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Original Article

JPX and LINC00641 ncRNAs expression in prostate tissue: a case-control study

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Abstract

Background and purpose: Prostate cancer (PC) is the second most prevalent cancer in men. Prostate-specific antigen (PSA) is the main biomarker for screening PC. An increase in PSA could lead to false-positive results. Thus, more appropriate markers should be investigated. In the present study, JPX and LINC00641 expression levels were measured in tumoral prostate tissue compared with the non-tumor tissue.
Experimental approach: 43 pairs of prostate tumoral and non-tumor tissue were prepared. The expression levels of JPX and LINC00641 were investigated by RT-qPCR.
Findings/Results: Significant upregulation of LINC00641 (2.47 ± 0.5 *vs* 1.41 ± 0.2) and downregulation of JPX (1.42 ± 0.6 *vs* 2.83 ± 1.0) were observed in PC tissues compared with the normal tissues (their adjacent non-tumoral tissues).
Conclusion and implications: Dysregulation of JPX and LINC00641 in PC patients could be used in the future as a prognostic biomarker in PC.

Keywords: Gene expression; JPX; LINC00641; Long non-coding RNA; Prostate cancer.

INTRODUCTION

Prostate cancer (PC) has been reported as the most prevalent malignancies in the male in the world. Many studies have shown that the incidence of PC is increasing significantly in recent years. About 42% of this type of cancer occurs in men over the age of 50 and most cases over the age of 60 years (1).

Commonly used PC screening methods are prostate-specific antigen (PSA) and digital rectal examination, and confirmation is based on biopsy (2). The Gleason scoring (GS) system is the most common PC grading system used, in which the cells with more transformation receive a higher score (3,4). Other clinical features reported with biopsy results include tumor involvement, PSA level, perineural invasion, prostatic intraepithelial neoplasia (PIN), apex and base, circumferential margins & capsule, and seminal vesicles (5). Usually, PSA levels above 4 ng/mL are assumed to be suspected. As levels increase higher than 10.0 ng/mL, the risk of cancer increases significantly. PSA level of 10 ng/mL is considered to be a cutoff level for performing the bone scan (6). However, none of them alone will be an absolute sign of progress, although GS is the most important item (7).

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Although the use of PSA as a marker is highly sensitive for diagnosing PC, it lacks sufficient specificity, as it can be high in benign prostate hyperplasia (BPH) and prostatitis. Therefore, PSA has a high false-positive rate leading to unnecessary biopsy and inappropriate treatment. Based on previous studies, it has been shown that two-third of the performed biopsies were not, in effect, necessary (1). Current techniques for the staging of PC cannot differentiate between indolent (which needs no treatment) and highly invasive, fatal tumors. Since there is no effective treatment available for patients with high-grade cancer, it is necessary to accurately specify the degree of malignancy of the PC (8).

The main mechanism(s) through which initiation of PC occurs are not well defined yet. The androgen receptor signaling pathway is one of the main pathways involved in PC initiation and progression. Three other signaling pathways that are common in PC include RAS/RAF, RB, and phosphoinositide 3-kinases (PI3K) pathways (9). The dysfunction of PI3K signaling pathway has been shown in 42% of patients with primary and in 100% of metastatic patients with PC. The protein kinase B (PKB/Akt) is the main intermediate of the oncogenic effect of PI3K. The two essential genes in this pathway with the highest mutations include *INPP4B* and *PTEN*. Mutation of *INPP4B* and *PTEN* are found in 42% and 47% of metastatic patients, respectively (9). PTEN is a tumor suppressor and antagonist of the PI3K/Akt pathway, which has an inhibitory function by the phosphatase effect in this pathway. The precise functional mechanism of INPP4B is not exactly determined, but some evidence suggests that it can also have a tumor suppressor role. Mutated *INPP4B* and *PTEN* lead to full activation of Akt (10).

In addition to studying markers such as receptors in cancer (11), one of the molecules that have been regarded recently to be involved in malignancy is long non-coding RNA (lncRNA). LncRNAs have been shown to perform key functions in the cell, including splicing, gene expression regulation, cell growth, differentiation, and epigenetic processes (12,13). Another role of lncRNAs is related to small non-coding RNAs including microRNAs (miRNAs). Recent studies emphasize that lncRNAs work as competing endogenous RNAs (ceRNAs) to control mRNAs through miRNAs, which indicates a novel layer of RNA crosstalk. However, the main regulation and characterization of this sort of crosstalk in cancers are still mainly unknown (14).

