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Research Article

Evaluation of the role of miR-424 in patients with prostate cancer

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Abstract - The prostate is a vital accessory organ in the male reproductive system, essential for producing secretions that promote sperm health. Positioned beneath the bladder [0.5p], prostate cancer is one of the five most common cancers among men and is the second most frequently diagnosed cancer globally, according to the International Agency for Research on Cancer in 2024 [P1]. In 2020, there were 1,414,000 new cases of prostate cancer, resulting in 375,304 deaths. Epigenetics examines heritable changes in gene expression that occur without modifying the DNA sequence itself (22). Epigenetic modifications include alterations in chromatin composition and structure, irregular DNA methylation patterns, disrupted histone posttranslational modification (PTM) patterns, and the regulation of non-coding RNA (23,24). miRNAs are an essential group of short noncoding RNAs, approximately 22 nucleotides (22 nt) in length, that negatively regulate gene expression at the messenger RNA (mRNA) level. These molecules also play a vital role in regulating essential biological processes such as cell division and growth proliferation (45-46). This study aims to investigate the role of non-coding RNA miR-424 in patients with prostate cancer, as well as in a control group. Sixty blood samples from individuals aged 45-60 years were collected from general hospitals, which included 40 prostate cancer patients and 20 healthy controls. Blood samples were used for DNA and RNA extraction. The NanoDrop device was used to measure the purity of miR-424, and the gene expression data were analyzed using Q-PCR.

Keywords: prostate cancer; epigenetic; non-coding RNA; miR-424; Q-PCR

Introduction

Prostate cancer ranks among the top five most common cancers in men and is currently the second most diagnosed cancer globally, according to the 2024 report from the International Agency for Research on Cancer [P1]. In 118 countries, it was identified as the most frequently diagnosed cancer, with lung cancer coming in second, followed by liver, colorectal, and stomach cancers [p3]. Preliminary data from 2022 indicated that prostate cancer (PC) incidence across 185 nations was highest in northern Europe, with a

rate of 82.8 per 100,000 men. In comparison, southern Africa had the highest mortality rate at 29.7 per 100,000 men. The lowest rates were observed in south-central Asia, where the incidence and mortality rates stood at 6.4 and 3.1 per 100,000 men, respectively [p77]. Growth in interest surrounding epigenetics has surged within various scientific fields over the last decade, leading to an increased number of publications and research projects dedicated to the topic. Consequently, the term "epigenetics" has gained significant visibility (21.5). Epigenetics refers to the heritable changes in gene expression that occur without alterations to the DNA sequence itself. These epigenetic modifications have been associated with cancer progression and may offer insights into discovering cancer biomarkers for risk assessment, prognosis, and early diagnosis. The development and progression of prostate cancer (PC) is particularly influenced by these epigenetic alterations (22). Epigenetic alterations include abnormal DNA methylation patterns, disrupted histone posttranslational modification (PTM) patterns, non-coding RNA control, and changes in chromatin composition and/or structure (23,24). Non-coding RNAs are non-coding sequences that does not code for proteins. (ncRNAs) can influence gene expression through multiple mechanisms. For example, by interacting with epigenetic factors, ncRNAs may target chromatin to trigger gene silencing. Additionally, ncRNAs can work with transcription factors to either boost or reduce the expression of target genes. Moreover, through a process called RNA-induced silencing, ncRNAs bind directly to mRNA targets to inhibit gene expression (32). a significant class of short noncoding RNAs around 22 nucleotides (22 nt) that adversely control gene expression at the messenger RNA (mRNA) level, and can control critical biological processes like cell division, growth, and proliferation (45-46).

Materials and Methods

Between September and November 2024, sixty men aged 45 to 60 were examined. They were referred to Duhok Hospital through private pathological investigations conducted at Duhok facilities. We categorized the samples into two groups: the first group included 40 men diagnosed with prostate cancer, and the second group comprised 20 control samples.

