

Evaluation of specific lncRNAs, miRNAs, and mRNAs in different groups of prostate cancer

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Abstract

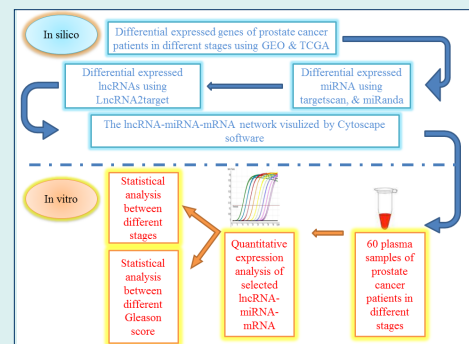
Introduction: lncRNAs interact with miRNAs and mRNAs that can have a special expression pattern in a specific cell type. We investigated the specific lncRNAs, miRNAs, and mRNAs in different groups of prostate cancer (PC).

Methods: The mRNAs with significant expression differences were first analyzed using the GEO and TCGA databases. The lncRNAs and miRNAs were then identified in the miRWalk2, miRmap, OncomiR, miRgator 3.0, miRCancerDB, LncRNA2target, TANRIC, LncRNADisease, Lnc2Cancer v3.0, and LncBase. Seventy subjects, including sixty PC

patients classified as local, locally advanced, biochemical relapse, metastatic, and benign groups, as well as ten normal individuals, were then included. Finally, real-time PCR determined the expression of the candidate biomarkers.

Results: The bioinformatics analysis detected candidate 6 miRNAs, 6 lncRNAs, and 6 mRNAs in different groups of PC patients. Unlike the significant decrease in candidate tumor suppressors, the expression levels of specific onco-lncRNA, onco-miRNA, and oncogenes exhibited a substantial increase in different groups of the patients compared to the normal group. The expression of lncRNAs, including LINC01128 ($P=0.0182$), LINC02246 ($P<0.0001$), and LINC02288 ($P<0.0001$), LINC00857 ($P<0.0001$), GNAS-AS1 ($P<0.0001$), and LINC02371 ($P<0.0001$), the expression of miRNAs, including miR-217 ($P<0.0001$), miR-375 ($P<0.0001$), miR-203a ($P<0.0001$), miR-876 ($P=0.0046$), miR-27b ($P<0.0001$), and miR-152 ($P<0.0001$), and the expression of oncogenes, including ST14 ($P<0.0001$), CD24 ($P<0.0001$), CDH1 ($P<0.0001$), DSC2 ($P<0.0001$), TGFB3 ($P<0.0001$), and MYL2 ($P=0.0186$) had significant changes at different groups of PC patients.

Conclusion: Our results identified promising biomarkers that play a role in specific groups of prostate cancer patients. Detecting specific biomarkers may be an effective strategy for different groups of PC patients.



Introduction

In recent decades, many investigations have been conducted to identify biomarkers related to prostate cancer (PC) development.¹ The ceRNA hypothesis describes a complex regulatory network of lncRNA, miRNA, and mRNA that can interact in PC.² In this

setting, lncRNAs interact with miRNAs and modify their availability as endogenous mRNAs. The essential point is that lncRNAs may have a special expression pattern in a specific cell type.² However, the diversity in their functions, from regulating protein activities to modulating other noncoding RNAs, indicates that we



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are beginning to understand the importance of lncRNAs in different biochemical and cellular processes.³ More importantly, the role of lncRNA in some malignancies as a robust prognosis biomarker has been revealed.⁴ There are reports of some lncRNA sequences that compete with miRNA.⁵ This complicated interaction between lncRNAs and miRNAs finally determines the level of protein-coding mRNAs.⁶ Therefore, evaluation of miRNA levels can be applied to cancer classification, detection of tumor development, monitoring treatment response, remission after surgery, prognosis of tumor relapse, etc. MiRNAs' pivotal role, stability, availability, quantification ability, and direct association with the stage of the disease have made them a candidate as an informative cancer biomarker.⁷ In this regard, miRNAs are considered one of the most defining elements controlling cancer signaling pathways.⁸ Thus, the secretion of miRNAs from tumor cells into body fluids such as blood and urine has made them a suitable diagnostic biomarker.⁹

Adopting bioinformatics approaches to explore the lncRNA-miRNA-mRNA interaction in PC patients has been a concern in recent years. In a bioinformatics study by Taheri et al, a ceRNA network including 5 lncRNAs, 5 miRNAs, and 17 mRNAs was constructed to detect prostate cancer.¹⁰ Another bioinformatics study reported lncRNA-miRNA-mRNA regulatory networks and their interaction with transcription factors to explore involved genes and pathways in prostate cancer.¹¹ However, the application of these networks in detection and therapy still needs to be improved. The present bioinformatics and experimental study analyzed the significant differential expression of lncRNAs, miRNAs, and mRNAs in PC patients along with Gleason scores. In this setting, specific candidate expressions of lncRNAs, miRNAs, and mRNAs in different prostate cancer groups were identified that would provide opportunities for better biomarker estimations for diagnostic purposes.