LncRNAs through their sponge role cause trapping and destruction of miRNAs. Consequently, they inhibit miRNA function (15). Also, lncRNAs with no protein-coding capability have key roles in the initiation and progression of tumors through modifying oncogenic and tumor-suppressing pathways (16). They can affect multiple signaling pathways for tumor invasion, progression, metastasis, and chemotherapy resistance. The characteristics of these molecules have led researchers to investigate the lncRNA-disease association, especially in cancers (17,18). In recent years, *in silico* bioinformatic studies have been applied to clarify the etiology and treatment of complex disorders such as cancer (19,20). Here, we used a customized pipeline to select and identify lncRNA by using a variety of bioinformatics software and databases such as HMDD (www.cuilab.cn/hmdd), starBase (www.starbase.sysu.edu.cn/), MNDR (www.rna-society.org/mndr/), LncRNADisease (www.cuilab.cn/lncrnadisease), DIANATOOLS (http://diana.imis.athena-innovation.gr/DianaTools/index.php), Lnc2Cancer (www.bio-bigdata.net/lnc2cancer/).

Finally, in this study, a couple of lncRNA including LINC00641 and JPX revealed through *in silico* analysis, were selected for expression analysis. Both of them were selected for expression analysis in the prostate tumor tissue compared to the non-tumoral adjacent tissue. Furthermore, the correlation between lncRNAs and pathologic factors of PC was investigated to assess their potential as tumor markers for PC detection. LncRNAs were selected according to a customized pipeline, which involved a combination of two methods. Method A was based on the important signaling pathway in the PC. Method B was performed with HGLDA, a computational model based on the selection of lncRNAs. We aimed to evaluate

the dysregulation of LINC00641 and JPX in PC tumor tissues and their adjacent tissues and to assess their correlation with other characteristics of PC to eventually evaluate their potential as tumor markers for PC tracing in future studies.

MATERIALS AND METHODS

In silico study

According to the designed pipeline in our previous study (unpublished results), several lncRNAs relevant to PC with *P*-values less than 0.05 were selected. Based on the NONCODE database, LINC00641 and JPX showed an appropriate expression in the prostate. In addition, both of them have many target miRNAs in the PI3K/AKT pathway. In this experimental study, these lncRNAs were selected for expression analysis in prostate tumor tissue of affected individuals and adjacent non-tumoral tissue. Table 1 presents the sequences of our designed oligonucleotide primers.

Tissue sample collection

In this study, after collecting informed consent, all patients were selected based on the inclusion criteria: the histopathological results of the needle biopsy specimens supported PC or BPH, with no chemotherapy administered prior to surgery. Table 2 presents the characteristics of the patients. Fresh tumor tissues and their adjacent non-tumoral tissues were obtained from PC patients and were directly frozen in liquid nitrogen and then stored at -80 °C until RNA extraction.

Table 1. Nucleotide sequences of the primers used for the real-time polymerase chain reaction.

Primer name	Sequences	Product size (bp)
Linc00641	Forward: AAATTCTGATGTCCAGTGGCATG	268
Linc00641	Reverse: CAGATGAACAGCCGGGCAC	
JPX	Forward: GTCCGAAGTATGAGTCCACTAAC	147
JPX	Reverse: CGAACTCCATCTTCTGCAACT	
ACTB	Forward: AGAGCTACGAGCTGCCTGAC	184
ACTB	Reverse: AGCACTGTGTTGGCGTACAG	

Table 2. Clinical characteristics of patients.