Blood sample collection: Five milliliters of venous blood were collected from each male and divided into two groups. The first group was preserved in a tube with EDTA for methylation and DNA extraction, while the second group was placed in a tube with Trizol for RNA extraction.

The q-PCR technique was employed to assess the gene expression level of miR-424 genes, and the procedure involved many stages:

RNA extraction: After collecting blood from the participants, 750 µL of Trizol was combined with 250 µL of blood, ensuring thorough mixing for both patient and control samples. The RNA was extracted using a Transgen Biotech kit, and its purity was assessed with a NanoDrop device**.**

The process of Non-coding RNA extraction

Poly(A) tails were incorporated into RNA for cloning and affinity purification. For instance, non-coding RNA was appended to Poly(A) to create oligo-dT primer binding sites, facilitating cDNA synthesis. This approach enhances the translation efficiency of RNA in eukaryotic organisms' cells.

Definition of the unit: One active unit (U) refers to the amount of enzyme required to transform one nmol of AMP into RNA within ten minutes at 37 °C.

Operation method: Assemble the reaction system with these elements: a total volume of 20µl. The PCR reaction includes 2µl of Master mix poly(A), 1µl each of ATP and GMP, 10µg of RNA TEMPLATE, 1µl of Poly(A) polymerase, and 6µl of RNase-free water.

Converting extracted mRNA to cDNA: The isolated RNA was transformed into cDNA using the Transgen Biotech kit protocol.

qPCR reaction: The table below shows the selected housekeeping genes along with their corresponding gene primers.

Table 1: Primers utilized in the RT-PCR process

Primer	Primer sequence
DNMT 1(RT-PCR) analysis forward	5'-GCC CTA CAC CGT GGT CTA TT-3
DNMT 1(RT-PCR) analysis reverse	5'-GAC GCA GGA TGG TAT TGG A-3
Lnc RNA RT PCR forward	GTGGCTGTTGGAGTCGGTAT
Lnc RNA RT PCR reverse	TAACAAACCACGGTCCATGA
SNORA42 RT PCR forward	TGGATTTATGGTGGGTCCTT
SNORA42 RT PCR reverse	GCTCACAGCCCACAGGTAAG

Table (2): This table presents the final reaction volume, recorded at 20 µl.

The Component	The Volume
Ultra sybr q-PCRMIX	10 µl
Forward RT primer	0.5 µl
RT Reversed primer	0.5 µl
cDNA templet	4 µl
D.W	5 µl

Table (3): The q-PCR reaction software

Stage	Process time	Process Temperature
Before denaturation	10 min	95 °C
Denaturation	15 sec	95 °C
Annealing /Extension	1 min	60 °C
Melting curve analysis	15 sec 1 min 15 sec 15 sec	95 °C 60 °C 95 °C 60 °C

calculate the rate of gene expression: (levak calculate)

The following guidelines state that the rate of gene expression for both patient and control samples is determined by the CT value of the target gene inside the housekeeping gene.

CT (target/test) - CT (ref/test) = ΔCT (test).

CT (target/control) - CT (ref/control) = ΔCT (control).

The DNMT1 gene's mRNA transcripts from patient samples are displayed on the CT of the (target/test).

CT (ref/test) displays the mRNA transcripts of the housekeeping gene from patient samples.

CT (target/control) shows the mRNA transcripts of the CDKN2B gene in control samples.

CT (ref/control) displays the mRNA transcripts of the housekeeping gene in control samples.

Methylation protocol

DNA extraction

As outlined in the Transgen Biotech kit instructions, which convert unmethylated cytosine to uracil while preserving methylated cytosine, sodium bisulfite was applied to the DNA sample for 24 hours. Before proceeding with the PCR experiment, the bisulfite-treated DNA was re-extracted using primers from the gene. After completing the PCR reaction, agarose gel electrophoresis was performed, revealing the band under a UV transilluminator.