Materials and Methods

Microarray data collection for PC patients

The mRNA expression profiles (GSE30994, GSE69223, GSE3325, GSE70770, and GSE35988) at different groups of PC were downloaded from the Gene Expression Omnibus (GEO) database.⁴ There were 6, 30, 12, 136, and 87 samples in each dataset, respectively. For further analysis, we chose 3 prostate tumors and 3 normal tissues from GSE30994, 15 prostate tumors and 15 adjacent normal samples from GSE69223, 6 prostate tumors and 6 adjacent normal samples from GSE3325, 63 prostate tumors and 73 adjacent normal samples from GSE70770: GPL10558, and 59 prostate tumors and 28 normal tissues from GSE35988: GPL9075. Using GEO2R, the data was analyzed, and genes with significant differential expressions ($|\log_2FC| > 1$ and adjusted p -value < 0.05) were selected. Likewise, GEPIA2 analyzed differentially expressed genes

(DEGs) from the TCGA.¹² MiRNAs of candidate genes were identified in the miRWalk2, miRmap, OncomiR, miRgator 3.0, and miRCancerDB databases. Moreover, lncRNA2target, TANRIC, lncRNADisease, lnc2Cancer v3.0, and lncBase of the TCGA dataset were collected to identify candidate lncRNAs. The bioinformatics analyses are shown in Supplementary file 1.

Gene ontology (GO) and pathway enrichment analysis

The GO term enrichment analysis of DEG was performed using the Enricher tool. The pathway enrichment analysis of the same genes was explored using the Reactome and WikiPathway databases. The P value < 0.05 was considered significant in the data.¹³

Protein-protein interaction (PPI) network

The PPI network of DEGs was constructed from the STRING online database (<https://string-db.org/>) with medium confidence (0.400) as the minimum required interaction score. Candidate hub genes were identified by Cytohubba (Degree=4) and the Molecular Complex Detection (MCODE) plugin of Cytoscape software. The degree cut-off value of 3 and the number of nodes > 4 were set as definitive criteria.

Sample collection

As previously reported, plasma, serum, and peripheral blood mononuclear cells were collected from 60 PC patients and 10 normal individuals at the Cancer Institute of Iran and Imum-Khomaini Hospital (Tehran, Iran) from September 2019 to September 2020.² According to their pathological reports and clinical exams, prostate cancer patients (KPS ≥ 70 or ECOG=0-1) were divided into five groups, including local ($n=22$), locally advanced ($n=9$), biochemical relapse ($n=11$), metastatic ($n=8$), and benign prostatic hyperplasia ($n=10$) tumors. The routine treatments are considered radical therapy and prostatectomy for patients with local prostatic cancer; radiotherapy, androgen therapy, and radical prostatectomy for patients with locally advanced prostatic cancer; post-radical prostatectomy, salvage radiotherapy with or without androgen therapy for patients with biochemical relapse prostatic cancer; and anti-androgen therapy, chemotherapy, radiotherapy, and target therapy for patients with metastatic prostatic cancer.^{14,15} Exclusion criteria were younger than 50 or older than 75. In addition, the participants were excluded with diabetes, hypertension, acute or chronic heart disease, hepatitis, autoimmune disease, human immune deficiency disease, and other cancers.

Total RNA isolation and cDNA synthesis

The total RNA was extracted from 1 mL plasma samples using TRIzol reagent and incubated at -80 °C. RNA quality, purity, and concentration were examined with a

NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies). Reverse transcription of RNA was then carried out using a Fermentase cDNA synthesis kit according to manufacturer instructions.

Quantitative real-time PCR analysis

According to the manufacturer's protocol, gene expression quantification was performed with a Syber-Green Real-Time PCR kit. The reaction mixture contained 10 μ L SYBR green master mix, 2 μ L of cDNA template, forward and reverse primers (final concentration of 0.2 μ M for each primer), and nuclease-free distilled water to reach a total volume of 20 μ L. The program was: 1 cycle of pre-denaturing at 95 $^{\circ}$ C for 10 seconds, then 40 cycles containing 3 steps of denaturation for 5 seconds at 95 $^{\circ}$ C, annealing for 20 seconds at 60 $^{\circ}$ C, and extension for 30 seconds at 72 $^{\circ}$ C. The $-\Delta\Delta CT$ was used to analyze the data, and beta-actin and U6 were used to normalize mRNA and miRNA genes, respectively. The list of used primers is shown in Supplementary file 2.

Statistical tests

GraphPad Prism 7.0 was used for statistical analysis. Kolmogrov-Smirnov evaluated the natural distribution of the data. T-test and Mann-Whitney were used to analyze the parametric and nonparametric data of the two groups.

A *P* value less than 0.05 was considered statistically significant.

Results

Identification of DEGs in different groups of PC patients

Data analysis determined 1 up-regulated and 6 down-regulated genes in the local group, 2 up-regulated and 16 down-regulated genes in the locally advanced group, 106 up-regulated and 297 down-regulated genes in the metastatic group, 48 up-regulated and 19 down-regulated genes in biochemical relapse and 29 up-regulated and 19 down-regulated genes in the benign group. Van diagram (Fig. 1) demonstrated the DEGs between different PC patient groups. The list of hub genes, lncRNAs, and miRNAs were included in Supplementary file 3.

GO enrichment analysis

GO enrichment analyses were performed to better understand the role of specific hub genes in different prostate cancer groups. The most significant terms in biological process, cellular components, and molecular function regarding DEGs of various groups have been reported in Supplementary file 4. Table 1 indicates the selected lncRNAs, miRNAs, and mRNAs in different prostate cancer groups. The selected pathways in hub genes at each PC group were obtained through WikiPathway and Reactome pathway analysis (see Supplementary file 4).

