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ORIGINAL ARTICLE

Identification of Potential miRNAs Biomarkers for High-Grade Prostate Cancer by Integrated Bioinformatics Analysis

Laura Foj [1] **· Xavier Filella** [2]

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Abstract

The increasing number of datasets available in the GEO database offers a new approach to identify new miRNAs related to PCa. The aim of our study was to suggest a miRNA signature for the detection of high-grade PCa (Gleason score ≥ 7) using bioinformatics tools. Three mRNA datasets (GSE26022, GSE30521, GSE46602) were selected to identify the differentially expressed genes (DEGs) in high-grade PCa. Furthermore, two miRNA datasets (GSE45604, GSE46738) were analyzed to select the differentially expressed miRNAs (DEMs). Functional and pathway enrichment analysis was performed using DAVID and a protein-protein interaction network (PPI) was constructed through STRING. Besides, miRNAs which regulate hub genes were predicted using microRNA.org. A total of 973 DEGs were identified after the analyses of the mRNA datasets, enriched in key mechanisms underlying PCa development. Furthermore, we identified 10 hub genes (*EGFR, VEGFA, IGF1, PIK3R1, CD44, ITGB4, ANXA1, BCL2, LPAR3, LPAR1*). The most significant KEGG Pathway was PI3K-Akt signaling pathway, involved in cell proliferation and survival. Moreover, we identified 30 common miRNAs between significant DEMs and the predicted hub gene regulators. Twelve of these miRNAs (miR-1, −365, −132, −195, −133a, −133b, −200c, −339, −222, −21, −221, −708) regulate two or more hub genes identified in our study. We suggested a signature including these 12 miRNAs for high-grade PCa detection. These miRNAs have been associated with aggressive PCa, poor survival and resistance to treatment in the last years.

Keywords miRNAs · Bioinformatics analysis · Differentially expressed genes · Protein–protein interaction network · Prostate cancer

Introduction

Prostate cancer (PCa) is the second most common cancer in men after lung cancer (excluding non-melanoma skin cancers) and the fifth cause of cancer-related death in men worldwide [1]. Furthermore, PCa is a very heterogeneous disease, with high differences in patients' evolution, including patients having low-risk of progression and those with lethal castration resistant PCa (CRPC). Different risk classification systems based on clinicopathological information have been developed to distinguish patients with early PCa according to the prognosis, among them the D'Amico classification system, the Cancer of the Prostate Risk Assessment (CAPRA) score, and the National Comprehensive Cancer Network (NCCN) risk-groups classification. All these systems recognize a low-risk of progression for patients with a biopsy Gleason score 6 or lower [2]. However, risk of misclassification using these systems is not negligible. Understanding the biological bases of the clinical heterogeneity of PCa could lead to improve the management of PCa patients.

MicroRNAs (miRNAs) are small (17–22 nucleotides) non-coding RNA molecules that negatively regulate the gene expression through the binding to their corresponding mRNA targets. The expression of aberrant miRNAs has been demonstrated in PCa, playing a critical role in tumor initiation and development [3]. In addition, significant pathways involving miRNAs have also been determined to exhibit critical roles in PCa progression. In this regard, the interactions of miRNAs with androgen receptor (AR) play a determinant role in the transition from castration sensitive PCa to an incurable CRPC [4, 5].

✉ Xavier Filella
xfilella@clinic.cat

[1] Labco Diagnostics, SYNLAB Group, Barcelona, Catalonia, Spain

[2] Department of Biochemistry and Molecular Genetics (CDB), Hospital Clínic, IDIBAPS, C/ Villarroel, 170, 08036 Barcelona, Catalonia, Spain

Many efforts based on microarray or next generation sequencing (NGS) technologies have been done in order to select the miRNAs associated with PCa. Therefore, several miRNAs profiles have been proposed as biomarkers for PCa management in recent years [6]. The increasing number of datasets involving mRNAs and miRNAs available in the Gene Expression Omnibus (GEO) database offers a new approach to accurately identify miRNAs and mRNAs related to high-grade PCa. In silico studies provide scientists with some criteria to hierarchize trials to later validate in vitro the predicted networks and discover novel biomarkers related with PCa aggressiveness.

The aim of our study was to suggest a miRNAs signature useful for PCa detection and prognosis, also providing valuable information at molecular level for PCa patients' management. Three mRNA datasets were selected to identify the differentially expressed genes (DEGs) in high-grade PCa (defined by Gleason score ≥ 7 or ISUP Grade group ≥2 in the novel nomenclature). Furthermore, two miRNA datasets were analysed to select the differentially expressed miRNAs (DEMs). Functional and pathway enrichment analyses were performed.

Materials and Methods

Collection and Inclusion Criteria of Studies

GEO is a public repository for data storage, such as microarray and NGS, which is freely available to users. We searched the GEO database (https://www.ncbi.nlm.nih.gov/geo/) for publicly available studies using the following keywords: "RNA", "prostate cancer", "Gleason", "*Homo sapiens*" (organism), and "tissue" (attribute name). The inclusion criteria for studies were as follows: 1) PCa samples obtained from radical prostatectomy (RP) and normal or benign tissue samples, 2) messenger RNA (mRNA) expression profiling, and 3) Gleason score determined. After a systematic review, three gene expression GSE datasets were selected. Besides, available studies in GEO database related to miRNAs were searched using the following keywords: "miRNA", "prostate cancer", "Gleason", "*Homo sapiens*" (organism), and "tissue" (attribute name). The inclusion criteria for studies were the same as for mRNA. According to the inclusion criteria, two miRNAs GSE studies were selected for analysis. The bioinformatics pipeline with the followed steps is represented in Fig. 1.

