Berita Biologi
LIPI
Jurnal ilmu-ilmu hayati

Berita Biologi 24(3): 427–443 (2025)
https://ejournal.brin.go.id/berita_biologi ISSN 2077 7019
DOI: 10.55981/berita_biologi.2025.11311

P-ISSN 0126-1754
E-ISSN 2337-8751

ARTIKEL

EXPRESSION OF HSA-MIR-22-3P IN THE URINE OF PROSTATE CARCINOMA PATIENTS AS A NON-INVASIVE BIOMARKER

[*Ekspresi Hsa-Mir-22-3p Pada Urin Pasien Karsinoma Prostat Sebagai Biomarker Non-Invasif*]

Angga Dwi Prasetyo*[1], R. Danarto[2], Sofia Mubarika Haryana[2], Indwiani Astuti[2]

[1]Biotechnology Study Program, Universitas Islam Negeri Raden Mas Said Surakarta, Jalan. Pandawa, Dusun IV, Pucangan, Kec. Kartasura, Kabupaten Sukoharjo, Jawa Tengah.
[2]Public Health and Nursing, Faculty of Medicine, Universitas Gadjah Mada, Jalan. Farmako, Sekip Utara, Depok, Sleman, Yogyakarta

ABSTRACT

Prostate carcinoma is one of the prostate diseases with the highest prevalence in men. Many factors cause the disease; some are androgen receptor disorder, mutation of genes, age, epigenetics, and environment. Currently, the detection of the disease is done by Prostate-Specific Antigen (PSA), Transurethral Resection of Prostate (TURP), and Digital Rectal Examination (DRE) tests; all of which are invasive to the patients. The microRNA that exists in urine exosomes can be used to detect non-invasive prostate carcinoma. Hsa-miR-22-3p with Gleason Score. This study aimed to examine the expression potential of Hsa-miR-22-3p in urine samples of prostate carcinoma exosomes as a non-invasive biomarker to determine the correlation between the expression of Hsa-miR-22-3p and the value of the Gleason Score. This study is of cross-sectional observational analysis. Urine samples were obtained from RSUP dr. Sardjito Yogyakarta dan RSUP dr. Soeradji Tirtonegoro. The exosome isolation was then carried out, followed by RNA isolation, cDNA synthesis, and quantification using qRT-PCR. Based on the result of the study, there was a decrease in the expression of Hsa-miR-22-3p by 6.6 times in prostate carcinoma; there was a significant difference between the samples of prostate carcinoma and healthy individuals (P = 0,031), and there was a correlation between the expression level of Hsa-miR-22-3p and the value of Gleason Score. Therefore, Hsa-miR-22-3p has the potential to be used as a biomarker for prostate carcinoma patients.

Keywords: Carcinoma Prostate, PSA, TURP, MicroRNA, qRT-PCR, Hsa-miR-22-3p

Diterima: 29 April 2025; **Diperbaiki:** 27 Agustus 2025; **Disetujui:** 1 September 2025
***Penulis untuk Korespondensi:** e-mail – angga.dwiprasetyo@staff.uinsaid.ac.id

ABSTRAK

Karsinoma prostat merupakan salah satu penyakit prostat dengan prevalensi tertinggi bagi pria. Banyak faktor yang menyebabkan penyakit; Beberapa adalah gangguan reseptor androgen, mutasi gen, usia, epigenetik, dan lingkungan. Saat ini, deteksi penyakit tersebut dilakukan dengan tes Antigen Spesifik Prostat (PSA), Reseksi Transuretra Prostat (TURP), dan Pemeriksaan Rektal Digital (DRE); ketiganya invasif bagi pasien. MicroRNA yang ada dalam eksosom urin dapat digunakan untuk mendeteksi karsinoma prostat non-invasif. Hsa-miR-22-3p dengan Skor Gleason. Penelitian ini bertujuan untuk mengkaji potensi ekspresi Hsa-miR-22-3p pada sampel urin eksosom prostat karsinoma sebagai biomarker non-invasif untuk mengetahui korelasi ekspresi Hsa-miR-22-3p dengan nilai Gleason Score. Penelitian ini adalah analisis observasional cross-sectional. Sampel urin diperoleh dari RSUP dr. Sardjito Yogyakarta dan RSUP dr. Soeradji Tirtonegoro. Isolasi eksosom kemudian dilakukan, dilanjutkan dengan isolasi RNA, sintesis cDNA, dan kuantifikasi masing-masing menggunakan qRT-PCR. Berdasarkan hasil penelitian, terjadi penurunan ekspresi Hsa-miR-22-3p sebesar 6,6 kali pada karsinoma prostat; ada perbedaan yang signifikan antara sampel karsinoma prostat dan individu sehat (P = 0,031), dan ada korelasi antara tingkat ekspresi Hsa-miR-22-3p dan nilai Skor Gleason. Oleh karena itu, Hsa-miR-22-3p berpotensi sebagai biomarker bagi pasien karsinoma prostat.

Kata Kunci: *Kanker Prastat, PSA, TURP, MicroRNA, qRT-PCR, Hsa-miR-22-3p*

INTRODUCTION

Carcinoma is a disease caused by the uncontrolled, invasive, and metastatic cell proliferation process that loses the apoptosis ability; the causes of carcinoma vary, such as genetic disorders, carcinogenic factors, virus infection, and environmental and lifestyle (Foster *et al*., 2018; Sriharikrishnaa *et al*., 2023). *The International Agency for Research on Cancer* (IARC) reported that the increase in carcinoma patients had become a potential issue for human development worldwide. There were 14.1 million new cases of carcinoma, 8.2 million deaths from carcinoma, and 32.6 million people suffered from carcinoma in 2012 based on a five-year diagnosis worldwide, with the average occurrence of standard age reached almost 25% higher in men than women, 205 and 165 per 100,000 individuals, respectively. One of the most significant carcinoma types contributing to men's highest death rate is prostate carcinoma.