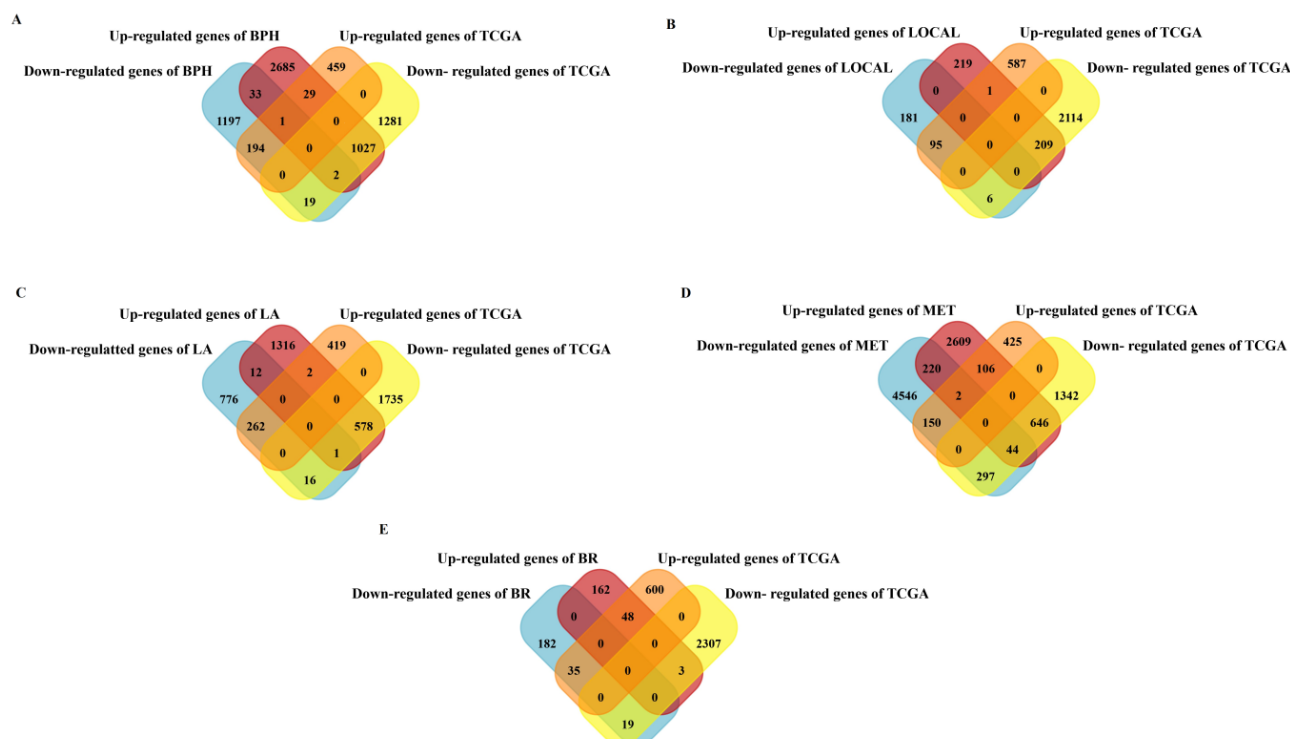


Fig. 1. Identification of DEGs in different groups of PC patients. The Van diagram shows the DEGs between different groups of PC patients. (A) 48 genes (29 up-regulation and 19 down-regulation) in the Benign Prostatic Hyperplasia group, (B) 7 genes (1 up-regulation and 6 down-regulation) in the Local group, (C) 18 genes (2 up-regulation and 16 down-regulation) in the Locally Advanced group, (D) 403 genes (106 up-regulation and 297 down-regulation) in the Metastatic group, and (E) 67 genes (48 up-regulation and 19 down-regulation) in the Biochemical Relapse group were differentially expressed compared to the normal samples. A) BPH: Benign Prostatic Hyperplasia, B) L: Local, C) LA: Locally Advanced, D) BR: Biochemical Relapse, E) MET: Metastatic.

PPI network

Cytoscape software showed the interaction networks of selected lncRNA-miRNA-mRNA in each of the different groups of prostate cancer (see Supplementary file 5). Besides, CIMminer software drew a heatmap of candidate lncRNAs', miRNAs', and mRNAs' expressions (see Supplementary file 6).

Clinical features of the patients

The previous study 2 reported clinicopathologic features of participants. There was no significant difference in age, BMI, and prostate volume between all PC groups. The majority of patients in each group had adenocarcinomas. Gleason scores and clinical tumor stages were significantly increased in different groups of PC patients with more aggressive disease.

PSA levels at different groups of PC patients

Except for the biomedical relapse group (0.5 ± 0.1 pg/mL), serum PSA level was statistically significantly higher in patients with benign prostatic hyperplasia (4.5 ± 2.3 pg/mL), local (13.2 ± 10.2 pg/mL), locally advanced (45.9 ± 14.3), and metastatic (59.5 ± 14.6 pg/mL) groups than in normal individuals (1.5 ± 0.4 pg/mL) ($P < 0.05$). This increase was more significant in patients with more advanced stages than in the early stages.

Specific lncRNA, miRNA, and mRNA expression in different groups of PC patients

Results revealed that the expression of onco-lncRNAs, including LINC01128 ($P = 0.0182$) in the locally advanced group, LINC02246 ($P < 0.0001$) in the local group, and LINC02288 ($P < 0.0001$) in the metastatic group demonstrated significant changes compared to the normal group (Table 2). Expression of candidate tumor suppressor lncRNAs, including LINC00857 ($P < 0.0001$) in the biomedical relapse group, GNAS-AS1 ($P < 0.0001$) in the metastatic group, and LINC02371 ($P < 0.0001$) in the benign prostatic hyperplasia group had significant changes compared to the normal group (Table 2).