Microarray Data

In this study, three mRNA expression profiles (GSE26022, GSE30521, GSE46602) and two miRNA expression profiles (GSE45604, GSE46738) were obtained from GEO database. From the mRNA expression dataset GSE26022 (study not published), we selected 10 normal samples and 113 high-grade PCa samples obtained from formalin-fixed paraffin-embedded (FFPE) RP specimens. From the expression profile of GSE30521 [7] dataset, our analysis included 5 normal human prostate tissue samples and 11 high-grade PCa FFPE or frozen samples obtained from RP specimens. The GSE46602 dataset [8] included 10 normal prostate tissue samples and 19 high-grade PCa, obtained all from laser micro dissected prostate tumor tissue. The platforms used in each case were Illumina Custom Prostate Cancer DASL Panel 1.5 K expression beadchip for GSE26022; Affymetrix Human Exon 1.0 ST Array for GSE30521; and Affymetrix Human Genome U133 Plus 2.0 Array for GSE46602.

The miRNA expression dataset GSE45604 [9] was collected from 50 patients with PCa treated by RP, including 35 high-grade PCa. Besides, 10 tissue samples of normal prostate tissue from patients undergoing radical cystectomy were analyzed as controls. Finally, the miRNA expression profile of GSE46738 (study not published) included 4 samples from patients with benign prostatic hyperplasia and 38 high-grade PCa. All samples were obtained from excised prostate tissue obtained after gland removal. The platforms used in each case were Affymetrix Multispecies miRNA-2 Array for GSE45604; and Affymetrix Multispecies miRNA-1 Array for GSE46738 database.

Data Processing

The GEO database archives a large number of high-throughput functional genomics studies that contain data that are processed and normalized using various methods. GEO2R is an interactive web tool that compares two groups of samples under the same experimental conditions [10]. GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) was used to evaluate the DEGs and DEMs between normal controls and high-grade PCa samples. The adjusted P values (adj. P) using Benjamini and Hochberg (BH) method were applied to correct for the occurrence of false positive results. Genes with and an adj. $P < 0.05$ and $|\log_2$ fold change (FC)$|$ >1.5 were selected as DEGs. MiRNAs with an adj. $P < 0.05$ and $|\log_2 FC| > 1$ were selected as DEMs.

Functional and Pathway Enrichment Analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) is an online bioinformatics program that provides a comprehensive set of functional annotation tools for researchers to understand the biological meaning from a large quantity of genes [11]. Gene ontology (GO) is a tool for annotating genes, by using a defined and structured vocabulary [12]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is an integrated database used to assign genome sequences to specific pathways [13]. GO and KEGG analyses were performed for identified targets using DAVID. A p value lower than 0.05 was used as the threshold value.

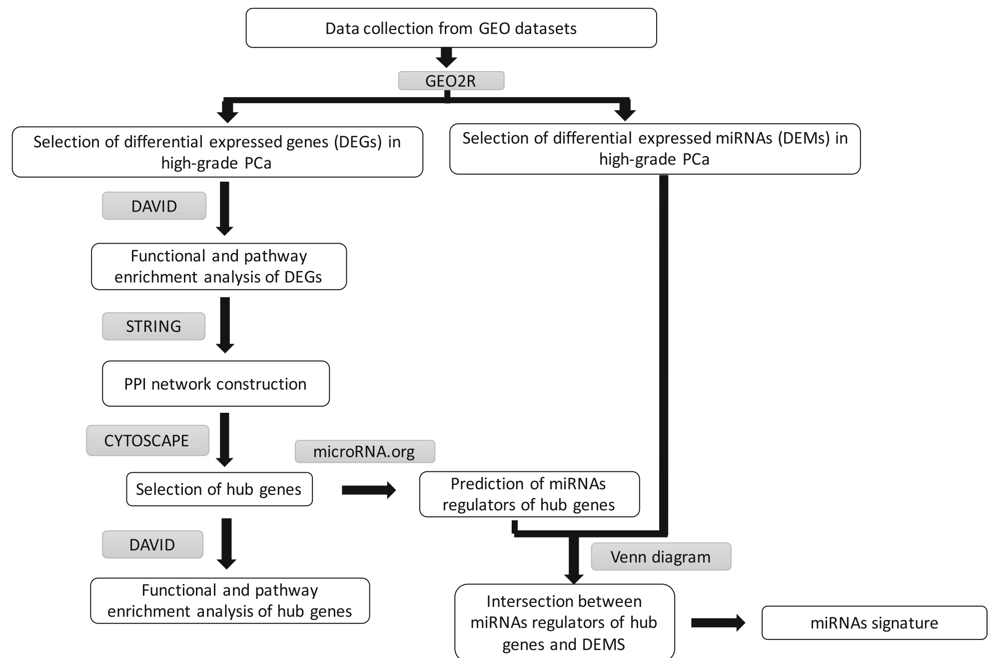

Fig. 1 Illustration of methodology flow sheet

Protein–Protein Interaction Network Construction

The functional interactions between proteins can provide context in molecular mechanism of cellular processing. In the present study, protein–protein interaction (PPI) network of DEGs was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING, http://string.embl.de/) database [14]. A confidence score ≥ 0.7 was considered the threshold value. The network was then visualized and analyzed using Cytoscape [15] and a degree of 15 or higher was set as the cut-off value to select the hub genes.

Prediction of miRNAs Regulators of Hub Genes

The online tool microRNA.org (http://www.microrna.org) was applied to predict the miRNAs that regulate the hub genes. The miRNAs were ordered by sum of mirSVR scores [16]. The intersection between the predicted miRNAs of the hub genes and DEMs obtained from GEO datasets was performed using Venn diagrams and the common miRNAs from both groups were selected.

Results

Identification of DEGs

A total of 973 DEGs were identified after the analyses of the GSE26022, GSE30521, GSE46602 datasets, consisting of 617 down-regulated genes and 356 up-regulated comparing controls with high-grade PCa samples.

