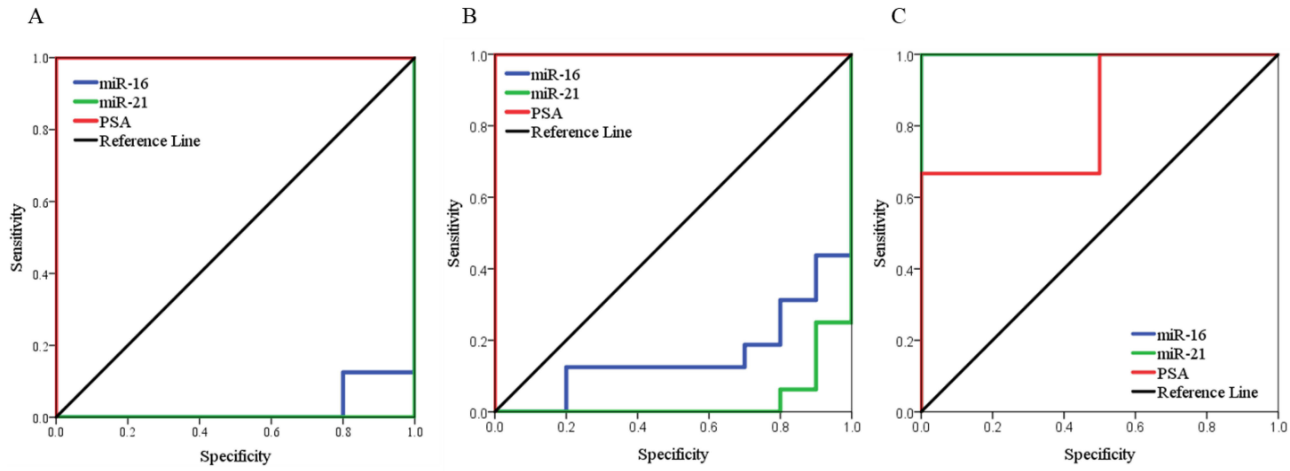

Figure 3 ROC curve analysis suggests miR-16 and miR-21 as potential clinical biomarkers, similar to PSA. (**A**) Both markers showed significant expression differences at tumor stage II versus healthy controls. (**B**) Significant differences were also observed at stage III compared to healthy subjects. (**C**) A notable distinction was found between stage II and IV.

remains the most widely used clinical marker, primarily because early-stage PCa is often asymptomatic. While PSA is a highly sensitive indicator, its diagnostic specificity is still limited. Elevated PSA levels can result from both malignant and benign conditions such as BPH and inflammation, increasing the risk of false positives and unnecessary clinical interventions. Conversely, a low PSA level does not necessarily rule out the presence of cancer. Notably, the only case diagnosed with stage I PCa in this study exhibited extremely low PSA levels both preoperatively (0.27 ng/mL) and postoperatively (0.008 ng/mL), with the diagnosis confirmed through a PSA-independent method. This case underscores a clear limitation of PSA as a stand-alone screening tool. Importantly, the expression levels of miR-16 and miR-21 in the patient's urinary exosomal samples were comparable to those observed in other confirmed cancer cases, further supporting the potential of miR-16 and miR-21 as alternative or complementary biomarkers for early PCa detection.

Given the diagnostic ambiguity associated with PSA, we therefore did not consider it the gold standard for evaluating the clinical value of miRNAs in this study. Instead, we relied on tumor staging confirmed by postoperative histopathological diagnosis, as it more accurately reflects the true clinical status of the disease. The resulting data further support the significance of urinary exosomal miR-16 as a promising complementary biomarker to PSA, with the potential to enhance diagnostic accuracy and reduce both false-positive and false-negative results associated with PSA testing alone. In addition, there is no general consensus among clinicians regarding the exact PSA threshold that warrants further diagnostic intervention, such as prostate biopsy or treatment. Nonetheless, significant fluctuations in PSA levels often cause patients to experience considerable anxiety. For individuals undergoing active surveillance or observation, PSA levels are monitored periodically; however, the addition of additional ancillary testing during this time can provide a more comprehensive assessment of disease progression. This complementary diagnostic helps determine if cancer is progressing and whether clinical intervention should be initiated.