Characteristics	Group 1	Group 2	Group 3	Group 4	Group 5
Gleason score (GS)	Non-cancer	benign prostate hyperplasia	GS 6	GS 7	GS 8-10
Sample size	43	5	10	23	10
Age(min-max)	52-83	60-78	56-77	52-81	56-83
≤ 60	11	0	3	6	2
> 60	32	5	7	17	8
Tumor involvement					
One side	-	-	4	8	3
Two side	-	-	6	15	7
PSA level (ng/mL)					
PSA < 10	-	-	8	15	6
PSA ≥ 10	-	-	2	8	5
Perineurial invasion					
Negative	-	-	4	2	2
Positive	-	-	6	21	8
Prostatic intraepithelial neoplasia					
Negative	-	-	2	5	2
Positive	-	-	8	18	8
Apex and base					
Free	-	-	8	19	7
Involved	-	-	2	4	3
Circumferential margins and capsule					
Free	-	-	7	21	7
Involved	-	-	3	2	3
Seminal vesicles					
Free	-	-	8	20	6
Involved	-	-	2	3	4

Table 3. Used material in real-time polymerase chain reaction.

material	Volume (µL)
2X SYBR Green master mix	5 µL
cDNA samples	1 µL
Forward and reverse primers (2 pmol)	0.5 µL
Nuclease-free water	3 µL

Table 4. Thermal cycling conditions.

Initial activation	Denaturation (40 cycles)	Annealing/extension (40 cycles)
30 s at 95 °C	5 s at 95 °C	20 s at 60 °C

All tests were accomplished in conformity with relevant instructions. First, from each patient, written informed consent was received. All the clinical samples were obtained from Imam Khomeini Hospital of Tehran University of Medical Sciences. The Review Board of Isfahan University of Medical Sciences approved this study (Ethics code: IR.MUI.REC.1396.3.123).

RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction

According to the manufacturer's instructions, the total RNA purification kit (Norgen, Canada), total RNA isolation was performed from tissue samples. The quantity of RNA was evaluated spectrophotometrically by Nanodrop ND-2000 (Thermo Fisher Scientific, USA). Then, 1 µg of the extracted RNA was reverse transcribed into cDNA using the PrimeScript II first-strand cDNA synthesis kit (Takara, Japan), according to the instructions of the manufacturer. Quantitative real-time polymerase chain reaction (qRT-PCR) was assessed on the Rotor-Gene Q 5plex HRM system (Qiagen, Germany) using SYBR Premix Ex Taq II (Tli RNaseH Plus, Takara, Japan). The protocol detailed in Table 3 was applied to perform PCR in 10 µL reaction volumes. Thermal cycling conditions were set as shown in Table 4. The specificity of the target PCR product was confirmed by visualizing a single peak in the melting curve analysis. Beta-actin (ACTB) was used as a reference gene. qRT-PCR data analysis was accomplished based on the $2^{-\Delta\Delta ct}$ comparative expression method (21).

Statistical analysis

All tests were performed in duplicate and the mean values were calculated. In the result section, each column of data has been shown as mean ± SEM. Wilcoxon, Mann Whitney U, and Kruskal-Wallis tests were used for comparison of the control and tumor groups in terms of different grades of the tumor using SPSS (version 25.0), and GraphPad Prism (version 6.0) software tools. In all tests, $P < 0.05$ indicated a significant difference.

RESULTS

Upregulation of LINC00641 in prostate tissue of patients

Levels of LINC00641 expression were evaluated in the tissue samples of 43 patients diagnosed with different grades of PC and in their adjacent non-tumoral tissues. Also, they were assessed in 5 tissue samples of patients diagnosed with BPH. As shown in Fig. 1A, LINC00641 expression levels were significantly higher in the PC group (case) than in the non-tumoral group (control) (2.47 ± 0.5 *vs* 1.41 ± 0.2). To investigate the prognostic significance of LINC00641 in PC patients, the mean expression values were evaluated compared with available clinicopathological features (Table 5). The expression levels of LINC00641 were investigated in four groups including cancerous tissues with GSs 6, 7, 8-10 and BPH. LINC00641 showed the highest expression levels in the BPH group. In cancerous tissues, this increase was found to be higher as Gleason scores advanced (Fig. 1B). The expression levels significantly differed between GS 6 and BPH groups, between GS 7 and BPH and finally between GSs 8-10 and BPH. In other words, based on the results illustrated in Fig. 1C and considering all cancer samples in a group, the LINC00641 could differentiate cancer samples from BPH. Fig. 1D shows LINC00641 expression in control and case groups in low GS in PC tissues.

Downregulation of JPX in the prostate tissue

JPX expression levels were evaluated in tissue samples of 43 patients diagnosed with different grades of PC and in their adjacent non-tumoral tissues. Also, they were assessed in 5 tissue samples of patients diagnosed with BPH.

