

## Original Article

# Expression levels of miRNA 22-5p and miRNA 337-5p in bladder cancer

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**Background and objectives:** MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression at the post-transcriptional level and play critical roles in tumour development and progression. Bladder cancer requires reliable molecular biomarkers for diagnosis and prognosis; the roles of certain miRNAs remain insufficiently explored. This study aimed to investigate the expression profiles of miR-22-5p and miR-337-5p in bladder cancer and to evaluate their associations with clinicopathological characteristics.

**Methods:** Paired tumour and adjacent non-tumorous bladder tissue samples were collected from 50 patients undergoing transurethral resection of bladder tumour. Quantitative PCR was performed using RNA U6 as the reference gene. Expression differences were analysed, and correlations with clinicopathological features were assessed.

**Results:** Both miR-22-5p and miR-337-5p were downregulated in tumour tissues compared to normal tissues. miR-22-5p expression showed a marked reduction in bladder cancer, while miR-337-5p downregulation reached borderline statistical significance. Correlation analyses revealed no association between miR-22-5p expression and clinical variables; however, miR-337-5p expression was significantly correlated with patient age and disease duration.

**Interpretation and conclusions:** Altered expression of miR-22-5p and miR-337-5p may contribute to bladder cancer pathogenesis. miR-22-5p appears as a potential tumour suppressor, while miR-337-5p expression is influenced by clinical parameters such as age and disease duration, highlighting their potential roles as prognostic and therapeutic biomarkers.

**Keywords** Biomarker; Bladder cancer; Microrna; Mir-22-5p; Mir-337-5p; Prognosis

Bladder cancer is the ninth most common malignancy worldwide and is particularly common among men in Southern and Western Europe, North America, North Africa, and Western Asia<sup>1</sup>. Although incidence varies by geography and gender, the disease is generally more prevalent in men. According to 2022 WHO data, the incidence is 19.1 per 100,000 in Europe, 3.1 in Asia, and 10.7 in Turkey<sup>2</sup>. Bladder cancer is often diagnosed at advanced stages, limiting treatment options and complicating disease management.<sup>3</sup> Therefore, understanding its molecular biology is essential for developing effective treatment strategies.

Recent studies have highlighted the important roles of microRNA's (miRNAs) in cancer development and progression.<sup>4</sup> miRNAs regulate gene expression

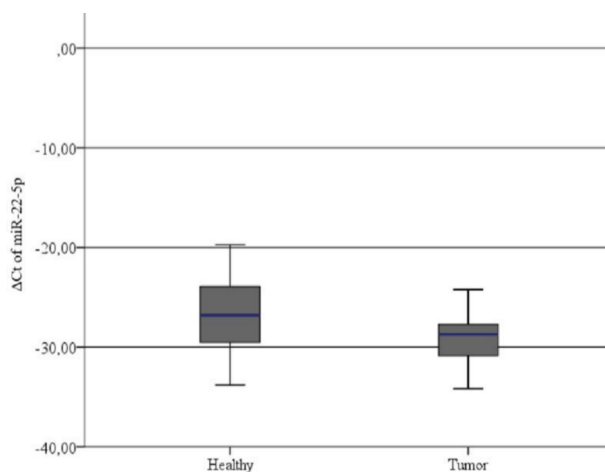
at the post-transcriptional level and affect key cancer-related processes such as cell proliferation, apoptosis, metastasis, and epithelial–mesenchymal transition (EMT).<sup>5</sup> EMT enables epithelial cells to gain mesenchymal features, increasing invasiveness, and is largely regulated by transcription factors such as Snail. Dysregulation of specific miRNAs has been linked to EMT activation and increased expression of EMT-related transcription factors, suggesting a relationship between miRNA expression and tumour aggressiveness.<sup>5-7</sup>

miRNAs may act as oncogenes or tumour suppressors in lung, prostate, and colorectal cancers. Similar mechanisms are being investigated in bladder cancer. They influence proliferation, invasion, metastasis, and