Our results also demonstrated that the expression of onco-miRNAs, including miR-217 ($P < 0.0001$) in the metastatic group, miR-375 ($P < 0.0001$) in the benign prostatic hyperplasia group, and miR-203a ($P < 0.0001$) in the biomedical relapse group, changed significantly compared to the normal group (Table 3). Moreover, the expression of tumor suppressor miRNAs, including miR-876 ($P = 0.0046$) in the local group, miR-27b ($P < 0.0001$) in the locally advanced group, and miR-152 ($P < 0.0001$) in the metastatic group, demonstrated significant changes compared to the normal group (Table 3).

Our results also demonstrated that the expression of oncogenes, including ST14 ($P < 0.0001$) in the locally advanced group, CD24 ($P < 0.0001$) in the benign

Table 1. A list of specific lncRNAs, miRNAs, and mRNAs in different groups of PC patients

Groups	Markers					
	lncRNAs	P-value	miRNAs	P-value	mRNAs	P-value
BPH	LINC02371	5.12e-04	miR-375	1.62e-12	CD24	9.85e-15
LA	LINC01128	1.07e-15	miR-27b	1.63e-12	ST14	3.95e-62
L	LINC02246	6.15e-04	miR-876	1.10e-03	MYL2	7.62e-4
BR	LINC00857	1.88e-10	miR-203a	1.62e-12	TGFB3	2.59e-19
MET	GNAS-AS1	1.94e-06	miR-217	4.37e-08	CDH1	3.27e-39
	LINC02288	9.77e-09	miR-152	6.65e-10	DSC2	2.45e-53

BPH: Benign Prostatic Hyperplasia, L: Local, LA: Locally Advanced, BR: Biochemical Relapse, MET: Metastatic.

Table 2. Changes in the expression level of specific lncRNAs in different groups of PC patients

lncRNAs	Groups										
	Normal	BPH	P-value	L	P-value	LA	P-value	BR	P-value	MET	P-value
Onco-lncRNA											
LINC01128	-14.5 ± 1.2	-13.4 ± 1.1	0.0523	-13.7 ± 1.2	0.0940	-13.1 ± 1.2	0.0182*	-13.6 ± 1.1	0.0766	-13.5 ± 1.2	0.1051
LINC02246	-14.6 ± 0.6	-15.4 ± 1.2	0.0931	-13.2 ± 0.8	< 0.0001*	-14.9 ± 1.0	0.3171	-13.9 ± 1.1	0.1410	-13.3 ± 2.6	0.1515
LINC02288	-14.0 ± 1.3	-14.3 ± 1.0	0.4772	-13.2 ± 1.4	0.1740	-14.2 ± 1.0	0.7281	-13.5 ± 1.6	0.4963	-5.3 ± 0.7	< 0.0001*
T.S lncRNA											
LINC00857	-6.1 ± 1.5	-5.4 ± 1.7	0.3495	-6.8 ± 2.3	0.4324	-6.4 ± 1.9	0.7077	-17.8 ± 0.9	< 0.0001*	-5.8 ± 1.4	0.6583
LINC02371	-4.0 ± 1.2	-13.6 ± 1.3	< 0.0001*	-4.3 ± 1.0	0.5621	-4.8 ± 1.2	0.2345	-4.8 ± 0.8	0.1011	-5.0 ± 0.7	0.0837
GNAS-AS1	-6.5 ± 1.2	-5.7 ± 1.0	0.1242	-5.7 ± 1.0	0.0656	-6.0 ± 1.5	0.4098	-5.8 ± 1.2	0.2055	-16.9 ± 1.2	< 0.0001*

BPH: Benign Prostatic Hyperplasia, L: Local, LA: Locally Advanced, BR: Biochemical Relapse, MET: Metastatic.

* $P < 0.05$ compared to normal groups.

Table 3. Changes in the expression level of specific miRNAs in different groups of PC patients

MiRNAs	Groups										
	Normal	BPH	P-value	L	P-value	LA	P-value	BR	P-value	MET	P-value
Onco-miRNAs											
miR-203a	-14.7 ± 1.8	-13.4 ± 1.0	0.0751	-14.9 ± 1.6	0.7216	-13.5 ± 1.2	0.1197	-5.2 ± 1.3	< 0.0001*	-13.3 ± 0.5	0.0574
miR-217	-12.0 ± 2.2	-12.2 ± 1.8	0.8372	-11.9 ± 2.8	0.9219	-11.1 ± 2.2	0.3955	-11.2 ± 2.4	0.4879	-2.1 ± 0.9	< 0.0001*
miR-375	-13.2 ± 1.0	-2.2 ± 0.8	< 0.0001*	-14.1 ± 1.5	0.0846	-12.9 ± 1.5	0.6912	-12.1 ± 1.2	0.5546	-12.2 ± 1.0	0.0662
T.S miRNAs											
miR-876	-5.6 ± 1.0	-5.2 ± 1.1	0.4276	-6.9 ± 1.1	0.0046*	-5.3 ± 1.1	0.1190	-6.4 ± 0.9	0.1797	-6.4 ± 0.9	0.1047
miR-27b	-6.4 ± 0.7	-7.0 ± 1.4	0.3696	-7.2 ± 1.2	0.0742	-14.2 ± 2.0	< 0.0001*	-7.1 ± 1.1	0.1328	-7.2 ± 1.1	0.1229
miR-152	-6.3 ± 0.8	-6.8 ± 0.9	0.2045	-6.2 ± 1.7	0.8134	-7.0 ± 2.0	0.3463	-6.8 ± 0.8	0.1848	-14.5 ± 0.9	< 0.0001*

BPH: Benign Prostatic Hyperplasia, L: Local, LA: Locally Advanced, BR: Biochemical Relapse, MET: Metastatic.