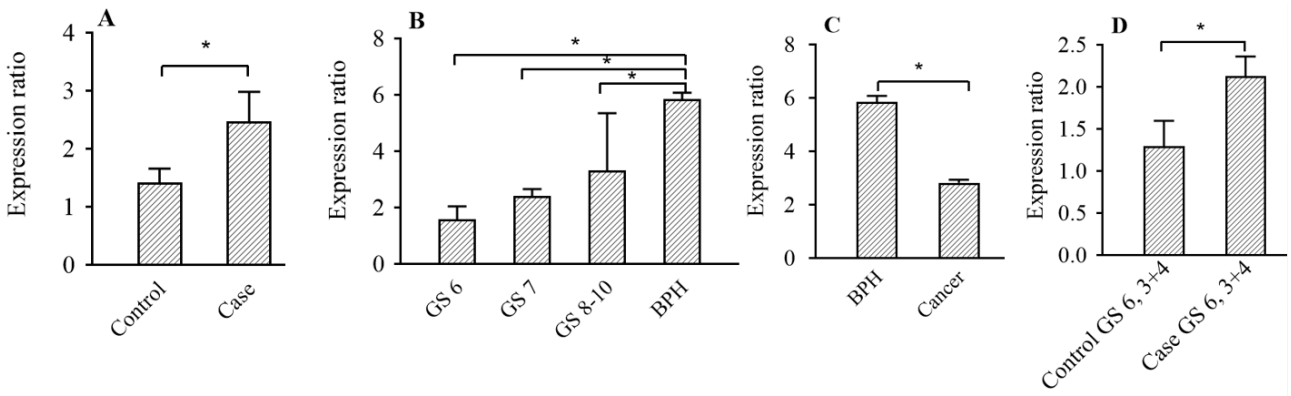

Fig. 1. (A) LINC00641 expression in prostate cancer tissues and their adjacent noncancerous tissues; (B) the expression levels of LINC00641 in different groups with different GSs; (C) the expression levels of LINC00641 in cancerous tissues and BPH; and (D) LINC00641 expression in control and case groups in low GS in prostate cancer tissues. Values represent means ± SEM. **P* < 0.05 shows significant differences between indicated groups. BPH, Benign prostate hyperplasia; GS, Gleason scores.

Table 5. Association of LINC00641 expression levels and clinicopathological characteristics of prostate cancer samples. Values represent means ± SEM.

Characteristics	Mean ± SEM	*P*-value
Age(min-max)		0.7
≤ 60	3.74 ± 1.7	
> 60	2.0 ± 0.2	
Tumor involvement		0.2
One side	2.96 ± 1.4	
Two side	2.21 ± 0.2	
Prostate-specific antigen level (ng/mL)		0.3
PSA < 10	2.87 ± 1.0	
PSA ≥ 10	2.16 ± 0.38	
Perineurial invasion		0.1
Negative	1.205 ± 0.35	
Positive	2.73 ± 0.6	
Prostatic intraepithelial neoplasia		0.9
Negative	2.07 ± 0.5	
Positive	2.53 ± 0.5	
Apex and base		0.1
Free	2.46 ± 0.6	
Involved	2.58 ± 0.3	
Circumferential margins and capsule		0.3
Free	2.56 ± 0.5	
Involved	1.33 ± 0.6	
Seminal vesicles		0.8
Free	2.52 ± 0.5	
Involved	1.97 ± 0.7	

As shown in Fig. 2A, JPX expression level was significantly lower in the PC group (case) than in non-tumoral samples (control) (1.42 ± 0.6 *vs* 2.83 ± 1.0).

To investigate the prognostic significance of JPX in PC patients, we evaluated the association of its mean expression with available clinicopathological features of our patients (Table 6).

The expression level of JPX was investigated in four groups including cancerous tissues with GSs 6, 7, 8-10 and BPH. Fig. 2B shows that the amount of JPX expression level in the BPH group is the lowest among the groups, and its expression in cancerous tissues varies; while it is low in tissues with GS 6, the expression is increased in samples with GS 7 but descends in GS 8-10.