The change in lifestyle and difficulty of early detection for this type of carcinoma, primarily due to the anatomical location and nonspecific symptoms, causes the disease's high incidence and mortality rates. Prostate carcinoma does not demonstrate any symptoms at the early stage. The symptoms only appear when the cell grows bigger or is marked by swelling that affects the urethra. Prostate carcinoma is of a multi-factorial type as it is induced by many factors, such as lifestyle (smoking habit), age, family history, race, genetics, obesity, inflammation, virus infections of *Epstein-Barr* and *Human Papillomavirus,* and infection during sexual intercourse (Yuri *et al*., 2015; Wei *et al*., 2023).

The current medical treatment for prostate carcinoma management is commonly through *Transurethral Resection of Prostate* (TURP), SOB, chemotherapy, and radiotherapy, despite the many shortcomings, including high cost and high recurrence rate. The difficulty in diagnosing the symptoms of prostate carcinoma has caused most patients not to receive immediate treatment. The lack of quick response in treating the disease indicates the high mortality rate for prostate carcinoma. According to Zeng (2022), the common diagnosis to detect carcinoma prostate is by digital rectal examination, transrectal ultrasound test (TRUS), biopsy, and Prostate-Specific Antigen (PSA), all of them are of invasive nature which cause some pains for the patients; therefore, it requires a new non-invasive method (Dijkstra, 2014).

Malignancy level of carcinoma prostate can be measured and diagnosed by using *Gelasan Score* (GS), *Prostatic Intraepithelial Neoplasia* (PIN), *Atypical Small Acinar Proliferation* (ASAP); adenocarcinoma symptoms have been diagnosed by using the GS system since the 1960s. GS ranges from one to five according to the gland differentiation and structures viewed under a microscope. A high GS score indicates a more aggressive carcinoma nature. Two scores are given to represent the two most prominent patterns (or a single GS, which becomes twice as much if only one pattern is identified) to achieve a total score that ranges between 2 to 10 (Gordetsky & Epstein, 2016)

Prostate Intra-epithelial Neoplasia is a measurement based on the smallest changes in prostate gland cells in terms of size and form. Prostate Intra-epithelial Neoplasia is identified in around 5% of the initial prostate biopsy. *Prostatic intraepithelial neoplasia* is divided into high and low classes depending on the pattern of prostate cells. *Prostatic intraepithelial neoplasia* is related to molecular changes similar to prostate carcinoma without increasing the PSA value. If a high PIN is found in the biopsy, there is a 20% possibility that carcinoma has spread to other tissues in the prostate gland. ASAP has a high probability of indicating carcinoma in the prostate; thus, another biopsy will be carried out several times in the following couple of months (Pullar & Shah, 2016).

The current medical treatment for prostate carcinoma management is commonly through *Transurethral Resection of Prostate* (TURP), SOB, chemotherapy, and radiotherapy (Bunting, 2002; Ilic, 2013; Heijnsdijk, 2015) despite the many shortcomings, including high cost and high recurrence rate (Schalken, 2014). On the other hand, the treatment is also invasive for patients; therefore, an urge to develop a new method to detect the disease without invasive effects (non-invasive), such as urine observation.

Urine is a liquid by-product of metabolism in humans, excreted by the kidneys from the human body through the urination process, which contains exosomes (Wu *et al*., 2017; Liu *et al*., 2021). Exosomes in urine carry genetic information, such as DNA, RNA, mRNA, *microRNA*, and siRNA, and become a non-invasive substrate for biomarker (Hessels & Schalken, 2013; Prasetyo, 2022). MicroRNA is a single-stranded and non-coding RNA molecule (Cochetti *et al*., 2020). The microRNA has the potential of a biomarker to investigate the mechanism of a particular post-transcriptional process in a specific, sensitive, and non-invasive manner. The reason for this is that miRNA has a stable existence in cells and body fluids (Ajit, 2012). Some oncomir genes in prostate carcinoma and BPH occurrence are known as the target of *Hsa-miR-22-3p*; one of them is the *ATP Citrate Lyase* (ACLY) (Xin *et al*., 2016). Enzyme ACLY has an essential role in lipid de novo synthesis, which is significant in carcinoma occurrence. There is a decrease in the expression of *Hsa-miR-22-3p* in patients with prostate carcinoma; this increases the ALY function that causes excessive androgen production accumulated in the form of Androgen Receptor (AR), which, respectively, reduces the cell apoptosis ability and simultaneously increases the differentiation and proliferation of cells.

Hsa-miR-22-3p is located on chromosome 17 with pair 53 to 74 and consists of 22 nucleotides. The *Hsa-miR-22-3p* has a significant role as a tumor suppressor in many carcinoma cases, such as in the cervix, lung, bone, and prostate (Zhang *et al*., 2015). As a tumor suppressor gene, miR-22 suppresses or inhibits oncomir expressions that include AKT1, PTEN, PPPM1K, BMP7, PPARA, ESR1, HDAC4, so on (Mirtarbase, 2025). There were several studies conducted concerning the expression of *Hsa-miR-22-3p* in prostate carcinoma; according to Ren *et al*., (2024) the profiling result on prostate carcinoma tissues showed the role of *Hsa-miR-22-3p* and experienced expression decrease on the respective tissues compared to the BPH samples. *Hsa-miR-22-3p* experiences a reduction in expression in tissues with prostate carcinoma (Fan *et al*., 2016; Prasetyo, 2021). Several

previous studies related to *Hsa-miR-22-3p* in prostate carcinoma have proven that the change in expression of this miRNA is down-regulated compared to the normal control.