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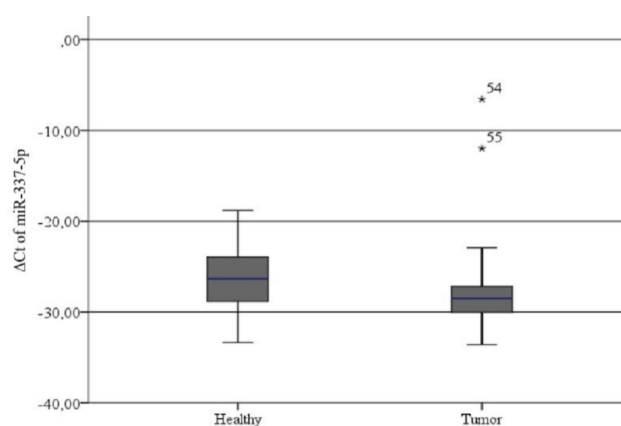
**Fig. 1.** Expression levels of miR-22-5p in normal and bladder tumour tissues shown as a box plot. Data are presented as median with interquartile range.

EMT. Lamy *et al*<sup>8</sup> showed that DNA copy number changes correlate with miRNA expression, while Shi *et al*<sup>9</sup> reported that bladder cancer is classified as non-muscle-invasive and muscle-invasive, the latter having higher mutation burdens and poorer prognosis. Xiao *et al*<sup>10</sup> found that one-third of patients develop muscle-invasive or metastatic disease, contributing to poor outcomes.<sup>10</sup> Despite surgery and systemic therapy, recurrence rates remain high, emphasising the need for personalised approaches and early detection strategies.<sup>11</sup> The present study investigates the regulatory levels of miR-22-5p and miR-337-5p in bladder cancer to clarify their potential roles in diagnosis and prognosis.

### Methods

This study was undertaken by the Department of Urology, Gaziantep University, Turkey, and supported by the Scientific Research Projects Unit (BAP), Gaziantep University, after obtaining ethical clearance from the Institute Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki, and informed consent was obtained from all participants.

**Sample collection:** Tissue samples were collected between October 1, 2022, and December 11, 2023, in the operating room of the Urology department, Gaziantep University Medical Faculty Research Hospital, using the Transurethral resection (TUR) procedure. A total of 100 samples were obtained, including 50 bladder tumour tissues and 50 morphologically normal (non-tumorous) bladder tissues. All samples were stained with haematoxylin–eosin and subjected to



**Fig. 2.** Expression levels of miR-337-5p in normal and bladder tumour tissues shown as a box plot. Data are presented as median with interquartile range.

histopathological evaluation. Microscopic images of normal and tumour tissues were also documented. Microscopic examination of the tumour tissues confirmed neoplastic morphology and characteristic changes consistent with malignancy.

**Analysis of miRNA expression in tissue samples:** Following sample collection, all tissues were processed for RNA isolation using mechanical and enzymatic lysis methods. Amplification curves for miR-22-5p are provided in **Supplementary Figure 1A** for normal tissues and **Supplementary Figure 1B** for tumour tissues. Amplification curves for miR-337-5p are presented in **Supplementary Fig. 2A** for normal tissues and **Supplementary Fig. 2B** for tumour tissues. The box plots in **Figs. 1 and 2** demonstrate that both miR-22-5p and miR-337-5p are significantly downregulated in tumour tissues compared to normal tissues.<sup>12</sup>

Raw Ct values were normalised to the endogenous control RNU6 to account for inter-sample variation, generating  $\Delta$ Ct values.  $\Delta\Delta$ Ct values were calculated by subtracting the  $\Delta$ Ct of the corresponding normal tissue from that of the tumour tissue. Relative expression (fold-change) was determined using the formula:  $2^{-\Delta\Delta Cq}$ . A  $2^{-\Delta\Delta Cq}$  value  $>1$  indicates upregulation in tumour tissues, whereas a value  $<1$  indicates downregulation relative to normal tissues. This method provides a robust and quantitative assessment of differential miRNA expression. Statistical analyses were conducted on raw  $\Delta$ Ct values, while figures display relative expression (fold-change) values derived using the  $2^{-\Delta\Delta Cq}$  method.<sup>13</sup>