* $P < 0.05$ compared to normal groups.

Table 4. Changes in the expression level of specific genes in different groups of PC patients

mRNAs	Groups										
	Normal	BPH	P-value	L	P-value	LA	P-value	BR	P-value	MET	P-value
Oncogenes											
ST14	-13.5 ± 1.3	-14.3 ± 1.6	0.2245	-14.6 ± 1.5	0.0773	-5.5 ± 1.1	< 0.0001*	-12.9 ± 2.1	0.4237	-13.0 ± 1.5	0.4317
CD24	-13.7 ± 1.1	-5.2 ± 1.4	< 0.0001*	-12.8 ± 2.0	0.5171	-13.4 ± 1.2	0.9162	-12.7 ± 1.1	0.0551	-13.2 ± 1.2	0.8443
CDH1	-13.5 ± 1.5	-13.3 ± 0.7	0.7234	-12.3 ± 1.8	0.0739	-12.2 ± 1.6	0.0868	-14.2 ± 1.4	0.3413	-3.2 ± 1.4	< 0.0001*
DSC2	-13.0 ± 1.2	-13.2 ± 1.2	0.7553	-12.4 ± 1.2	0.1742	-12.5 ± 1.0	0.2750	-13.7 ± 0.9	0.2009	-3.3 ± 1.0	< 0.0001*
T.S genes											
MYL2	-3.6 ± 1.3	-3.7 ± 1.4	0.8188	-4.9 ± 1.3	0.0186*	-2.9 ± 0.8	0.1925	-4.6 ± 0.9	0.0657	-4.6 ± 1.0	0.0915
TGFB3	-3.1 ± 1.4	-3.4 ± 1.5	0.6934	-4.0 ± 1.0	0.0573	-4.3 ± 1.0	0.0597	-14.0 ± 1.6	< 0.0001*	-4.0 ± 0.9	0.1519

BPH: Benign Prostatic Hyperplasia, L: Local, LA: Locally Advanced, BR: Biochemical Relapse, MET: Metastatic.

* $P < 0.05$ compared to normal groups.

prostatic hyperplasia group, and CDH1 ($P < 0.0001$) and DSC2 ($P < 0.0001$) in the metastatic group, increased significantly compared to the normal group (Table 4). A significant decrease was observed in the expression of candidate tumor suppressor mRNAs, including TGFB3 ($P < 0.0001$) in the biochemical relapse group and MYL2 ($P = 0.0186$) in the local group, compared to the normal group (Table 4).

Association of specific lncRNA, miRNA, and mRNA biomarkers with Gleason scores

Unlike the significant decrease in candidate tumor suppressors, the expression levels of specific onco-miRNA, onco-lncRNA, and oncogenes exhibited a substantial increase in PC patients with higher Gleason scores than in normal ones (Figs. 2-4). The expression of onco-lncRNAs such as LINC02288 ($P = 0.0002$) increased significantly in PC patients with higher Gleason scores compared to lower ones (Fig. 2). The expression of tumor suppressor lncRNAs LINC00857 ($P < 0.0001$) and GNAS-AS1 ($P < 0.0001$) decreased significantly in PC patients with higher Gleason scores compared to lower ones (Fig. 2).

Our results also showed that the expression of candidate onco-miRNAs such as miR-203a ($P < 0.0001$), miR-217 ($P = 0.0003$), and miR-375 ($P = 0.0003$) increased

significantly in PC patients with higher Gleason scores compared to lower ones (Fig. 3). The expression of tumor suppressor miRNAs such as miR-27b ($P < 0.0001$) and miR-152 ($P < 0.0001$) decreased significantly in PC patients with higher Gleason scores compared to lower ones (Fig. 3).

Our results also showed that the expression of oncogenes such as CDH1 ($P = 0.0172$) and DSC2 ($P = 0.0030$) increased significantly in PC patients with higher Gleason scores compared to lower ones (Fig. 4). The expression of tumor suppressor genes such as TGFB3 ($P < 0.0001$) decreased significantly in PC patients with higher Gleason scores compared to lower ones (Fig. 4).

Furthermore, CIMminer software was used to create a heatmap showing the expression of specific lncRNAs, miRNAs, and mRNAs in PC patients. This issue was based on Gleason scores' clinicopathologic status. We showed that the high expression of the onco-lncRNAs, onco-miRNAs, and oncogenes and the low expression of the tumor suppressors led to increases in Gleason scores (see Supplementary file 6).