MATERIALS AND METHODS

Materials

The urine samples of patients with prostate carcinoma in the form of supernatant are collected from the RSUP. Dr. Sardjito and RSUP. dr. Soeradji Tirtonegoro. The exosomes isolation kits were *miRCURY Exosome Isolation Kit-Cells*, *Urine and CSF*, 12-80 rxnx (Exiqon). The RNA isolation kits included *miRCURY RNA Isolation Kit-Cell and Plant*, (Exiqon). The cDNA isolation kits were *Universal cDNA Synthesis kit II*, 8-64 rxnx (Exiqon) that included 5x *reaction buffer, nuclease-free water, enzyme mix, and spike in* (sp6). *The ExiLent SYBR Green master mix*, 2.5 mL, 500rxnx. (Exiqon) was used to detect the miRNA expression. *miRCURY LNA Universal RT microRNA PCR primer set: Hsa-miR-22-3p* mimics (miR-22-3p; 5′-AAGCUGCCAGUUGAAGAACUGU-3′, TaqMan Gene Expression Assay ID PM10203) was applied as the primary target. *miRCURY LNA Universal RT microRNA PCR primary set: Hsa-miR-191-5p* was applied as the *reference gene*.

Research Design

This research is a cross-sectional observational analysis, that information about patients or participants is collected at a single point in time, rather than being followed over a longer period. This approach allows researchers to assess the prevalence of certain health conditions, risk factors, or clinical characteristics within a defined population. (Dahlan, 2010). The *Hsa-miR-191-5p* is used as the internal control of *Hsa-miR-22-3p* (Egidi *et al*., 2015). The housekeeping gene's internal control is highly recommended as it validates each cell that demonstrates no changes due to the experiment treatments (Livak dan Schmittgen, 2008). The ethical clearance (KE/FK/0515/EC/2017) was obtained from the Health Research Ethics Committee of UGM School of Medicine – RSUP Dr. Sardjito Yogyakarta.

Sample Collection and Handling

Urine samples were obtained from prostate cancer patients who had been diagnosed in terms of clinical pathology and histopathology. The samples used were obtained from male patients with an age range ≥of 50-90 years. Twenty urine samples used for the research included ten samples of patients diagnosed with positive prostate carcinoma that had undergone the Anatomical Pathology (PA), resulting in PSA value and TURP result, and ten of healthy patients as the normal control; all of the samples were stored at -80ºC. Urine samples were collected from RSUP. Dr. Sardjito Yogyakarta and RSUP. dr. Soeradji Tirtonegoro underwent centrifugation at 10,000xg for 5 minutes; its supernatant was stored in a freezer at -80ºC located in the Bio Molecular Laboratory of the Faculty of Medicine of Universitas Gadjah Mada before testing.

The experiment was held at the BioMolecular Laboratory, which included exosomes and RNA isolations, and cDNA synthesis, while the expression test of *Hsa-miR-22-3p* using the qRT-PCR method was conducted at the Bio-Chemical Laboratory of the Medicine Faculty of Universitas Gadjah Mada. CFX manager 96 application and GenEX were used to analyze the *Hsa-miR-22-3p* expression result, and the Livak method (or known as the 2(-Delta Delta C(T)) method) was used to determine the quantitative expression analysis. Samples were obtained from RSUP. Dr. Sardjito and RSUP. dr. Soeradji Tirtonegoro.

Hsa-miR-22-3p Expression Result Analysis

The Biorad CFX Manager Software was employed to analyze the *Hsa-miR-22-3p* expression result in terms of the *Quantification cycle (Cq) value*, *quantification curve*, *melting curve,* and qPCR result.

ExiLENT SYBR Green Master Mix 2.5 mL (Exiqon) was employed in the qPCR process with microRNA as the primary target and the cDNA of the synthesis result. The cDNA samples were taken from the cooler at -20°C and put in the tube cooler. After defrosting, the samples were vortexed and spin down. The next step was to dilute cDNA samples with nuclease-free water before underwent homogenization for tube cooler requirement. The PCR Master Mix and PCR Primer mix (primer microRNA miR-22) were homogenized to form a qPCR Mix and put in the tube strip. 4 µL cDNA was put in the tube strip and underwent homogenization by conducting resuspension of cDNA using a micropipette for the qPCR Mix. The next step was setting the CFX 96 Real-Time System C1000 Therma Cycle program for the running process.

Analysis of Data Statistics

A normality test is conducted to determine the distribution of *Hsa-miR-22-3p* in groups of prostate carcinoma patients and normal controls; it investigates the normal distribution using *the Shapiro-Wilk* test. The normality test was carried out with the following requirement: $p > 0.05$ refers to normal data distribution, $p < 0.05$ refers to abnormal data distribution.

The independent T-test determines whether there are significant differences between samples of prostate carcinoma and the normal control. The samples of carcinoma prostate and normal control are significantly different if $p < 0.05$ and are not significantly different if $p > 0.05$.