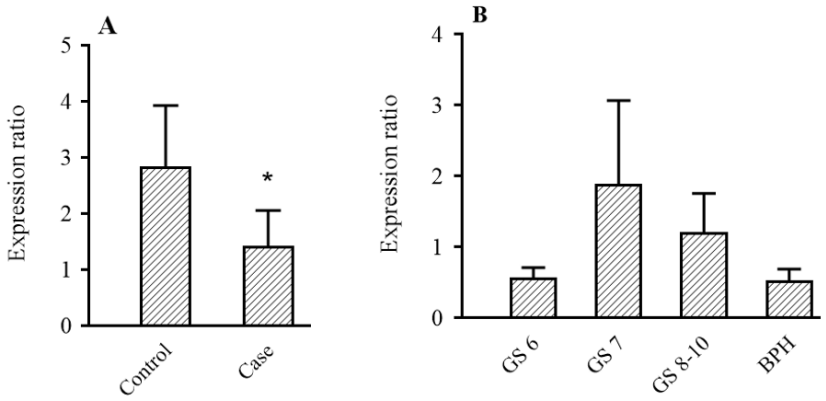

Fig. 2. (A) JPX expression in prostate cancer tissues and their adjacent noncancerous tissues; and (B) the expression levels of JPX in different groups. Values represent means ± SEM. **P* < 0.05 indicates significant differences in comparison with the control group.

Table 6. Association of JPX expression levels and clinicopathological characteristics of prostate cancer samples. Values represent means ± SEM.

Characteristics	Mean ± SEM	*P*-value
Age (min-max)		0.5
≤ 60	1.07 ± 0.4	
> 60	1.5 ± 0.8	
Tumor involvement		0.2
One side	0.6 ± 0.2	
Two side	1.84 ± 0.9	
PSA level (ng/mL)		0.5
PSA < 10	2.02 ± 1.1	
PSA ≥ 10	0.5 ± 0.1	
Perineurial invasion		0.5
Negative	0.65 ± 0.1	
Positive	1.5 ± 0.7	
Prostatic intraepithelial neoplasia		0.07
Negative	0.2 ± 0.05	
Positive	1.6 ± 0.7	
Apex and base		0.06
Free	1.37 ± 0.8	
Involved	1.61 ± 0.5	
Circumferential margins and capsule		0.4
Free	1.48 ± 0.7	
Involved	0.8 ± 0.2	
Seminal vesicles		0.6
Free	1.4 ± 0.6	
Involved	1.6 ± 0.9	

DISCUSSION

PC is the most prevalent malignancy in men. Several studies have revealed that the incidence has increased dramatically in recent years (1). PC is caused by a combination of both genetic and epigenetic changes converting the natural gland epithelium to pre-neoplastic lesions and ultimately to invasive cancer (17,22). PSA and digital rectal examination are screening tests for PC. However, in some conditions such as BPH, PSA levels are also increased, which may lead to unnecessary prostate biopsy resulting in additional costs, anxiety, and pain. Thus, other biomarkers are needed to be sought for possible diagnosis, prognosis, and management of PC (17).

One of the emerging biomarkers is lncRNA, which plays a key role in biological functions and regulating gene expression at different levels. lncRNAs have remarkable advantages over proteins. Although they have low

expression levels, they are of high tissue specificity and can be detected even at very low levels and in any type of sample (23). Also, many lncRNAs are related to different types of cancers including breast cancer, glioblastoma, colorectal cancer, liver cancer, and leukemia (24). The PI3K/Akt/mTOR pathway has multiple cellular activities including cell survival, growth, proliferation, cell migration, and invasion (25). Experimental studies indicate that the pathway is necessary for the survival of the cancer stem cell population and participates in epithelial-to-mesenchymal transition (EMT) in PC cells. Also, many studies have shown that the PI3K/AKT pathway is one of the most common pathways altered in cancers such as PC (26). PTEN and INPP4B are two components of the PI3K/AKT signaling pathway whose loss of function leads to PC (27,28). Therefore, the PI3K/AKT/mTOR pathway is essential to the metastatic potential of PC and presents considerable evidence for targeting the PI3K/AKT/mTOR pathway in this disease (29).