Table 4 displays the DNA methylation primers.

Primers	Sequences
DNMT1methylation F	5′-GGAGTGTAGTGGTATGATTTTGGTT- 3′ (25 mer)
DNMT1 methylation R	5′- ACTCCTCCAAACTAACCTTCCTAAA-3′ (25 mer)

PCR Methylation-specific PCR (MSP):

Table 5 demonstrates that 10 µl was the final volume of the PCR reaction utilized for DNA methylation.

Component	Volume
The DNA	2 microliter
For. primer	0.5 microliter
Rev. primer	0.5 microliter
Master mix of PCR	5 microliter
Distilled water	2 microliter

Table 6 displays the methylation program for a particular PCR.

Stages	Temperatures	Timing	Number of the cycle
Initial denaturation	98°C	2 min	1
Denaturation	94 °C	15 sec	25
Annealing	55°C	15 sec	
Extension	72°C	15 sec	
Last extension	72 C°	2 min	1
Reaction termination	4 C°	4 min	1

Results and discussion

4-1 Analyze the gene expression level of miR-424 in study group

The study's results show that patients' miR-424 gene expression levels differ from those of the control group.

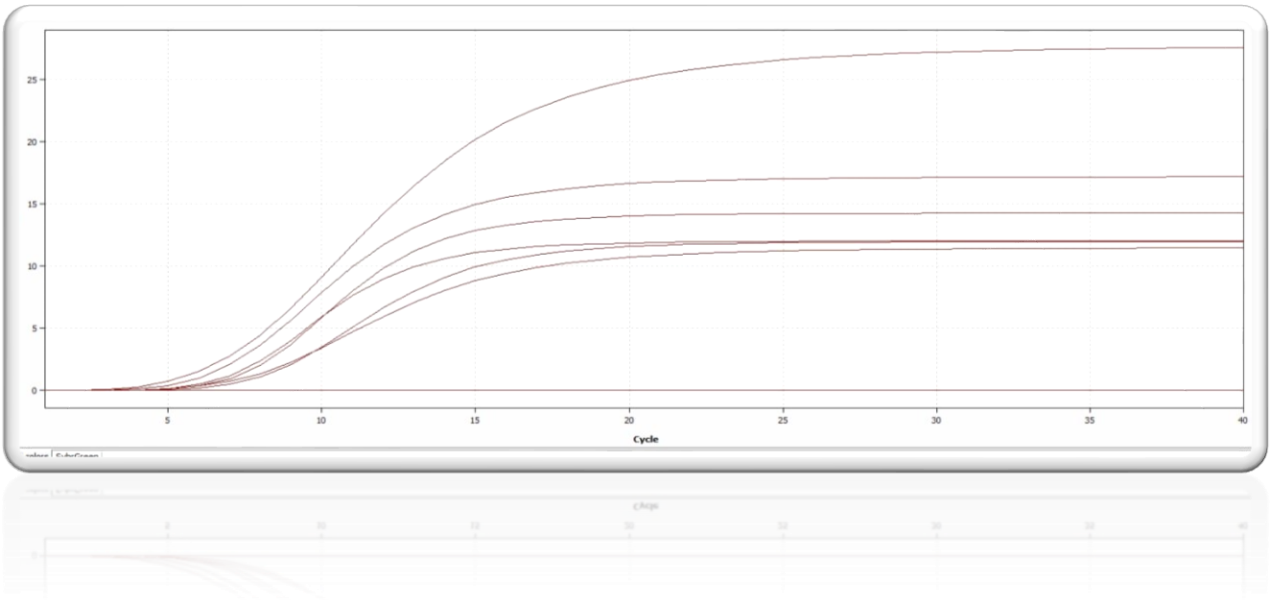

Figure 4-1: The melting curve level of prostate cancer patients is shown in this figure

The table below shows the variation in CT values of housekeeping genes and miR-424 among the research groups. The control group's CT values for the patient's target gene were 36.45 and 33.682.