Quantitative Analysis of qPCR Real-time Result by using the LIVAK Method

The expression difference of *Hsa-miR-22-3p* in urine samples of BPH patients compared to those of reference genes expression (*Hsa-miR-191-5p*), Reference genes were determined based on the results of optimization with 3 types of *reference gene* candidates tested, namely *Hsa-miR-16-5p*, *Hsa-miR-191-5p* and Let-7. Based on the optimization results, *Hsa-miR-191-5p was obtained as a reference gene.* was achieved by using the following Livak method (Livak & Schmittgen, 2008):

$$\Delta C_{q\mathit{Calibrator}} = C_{q(\text{Target, } \mathit{Calibrator})} - C_{q(\text{Ref, } \mathit{Calibrator})}$$

$$\text{Expression change multiplication} = 2^{-\Delta\,(\text{Cq. } \mathit{Treated} - \text{Cq. } \mathit{Untreated})} = 2^{-\Delta Cq\,(\mathit{Treated} - \mathit{Untreated})} = 2^{-\Delta\Delta Cq}$$

RESULT

qPCR Value and Value Comparison of Cq Samples of Carcinoma Prostate and Normal Control

The average measurement of the exosomes Cq sample of BPH urine and the normal control showed 34.63 for the prostate carcinoma sample and 31.63 for the normal control. The next step was to compare each group's average value to determine that the carcinoma prostate group had a higher Cq value than the normal control.

The Shapiro-Wilk test was done to determine the distribution of *Hsa-miR-22-3p* data in carcinoma prostate and normal control groups, whether it was normally distributed or not. The normality test showed that the Cq miR-2 data in both groups were distributed normally, as its

significance value $p > 0.05$. Further, the independent t-test result showed the significance (2-tailed) $p = 0.031$ or $p < 0.05$, which means both samples had a significant difference.

Table 1. Samples characteristics of prostate carcinoma urine exosomes (*Karakteristik sampel eksosom urin karsinoma prostat*).

Variable (*Variabel*)	**Classification** (*Klasifikasi*)	**Frequency** (*Frekuensi*)	**Percentage** (*Persentase*)
Gleason Score (*Skor Gleason*)	*Group* 1(≤ 6)	1	10 %
	Group 2 (3+4 = 7)	1	10 %
	Group 3 (4+3 = 7)	-	-
	Group 4 (4+4 = 8)	3	30 %
	Group 5 (9-10)	3	30 %
	Missing data	2	20 %
Age (*Usia*)	50-59 year	-	-
	60-69 year	6	60 %
	70-79 year	2	20 %
	80-89 year	2	20 %
	Missing data	0	0 %
Metastasis	Positive	3	30 %
	Negative	6	60 %
	Missing data	1	10%
Chemoterapy (*Kemoterapi*)	Positive	4	40 %
	Negative	5	50 %
	Missing data	1	10%

Table 2. Samples characteristics of normal control urine exosomes (*Sampel karakteristik eksosom urin kontrol normal*).

Variable (*Variabel*)	**Classification** (*Klasifikasi*)	**Frequency** (*Frekuensi*)	**Percentage** (*Persentase*)
Age (*Usia*)	50-59 year	8	80 %
	60-69 year	2	20%
	70-79 year	-	-
	80-89 year	-	-

Table 3. Calculation result of the mean cq value prostate carcinoma urine exosome samples and normal controls (*Hasil perhitungan nilai cq rata-rata karsinoma prostat, sampel eksosom urin dan kontrol normal*).

Healthy Individual (*Individu yang Sehat*)		**An individual with Carcinoma Prostate** (*Seorang individu dengan Karsinoma Prostat*)	
Sample (*Sampel*)	**Cq Value** (*Nilai Cq*)	Sample **(*Sampel*)**	**Cq Value** (*Nilai Cq*)
N1	32,39	P2	39,2
N2	30,74	P3	37,72
N3	31,53	P5	38,59
N4	29,1	P6	39,44
N5	32,92	P9	28,3
N6	29,09	P11	30,4
N7	31,03	P14	33,24
N8	30,71	P15	30,31
N9	32,78	P16	35,18
N13	36,02	P17	34,1
Average (*Rerata*)	**31,63**		**34,65**

Table 4. Normality test results of the cq mir-22 in the prostate carcinoma group and normal controls (*Hasil uji normalitas cq mir-22 pada kelompok karsinoma prostat dan kontrol normal*).

MicroRNA	**Shapiro-Wilk** ***P Value**** (*Nilai P*)
miR-22 Ca. Prostate	0.383
miR-22 Kontrol sehat	0.332
***p>0.05: data is normally distributed/ not significantly different** (*data biasanya didistribusikan/tidak berbeda secara signifikan*)	

Table 5. Independent t-test results of the cq mir-22 in the prostate carcinoma group and the normal controls (*Hasil uji-t independen dari cq mir-22 pada kelompok karsinoma prostat dan kontrol normal*).