In this study, two lncRNAs, JPX and LINC00641, identified *via in silico* methods, were investigated in PC. After the construction of the ceRNA network *via* the Cytoscape v3.2.1 software, based on the interaction between genes, miRNAs, and lncRNAs associated with the disease, the selected lncRNAs are expected to be associated with the progression of PC (Fig. 3). All items in the network have been reported in association with cancer, especially PC. Several important aspects of the network are discussed below.

Within the nucleus, forkhead box transcription factors (FOXO) lead to apoptosis through the induction of death genes such as the FasL gene. Studies suggest that FOXOs are the main mediators of tumor suppression downstream of PTEN, and are dysregulated in many tumor types including breast cancer, PC, glioblastoma, rhabdomyosarcoma, and leukemia (30).

Improper expression of anti-apoptotic members of the *BCL2* gene family has also been shown in human cancers and leukemias. BCL2 family proteins control all main types of cell death, such as necrosis, autophagy, and apoptosis, hence playing a role as a major factor in several pathways associated with oncology. The upregulation of *BCL2* has been documented in many cancer types. BCL2 inhibits apoptosis by interacting with pro-apoptotic members of the BCL2 family, such as BAX, BAD, and BID. *BCL2* is overexpressed in androgen-independent PC (31).

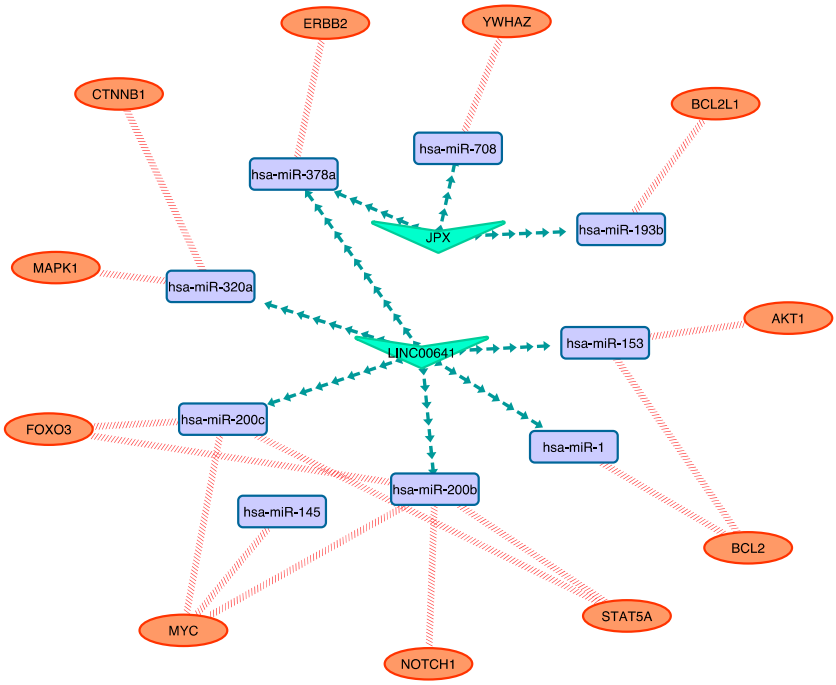

Fig. 3. Predicted lncRNAs-related ceRNA network in prostate cancer by Cytoscape v3.2.1 software. The V shapes represent lncRNAs.

ERBB2 encodes a member of the epidermal growth factor receptor family of receptor tyrosine kinases. Overexpression of this gene has been shown in many cancers, including ovarian and breast tumors. It has been indicated that ERBB2 activates androgen receptor signaling at the molecular level, even in the presence of low levels of androgen (32).

Notch signaling is involved in controlling the balance between apoptosis, cell proliferation, and differentiation. The *Notch* gene is aberrantly overexpressed in many human cancers. It has been demonstrated that the Notch signaling participates in PC cell survival and *Notch*-related genes are overexpressed in PC. In human PC tissues, *Notch* expression level is raised with increasing tumor grade (33).

MYC is a proto-oncogene and encodes a nuclear phosphoprotein participating in the cell cycle progression, apoptosis, and cellular transformation. Changes in chromosome 8q24 engulfing *MYC* have also been associated with disease severity. *MYC* appears to be overexpressed at the earliest phases of PC, from prostatic intraepithelial neoplasia to advanced prostatic adenocarcinoma (34).