Identification of DEMs

Nineteen miRNAs were selected from GSE45604 dataset (14 down and 5 up-regulated), comparing controls with high-grade PCa. Seventy-three DEMs were selected from GSE46738 (50 down and 23 up-regulated). Five DEMs were common between the two datasets: miR-182, miR-183, miR-184, miR-200c and miR-375. The 20 most significant DEMs obtained from both datasets are presented in Table 1.

Functional and Pathway Enrichment Analysis of DEGs

A functional and pathway enrichment analysis was performed using DAVID in order to get more information about the function of the identified genes. The analysis showed that down-regulated genes were mainly involved in biological processes of angiogenesis, hemidesmosome assembly, negative regulation of epithelial cell proliferation and cell adhesion, while up-regulated genes were mainly enriched in the biological processes of extracellular matrix organization, protein transport, oxidation-reduction process and cell division. Moreover, several KEGG pathways were overrepresented in down-regulated genes, including glutathione metabolism, focal adhesion and pathways in cancer. Metabolic pathways and ECM-receptor interaction were the significant KEGG pathways for up-regulated genes (Table 2).

PPI Network Construction

The PPI network of the DEGs consisted of 305 nodes and 660 edges (Fig. 2). The network was downloaded from STRING

Table 1 The 20 most significant DEMs in high-grade PCa obtained from GEO datasets

miRNA ID	Adj. *p* value	Up/down-regulation	Log FC
hsa-miR-125a-5p	2.53E-07	Down-regulated	−4.25
hsa-miR-224	1.69E-05	Down-regulated	−1.14
hsa-miR-221	4.02E-05	Down-regulated	−1.38
hsa-miR-184	4.43E-05	Up-regulated	3.81
hsa-miR-187	8.11E-05	Down-regulated	−2.65
hsa-miR-1307	8.59E-05	Down-regulated	−3.10
hsa-miR-1231	1.38E-04	Down-regulated	−3.68
hsa-miR-182	1.53E-04	Up-regulated	1.98
hsa-miR-646	3.03E-04	Up-regulated	1.30
hsa-miR-633	4.46E-04	Up-regulated	1.33
hsa-miR-20a	5.74E-04	Up-regulated	2.05
hsa-miR-513a-5p	5.86E-04	Up-regulated	1.35
hsa-miR-1229	7.63E-04	Up-regulated	2.17
hsa-miR-339-5p	8.63E-04	Down-regulated	−2.94
hsa-miR-92a	1.52E-03	Down-regulated	−2.63
hsa-miR-519c	1.52E-03	Up-regulated	1.28
hsa-miR-331	1.60E-03	Down-regulated	−2.20
hsa-miR-409	1.60E-03	Down-regulated	−1.76
hsa-miR-27b	1.72E-03	Down-regulated	−1.08
hsa-miR-375	2.07E-03	Up-regulated	1.96

and visualized with Cytoscape software. The network was then analysed and a degree ≥15 was set as the threshold. A total of 10 genes were selected as hub genes, including *EGFR, VEGFA, IGF1, PIK3R1, CD44, ITGB4, ANXA1, BCL2, LPAR3* and *LPAR1*. The hub genes were mainly enriched in the biological processes of negative regulation of apoptotic process and positive regulation of cell migration. Furthermore, the most significant KEGG Pathway was PI3K-Akt signaling pathway (Table 3). The role of hub genes in PCa is schematized in Fig. 3.

Common miRNAs between Significant DEMs and Predicted Hub Gene Regulators

A total of 165 miRNAs were predicted to be hub genes regulators using microRNA.org (Table 4). Thirty miRNAs were common between significant DEMs and predicted hub gene regulators using Venn diagram (Fig. 4a). We selected a signature of 12 miRNAs regulating 2 or more hub genes, constituted by miR-1, −365, −132, −195, −133a, −133b, −200c, −339, −222, −21, −221 and − 708. The hub genes regulated for these miRNAs is shown in Fig. 4b.

Discussion

PCa remains a leading cause of cancer-related deaths among men worldwide, despite continuously improved detection and treatment strategies. Due to microarray technology, it is easier to analyze the genetic alterations underlying PCa development and progression. In addition, through bioinformatics tools it is possible to identify new biomarkers and to construct networks that could be valuable for the management of PCa patients.

In our study, a total of 973 DEGs were identified from 3 datasets, consisting of 617 down-regulated genes and 356 up-regulated genes in high-grade PCa compared to control samples. Moreover, by constructing the PPI, high degree genes were identified, such as Epidermal growth factor receptor (*EGFR)*, which was found to have close interactions with *PIK3R1, ITGB4* and *ANXA1*. EGFR is the first member of ErbB family of transmembrane receptor tyrosine kinases and a proto-oncogene overexpressed in several cancers, including PCa [17]. It has been estimated that nearly 30% of PCa cases overexpress EGFR and that deregulation of EGFR-mediated signaling pathways is associated with high-grade PCa, poor prognosis and reduced survival rate, thus contributing to CRPC and progression to metastasis [18–20]. Consequently, EGFR has been suggested as an important anti-tumor target, but therapies against EGFR using tyrosine kinase inhibitors such as Lapatinib have been shown to have limited effectiveness in PCa [21]. Therefore, it has been proposed that blocking more than one key pathways at the same time in PCa could be more effective. Because EGFR overexpression mediates the cell proliferation via AR-independent growth signaling mechanisms in CRPC, it has been postulated that the simultaneous suppression of EGFR and AR could be an effective strategy for the treatment of advanced PCa. In this sense, recently Brizzolara et al. [22] suggested that the COX-2 inhibitor Celecoxib is useful for the clinical management of PCa, due to its ability of modulating the EGFR-AR signaling pathway in androgen-dependent PCa cells. Similarly, Thamilselvan et al. [23] found that combination of carmustine and selenite effectively induces apoptosis and growth inhibition by targeting AR in CRPC cells. Furthermore, in a posterior study, the authors showed that the combination of carmustine and selenite inhibits EGFR mediated growth signaling and induces apoptosis in androgen independent PCa cells, suggesting a potential candidate for the treatment of CRPC. The results obtained in our study underlined the involvement of EGRF in the development of PCa. Furthermore, AR plays a key role closely associated with the miRNA-gene networks demonstrated in this study.