MicroRNA	ΔCq Average (*Rerata ΔCq*)	P Value* (*Nilai P*)
MiR-22 Ca. Prostate	-3.33	
MiR-22Normal Control	1.78	0.031
***p>0.05: data is normally distributed/ not significantly different** (*data biasanya didistribusikan/tidak berbeda secara signifikan*)		

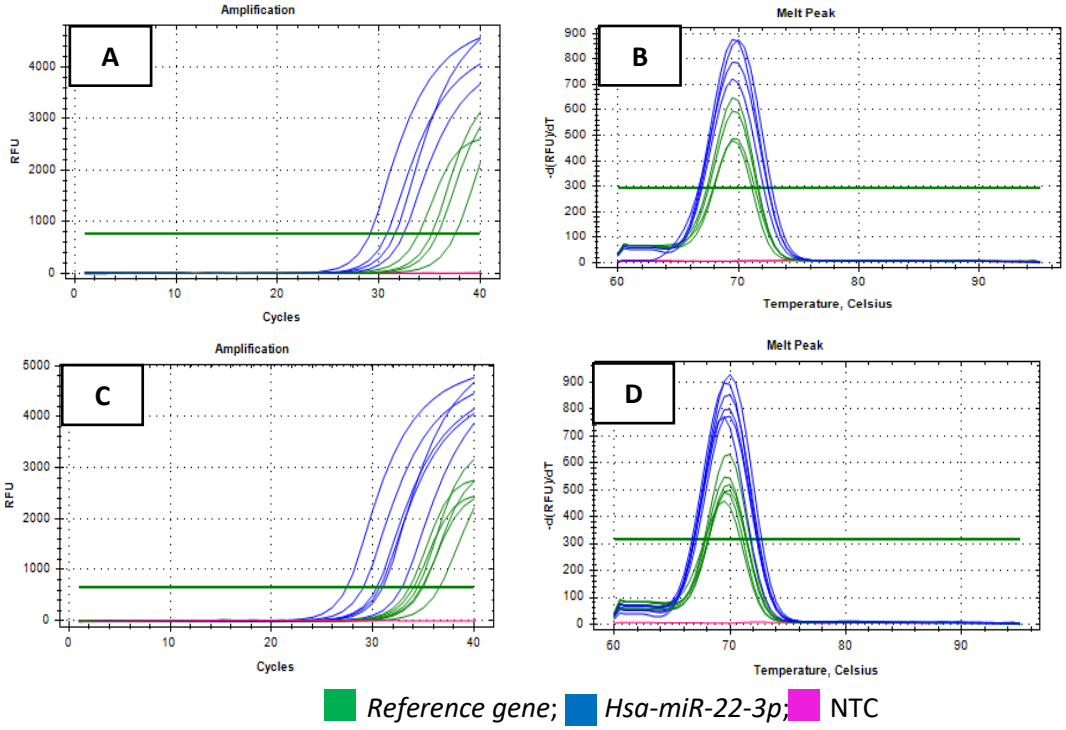

Figure 1. qRT-PCR results from the urine exosomes sample of the normal control and ca. prostate, (A & C) amplification curve; (B & D) melt peak curve. no template control (NTC); green line (threshold) (*qRT-PCR hasil dari sampel eksosom urin dari kontrol normal dan ca. prostat, kurva amplifikasi (A & C); (B & D) kurva puncak leleh. tidak ada kontrol templat (NTC); garis hijau (ambang batas)*).

Hsa-miR-22-3p Expression Test

A quantitative analysis was done towards qPCR. The fold change calculation result showed that the miR-22 expression experienced a decrease of 6.6 times compared to the normal control on average. The reduction showed decreasing miR-22 expression (down-regulated) in the prostate carcinoma samples.

Table 6. miR-22 Relative Expressions between the Prostate Carcinoma and Normal Control (*miR-22 Ekspresi Relatif antara Karsinoma Prostat dan Kontrol Normal*).

Sample (*Sampel*)	**ΔCq miR-22**	**ΔΔCq miR-22**	**Fold Change ($2^{-\Delta\Delta Cq}$) (-1/FC)**
Ca. Prostate	-0,6	2,725	0,151 (-6,6)
Normal Control	-3,33		

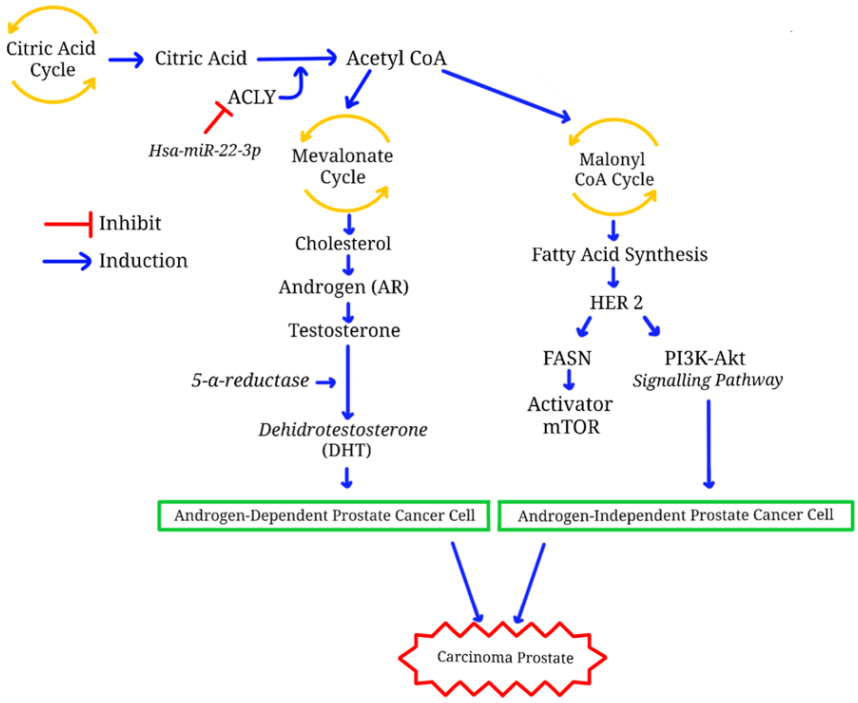

Figure 2. Propose interaction between Hsa-miR-22-3p and mRNA ACLY on the incidence of prostate carcinoma (*Mengusulkan interaksi antara Hsa-miR-22-3p dan mRNA ACLY pada kejadian karsinoma prostat*).