To address the need for efficient biomarker detection, we developed a rapid and user-friendly urine exosome miRNA extraction kit and subsequently evaluated the diagnostic potential of two candidate biomarkers, miR-16 and miR-21, for PCa. Encouragingly, our findings align with previous studies conducted on PCa tumor tissues, supporting the premise that the expression levels of these miRNAs in urinary exosomes mirror their abundance in tumor tissue. This correlation strengthens the case for urine-based exosomal miRNA analysis as a non-invasive surrogate for profiling tumor-associated molecular signatures. In this study, we introduce a simplified five-step protocol for extracting urinary exosomal miRNAs, utilizing the high water-absorbing capacity of PEG in combination with 0.1% SDS and 0.1% DEPC-treated water. This formulation reduces the hydrophilicity of urinary exosomes and, through brief centrifugation, disrupts the phospholipid bilayer, enabling efficient and rapid release of miRNAs. The streamlined procedure reduces total extraction time to approximately 1.08 hours, thereby minimizing the risk of degradation of single-stranded miRNAs once they are released from the protective exosomal membrane. This approach not only

improves miRNA yield but also enhances the accuracy of downstream molecular analyses. Moreover, as a complementary tool to serum PSA testing, this method holds promise in reducing dependence on expensive MRI imaging or invasive prostate biopsies. With an estimated cost of just $0.13 per test, it represents a highly cost-effective strategy for optimizing diagnostic workflows in PCa screening and monitoring.

Moreover, our results corroborate previous studies demonstrating that the expression levels of miR-16 and miR-21 in urine align with those observed in PCa tumor tissues. Urinary miR-16 and miR-21 play significant roles in PCa. miR-16, a tumor suppressor, is often downregulated in PCa and can be used for tumor staging and monitoring disease progression. In contrast, miR-21 is an oncogenic miRNA that is overexpressed in PCa, promoting cancer cell proliferation and metastasis, and serving as a potential diagnostic and prognostic biomarker. These findings supported the potential of urinary miR-16 and miR-21 as reliable biomarkers for monitoring PCa tumor progression. Indeed, significant differences in miR-16 expression were observed across PCa patients at various stages (all $p < 0.05$), indicating that miR-16 could serve as an effective marker for tumor staging. This would be valuable in tailoring individualized treatment plans for patients, contributing to the advancement of precision medicine in the management of PCa. Interestingly, while some studies report miR-16 upregulation in PCa, our findings show its downregulation. This discrepancy may stem from differences in sample types or heterogeneity of PCa. Besides, prior studies often analyzed tumor tissue, our study focused on urinary exosomes; miR-16 may be upregulated locally in the tumor microenvironment but downregulated systemically, as reflected in urine. Factors such as tumor progression, therapeutic response, or selective miRNA release may contribute to the observed downregulation.

To assess the diagnostic potential of miR-16, ROC curve analysis was conducted across different stages of PCa. Significant correlations were observed between miR-16 expression and stages II and III, indicating its potential as a biomarker for intermediate-stage PCa, and supporting the role in tumor progression. However, no significant association was found for stage IV, likely due to the small sample size and increased molecular heterogeneity typical of advanced disease, which may obscure expression patterns. Future studies with larger, more diverse cohorts are warranted to validate miR-16 as a stage-specific biomarker (Table 1 and Figure 3).

Mobile biosensors represent a promising direction for the advancement of telemedicine technology, particularly in both developed and developing countries with rapidly aging populations. In these settings, there is a growing need for diagnostic tools that require minimal human intervention, are easy to operate, and provide reliable results. While ensuring the stability of test results is crucial, it is equally important to consider the quality of the samples obtained, as the compatibility between the sample collection method and the measurement instrument is a key factor influencing the accuracy and reliability of the detection values. Therefore, an efficient and user-friendly extraction kit is essential to enable easy operation by users and seamless integration with the detection sensor. Additionally, the integration of complementary biomarkers or the application of multi-omic approaches may offer a more comprehensive understanding of tumor biology, thereby enhancing diagnostic accuracy across all stages of the disease.

