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RESEARCH ARTICLE

MAPK and JAK/STAT pathways targeted by miR-23a and miR-23b in prostate cancer: computational and in vitro approaches

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Abstract The long-lasting inadequacy of existing treatments for prostate cancer has led to increasing efforts for developing novel therapies for this disease. MicroRNAs (miRNAs) are believed to have considerable therapeutic potential due to their role in regulating gene expression and cellular pathways. Identifying miRNAs that efficiently target genes and pathways is a key step in using these molecules for therapeutic purposes. Moreover, computational methods have been devised to help identify candidate miRNAs for each gene/pathway. MAPK and JAK/STAT pathways are known to have essential roles in cell proliferation and neoplastic transformation in different cancers including prostate cancer. Herein, we tried to identify miRNAs that target these pathways in the context of prostate cancer as therapeutic molecules. Genes involved in these pathways were analyzed with various algorithms to identify potentially targeting miRNAs. miR-23a and miR-23b were then selected as the best potential candidates that target a higher number of genes in these pathways with greater predictive scores. We then analyzed the expression of candidate miRNAs in LNCAP and PC3 cell lines as well as prostate cancer clinical samples. miR-23a and miR-23b showed a significant downregulation in cell line and tissue samples, a finding which is consistent with overactivation of these pathways in prostate cancer. In addition, we overexpressed miR-23a and miR-23b in LNCAP and PC3 cell lines, and these two miRNAs decreased IL-6R expression which has a critical role in these pathways. These results suggest the probability of utilizing miR-23a and miR-23b as therapeutic targets for the treatment of prostate cancer.

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Introduction

Prostate cancer is the second most common cancer and fifth cause of cancer death worldwide with an estimated 1,111,689 newly diagnosed cases and 307,471 deaths in 2012 [1]. Considering the global changes in the aging of the populations, it is estimated that the numbers will rise to 1,700,000 new cases and 499,000 deaths by 2030 [2]. Present therapies for prostate cancer have limited efficacy, and developing new therapeutic strategies and drugs for controlling prostate cancer is an active area of research [3].

MicroRNAs (miRNAs) are a class of small endogenous noncoding RNAs that play a crucial role in regulating gene expression in different cells and organisms [4]. Each miRNA can regulate a large number of genes, likely around several hundred genes in case of mammalian cells [5]. Indeed, this capability of miRNAs makes these molecules potent regulators of cellular processes, particularly when transcripts belong to the same pathway. This feature has brought miRNAs to the spotlight of molecular research for treatment of the human diseases [6]. Several miRNA-based therapeutics is currently under investigation and promising developments have been made in the context of infectious diseases as well as cancers. SPC3649, a miR-122 antagonist, which is designed to block the reproduction of HCV in hepatocytes, is presently undergoing a phase 2 clinical trial [7]. For cancer treatment, let-7 and miR-34 are being actively investigated as they show efficient in vitro and in vivo inhibition of cancer cells in culture and mouse cancer models, respectively [8]. Currently, let-7 is in preclinical development and miR-34, which displays antineoplastic activity in mouse cancer models including melanoma, lymphoma, lung, prostate, and pancreatic cancer, is undergoing phase 1 clinical trial in patients with liver cancer [9].

Identifying the appropriate miRNAs that target desired pathways and genes is one of the major challenges in using miRNAs as therapeutic agents [10]. Computational approaches are useful tools for the prediction of miRNAs that are more likely to target selected genes or signaling pathways. Nonetheless, these methods which work based on sequence complementarity between the miRNA and 3′UTR of messenger RNAs (mRNAs) usually generate a large number of predicted miRNA-mRNA interactions for each transcript. Several prediction algorithms have been developed including TargetScan, miRanda, PicTar, DIANA-microT, and RNA22 [11]. Furthermore, there are some databases, such as TarBase V 6.0, that present the experimentally validated interactions between miRNAs and mRNAs [12]. A frequently used strategy for target prediction is to combine the results of different prediction algorithms to find miRNA-mRNA interactions which are predicted by multiple algorithms and exhibit a greater combined score [13].

In order to find miRNAs that target prostate cancer, first, we should select strategic pathways that have a critical role in pathogenesis and progression of the disease. MAPK and JAK/STAT are two important pathways that are involved in tumorigenesis of prostate cancer [14]. The MAPK pathway exerts key roles in cell proliferation, gene expression, and differentiation in different cells. It has been reported that the MAPK pathway has also a major role in tumor growth and metastasis in prostate cancer [15, 16], and its inhibition prevents prostate cancer cell growth [17, 18]. The JAK/STAT pathway is another key signaling cascade involved in physiological processes such as cell differentiation, growth, and apoptosis [19]. This pathway is upregulated in a wide range of cancers including prostate cancer [20]. Moreover, previous studies have indicated that blocking this pathway suppresses prostate cancer cell proliferation and promotes apoptosis of neoplastic cells [21, 22]. The essential roles of these pathways in the prostate cancer initiation and progression have made them excellent candidates to be investigated in the context of this disease [14]. In this study, using a combination of computational and experimental approaches, we have tried to identify miRNAs that can efficiently downregulate these pathways through multitargeting of the pathway genes. We report that miR-23a and miR-23b might be excellent miRNA species for diminishing the activity of these disease-related pathways.