Correlation between Fold Change Value and Stages of Prostate Carcinoma based on Gleason Score value in patients with prostate carcinoma

Table 7. FC and GS values of prostate carcinoma patients (*Nilai FC dan GS pasien karsinoma prostat*).

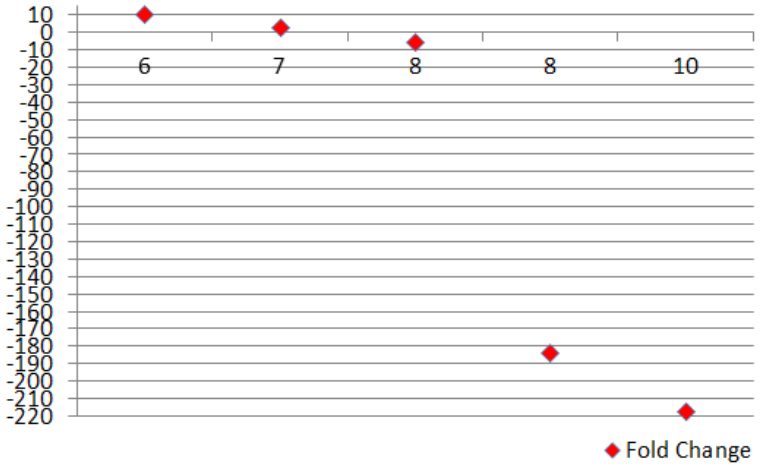

Figure 3. FC and GS correlation graph on miR-22 (*Grafik korelasi FC dan GS pada miR-22*).

Sample (*Sampel*)	**Fold Change** (*Lipat Perubahan*)	**Gleason Score** (Skor Gleason)
P9	10.37	6
P15	2.58	7
P17	-5.37	8
P2	-184.18	8
P3	-66.03	8
P5	-21.63	9
P6	-217.51	10
P11	2.42	10

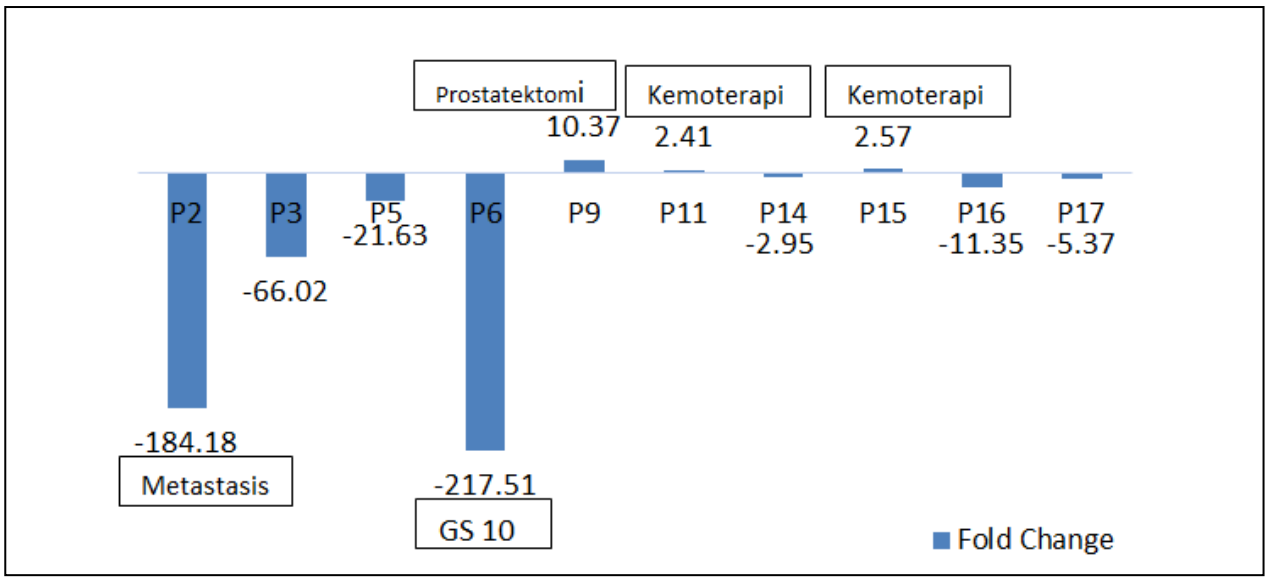

Figure 4. Correlation between FC value and clinical data on prostate carcinoma group (*Korelasi antara nilai FC dan data klinis pada kelompok karsinoma prostat*).

DISCUSSION

A Comparison Analysis of Cq Value on Urine Exosomes Samples of Carcinoma Prostate and Normal Control

Based on Table 3, the Cq average value on the prostate carcinoma sample group reached 34.65, while on the normal control was 31.63, which showed that the concentration of *Hsa-miR-22-3p* in urine exosome samples of prostate carcinoma was lower than the normal control, as the higher Cq value was, the lower copy number of target genes was amplified. The condition was in line with a theory proposing that *Hsa-miR-22-3p* experienced an expression decrease in the prostate carcinoma cases. According to Kumar & Lupold (2016) the expression of *Hsa-miR-22-3p* in prostate carcinoma tissues experienced a decrease; thus, the *Hsa-miR-22-3p* in the urine exosome sample of prostate carcinoma incident was lower than that of the normal control.