**RNA extraction and cDNA synthesis:** Total RNA was extracted from tumour and matched normal

**Table I. Demographic properties of the patients (N=50)**

Parameter	n (%)
Age ≥55 yr	9 (18)
Age ≤ 55 yr	41 (82)
Males	46 (92)
Female	4 (8)
Cigarette smoking yes	19 (38)
Cigarette smokin no	31 (62)
Duration of diagnosis >5 yr	5 (10)
Duration of diagnosis <5 yr	45 (90)
Existing diseases yes	27 (54)
Existing diseases no	23 (46)
Tumour stage high	29 (58)
Tumour stag low	21 (42)
Tumour invasive	19 (38)
Tumour noninvasive	31 (62)

bladder tissues. miRNA reverse transcription was performed using the A.B.T.<sup>™</sup> miR-cDNA Synthesis Kit (A.B.T, Turkey). Each reaction contained 1 µL RNA and 9 µL synthesis mix (total 10 µL). The RT program was: 25°C for 10 min, 37°C for 20 min, 85°C for 5 min, followed by 4°C hold. qPCR was performed using SYBR Green chemistry. Primer sequences were as follows: miR-22-5p: Forward: CAGAGTTCTTCAGTGGCAAG; Reverse: GGTCCAGTTTTTTTTTTTTTTTAAAGC; miR-337-5p: Forward: CAGGAACGGCTTCATACAG; Reverse: GGTCCAGTTTTTTTTTTTTTTTAACTC; RNU6 (reference) Forward: GCAGAACGCTTCACGA; Reverse: TCCAGTTTTTTTTTTTTTTTACGCA. Each qPCR reaction (10 µL) consisted of 9 µL miR-qPCR MasterMix and 1 µL cDNA. Reactions were run on the StepOnePlus system using the following protocol: Initial denaturation: 95°C, 10 min 40 cycles: 95°C, 15 s; 60°C, 60 sec. All reactions were performed in duplicate; no-template controls were included in every run. Primer efficiencies were determined by standard curves from serial cDNA dilutions and were considered acceptable within 90-105%, in agreement with MIQE recommendations.<sup>14,15</sup> Ct values were normalised to RNU6,<sup>16</sup> and relative expression was calculated using the 2<sup>-ΔΔCt</sup> method.

**Statistical analysis:** All statistical analyses were conducted using SPSS v25.0 (IBM Corp., Armonk, NY, USA). Paired t-tests were used to compare miRNA expression between tumour and normal tissues. Associations between miRNA expression and clinicopathological parameters were assessed

**Table II. Significance (P value) of associations between miR and clinicopathological variables**

Variable	miR-22		miR-337-5p	
	P value	After correction	P value	After correction
Age	0.180	Not significant	0.001	Significant
Gender	0.673	Not significant	0.463	Not significant
Cigarette	0.761	Not significant	0.763	Not significant
Diagnosis duration	0.279	Not significant	0.001	Significant
Existing diseases	0.273	Not significant	1.417	Not significant
Tumour stage	0.186	Not significant	0.150	Not significant
Tumour spread	4.401	Not significant	2.668	Not significant

using Pearson's Chi-Square or Fisher's Exact Test, as appropriate. Considering that seven statistical comparisons were performed for each miRNA, the Bonferroni-adjusted significance threshold was calculated as 0.05/7=0.0071. Accordingly, only results with  $P < 0.0071$  were considered statistically significant after correction.

## Results

Expression levels of miRNA-22-5p and miRNA-337-5p are shown in **Figures 1 and 2**, respectively, as compared to healthy tissue. Relationships between the expression levels of miR-22-5p and miR-337-5p and clinical-demographic variables in bladder cancer patients are shown in **Table I**. No significant association was found between miR-22-5p and any variable, whereas miR-337-5p showed significant associations with age ( $P < 0.001$ ) and duration of diagnosis ( $P < 0.001$ ). These results were confirmed by Bonferroni correction (**Table II**). However, for statistical analyses and graphical representations,  $\Delta Ct$  values ( $Ct_{\text{target}} - Ct_{\text{reference}}$ ) were used because they showed a more normal distribution suitable for parametric or non-parametric tests. The  $\Delta\Delta Ct$  values were calculated to determine the relative expression differences between tumour and control tissues, and the fold change ( $2^{-\Delta\Delta Ct}$ ) values were reported to represent the magnitude and direction of these changes. These findings suggest that miR-337-5p may have a stronger association with age and disease duration, as the increase in  $\Delta Ct$  values of miR-337-5p became more

**Table III. Correlation between miR-22\_5p and miR-337\_5p expression and age.**

		Age (yr)		Total	P
		≥55	<55		
miR-22_5p expression	Low	29	8	37	0.190
	High	12	1	13	
miR-337_5p expression	Low	27	6	33	0.001
	High	14	3	17	

pronounced compared to miR-22-5p with increasing age and disease duration (**Tables III and IV**). When compared with healthy tissues from the same patients, miR-22-5p was found to be significantly downregulated in tumour tissues ( $P < 0.001$ ), while miR-337-5p did not show a statistically significant difference ( $P = 0.054$ ).