Otherwise, the most significant KEGG pathway in our enrichment analysis study was PI3K-Akt signaling, which regulates several key cellular processes, such as metabolism, growth, proliferation, survival, transcription and protein synthesis. EGFR and other receptor tyrosine kinases such as insulin-like growth factor receptor 1

Table 2 Functional and pathway enrichment analysis of up-regulated and down-regulated genes in high-grade PCa

Term	Description	Count	*P* value
Down-regulated genes			
GO:0001525	Angiogenesis	24	7.89E-07
GO:0031581	Hemidesmosome assembly	7	8.07E-07
KEGG: hsa00480	Glutathione metabolism	11	4.52E-06
KEGG: hsa04510	Focal adhesion	21	1.15E-05
GO:1901687	Glutathione derivative biosynthetic process	7	4.96E-05
GO:0050680	Negative regulation of epithelial cell proliferation	10	6.40E-05
GO:0007155	Cell adhesion	32	7.53E-05
GO:0018916	Nitrobenzene metabolic process	4	1.26E-04
KEGG: hsa05200	Pathways in cancer	28	1.81E-04
GO:0050731	Positive regulation of peptidyl-tyrosine phosphorylation	11	2.74E-04
KEGG: hsa00982	Drug metabolism - cytochrome P450	10	3.35E-04
KEGG: hsa04151	PI3K-Akt signaling pathway	25	3.51E-04
GO:0006749	Glutathione metabolic process	9	3.77E-04
GO:0006928	Movement of cell or subcellular component	11	4.06E-04
GO:0001666	Response to hypoxia	16	4.17E-04
GO:0007165	Signal transduction	59	4.64E-04
KEGG: hsa04512	ECM-receptor interaction	11	5.05E-04
KEGG: hsa05215	Prostate cancer	11	5.54E-04
GO:0043066	Negative regulation of apoptotic process	29	7.42E-04
GO:0030512	Negative regulation of transforming growth factor beta receptor signaling pathway	9	9.40E-04
GO:0071456	Cellular response to hypoxia	11	9.73E-04
GO:0042178	Xenobiotic catabolic process	4	0.00103
KEGG: hsa05204	Chemical carcinogenesis	10	0.00112
GO:0051897	Positive regulation of protein kinase B signaling	10	0.00141
GO:0048672	Positive regulation of collateral sprouting	4	0.00161
GO:0002576	Platelet degranulation	11	0.00167
GO:0098869	Cellular oxidant detoxification	9	0.00170
GO:0042493	Response to drug	21	0.00190
KEGG: hsa05205	Proteoglycans in cancer	16	0.00224
GO:0030335	Positive regulation of cell migration	15	0.00238
KEGG: hsa00980	Metabolism of xenobiotics by cytochrome P450	9	0.00272
GO:0043393	Regulation of protein binding	5	0.00272
GO:0048661	Positive regulation of smooth muscle cell proliferation	8	0.00290
GO:0030154	Cell differentiation	27	0.00383
GO:0016337	Single organismal cell-cell adhesion	10	0.00498
GO:0000122	Negative regulation of transcription from RNA polymerase II promoter	37	0.00536
GO:0007010	Cytoskeleton organization	13	0.00557
GO:0007171	Activation of transmembrane receptor protein tyrosine kinase activity	4	0.00574
GO:0045717	Negative regulation of fatty acid biosynthetic process	4	0.00574
KEGG: hsa05222	Small cell lung cancer	9	0.00638
GO:0033138	Positive regulation of peptidyl-serine phosphorylation	8	0.00686
GO:0070372	Regulation of ERK1 and ERK2 cascade	5	0.00762
GO:0032570	Response to progesterone	6	0.00764
GO:0008285	Negative regulation of cell proliferation	23	0.00856
GO:0032355	Response to estradiol	9	0.00856
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	46	0.00931
Up-regulated genes			
GO:0030198	Extracellular matrix organization	10	0.00546

Table 2 (continued)

Term	Description	Count	*P* value
GO:0015031	Protein transport	15	0.00633
GO:0015949	Nucleobase-containing small molecule interconversion	4	0.00789
GO:0010873	Positive regulation of cholesterol esterification	3	0.00911
GO:0055114	Oxidation-reduction process	19	0.00992
KEGG: hsa01100	Metabolic pathways	35	0.01032
GO:0034374	Low-density lipoprotein particle remodeling	3	0.01361
GO:0051301	Cell division	13	0.01407
GO:0043967	Histone H4 acetylation	4	0.01433
GO:0006468	Protein phosphorylation	15	0.02010
GO:0030574	Collagen catabolic process	5	0.02150
GO:0032436	Positive regulation of proteasomal ubiquitin-dependent protein catabolic process	5	0.02150
GO:2000147	Positive regulation of cell motility	3	0.02180
GO:0007067	Mitotic nuclear division	10	0.02277
KEGG: hsa04512	ECM-receptor interaction	6	0.02363
GO:0006469	Negative regulation of protein kinase activity	6	0.02435
GO:0007076	Mitotic chromosome condensation	3	0.02488
GO:0007059	Chromosome segregation	5	0.02612
GO:0030199	Collagen fibril organization	4	0.02646
GO:0098609	Cell-cell adhesion	10	0.03670
KEGG: hsa04530	Tight junction	7	0.04402
GO:0016338	Calcium-independent cell-cell adhesion via plasma membrane cell-adhesion molecules	3	0.04664
GO:0006139	Nucleobase-containing compound metabolic process	4	0.04734

(IGF-1R) are downstream effectors of PI3K-Akt signaling. However, some studies in PCa cells suggest that basal activation of this pathway occurs independently of receptor tyrosine kinases [24].