Materials and methods

Bioinformatics studies

We prepared a list of the genes of MAPK and JAK/STAT pathways which their activation enhanced but not inhibited the activity of these pathways. In preparation of this list, we attempted to consider the key genes that had been reported as potential drug targets in prior studies concerning prostate cancer [14]. The final list included *MAP2K1*, *MAP3K1*, *MAP3K12*, *MAP3K3*, *MAP3K5*, *MAP3K7IP2*, *MAP3K8*, *PDGFA*, *PDGFRA*, *FAS*, *FGF1*, *FGF10*, *FGF12*, *FGF3*, *FGFR3*, *HRAS*, *MEK1*, *MEK2*, *BRAF*, and *RAF1* from the MAPK pathway and *JAK1*, *JAK2*, *JAK3*, *STAT3*, *IL6R*, *IL6*, *BRCA1*, *AR*, *PIK3R3*, *AKT*, and *mTOR* from the JAK/STAT pathway. We then analyzed these genes using validated target databases (i.e., TarBase 6) as well as several target prediction algorithms, such as DIANA-microT, miRanda, miRDB, miRWalk, RNAhybrid, PICTAR5, RNA22, and TargetScan. In addition, the list of the genes was analyzed in the miRNApath database [23].

Pathway analysis

DIANA mirPath v.2.0 was used to analyze the target genes for candidate miRNAs from MAPK and JAK/STAT pathways [24]. In this database, it is possible to analyze the miRNAs with a miRNA target prediction algorithm (DIANA-microT-CDS) or the experimentally validated miRNA targets (DIANA-TarBase v6.0). These predicted and validated interactions for different miRNAs can be combined and presented graphically for each pathway. For pathway analysis, the selected miRNAs were subjected to both DIANA-microT-CDS and DIANA-TarBase v6.0 algorithms.

Expression analysis

For the expression analysis, we used the mimiRNA database to investigate the expression of candidate miRNAs in prostate tissue and prostate cancer cell lines, LNCAP and PC3 [25].

Prostate cancer cell line preparation

LNCAP and PC3 prostate cancer cell lines were obtained from the National Cell Bank of Iran (Pasteur Institute of Iran, Iran). These cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) containing 10 % fetal bovine serum (FBS) (Gibco, USA) and antibiotics from Sigma-Aldrich, Germany. After proliferation and reaching 80 % confluence, cells were trypsinized and washed with DMEM.

Human sample preparation

Human samples were obtained from 16 patients in early stages of prostate cancer who had undergone radical prostatectomy at Tehran hospitals. All the samples confirmed by pathology tests and except three patients (samples 6, 13, and 14) who were in the T3 stage, the rest of the patients were in T2 stage. Fourteen patients had a Gleason score of 7 and the other patients had a Gleason score of 6 (samples 4 and 8). In addition, ten benign prostatic hyperplasia (BPH) samples were obtained from patients that were referred to Tehran hospitals in 2012. Written informed consent was taken from all of the patients. The ethical committee of the hospitals approved this study by project no. 1544.

Designing stem-loop, primers, and probe for miRNAs and primers for IL6R

The sequences of the selected miRNAs were obtained from miRBase (http://www.mirbase.org), and the sequence of IL6R gene was taken from NCBI Gene (http://www.ncbi.nlm.nih.gov/gene). The stem-loops, primers, and probes were designed based on the work from Mohammadi-Yeganeh et al. [26, 27]. The primers were designed using AlleleID 6 (PREMIER Biosoft International, USA), and the specificity of each primer was determined via NCBI BLAST (www.ncbi.nlm.nih.gov/BLAST). All the stem-loops, primers, and probes were synthesized by Macrogen (Seoul, Korea). Sequences of primers and probe are shown in Table 1.

miR-23a and miR-23b plasmid construction

DNA was extracted from human blood samples using QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instruction. For amplification of miR-23a and miR-23b, PCR was done using forward and reverse primers containing Xho1 and Mlu1 restriction sites (Table 1), respectively. This fragment was cloned into the lentiviral vector pLEX-JRed, which its turbo GFP were extracted by Xho1 enzyme (Open Biosystems, USA) (Fig. 1).