Discussion

Our bioinformatics results demonstrated that 6 lncRNAs, 6 miRNAs, and 6 mRNAs were differentially expressed

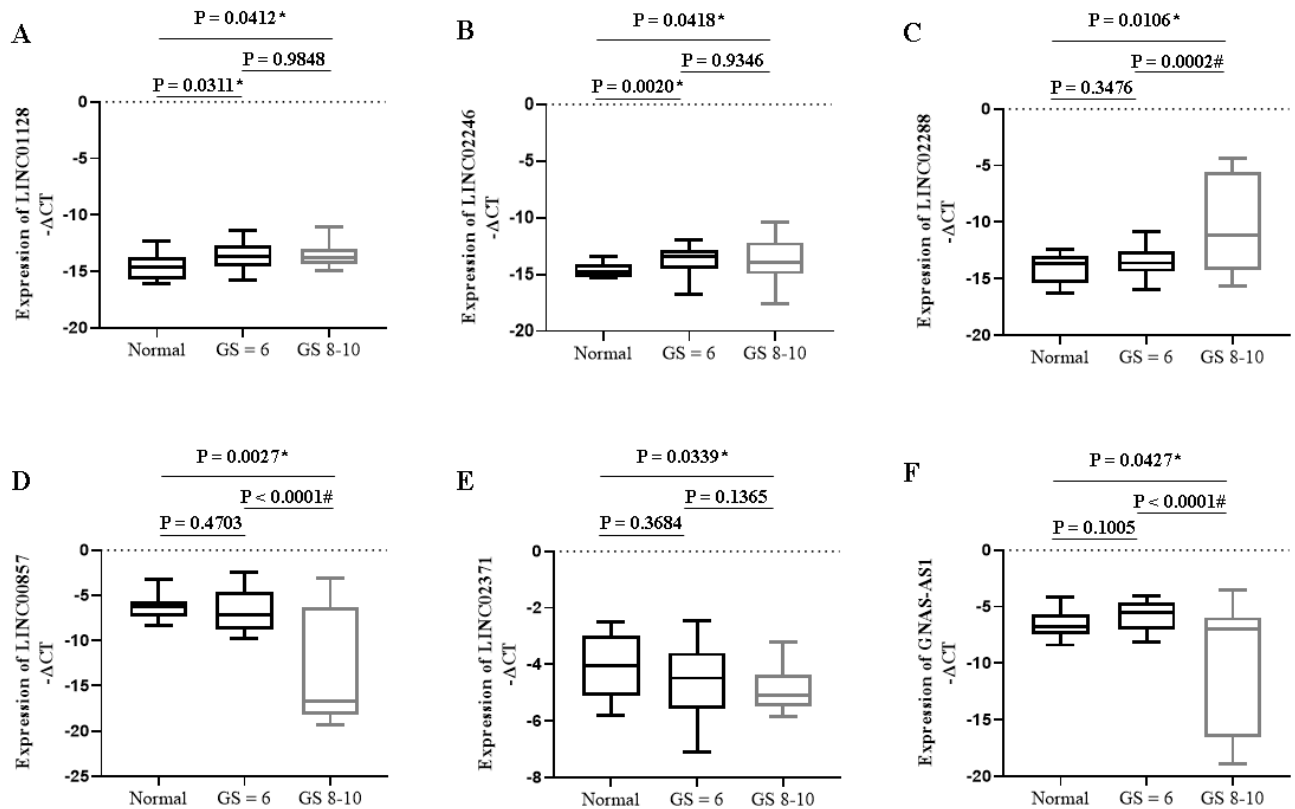


Fig. 2. Expression of specific lncRNAs in PC patients with different Gleason scores. * $P < 0.05$ compared to the normal group; # $P < 0.05$ compared to GS ≤ 6. GS: Gleason Score.