Notably, the tumor suppressor phosphatase and tensin homolog (*PTEN*) acts as the main inhibitor of Akt activity by dephosphorylating phosphatidylinositol trisphosphates (PIP3). *PTEN* deletion is an established prognostic biomarker in PCa tightly associated with high-grade PCa and an elevated rate of metastasis [25]. Furthermore, a recent metaanalysis including ten independent cohort studies demonstrated the association between *PTEN* deletion and biochemical recurrence [26]. Moreover, the activation of PI3K-Akt signaling pathway is related to resistance to androgen deprivation therapy [27]. CRPC is characterized by persistent tumor growth because of a continuous AR signaling despite castration levels of androgens. Reciprocal regulation between PI3K-Akt and AR signaling has been demonstrated, suggesting that both pathways coordinately support PCa cells survival [28].

Increasing evidence has shown that the deregulation of miRNAs is an important part of the pathogenesis of multiple cancer types, including PCa. MiRNAs regulate the expression of most genes and form a complex network of expression regulation which tightly interacts with known gene regulatory networks. Besides, miRNAs can be detected in body fluids such as serum or urine due to their stability.

We performed a double way to identify the most relevant miRNAs in high-grade PCa. On one hand, 87 DEMs were selected from GSE45604 and GSE46738 datasets according to the criteria to sort out miRNAs from GEO databases. On the other hand, 165 miRNAs were predicted to be hub genes' regulators using microRNA.org. Finally, 30 miRNAs were found to be common using Venn diagrams between the two groups of miRNAs. For instance, miR-7 was predicted as the main regulator of *EGFR* in our study. MiR-7 has been characterized as a tumour-suppressor miRNA in several human cancers by targeting a number of key signaling molecules, including *EGFR, IRS1* and *RAF1* [29, 30]. Moreover, Chang et al. [31] demonstrated that miR-7 is down-regulated in PCa cells, showing that the restoration of miR-7 suppresses the expression of the stemness factor KLF4 in PCa stems cells and inhibits prostate tumorigenesis by suppressing the PI3K-Akt pathway. Furthermore, miR-7 affects the activity of multiple oncogenic molecules in the EGFR signaling cascade, such as Akt, PI3K, ERK1/2 and mTOR [32, 33] in different cancers, demonstrating broad regulatory control over this signaling network.

MiR-21 has been widely investigated in PCa. It is usually overexpressed and plays an important role in PCa