Lentivirus packaging and transduction

Viral packaging cell line HEK 293T cells, were cultured in DMEM (Gibco, USA) containing 10 % FBS (Gibco, USA) and antibiotics (Sigma-Aldrich, Germany). Lentivirus packaging was performed using the standard calcium phosphate method for miR-23a-pLEX-Jred and miR-23b-pLEX-Jred constructs and intact pLEX-Jred as control. After the transfection step, supernatants were gathered and used for transducing cell lines. Media containing lentiviruses were replaced by fresh medium after 8 h. Two days after transduction, transduced cell lines were treated with 2 μg/ml puromycin for 2 days. The percentage of transduced cells was analyzed under fluorescent microscope.

mRNA and miRNA extraction

mRNA and miRNA extraction was performed using a modified RNX-Plus RNA extraction kit (SinaClon, Iran). In short, $2–3\times10^6$ of prostate cancer cells from LNCAP and PC3 cell lines were suspended in 1 ml RNX-Plus. For clinical samples, 100 mg of each sample was homogenized in 1 ml RNX-Plus using TissueLyser LT (Qiagen, Germany). After 5 min incubation at room temperature, 250 μl chloroform (Merck, Germany) was added to each tube and the tubes were shaken vigorously. The tubes were then centrifuged by MIKRO 200 R (Hettich, Germany) for 25 min at 12,000*g* at 4 °C. The upper phase was transferred to a new tube and an equal volume of 99 % ethanol (Merck, Germany) was added. The tubes were kept at −20 °C overnight and subsequently centrifuged for 45 min at 12,000*g* at 4 °C. The supernatant was then removed and 1 ml of 70 % ethanol was added. The tubes were centrifuged for 20 min at 12,000*g* at 4 °C. The supernatant was discarded and the RNA pellets were dried at room temperature. The quality and purity of the RNA was analyzed by BioPhotometer (Eppendorf, Germany). The RNA samples were stored at −80 °C until further use.

Table 1 miRNA RT stem-loops, primers and probe and expression and cloning primers

miRNA	Forward primer	RT stem-loop primer
hsa-miR-23a	ATCACATTGCCAGGGATTT	GGTCGTATGCAAAGCAGGGTCCGAGGTATC CATCGCACGCATCGCACTGCATACGACCGGAAAT
hsa-miR-23b	ATCACATTGCCAGGGATTA	GGTCGTATGCAAAGCAGGGTCCGAGGTATC CATCGCACGCATCGCACTGCATACGACCGGTAAT
Gene	Forward primer	Reverse primer
IL-6R	TGAGGAAGTTTCAGAACAGTC	GACGCACATGGACACTATG
miR-23a	*CCGCTCGAG*TTGATCAAAGGAAGCATCTGG	*CGACGCGT*AGCCACTGTGAACACGAC
miR-23b	*CCGCTCG*AGATATACCTCTAGGATGCCAAC	*CGACGCG*TTCACCAATCAGCTAAGCTC

Universal TaqMan probe: FAM 5′TCCATCGCACGCATCGCACT 3′BHQ-1; universal reverse primer: GAGCAGGGTCCGAGGT; the part of the sequences in italic is restriction site Xho1 and Mlu1, respectively

Reverse transcription

All the RNA samples were reverse transcribed using RevertAid Reverse Transcriptase (Thermo Fisher Scientific, USA). For each tube, 1.5 μl of stem-loop RT primer (50 nM) for miRNA or 1.5 μl of random hexamer primer (100 nM) for mRNA was added to 2 μl of the extracted RNA containing 2–5 μg RNA and 6.5 μl distilled water. The tubes were incubated in a peqSTAR Thermocycler (PEQLAB Biotechnologie GmbH, Germany) at 75 °C for 5 min. Then, 3 μl of 5× RT Buffer, 1.5 μl of dNTP Mix (10 mM), and 0.5 μl of RevertAid Reverse Transcriptase were added to each tube. The reverse transcription was performed by the following setting; 25 °C for 15 min, 37 °C for 15 min, 42 °C for 45 min, and 70 °C for 10 min for heat inactivation of polymerase.

Real-time PCR

Real-time PCR test for miRNAs was carried out in 10-μl reactions which consisted of 5 μl of QuantiTect Probe PCR Kit (Qiagen, Hilden, Germany), 0.2 μM of each primer, 0.1 μM of the probe, 2 μl of complementary DNA (cDNA), and 1.6 μl of distilled water. Real-time PCR for mRNAs was done in 13-μl reactions which consisted of 6.5 μl of SYBR® Premix Ex Taq™ Kit (Takara, Japan), 0.2 μM of each primer, 2 μl of cDNA, and 3.5 μl of distilled water. Amplification was performed according to the manufacturer's instructions using a Rotor-Gene 6000 machine (Corbett Life Science, Australia). SNORD47 (U47) gene was used as the internal control for miRNAs and GAPDH gene was used as the internal control for mRNA. All the reactions were performed in triplicate with positive, negative, no RT, and no-template controls included. Data were analyzed with ΔΔCt method.