Table (4-1): The table below shows the gene expression level of miR-424 and the housekeeping gene in control group and prostate cancer patients in the case study:

Sample	CT target gene	CT housekeeping gene	Δ CT target gene	Δ CT control	Δ Δ CT	Gene Expression folding
Controls mean	36.45	32.61	3.84	3.84	0	1
Patients mean	33.682	27.95	5.73	3.84	1.89	0.49

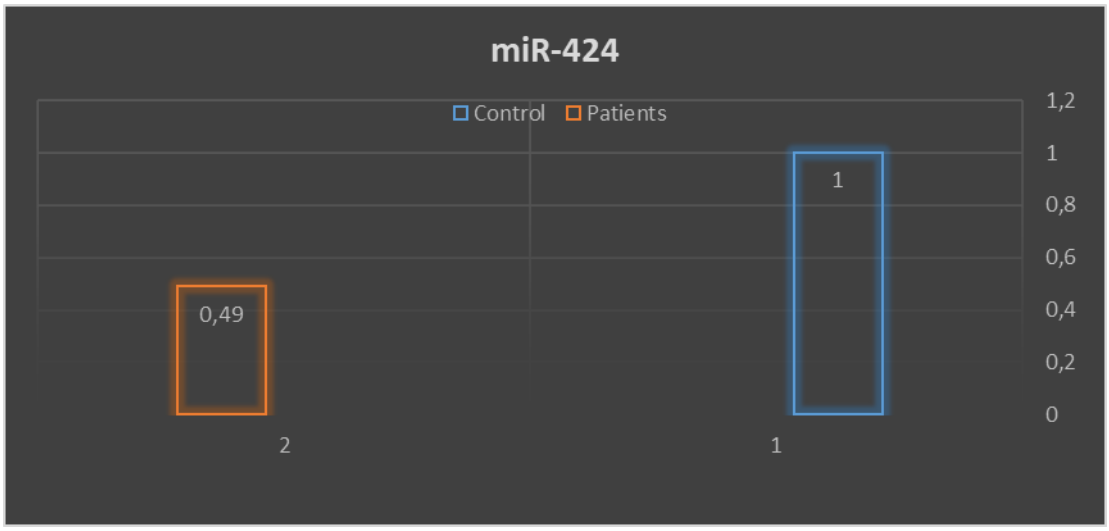

Figure 4-3: The qPCR results demonstrate that miR-424 is downregulated in prostate cancer patients compared to the control group. The expression level of miR-424 in patients with prostate cancer was 0.49 fold, in contrast to a level of 1 in the control group. These results indicate a 51% decrease in miR-424 expression among those with prostate cancer.

The downregulation of miR-424 observed in prostate cancer patients aligns with previous studies suggesting a tumor-suppressive role. For example, research conducted by Wei et al. (2021) demonstrates that miR-424 influences the STAT3 signaling pathway, which inhibits the growth of prostate cancer cells. This suggests that lower levels of miR-424 could result in increased STAT3 activity, thus promoting tumor progression. Furthermore, findings from Richardsen et al. (2019) emphasize that reduced miR-424 levels are linked to more aggressive forms of prostate cancer. Collectively, these results highlight the importance of miR-424 downregulation in understanding tumor behavior. Future studies should seek to investigate the mechanistic contributions of miR-424 to prostate cancer progression, consider its potential as a biomarker for early tumor detection, and assess its therapeutic applications.

Conclusion:

MiR-424 may act as a tumor suppressor, indicated by its significant downregulation in prostate cancer patients. This notable reduction in expression could influence the severity or progression of the disease. These findings suggest that miR-424 holds potential as a biomarker for diagnosing and prognosticating prostate cancer and may serve as a treatment target. Further research is essential to elucidate the molecular mechanisms regulated by miR-424 and to validate its therapeutic potential across broader patient populations.