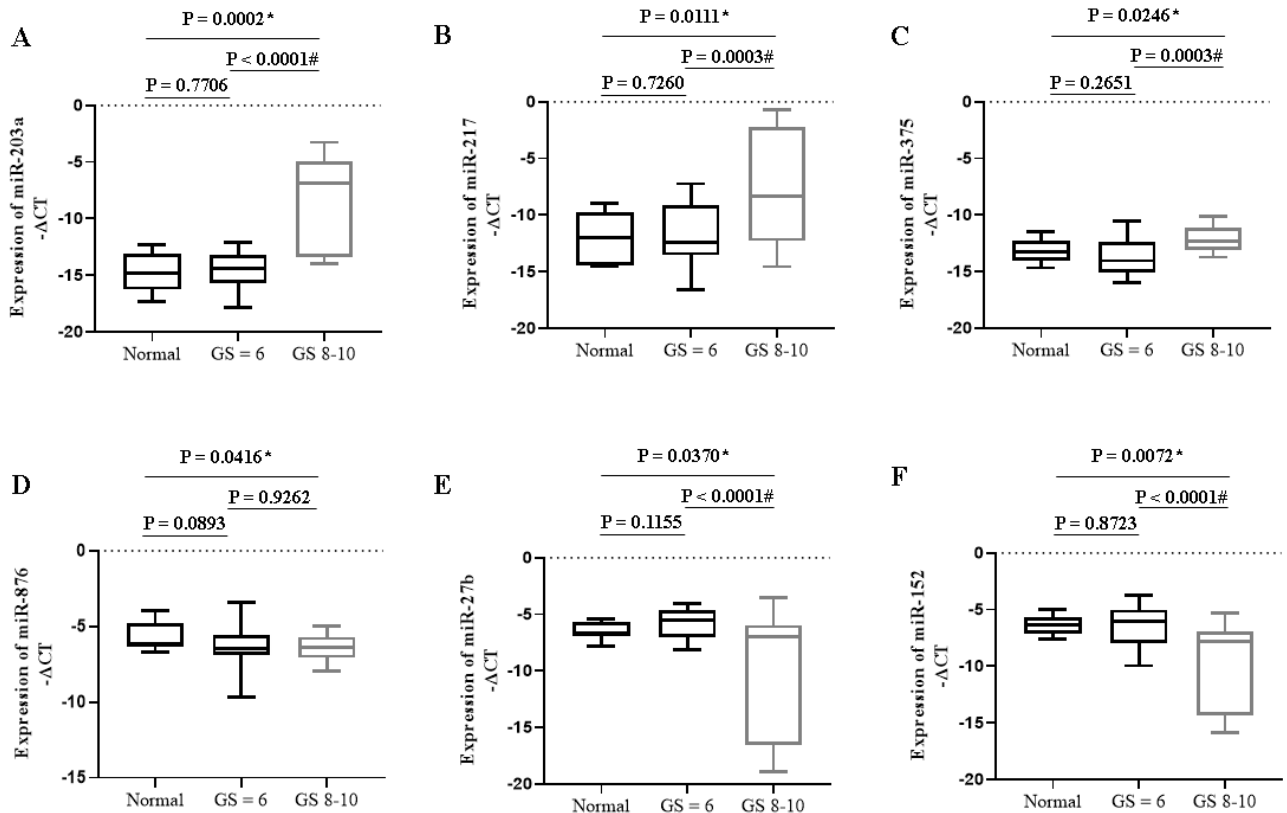


Fig. 3. Expression of specific miRNAs in PC patients with different Gleason scores. * $P < 0.05$ compared to the normal group; # $P < 0.05$ compared to GS ≤ 6. GS: Gleason Score.

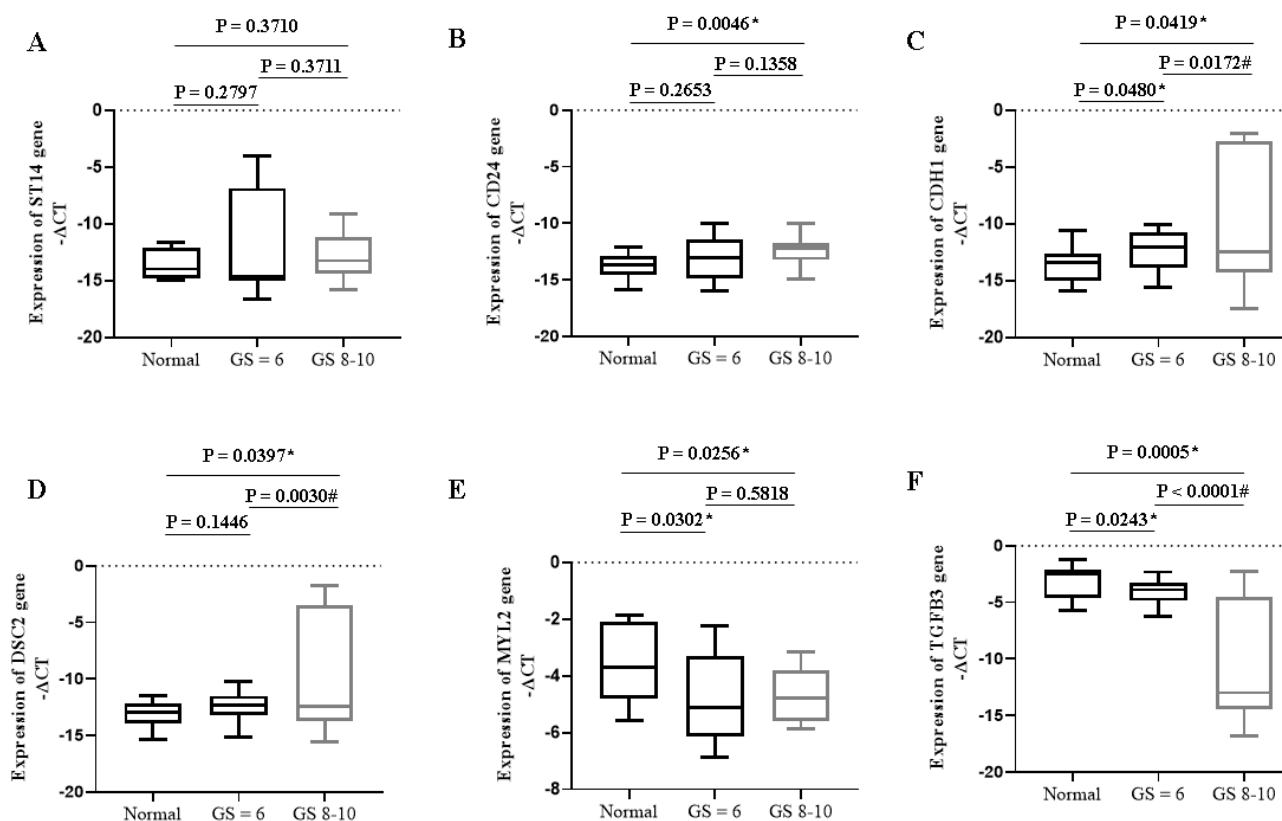


Fig. 4. Expression of specific mRNAs in PC patients with different Gleason scores. * $P < 0.05$ compared to the normal group; # $P < 0.05$ compared to GS ≤ 6. GS: Gleason Score.

in PC patients. Unlike candidate tumor suppressor expression, onco-lncRNA, onco-miRNA, and oncogene expression were significantly increased in PC patients compared to the normal group. Despite a substantial decrease in the tumor suppressors, the expression levels of specific onco-miRNAs, onco-lncRNAs, and oncogenes showed a considerable increase in the high Gleason scores compared to the lower ones.