An Analysis of the qRT-PCR result of the urine exosomes sample of prostate carcinoma and normal control

Based on Figure 1 (A and C), the average samples exhibited amplification around cycle 30, indicating a low concentration of the target gene, which required more cycles to amplify. Figure 1 (B and D) demonstrates a melt peak curve formed by the targeted genes. It can be seen that the temperature for each target gene does not significantly differ, and there is no significant shift; therefore, the product generated is of one target type. The condition demonstrated that the primer microRNA had been specific by the appearance of a single peak that produced one amplicon. The absence of a melting peak curve shift in the target gene amplification shows that it is a single product. This study's PCR product specificity is closely related to the Locked Nucleic Acid (LNA) structure's primary usage. The Locked Nucleic Acid is an analogue of RNA with high affinity with 2' oxygen dan 4' carbon atoms connected or locked by Methylene Bridge on its ring of ribose glucose (Hans *et al*., 2010; Dhara *et al*, 2025). The structure can increase the melting temperature to 2-8 ºC during hybridization and increase stability and the specificity of the formed duplex (Li *et al*., 2009; Dabin & Stirnemann, 2023). The reference gene and No-Template Control are used to determine the specificity of qRT-PCR.

The reference gene functions as the housekeeping gene of a target gene to undergo quantification for its stable expression; on the other hand, the NTC is used to monitor contamination and the formed primer dimer, which can create false positive results.

The normality test (Table 4) demonstrates that the Cq miR-22 data of the carcinoma prostate and normal control groups are normally distributed, as they achieved a significance value $p > 0.05$.

The independent t-test (Table 5) demonstrates the significance (2-tailed) $p = 0031$ (Table 5) or $p < 0.05$, which means that both samples have a significant difference. Therefore, the *Hsa-miR-22-3p* can be applied as a biomarker for prostate carcinoma treatment. Nevertheless, to achieve a significant difference, it requires data exclusion on sample P9, where the patient had a prostatectomy history that may affect the Cq value measurement. According to Camarillo dan Marchat (2013), there is an association between prostatectomy and miRNA concentration (oncomir) as miRNA concentration in patients with prostatectomy decreases.

Comparison Analysis of Fold Change (FC) value of Urine Exosomes Sample in Carcinoma Prostate Patients

Based on Table 6, on average, the miR-22 expression decreased compared to the normal control. The negative symbol demonstrates a decrease in microRNA expression (down-regulated). The finding is in line with a theory stating that *Hsa-miR-22-3p* acts as a tumor suppressor in the event of prostate carcinoma. A study by Pasqualini *et al*., (2015) investigated the role of miR-22and miR-29a on the androgen receptor of prostate carcinoma with LAMC1 and MCL1 targets; the samples used were cell lines of LNCaP and DuCaP using Chromatin immunoprecipitation coupled with deep-sequencing (ChIP-seq) that produced miR-22 as a tumor suppressor. The sequencing result by Szczyrba *et al*., (2010) using 33 miRNA on prostate cancer resulted in a decrease of miR-22 expression with an FC value of -2.86.

According to Wang *et al*., (2017) miR-22 inhibits tumor proliferation, invasion, and metastasis by promoting cellular senescence, and inhibits energy and angiogenesis metabolism. The miR-22 can inhibit tumor development and tumor cell energy, including proliferation, invasion, and metastasis. Kumar & Lupold (2016) demonstrated that in prostate cancer tissues, miR-22 expression decreased.

Based on the in-silico result, miR-22 targeted several genes in the prostate carcinoma, such as PTEN, AKT1, and ACLY. Hsa-miR-22-3p functioned as a tumor suppressor that inhibited ACLY expression in the de novo pathway of the lipid synthesis in prostate carcinoma, causing a decrease in energy supply to tumor cells, thus underlying the reduction of proliferation, differentiation, and increased apoptosis in the respective cells (Xin *et al*., 2016). In the event of prostate carcinoma, there is a decrease in miR-22 expression, underlying ACLY expression to increase, which produces energy supply for tumor cells, resulting in the tumor development, including proliferation, invasion, and metastasis of prostate carcinoma (His *et al*., 2014; Chen *et al*., 2024). The decrease of miR-22 expression increases ACLY expression (Figure 2), acting as an oncomir that targets citric acid, causing a significant increase in it. The citric acid through mevalonate can be transformed, a primary precursor of the androgen. A high androgen level correlates with a high testosterone level, forming Dihydrotestosterone (DHT) when bound with *5α reductase* enzyme. DHT will bind with the androgen receptor, which changes the conformation from prostate gene transcription regulation to the proliferation process and cell differentiation increase, resulting in prostate carcinoma and BPH through dependent androgen (Suburu & Chen, 2012). Meanwhile, citric acid can be transformed into fatty acid through the malonyl CoA cycle, which can induce the HER-2 gene as oncomir; therefore, it activates the PI3K/Akt and mTOR pathway that has a role in cell cycle progression, tumor

development, and apoptosis inhibition, resulting in prostate carcinoma and BPH through an independent androgen pathway (Zaidi *et al*., 2012).

Other factors that affect the miR-22 expression include sample characteristics (Tables 1 and 2), namely patient age, cancer cell metastasis, chemotherapy, and Gleason score value. According the database from *Surveillance, Epidemiology, and End Results Program* (SEER) *National Cancer Institute* (https://seer.cancer.gov), there is a correlation between age, incident, and mortality rate in carcinoma prostate; the older an individual is, the lower metabolism rate is, causing DNA repair process to decrease and methylation builds up in the DNA underlying the carcinoma prostate.