Statistical analysis

The gene expression analyses were done using Relative Expression Software Tool (REST 2009, Qiagen, Germany). *p*-values less than 0.05 were statistically considered significant.

Results

Bioinformatics analyses

Involved genes in MAPK and JAK/STAT pathways were analyzed with multiple miRNA-target prediction algorithms.

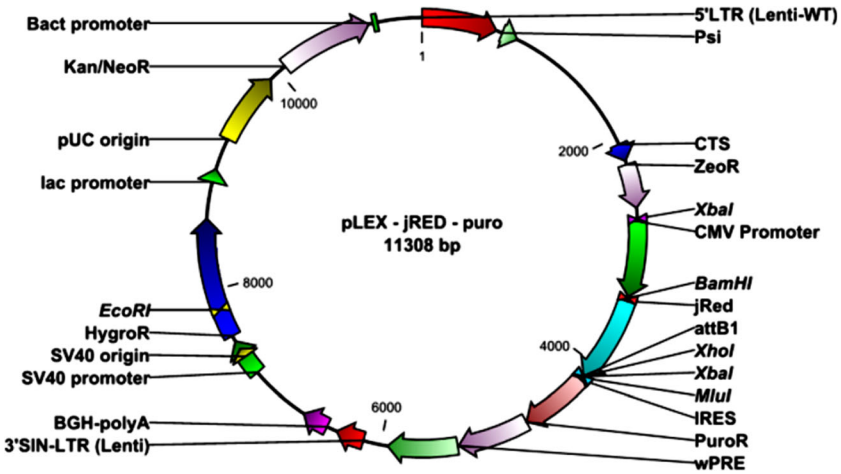

Fig. 1 Vector map of pLEX-JRed

The results of the prediction algorithms (DIANA-microT, miRanda, miRDB, miRWalk, RNAhybrid, PICTAR5, RNA22, and TargetScan) and the experimentally validated targets database (TarBase 6) led us to consider miR-23a and miR-23b as the best candidates. These miRNAs target the highest number of the genes in our list with the highest prediction scores. The results of the analysis for each gene by different algorithms are shown in Table 2. In addition, based on miRNApath results, miR-23a and miR-23b were the top miRNAs targeting the highest number of the genes.

Pathway analysis

The target genes of miR-23a-3p and miR-23b-3p are highlighted in MAPK and JAK/STAT pathway diagrams obtained from DIANA-miRPath database (Fig. 2a, b). In each pathway, several genes were targeted by miR-23a and miR-23b.

Expression analysis

In order to have an initial insight about the expression of miR23a/b in prostate cancer, we first explored previously measured miRNA levels as submitted to the mimiRNA database. To this end, we selected two well-established cell lines PC3 and LNCAP which are known to have high and low levels of metastatic potentials. Searching the database for the transcriptomic data for these cell lines showed lower levels of miR23a/b in all two cell lines than normal prostate tissue (Fig. 2c).

Confirmation of the construct and transduction

Recombinant vectors containing mir-23a and mir-23b were sequenced, and results showed that the inserted sequences were error free. LNCAP and PC3 prostate cancer cell lines were transduced with lentiviruses, and fluorescent microscope showed more than 90 % of prostate cancer cell lines were transducted. In addition, the overexpression of mir-23a and mir-23b in LNCAP and PC3 was confirmed using real-time PCR with fold changes from 2.5 to 23 in different cell lines. An empty pLEX-Jred vector was used to control cell transduction rate.

miRNA extraction, reverse transcription, and real-time PCR

To gain further information about the role of miR-23a and miR-23b in the context of prostate cancer, we examined the

Table 2 The result of different algorithms that predict miR-23a and miR-23b target list of genes

Gene	TarBase	DIANAmT	miRanda	miRDB	miRWalk	RNAhybrid	PICTAR5	RNA22	TargetScan	Sum
MAPK pathway genes										
FAS		+	+	+	+	+	+		+	7
MAP3K5		+	+		+	+	+	+	+	7
MAP3K1		+	+	+	+		+		+	6
MAP3K3		+	+		+	+	+		+	6
MAP3K12		+	+		+	+	+			5
MAP3K8		+	+		+		+		+	5
PDGFA		+	+		+		+		+	5
MAP2K1			+				+	+	+	4
FGFR3					+		+		+	3
MAP3K7IP2			+				+		+	3
FGF1			+		+					2
FGF12							+		+	2
PDGFRA			+				+			2
BRAF			+							1
JAK/STAT pathway genes										
IL6R	+	+	+	+	+	+	+		+	7
PIK3R3		+	+		+		+		+	5
JAK1		+	+				+		+	4
BRCA1			+				+		+	3
IL6			+				+		+	3
JAK2			+				+		+	3
MTOR			+					+		2
STAT3			+				+			2
AKT1							+			1