Conclusion

This study highlights the promising role of urinary exosomal miRNAs, particularly miR-16 and miR-21, as non-invasive biomarkers for PCa diagnosis, tumor staging, and disease monitoring. Compared to traditional PSA testing, these miRNAs offer improved specificity and diagnostic accuracy, especially in early-stage detection and active surveillance. The identification of miR-16 as a potential stage-specific marker further enhances its clinical relevance. With a simplified and cost-effective extraction method, this approach has the potential to streamline diagnostic workflows, reduce unnecessary medical interventions, and support personalized patient management. Future studies with larger, more diverse cohorts are essential to validate these findings and explore broader applications in oncology.

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Disclosure

The authors report no conflicts of interest to declare.

References

1. Decramer S, de Peredo AG, Breuil B, et al. Urine in clinical proteomics. *Mol Cell Proteomics*. 2008;7(10):1850–1862. doi:10.1074/mcp.R800001-MCP200
2. Harpole M, Davis J, Espina V. Current state of the art for enhancing urine biomarker discovery. *Expert Rev Proteom*. 2016;13(6):609–626. doi:10.1080/14789450.2016.1190651
3. Pisitkun T, Shen R-F, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci*. 2004;101(36):13368–13373. doi:10.1073/pnas.0403453101
4. Zhou Y, Yan X, Qian Y, et al. Lipidomic profiling of human milk derived exosomes and their emerging roles in the prevention of necrotizing enterocolitis. *Mol Nutr Food Res*. 2021;65(10):2000845. doi:10.1002/mnfr.202000845
5. György B, Szabó TG, Pásztói M, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci*. 2011;68(16):2667–2688. doi:10.1007/s00018-011-0689-3
6. Lee Y, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. *Human Molecular Genetics*. 2012;21(R1):R1. doi:10.1093/hmg/dds317
7. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. Exocarta 2012: database of exosomal proteins, RNA and lipids. *Nucleic Acids Res*. 2012;40(D1):D1241. doi:10.1093/nar/gkr828
8. Michael A, Bajracharya SD, Yuen PS, et al. Exosomes from human saliva as a source of microRNA biomarkers. *Oral Dis*. 2010;16(1):34–38. doi:10.1111/j.1601-0825.2009.01604.x
9. Otake K, Kamiguchi H, Hirozane Y. Identification of biomarkers for amyotrophic lateral sclerosis by comprehensive analysis of exosomal mRNAs in human cerebrospinal fluid. *BMC Med Genomics*. 2019;12(1):1–11. doi:10.1186/s12920-019-0473-z
10. Street J, Koritzinsky E, Glispie D, Star R, Yuen P. Urine exosomes: an emerging trove of biomarkers. *Adv Clin Chem*. 2017;78:103–122.
11. Vlassov AV, Magdaleno S, Setterquist R, Conrad R. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. *Biochim Biophys Acta General Sub*. 2012;1820(7):940–948. doi:10.1016/j.bbagen.2012.03.017
12. Wu C-X, Liu Z-F. Proteomic profiling of sweat exosome suggests its involvement in skin immunity. *J Invest Dermatol*. 2018;138(1):89–97. doi:10.1016/j.jid.2017.05.040
13. Johnstone RM, Adam M, Hammond J, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem*. 1987;262(19):9412–9420. doi:10.1016/S0021-9258(18)48095-7
14. Abels ER, Breakefield XO. Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. *Cell Mol Neurobiol*. 2016;36(3):301–312. doi:10.1007/s10571-016-0366-z
15. Cho S, Yang HC, Rhee WJ. Development and comparative analysis of human urine exosome isolation strategies. *Process Biochem*. 2020;88:197–203. doi:10.1016/j.procbio.2019.09.017
16. Hong C-S, Muller L, Whiteside TL, Boyiadzis M. Plasma exosomes as markers of therapeutic response in patients with acute myeloid leukemia. *Front Immunol*. 2014;5:160. doi:10.3389/fimmu.2014.00160
17. Whiteside TL. The potential of tumor-derived exosomes for noninvasive cancer monitoring. *Expert Revi Mol Diagnost*. 2015;15(10):1293–1310. doi:10.1586/14737159.2015.1071666
18. Kim Y, Shin S, Lee K-A. Exosome-based detection of egfr t790m in plasma and pleural fluid of prospectively enrolled non-small cell lung cancer patients after first-line tyrosine kinase inhibitor therapy. *Can Cell Inter*. 2021;21(1):1–8. doi:10.1186/s12935-021-01761-x
19. Bartel DP. microRNAs: genomics, biogenesis, mechanism, and function. *cell*. 2004;116(2):281–297. doi:10.1016/S0092-8674(04)00045-5
20. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. *Genomics Proteom Bioinform*. 2009;7(4):147–154. doi:10.1016/S1672-0229(08)60044-3
21. Karube Y, Tanaka H, Osada H, et al. Reduced expression of dicer associated with poor prognosis in lung cancer patients. *Cancer Sci*. 2005;96(2):111–115. doi:10.1111/j.1349-7006.2005.00015.x
22. Lee RC, Feinbaum RL, Ambros V. The c. Elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*. 1993;75(5):843–854. doi:10.1016/0092-8674(93)90529-Y
23. Liu J. Control of protein synthesis and mRNA degradation by microRNAs. *Curr Opin Cell Biol*. 2008;20(2):214–221. doi:10.1016/j.ceb.2008.01.006
24. Merritt WM, Lin YG, Han LY, et al. Dicer, drosha, and outcomes in patients with ovarian cancer. *N Engl J Med*. 2008;359(25):2641–2650. doi:10.1056/NEJMoa0803785
25. Leung W-H, Pang -C-C, Pang S-N, et al. High-sensitivity dual-probe detection of urinary mir-141 in cancer patients via a modified screen-printed carbon electrode-based electrochemical biosensor. *Sensors*. 2021;21(9):3183. doi:10.3390/s21093183
26. Asangani IA, Rasheed SA, Nikolova D, et al. microRNA-21 (mir-21) post-transcriptionally downregulates tumor suppressor pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*. 2008;27(15):2128–2136. doi:10.1038/sj.onc.1210856
27. Meng F, Henson R, Wehbe–Janek H, Ghoshal K, Jacob ST, Patel T. microRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*. 2007;133(2):647–658. doi:10.1053/j.gastro.2007.05.022
28. Rupaimoole R, Calin GA, Lopez-Berestein G, Sood AK. miRNA deregulation in cancer cells and the tumor microenvironment. *Cancer Discovery*. 2016;6(3):235–246. doi:10.1158/2159-8290.CD-15-0893
29. Shinde SS, Ahmed S, Malik JA, et al. Therapeutic delivery of tumor suppressor miRNAs for breast cancer treatment. *Biology*. 2023;12(3):467. doi:10.3390/biology12030467
30. Volinia S, Calin GA, Liu C-G, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci*. 2006;103(7):2257–2261. doi:10.1073/pnas.0510565103
31. Zhou H, Hao X, Zhang P, He S. Noncoding RNA mutations in cancer. *Wiley Interdiscip Rev RNA*. 2023;14(6):e1812. doi:10.1002/wrna.1812