The *Hsa-miR-22-3p* expression rate in the prostate carcinoma is also affected by the metastasis rate; the tumor cell movement from the primary sites to other tissues and organs is the main contributor to the lethal cancer effect (Steeg, 2016). Cancer metastasis can occur through the blood circulation and lymphoma gland, wherein prostate carcinoma, the metastasis generally occurs in the bone and colorectal. The expression decrease of *Hsa-miR-22-3p* in prostate cancer can increase the ACLY, AKT1, and mTOR activity, participating in cell proliferation, anti-apoptosis, and cell differentiation, increasing cell progression, even to the metastasis of prostate cancer.

Chemotherapy is one of the cancer treatments. The chemotherapy procedure is conducted by treating cancer thoroughly, preventing cancer from spreading, slowing down cancer cell development, killing the cancer cells that might have spread to other tissues, and reducing the cancer symptoms. Chemotherapy is usually applied together with operation, radiation therapy, or both treatments to reduce the tumor cells before operation or radiation, killing the remaining cancer cells after the operation and/ or radiation. Chemotherapy after operation, aiming at killing the remaining cancer cells, is called adjuvant therapy, while the treatment to reduce the size of the tumor before operation or radiation is called *neoadjuvant*. After treatment, the *Hsa-miR-22-3p* expression rate in prostate carcinoma will increase as cancer cells number is drastically decreases, increasing miR-22 expression as a tumor suppressor.

Correlation Analysis of Hsa-miR-22-3p with *Gleason Score* (GS) of Carcinoma Prostate

The differentiation level of prostate carcinoma is measured by the Gleason Grading System. The system determines prostate carcinoma based on the tumor structure pattern. The primary structure (the most occurring pattern found in tumor cells) and secondary structure (the second leading structure in tumor cells) are divided into five patterns, where pattern 1 shows the best differentiation and pattern 5 shows the worst. Tumor grading is determined by summing up two leading patterns and is reported in the Gleason score. Tumors with one most occurring structure will have their primary and secondary patterns similar.

Based on the FC value result of miR-22 in prostate carcinoma samples, there is a correlation between GS score and clinical data in prostate carcinoma patients (Figure 4). The patients' DS score is presented in Table 7. Based on Figure 3, the miR-22 expression can be affected by chemotherapy, metastasis, GS score, and prostatectomy. Several samples experienced expression increase (up-regulated); patients with expression increase have a chemotherapy and prostatectomy medical history. Chemotherapy, which aims to kill cancer cells, underlying the miR-22 expression as a tumor suppressor. Prostatectomy is an operation process to cure prostate cancer by removing the tumor or cancer tissues in the prostate; therefore, patients with a prostatectomy history will increase the miR-22 expression (*up-regulated*). Meanwhile, miR-22 expression increase is also affected by the patients' homeostasis mechanism through chemotherapy and prostatectomy. Patients who experienced metastasis and whose GS value is 10, resulting in the extreme reduction of miR-22 expression;

demonstrating that miR-22 can be used to determine the aggressiveness of prostate carcinoma. Patient data with chemotherapy and prostatectomy is deleted to determine FC and GS (Figure 4).

Figure 3 demonstrates the correlation among the Fold Change values that show the expression level in *Hsa-miR-22-3p* and *Gleason Score;* the higher GS score is, the lower miR-22 expression level is (down-regulated), showing the significant role of miR-22 in the initial stage of prostate carcinoma. The result is in line with Xin *et al*. (2016), suggesting that miR-22 targets ACLY, an enzyme that has an essential role in the de novo synthesis of lipids at the initial stage of prostate carcinoma.

The independent t-test result on ΔCq miR-22 found a significant difference (p = p.031) between the normal control and carcinoma prostate. The expression difference of microRNA in the normal control and carcinoma prostate samples described a particular tissue (Esquela-Kerscher & Slack, 2006; Schitcu *et al*, 2022). Therefore, the expression reduction of miR-22 potentially functions as a biomarker in diagnosing prostate carcinoma occurrence.

This research strengthens previous findings that miR-22 may potentially function as a biomarker in diagnosing prostate carcinoma. However, there are some limitations to the study, such as the need for in vivo research using test animals to confirm the results of the study, more samples are needed to be used to corroborate the findings.

CONCLUSION

There was a decrease in the expression level of *Hsa-miR-22-3p* in the urine exosomes sample from patients with prostate carcinoma; it was 6.6 times lower than the normal control. The *Hsa-miR-22-3p* can be a non-invasive biomarker candidate for prostate carcinoma based on the independent t-test result (p= 0.031). There was a correlation between the Gleason Score value and the expression level of *Hsa-miR-22-3p*. Therefore, it requires further study to investigate the interaction and mechanism of *Hsa-miR-22-3p* targeting the nRNA and protein in prostate carcinoma samples. Further, there is a need to optimise reference gene *Hsa-miR-22-3p* with more samples and compare RNA concentration using exosome samples of various urine, such as morning urine, temporary urine, and collected urine by sequencing to investigate the mutation possibility of *Hsa-miR-22-3p* in prostate carcinoma samples.

ACKNOWLEDGMENT

Thank you to the Faculty of Medicine, Public Health and Nursing (FK-KMK) Gadjah Mada University through the KPTU Damas Grant program, which has funded this research, the entire academic community of the UGM Biotechnology Study Program, and all the rights that have helped the implementation of this research.

AUTHOR CONTRIBUTIONS

ADP: conducting research and collecting research data, drafting articles, revising final manuscripts; RD: drafting research concepts, verifying research data, revising final manuscripts; SMH and IA: help prepare research concepts, provide input for article drafts, revise final manuscripts.

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