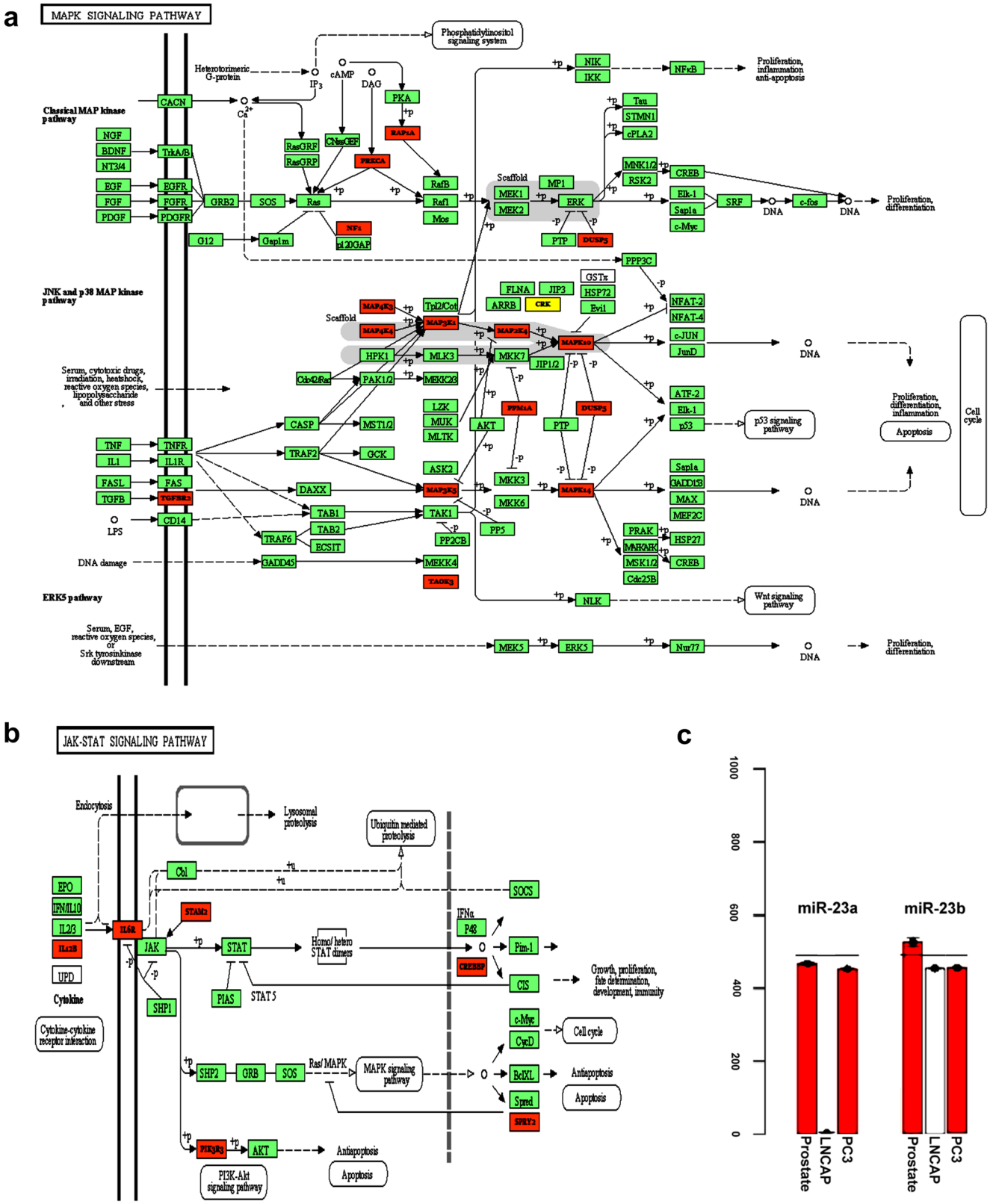

Fig. 2 The target genes of miR-23a-3p and miR-23b-3p in pathways and their expression in different cell lines. **a** genes targeted by miR-23a and miR-23b are highlighted in MAPK pathway. **b** JAK/STAT pathway. Both obtained from the *DIANA mirPath database*. **c** Expression of miR-23a and miR-23b in the prostate and different prostate cancer cell lines that took from the *mimiRNA database*