The diagnostic value of miR-378 is more than that of the commonly used markers including carcinoembryonic antigen and CA19-9, suggesting the high confidence of miRNA-378 as a primary diagnosing biomarker of malignancy. According to a meta-analysis, miRNA-378 has a specificity of 74% and a sensitivity value of 75% in differentiating individuals with cancer from healthy people. miRNA-378 is overexpressed and appears to act as an oncogene in ovarian cancer, liver cancer, nasopharyngeal carcinoma, human breast cancer, colorectal cancer, gastric cancer, and renal cell carcinoma. However, *in vitro* assays investigating the antiproliferative function of miRNA-378 in PC have revealed that the upregulation of miRNA-378 suppresses PC cell migration, invasion, and induces cell apoptosis (35).

Regarding the function of miRNA families in SLUG regulation, it has been reported that there is a mutually repressive interaction between miRNA-1 and miRNA-200 with SLUG, which is an anti-apoptotic protein. *SLUG* overexpression together with miRNA-1 and miRNA-200b downregulation are needed to induce EMT and mesenchymal differentiation in cancer. Also, studies show that miRNA-1 and miRNA-200 inhibit a variety of mesenchymal attributes and growth rates in invasive human PC cells. On the other hand, miRNA-200b and miRNA-200c transfections significantly diminished the expression of *Notch* (36).

miRNA-153 has been suggested as a promising tumor-associated miRNA and is dramatically dysregulated in cancers. The miRNA-153 expression is decreased in human oral cancer, glioblastoma, ovarian cancer, and breast cancer. In these cancers, miRNA-153 leads to cell apoptosis by directly suppressing anti-apoptosis family member BCL2. In PC, however, miRNA-153 represses *PTEN* expression to activate AKT kinase (37).

Hence, this network can provide evidence for molecular abnormalities and expression dysregulation in PC and suggests new candidate interactions that could be involved in PC etiology.

In our model, *PTEN* and *INPP4B* are the primary target genes of our selected lncRNAs. Thus, we evaluated the expression levels of lncRNAs with putative roles in the regulation of the PI3K/AKT signaling pathway. The results of the present study showed a significant upregulation of LINC00641 in PC samples compared to the adjacent non-cancerous tissues. Moreover, our data showed that the expression level of JPX is decreased in PC samples compared to the adjacent non-cancerous tissues. Notably, the prediction of these lncRNAs was initially based on the PI3K/AKT pathway (genes and related miRNAs). In our work, after adding other important genes and miRNAs associated with PC from the literature, as well as including the information from integrated databases such as TCGA, DIANA tool, starBase, and HMDD, other interactions and effects of these lncRNAs in the disease were inferred. In the other words, this 3-layer network consisting of PC-related mRNAs, miRNAs, and lncRNAs revealed the potential relationship between these components and pathways involved in the PC.

The use of lncRNAs as PC biomarkers is in its infancy. Currently, the only Food and Drug Administration (FDA)-approved test is PCA3. Marks *et al.* were the first to evaluate the PCA3 in 226 patients who had undergone a biopsy and showed its superiority to PSA. However, the PCA3 scores did not distinguish between high-grade and low-grade tumors. Besides, patients with low-grade tumors normally present too low PCA3 value to be detected (38).

JPX is located 10 kb upstream of *XIST* and activates it through making a balance between its activators and repressors (39). Therefore, the decrease in JPX expression could cause the loss of *XIST*, leading to X reactivation and altered expression of cancer-associated genes in the X chromosome, which may cause cancers. On the other hand, *XIST* is associated with β -catenin signaling, which induces EMT. Besides, EMT is involved in the progression of cancer metastasis. Hence, JPX may cause the development of cancers such as PC through the regulation of the *XIST* function (40,41). Thus far, few investigations have been performed on the association between JPX and cancer. In 2016, a study by Weijema *et al*. demonstrated a significant reduction in JPX expression in hepatocellular carcinoma. Their study was based on the investigation of the expression of this lncRNA in the tumoral tissue of the liver compared to the expression in the adjacent normal tissue (42). This study could be regarded as a clue for our findings. Also, overexpressed JPX increases the proliferation and invasion of human ovarian cancer cells, and prevents apoptosis by stimulating the PI3K/AKT/mTOR signaling (43). Recently, studies have been demonstrated that as an oncogene, JPX is significantly overexpressed in non-small-cell lung cancer tissues and is related to poor prognosis. JPX leads to cyclin D2 overexpression in the ceRNA mechanism by interacting with miRNA-145-5p leading to non-small-cell lung cancer development and progression (44). On the one hand, JPX acts as an oncogene to increase the risk of ovarian and lung cancer. On the other hand, JPX performs as a tumor suppressor gene to inhibit hepatocellular carcinoma development (45).