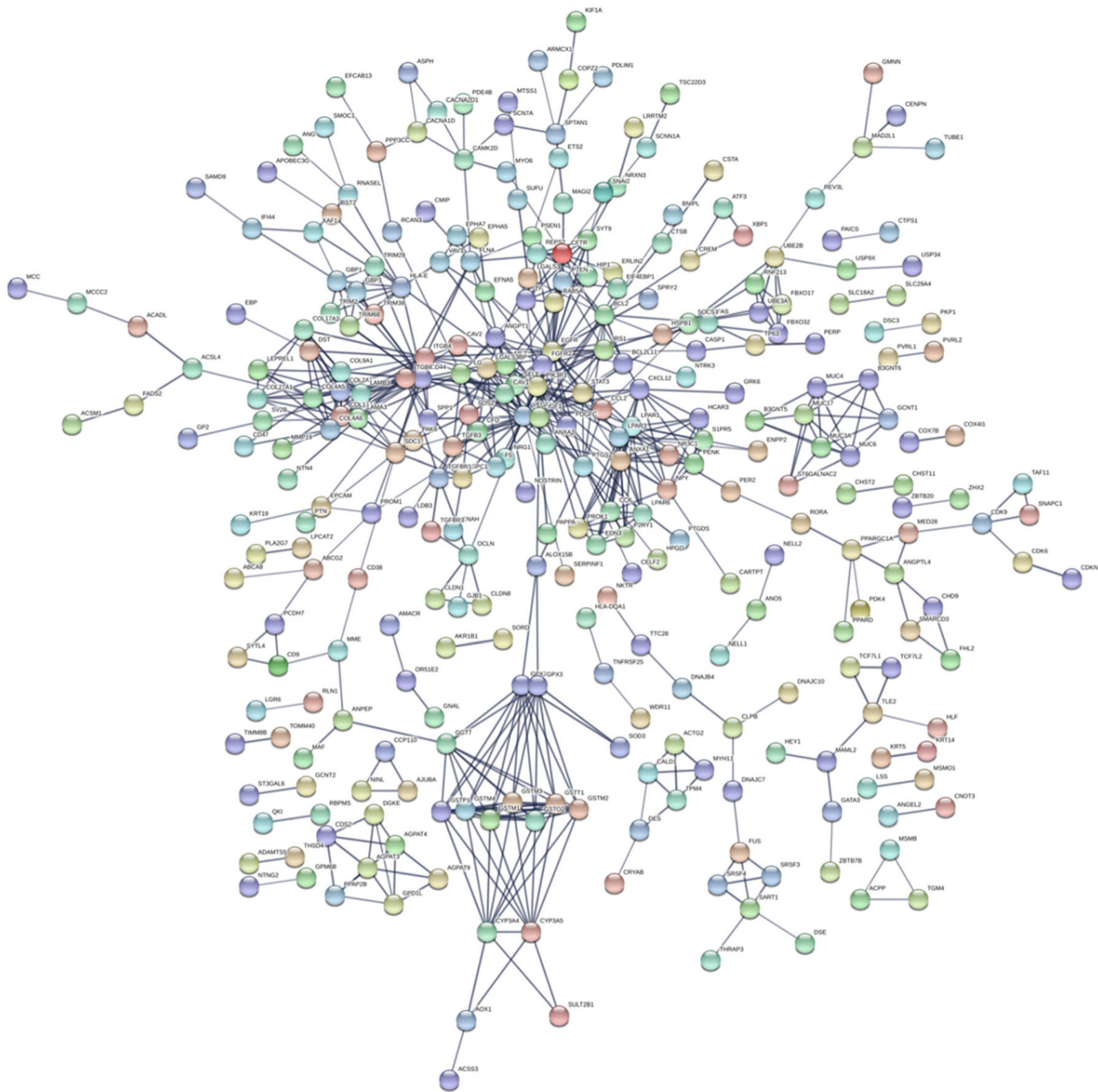

Fig. 2 PPI network of differential expressed genes in high-grade prostate cancer

tumorigenesis, promoting invasion and metastasis [6]. *PTEN* and *MARCKS* are targets of miR-21 and their down-regulation in PCa results in reduced apoptosis and aberrant proliferation [34, 35]. Besides, up-regulation of miR-21 in PCa also plays an important role in epithelial mesenchymal transition (EMT) by decreasing *BTG2* levels [36]. Another invasion related gene regulated by miR-21 is RECK, a matrix metalloproteinase inhibitor. Neutralizing miR-21 represses the matrix metalloproteinase levels and reverses the invasive phenotype. Besides, *AR* expression is regulated by several miRNAs, among them miR-21, while AR simultaneously regulates the expression of miR-21 [37].

Otherwise, miR-132 is down-regulated in PCa cells, enhancing cell proliferation through the increase of lactate production and glucose uptake [38]. Besides, Zhang et al. [39] showed that miR-195 promotes PCa progression by targeting *HMGA1* gene. The authors demonstrated that miR-195 expression levels were decreased positively correlated with prognosis, showing that *HMGA1* gene appears overexpressed in CRPC compared with androgen-dependent PCa. On the other hand, circulating miR-365 has recently been found significantly associated with PCa [40]. Furthermore, published data showed that miR-365 exhibited a greater negative regulatory effect on IL-6, a cytokine playing a key role in prostate carcinogenesis [41].

A cluster is a group of miRNAs that are activated by the same regulators or have the same gene targets and are often found working close to each other. One such example is the miR-221/222 cluster, whose down-regulation have been found in the tumor tissues of patients with CRPC [42], even when this cluster had been previously described to be up-regulated [43, 44]. This fact shows the dynamic status of

Table 3 Functional and pathway enrichment analysis of hub genes

Term	Description	Count	*P* value
KEGG: hsa04151	PI3K-Akt signaling pathway	8	5.59E-09
GO:0043066	Negative regulation of apoptotic process	7	3.00E-08
KEGG: hsa05200	Pathways in cancer	7	8.28E-07
KEGG: hsa04510	Focal adhesion	6	1.17E-06
KEGG: hsa04015	Rap1 signaling pathway	6	1.29E-06
KEGG: hsa04066	HIF-1 signaling pathway	5	2.55E-06
KEGG: hsa05205	Proteoglycans in cancer	5	4.35E-05
GO:0030335	Positive regulation of cell migration	4	1.04E-04
KEGG: hsa05215	Prostate cancer	4	1.07E-04
GO:0050679	Positive regulation of epithelial cell proliferation	3	4.45E-04
GO:0033138	Positive regulation of peptidyl-serine phosphorylation	3	6.05E-04
GO:0050731	Positive regulation of peptidyl-tyrosine phosphorylation	3	8.29E-04
GO:0048015	Phosphatidylinositol-mediated signaling	3	0.00138
GO:0000187	Activation of MAPK activity	3	0.00141
GO:0008284	Positive regulation of cell proliferation	4	0.00157
KEGG: hsa04014	Ras signaling pathway	4	0.00171

miRNAs in the development of PCa, regulating the same miRNA different targets depending on the point of the cancer progression. Two negative regulators of cell cycle progression, such as p27 and p57, have been described as specific targets of miR-221/222 [45]. The loss of the tumor-suppressive miR-221/222 cluster enhanced migration and invasion in PCa cells targeting Ecm29, which is involved in cancer cells invasion.

Kojima et al. [46] analyzed the cluster miR-1/133a in PCa cells, finding that both miRNAs were down-regulated in PCa compared with non-PCa tissues. Furthermore, when the expression was restored in PCa cells, there was a significant inhibition of proliferation, migration and invasion through the regulation of *PNP* gene. Besides, decreased miR-1 levels correlated with enhanced expression of EGFR, leading to promote bone metastasis [47].