32. Borkowetz A, Lohse-Fischer A, Scholze J, et al. Evaluation of microRNAs as non-invasive diagnostic markers in urinary cells from patients with suspected prostate cancer. *Diagnostics*. 2020;10(8):578. doi:10.3390/diagnostics10080578
33. Bonci D, Coppola V, Musumeci M, et al. The mir-15a–mir-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. *Nat Med*. 2008;14(11):1271–1277. doi:10.1038/nm.1880
34. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci*. 2004;101(9):2999–3004. doi:10.1073/pnas.0307323101
35. Cissell KA, Deo SK. Trends in microRNA detection. *Anal Bioanal Chem*. 2009;394(4):1109–1116. doi:10.1007/s00216-009-2744-6
36. Lange T, Stracke S, Rettig R, et al. Identification of mir-16 as an endogenous reference gene for the normalization of urinary exosomal miRNA expression data from ckd patients. *PLoS One*. 2017;12(8):e0183435. doi:10.1371/journal.pone.0183435
37. Yang T-Q, Lu X-J, Wu T-F, et al. microRNA-16 inhibits glioma cell growth and invasion through suppression of bcl2 and the nuclear factor-κb1/mmp9 signaling pathway. *Cancer Sci*. 2014;105(3):265. doi:10.1111/cas.12351
38. Chiou Y-E, Yu K-J, Pang S-N, Yang Y-L, Pang S-T, Weng W-H. A highly sensitive urinary exosomal miRNAs biosensor applied to evaluation of prostate cancer progression. *Bioengineering*. 2022;9(12):803. doi:10.3390/bioengineering9120803