### Discussion

The present study focused on miR-22-5p and miR-337-5p, two miRNAs with tumour-suppressive roles in various cancers but insufficiently explored in bladder cancer.<sup>17</sup> Previous studies indicate that miR-22 regulates chemoresistance and inhibits EMT, suggesting that its loss may contribute to tumour progression.<sup>18</sup> miR-22-5p downregulation has been documented in hepatocellular, breast, and colorectal cancers, where it is associated with poor outcomes.<sup>19,20</sup> Similarly, miR-337-5p functions as a tumour suppressor in gastric, pancreatic, and lung cancers.<sup>21-24</sup> Reduced miR-337-5p promotes invasion and metastasis, partly through dysregulation of oncogenic pathways such as STAT3 and HOXB7. Despite these findings, data on miR-337-5p in bladder cancer are limited, making the present results an important early contribution.<sup>25</sup>

Consistent with reports from other tumour types, miR-22-5p was significantly downregulated in bladder cancer tissues. miR-337-5p also showed reduced expression, although with borderline statistical significance. This variability suggests that miR-337-5p expression may depend on tumour-specific molecular features. A review by Yarahmadi *et al*<sup>26</sup> reported strong anti-proliferative and anti-metastatic functions for the miR-337 family, supporting our observations.<sup>27</sup> Analyses of clinical and demographic variables revealed no significant associations for most parameters, including smoking status, tumour stage, and metastasis.<sup>28</sup> However, miR-337-5p expression showed a meaningful relationship with age, with lower levels observed in patients aged ≥55 years. Aging-related genetic and epigenetic changes likely contribute to this pattern.<sup>29,30</sup> After Bonferroni correction, the

**Table IV. Diagnosis duration status between miR-337\_5p and miR-22\_5p expression**

		Diagnosis duration		Total	P
		<5 yr	>5 yr		
miR-22_5p expression	Low	32	5	37	0.279
	High	12	1	13	
miR-337_5p expression	Low	30	4	34	0.001
	High	14	2	16	

associations between miR-337-5p and both age and disease duration remained statistically significant, confirming the robustness of the findings.

Overall, the findings support tumour-suppressive roles for miR-22-5p and miR-337-5p in bladder cancer, with stronger evidence for miR-22-5p.<sup>31</sup> Further functional studies are needed to clarify the molecular mechanisms regulated by these miRNAs and to examine their potential as biomarkers and therapeutic targets.

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**Conflicts of Interest:** None.

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### शोध-संदेश

माइक्रोआरएनए (miRNA) छोटे, non-coding RNA अणु होते हैं जो जीन की अभिव्यक्ति को नियंत्रित करते हैं और कैंसर के विकास में महत्वपूर्ण भूमिका निभाते हैं। इस अध्ययन में मूत्राशय कैंसर (Bladder cancer) के रोगियों में miR-22-5p और miR-337-5p के अभिव्यक्ति स्तरों का अध्ययन किया गया तथा उनका रोग की क्लिनिकल विशेषताओं से संबंध जांचा गया। अध्ययन के परिणामों से पता चला कि इन दोनों miRNAs की अभिव्यक्ति में बदलाव मूत्राशय कैंसर के विकास में भूमिका निभा सकता है। miR-22-5p संभावित रूप से ट्यूमर को दबाने वाले के रूप में (tumour suppressor) कार्य कर सकता है, जबकि miR-337-5p की अभिव्यक्ति रोगी की आयु और बीमारी की अवधि जैसे क्लिनिकल कारकों से प्रभावित होती है। ये निष्कर्ष दर्शाते हैं कि ये miRNAs भविष्य में मूत्राशय के कैंसर की पहचान, पूर्वानुमान, और उपचार के लिए उपयोगी जैविक संकेतक (biomarkers) सिद्ध हो सकते हैं।

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