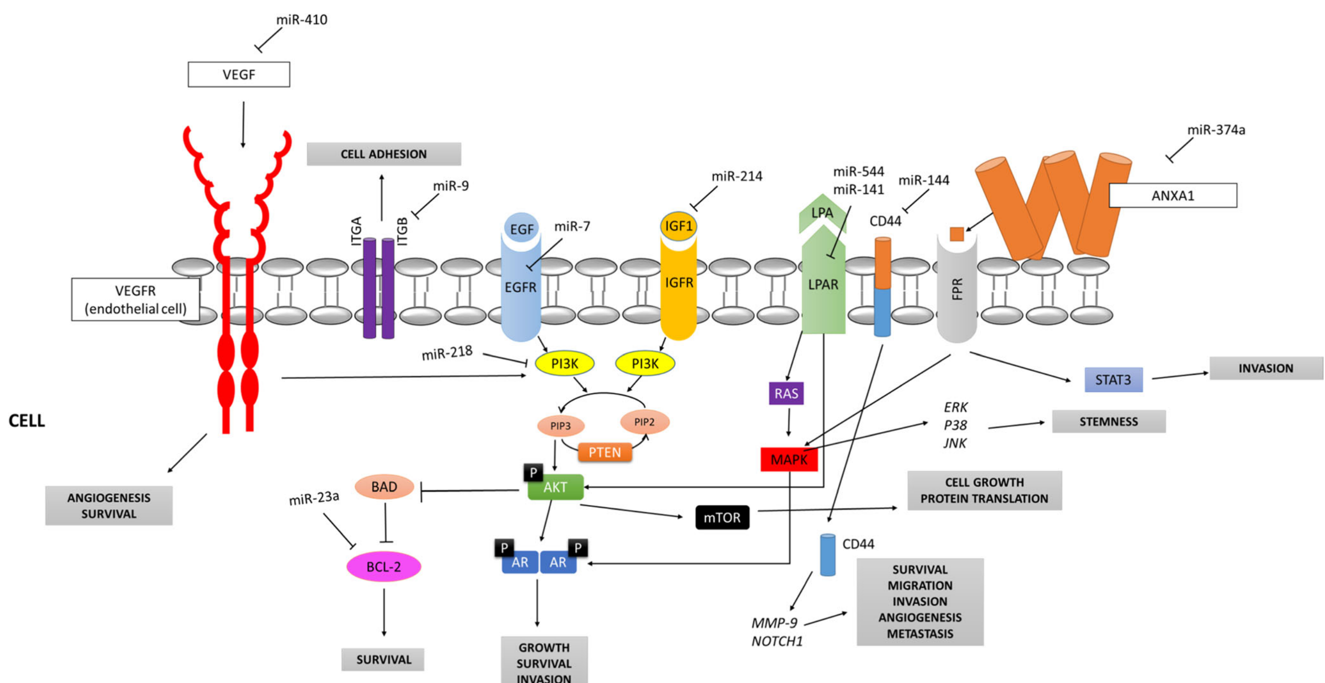

Fig. 3 A schematic representation of the role of the proteins codified by hub genes in prostate cancer and their regulator miRNAs. The hub genes found in our study (*EGFR, VEGFA, IGF1, PIK3R1, CD44, ITGB4, ANXA1, BCL2, LPAR3* and *LPAR1*) are involved in several cell signaling pathways promoting stemness, growth, survival, angiogenesis, invasion, metastasis in prostate cancer

Table 4 Hub genes and their predicted miRNAs regulators

Gene		Degree	Predicted miRNAs
EGFR	Epidermal growth factor receptor	31	miR-7, −875-5p, −134, −27a, −27b, −200a, −141, −539, −520e, −520d, −302e, −520b, −373, −302a, −302b, −302c, −302d, −520a, −520c, −372, −155, −491-5p, −133a, −133b, −450a, −874, −103, −107, −365, −129-5p, −370, −342, −299, −455-5p
VEGFA	Vascular endothelial growth factor A	26	miR-410, −590, −374a, −429, −185, −383, −361-5p, −186, −300, −381, −424, −29a, −29b, −29c, −497, −15b, −374b, −15a, −200b, −200c, −16, −195, −299, −494, −140-5p, −205, −134, −340, −495, −1, −206, −613, −339-5p, −329, −362, −141, −203, −382, −20a, −20b, −93, −106a, 106b, −17, −519d, −144, −505, −503, −543, −103, −107, −23a, −23b, −486-5p, −342, −125a-5p, −125b, −150, −199b-5p, −200a, −199a-5p, −101, −373, −520e, −125a, −302a, −302b, −302c, −372, −520a, 302e
IGF1	Insulin Like Growth Factor 1	25	miR-214, −544
PIK3R1	Phosphoinositide-3-Kinase Regulatory Subunit 1	24	miR-218, −496, −16, −195, −497, −424, −15a, −15b, −222, −92a, −92b, −367, −25, −363, −136, −32, −486-5p, −544, −361-5p, −212, −132, −129-5p, −448, −153, −542, −371-5p, −221, −590-5p, −21, −103, −107, −216a, −150, −194, −29a, −29b, −29c, −376a, −376b, −876-5p, −488, −491-5p, −1271, −96, −199a-5p, 199b-5p, −431, −155
CD44	CD44 Antigen	23	miR-144, −590, −302a, −302b, −302c, −302d, −520e, −302e, −373, −372, −520a, −520b, −520c, −520d, −340, −216a, −653, −124, −506, −485-5p, −145, −28-5p, −708, −326, −330-5p, −143, −410, −202, −216b, −132, −185, −204, −211
ITGB4	Integrin Subunit Beta 4	18	miR-9
ANXA1	Annexin A1	16	miR-374a, −374b, −21, −590-5p, −221, −222, −376c, −758, −653, −410, −296, −340, −1, −206, −613, −384, −196a, −196b, −431
LPAR1	Lysophosphatidic Acid Receptor 1	15	miR-544, −374b, −374a, −217, −200b, −429, −200c, −129-5p, −23a, −23b, −384, −9, −205, −144, −296, −186, −488, −192, −215, −224, −211, −204, −873, −335, −212, −382, −1297, −371-5p, −26a, −26b, −33a, −33b, −155, −613, −206, −1, −543, −365, −496, −485-5p, −132, −339-5p, −342, −361-5p, −433, −758, −324-5p, −299, −486-5p
LPAR3	Lysophosphatidic Acid Receptor 3	15	miR-141, −200a, −15a, −15b, −1297, −26a, −26b, −376c, −218, −186, −133a, −133b, −214, −543, −590, −155, −18a, −18b, −28-5p, −708, −24
BCL2	B cell lymphoma 2	15	miR-23a, −23b, −384, −181a, −181b, −181c, −181d, −448, −874, −371-5p, −590, −342, −383, −216a, −205, −136, −204, −211, −374a, −433, −206, −1, −613, −135a, −135b, −365, −424, −16, −195, −185, −15b, −497, −15a, −203, −219-5p

Predicted miRNAs are ordered by sum of mirSVR scores

Different miRNA-related therapies have been developed in recent years [48] based on targeting or mimicking the miRNAs involved in cancer. Some of these strategies are based on the use of small molecules inhibitors blocking the activity of protooncogenic miRNAs. Thus, Chen et al. [49] showed the inhibition of the proliferation of PCa cells both in vitro and in vivo animal experiments using a polymeric vector-mediated strategy for a miR-21 inhibitor (i.e. antisense oligonucleotides for miR-21). On the other hand, Mercatelli et al. [50] demonstrated that the anti-miR-221/222 antagomir treatment of established subcutaneous tumors derived from the PC3 cell line -a high miR-221/222 expressing prostate carcinoma cell line- reduces tumor growth in an experimental animal model.