expression of the mature forms of these two miRNAs in the abovementioned prostate cancer-derived cell lines. When compared with BPH samples, the expression levels of both miR-23a and miR-23b were substantially reduced in LNCAP and PC3 cancer cell lines (p value <0.001) (Fig. 3a, b). We then analyzed the expression of miR-23a and miR-23b in surgically removed tissues from prostate cancer cases, as described in the "Materials and methods" section. Real-time PCR analyses of clinical samples also revealed significant downregulation of both miR-23a and miR-23b compared with BPH samples (Fig. 3c). Overall, these findings support a potential role for miR23a/b in neoplastically transformed prostate epithelial cells. In the next step, we analyzed the expression of miR-23a and IL-6R in mir-23a-transduced LNCAP and PC3 cell lines (Fig. 4a). Moreover, we evaluated miR-23b and IL-6R in mir-23b-transduced LNCAP and PC3 cell lines (Fig. 4b). Finally, the results of cell line models were complemented with the gene expression data of miR-23a, miR-23b, and IL-6R in all clinical samples totally (Fig. 4c). The expression of miR-23a, miR-23b, and IL-6R in each sample is displayed in Fig. 5.

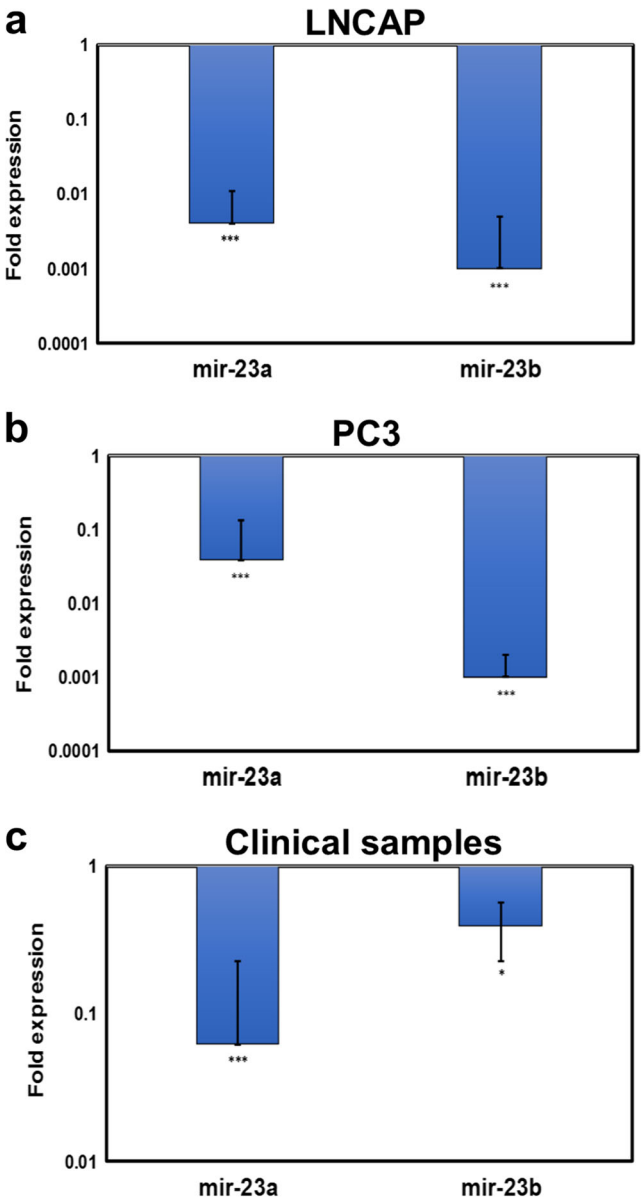

Fig. 3 miR-23a and miR-23b expression in prostate cancer cell lines and clinical samples. Expression in **a** LNCAP, **b** PC3, and **c** clinical samples. All the samples normalized with BPH samples. *P* values less than 0.05, 0.01, and 0.001 are marked with an asterisk (*), two asterisks (**), and three asterisks (***), respectively