In this study, we assessed the correlation between JPX and the tumor features of PC through the GS. Although the reduced expression was not statistically significant, the reduction trend was more pronounced in the initiation (tumors of the lowest GSs) and advanced forms (tumors of highest GSs) of PC. In case this is established in a future study with more sample size, it could be concluded that JPX plays a role in the initiation and invasion of cancer. As there is a preference for the increased expression of biomarkers over their reduction, in future studies, the expression of molecules such as miRNAs that are either upstream or downstream of JPX can be evaluated. On the other hand, many studies have suggested markers showing reduced expression in the disease (46).

There was no significant difference between clinicopathology subcategories in terms of JPX expression. However, JPX reducing expression trend was seen in PSA ≥ 10 (ng/mL) group and the involved circumferential margins and capsule group compared to PSA < 10 (ng/mL) and free circumferential margins and capsule groups.

LINC00641 has been reported to be one of the 20 differentially expressed LncRNAs and mRNAs dysregulated through *HOXA10* overexpression; for example, in one study, it showed downregulation with fold changes ≥ 2 and $P < 0.05$ (47). On the other hand, much evidence shows that several homeobox genes such as *HOXA10* are dysregulated in cancers, significantly contributing to tumor progression. For example, Li *et al.* demonstrated that a decrease *HOXA10* expression in endometrial carcinomas was significantly associated with increased tumor grade and was related to the modification of the *HOXA10* promoter (48). Thus, it could be concluded that increased expression of LINC00641 may occur in cancers such as PC. Also, LINC00641 can act as a stimulant in the acute myeloid leukemia carcinogenic process, according to a recent study (49). Some study results are not in line with other findings. For example, Li *et al.* and Liang *et al*. reported that LINC00641 is acting as a tumor suppressor in bladder and glioblastoma patients, and a decrease in its expression was observed (50,51). Also, concerning breast and cervical cancer similar results have been attained (52,53). Therefore,

all the above studies suggest that the function of LINC00641 may vary depending on the type of cancer. In our study, LINC00641 expression in cancer samples showed a significant difference compared with BPH samples, indicating the strength of this marker in differentiating cancer from BPH (Fig. 1C). Despite a 60- to 70-fold increase in PCA3 expression in PC tumors compared to the normal tissues, one of its limitations included its failure to distinguish between the high and low-grade tumors. While in the present study, LINC00641 showed only a 1.7-fold increase in prostate tumors compared to the normal adjacent tissue, after categorizing samples into control and case groups in low GS in PC tissues, we were able to differentiate between the control and case in low grade with $P < 0.05$ (Fig. 1D). This suggests the importance of LINC00641 in early diagnosis.

There was no significant difference between clinicopathology subcategories in terms of LINC00641 expression. However, LINC00641 increasing expression trend was seen in the positive perineurial invasion, positive prostatic intraepithelial neoplasia, involved apex and base groups compared to negative perineurial invasion, negative prostatic intraepithelial neoplasia, free apex and base groups.

CONCLUSION

In conclusion, our investigation provides insights into the levels of JPX/LINC00641 expression in PC. We clarified that the expression of JPX is significantly lower and expression of LINC00641 is dramatically higher in PC tissues than in the adjacent noncancerous tissues and in relation to GS. The data presented here suggest that JPX/LINC00641 could be a novel component of gene signatures for the detection of PC. The limited sample size is one of the restrictions of our study. Hence, our findings should be validated in investigations with more cases in future research. According to our results, as well as recent findings, one single biomarker is insufficient and a panel of them might be required to assess PC diagnosis, prognosis, and management.

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Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contribution

M.A. Tabatabaiefar and M.H. Modarressi contributed to the design of the work, interpretation of the results of the study, approval of the manuscript. R.S. Sajjadi performed the experiments and contributed to the analysis of the results and prepared the draft of the manuscript. The manuscript has been read and approved by all the authors.

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