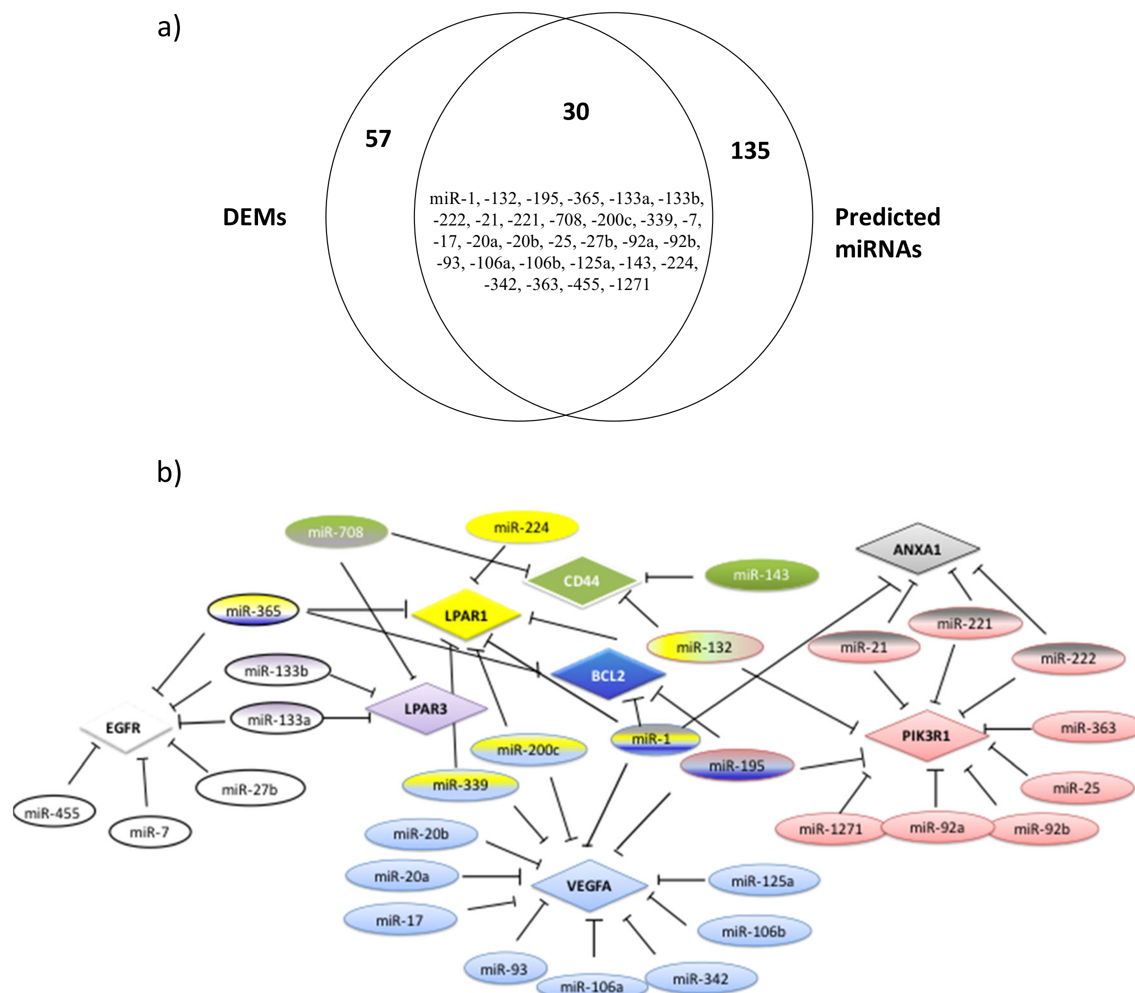

Fig. 4 Venn diagram showing common miRNAs between significant differentially expressed miRNAs (DEMs) and predicted hub gene regulators (**a**) and the common miRNAs that regulate 2 or more hub genes (**b**)

Results were confirmed by the authors checking that p27 levels were increased, as compared to untreated tumors.

The current study was intended to identify miRNAs with comprehensive bioinformatics analysis to find the potential biomarkers for PCa detection and prognosis. The analysis of this miRNA signature at the moment of biopsy could aid to select the most appropriate treatment. Our data suggest that PI3K-Akt signaling is one of the most critical PCa-promoting pathways through up-regulation of growth factor receptors, specifically EGFR, or through PTEN inactivation. Several new therapies have been developed in recent years using these targets. Furthermore, miRNA-based therapies appear as a new way in the management of PCa. Our study suggests some miRNAs as targets for new treatments.

The common miRNAs found in our study are involved in the development of PCa through the regulation of their corresponding target genes, and particularly miR-1, miR-365, miR-132, and miR-195 are involved in the regulation of 3 or more hub genes. Furthermore, we discussed the value of the miRNAs selected in our study as targets for new treatments in PCa. Finally, we suggested a signature defined by 12 miRNAs regulating 2 or more hub genes identified in our study. Further in vitro validation analysis will be necessary to confirm our hypothesis.

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