Sigel et al showed that most PC patients in advanced stages are resistant to treatment.¹⁶ In this setting, many morphological, immunological, and molecular characteristics are employed to assess the progression or prognosis of prostate cancer.^{13,17} Considering this approach, our study found specific ceRNA networks of PC in different groups that may be used as diagnostic or prognostic values. In the benign prostatic hyperplasia group, we reported LINC02371, miR-375, and CD24 biomarkers, which can be detected in the earliest stages of PC patients. LINC02371 was not previously reported, but similar to our results, some studies have reported over-expression of miR-375 and CD24 in PC patients.^{18,19} As differential markers of the locally advanced group, LINC01128, and miR-27b have been discovered in PC patients. However, a report showed the role of LINC01128 in pancreatic cancer progression.²⁰ Here, we reported ST14 overexpression in the locally advanced group. Similarly, a higher expression level of ST14 in prostate cancer cell

lines has been observed in another study.²¹ The specific markers identified for the current study's local group of PC patients included LINC02246, miR-876, and MYL2, while LINC02246 and MYL2 were found. In line with our findings, Aakula et al reported the down-regulation of miR-876.²² Regarding the biochemical relapse group, LINC00857, miR-203a, and TGFB3 could differentiate in PC patients with more advanced groups of the disease. Similarly, Gong et al showed a lower LINC00857 level with a poorer survival rate in PC patients.²³ However, we observed LINC00857 and miR-203 as onco-lncRNA and oncomiR, respectively, which were overexpressed in the biochemical relapse samples. In this setting, the potential role of TGFB3 as a specific marker of the biochemical relapse group has been reported in PC patients. As a result of this study, we reported several specific markers that could be used to distinguish metastatic PC patients from other groups. GNAS-AS1 and LINC02288 have been found in the metastatic group of PC patients. However, their role has been mentioned in different conditions, including osteoarthritis and lung adenocarcinoma.^{24,25} Moreover, miR-217 and miR-152 were identified as two miRNAs that distinguish metastatic group of the disease. In this setting, Zhu et al. identified miR-152 as a tumor suppressor in PC, which aligns with our findings.²⁶ CDH1 and DSC2 are other genes whose differential expression was specifically observed in the metastatic

Research Highlights

What is the current knowledge?

- LncRNA-miRNA-mRNA regulatory networks and their interaction are explored in relation to the expression of genes and signaling pathways in prostate cancer progression.
- The application of these networks in detection and therapy still needs to be improved.

What is new here?

- The high expression of onco-lncRNAs, -miRNAs, and -mRNAs and the low expression of the tumor suppressors are associated with high malignancy in prostate cancer.
- The specific lncRNAs, miRNAs, and mRNAs biomarkers may be targets for developing effective diagnostic and therapeutic strategies.

group of PC patients. CDH1 (E-cadherin expressing gene) significantly increased in metastatic PC compared with other groups.²⁷ Another oncogene was DSC2 (Desmocollin-2), a DSC family member. Similar to our results, Jiang et al reported overexpression of DSC2 in DU-145, VCaP, and LNCaP PC cell lines compared with RWPE-1, a normal prostate cell line.²⁸

The specific biomarkers in this study were also linked to Gleason scores in different PC groups. Using these biomarkers shows that, unlike candidate tumor suppressors, PC patients with higher Gleason scores onco-lncRNA, onco-miRNA, and oncogenes displayed a significant increase compared to the patients with lower ones. Brase et al demonstrated that miRNAs such as miR-375 were associated with higher Gleason scores, which aligns with our study.²⁹ Thus, candidate lncRNAs, miRNAs, and mRNAs could be used to diagnose PC patients. These biomarkers would be a novel strategy with effective targets for developing treatments in the future.

The study's limitations relate to the limited sample size; however, significant effects have been seen in many biomarkers. On the other hand, we set the exclusion criteria high to minimize confounding factors. Additionally, the treatment traits were selected based on a statistical profile of group individuals. However, it should be noted that the sampling was done before the start of treatment and after the diagnosis and placement of patients in different stages of the disease.

Conclusion

Our results showed that these biomarkers could be distinguished in different groups of PC patients. The candidate lncRNAs, miRNAs, and mRNAs may be targets for developing effective therapeutic strategies.

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Authors' contribution

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Competing interests

The manuscript's authors have no conflicts of interest and are responsible for the paper's content.

Data availability statement

The data supporting this study's findings are available from the corresponding author, AMA, upon reasonable request.

Ethical statement

All methods were performed under the relevant guidelines and regulations. All procedures performed in studies involving human participants were under institutional and/or national ethical standards. In addition, they were subject to the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. A review board committee approved the experimental procedures and care protocols of the Iran University of Medical Sciences (NO: IR.IUMS.FMD.REC.1401.100). Written informed consent was obtained from each participant before sample collection.

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Supplementary files

Supplementary file 1. A flowchart diagram for analyzing bioinformatics data.

Supplementary file 2. The sequence of real-time PCR primers.

Supplementary file 3. The identification of hub genes in different groups of prostate cancer patients.

Supplementary file 4. Gene ontology (GO) enrichment analysis.

Supplementary file 5. Protein-protein interaction network. A) Hub genes of the benign prostatic hyperplasia group, B) hub genes of the local group, C) hub genes of the locally advanced group, D) hub genes of the metastatic group, and E) hub genes of the biochemical relapse group. Protein-protein interaction constructed with hub genes from GEO and TCGA datasets that show the interaction between hub genes using STRING and Cytoscape software.

Supplementary file 6. A plot heatmap showing the gene expression profile of hub genes in both (A) bioinformatics and (B) experimental data ($-\Delta\Delta CT$), and (C) Gleason score (≤ 6 and 8-10) in prostate cancer patients. The green and red color indicates down- and up-regulated lncRNA-miRNA-mRNA interactions, respectively between tumor and normal samples.

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