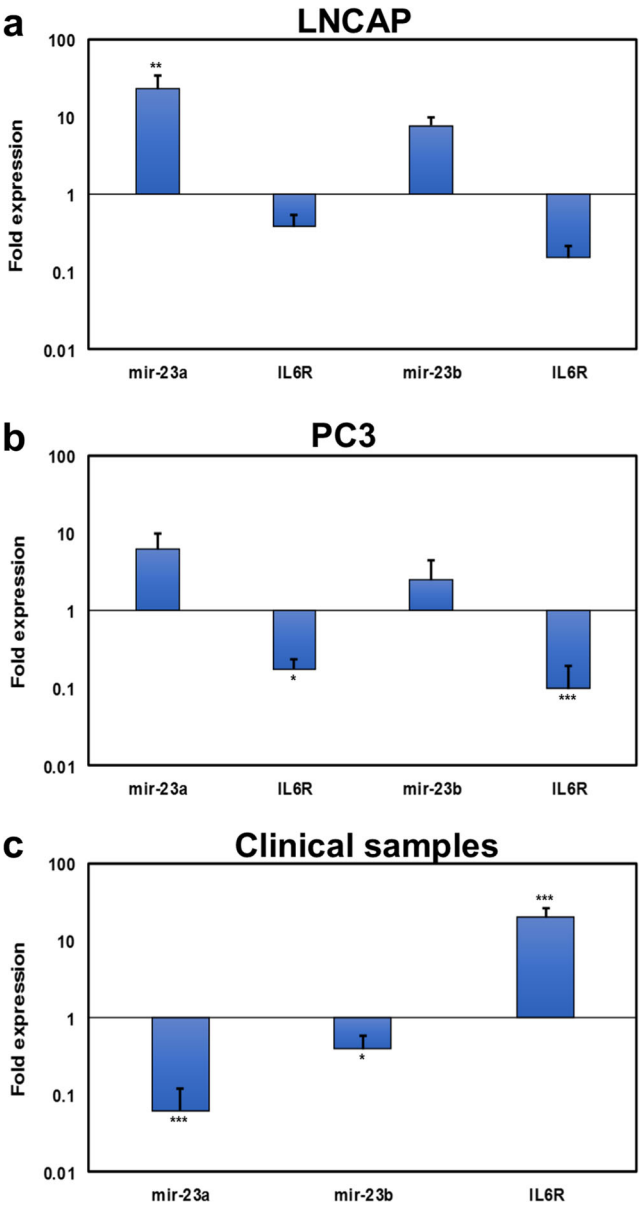

Fig. 4 miR-23a, miR-23b, and *IL-6R* expression in miR-transduced prostate cancer cell lines and clinical samples. **a** Expression of miR-23a and *IL-6R* in mir-23a-transduced LNCAP and expression of miR-23b and *IL-6R* in mir-23b-transduced LNCAP. **b** Expression of miR-23a and *IL-6R* in mir-23a-transduced PC3 and expression of miR-23b and *IL-6R* in mir-23b-transduced PC3 and **c** expression of miR-23a, miR-23b, and *IL-6R* in clinical samples. All the samples normalized with BPH samples. *P* values less than 0.05, 0.01, and 0.001 are marked with an asterisk (*), two asterisks (**), and three asterisks (***), respectively

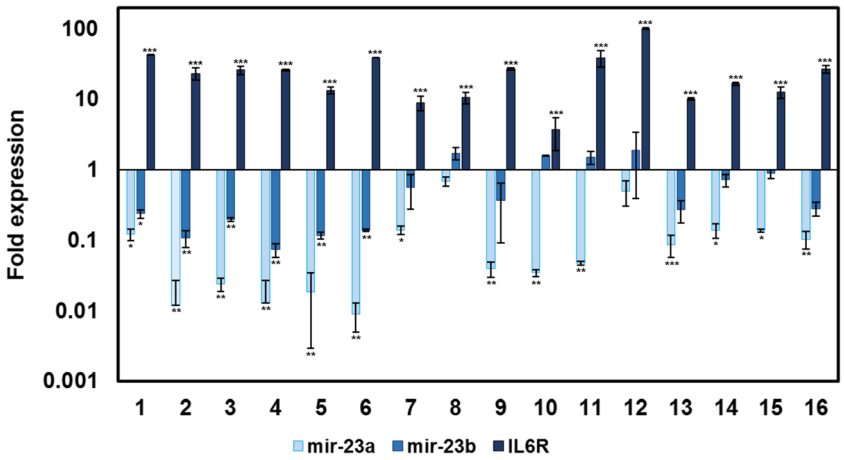

Fig. 5 Expression of miR-23, miR-23b, and *IL-6R* in each clinical sample. *P* values less than 0.05, 0.01, and 0.001 are marked with an asterisk (*), two asterisks (**), and three asterisks (***), respectively

Discussion

According to International Agency for Research on Cancer (IARC) in 2012, 15 % of the new cancer cases and 6.6 % of the cancer deaths among men worldwide are related to prostate cancer [1]. Indeed, the high prevalence rate of this cancer together with the low efficacy of the current treatments necessitates the development of novel therapies which can reduce the mortality rate of the disease [3]. miRNAs play critical roles in posttranscriptional regulation of gene expression and have recently received considerable attention for therapeutic use [8]. The distinctive ability of miRNAs to control multiple genes and pathways simultaneously makes them plausible candidates to be investigated for therapeutic purposes in the context of cancer. Some reports indicate that miRNA-mimicking sequences can efficiently prevent tumor growth in vitro and in vivo [28]. Liu et al. showed that miR-34a can inhibit prostate cancer stem cell proliferation and subsequent spread and metastasis in a mouse model of the disease [29]. Interestingly, miR-34, which could be labeled as the first anticancer therapeutic miRNA, is currently undergoing a phase 1 clinical trials for liver cancer. Several other miRNAs are also in preclinical development phase [9].

Identification of miRNAs which can target specific transcripts is a challenging step in developing miRNA-based drugs, and computational algorithms have proven to be valuable in this context [11]. In a study conducted by Hen et al., computational analysis of pathways associated with pancreatic cancer indicated that miR-548d targeted several nodes of the pathogenesis network. Overexpression of this miRNA in pancreatic cancer cells led to cell cycle arrest and increased apoptosis [10]. In the current study, we selected several genes from MAPK and JAK/STAT pathways and analyzed each gene by different algorithms. Finally, miR-23a and miR-23b were selected as they had been previously predicted to target various elements in the pathways.

Our results demonstrated that the expression of miR-23a and miR-23b in two different prostate cancer cell lines were substantially lower compared with BPH. These multiple prostate cancer cell lines have been derived from different stages of the disease and have distinctive characteristics. For instance, PC3 cells are extremely tumorigenic and metastatic, while LNCAP cells are poorly tumorigenic and metastatic [30]. In the clinical samples, the expression levels of miR-23a and miR-23b were significantly lower compared with prostate tissue derived from BPH cases, a finding which was consistent with our in vitro cell line data. Several other studies have also shown decreased levels of miR-23a and miR-23b in prostate cancer samples [31, 32]. Nonetheless, while our clinical samples were similar in terms of pathology, some samples demonstrated an increased expression of the miRNAs. The heterogeneity in miR-23b levels in prostate cancer tissues has been previously reported. For example, Majid et al. [33] and Sun et al. [34] reported enhanced levels of miR-23b in some cancer tissues. The increased expression of miR-23a and miR-23b in some clinical samples may be a consequence of individual genetic polymorphisms. Interestingly, it has been recently reported that in vivo intratumoral delivery of miR-23b mature sequences in a murine model of prostate cancer can considerably reduce tumor mass in animals, an effect attributed to the suppression of proto-oncogenic pathways in neoplastic cells [33].

While in this work we have focused on MAPK and JAK/STAT pathway genes and their potential targeting by miRNAs in the context of prostate cancer, soluble growth factors and cytokines that activate these pathways could be equally important in disease pathogenesis. IL-6 is one of the cytokines which signals through the JAK/STAT pathway, and several reports have also pointed to the role of IL-6 in promoting prostate cancer [35, 36]. The expression of IL-6 and its receptor, IL-6R, is upregulated in prostate cancer cells, and suppressing IL-6 levels in prostate cancer cell lines using small interfering RNAs decreases cell proliferation [37]. Considering the importance of IL-6R in prostate cancer, we selected this gene for analysis, and our results show that overexpression of both mir-23a and mir-23b

decreased IL-6R expression in LNCAP and PC3 cell lines. In addition, IL-6R in all clinical samples was upregulated unlike mir-23a and mir-23b that were downregulated. Interestingly, IL-6R is also a confirmed target of miR-23a [38]. The role of IL-6 signaling in the pathogenesis of prostate cancer, together with the inhibitory effect of miR-23a on IL-6 receptor, gives more weight to miR-23a as a candidate for prostate cancer therapy.

Conclusion

miRNA-based therapy is an attractive strategy for therapeutic gene silencing. miRNAs can affect cellular processes through controlling the gene expression machinery. Using a miRNA as therapeutic target may influence several cellular pathways at once and exert a more robust effect compared with the targeting of individual protein-coding genes. That said, this might also lead to nonspecific effects which need to be evaluated prior to clinical use of miRNAs. In this study, we provided some evidence showing that miR-23a and miR-23b can target IL-6R which signals through the JAK/STAT pathway, and in turn, this pathway controls cell proliferation, development, and survival in the context of cancer. Moreover, we confirmed that these miRNAs are downregulated in prostate cancer cells. Altogether, our results represent the possibility of using miR-23a and miR-23b as therapeutic targets in prostate cancer.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Authors' contributions Seyed Hamid Aghaee-Bakhtiari carried out the practical tests and drafted the manuscript. Ehsan Arefian carried out the study design and drafting. Mahmood Naderi participated in the design of the study and drafting. Farshid Noorbakhsh participated in study design, data analysis, and draft preparation. Vahideh Nodouzi helped in clinical sample gathering and assisted in data analysis. Mojgan Asgari aided sample gathering and analyzed the stage of tumor samples. Pezhman Fard-Esfahani participated in data analysis and assisted in drafting the manuscript. Reza Mahdian helped in study design and aided in manuscript editing. Masoud Soleimani assisted in study design, data analysis, and draft preparation. All authors read and approved the final manuscript.

Conflicts of interest None.

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