Integrative Analysis of four miRNA prognostic signatures of prostate cancer

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Abstract

Background

Prostate cancer (PCa) is the most common urological cancer among men, having a poor prognosis, which is hard to accurately evaluate based on the present methods. MicroRNAs (miRNAs), a class of internal non-coding small RNA, can involve in the regulation of tumor biological function. So far, many researchers have tried to explore the relationship of malignant progress of PCa with miRNA, while there are just limited studies conducting the comprehensive analysis of miRNA in PCa clinical significance.

Methods

The data of miRNA and mRNA expressions in PCa were downloaded from TCGA database, and were performed the overall survival (OS) analysis using Survival package of R software to harvest the differentially expressed miRNAs (DEMs) and differentially expressed genes (DEGs). The bioinformatics tools such as TargetScan, miRDB, and miRanda were also conducted to forecast the desired target genes related with prognostic DEMs. In addition, both GO and KEGG analyses were used to uncover the fundamental signaling pathways and cellular processes in PCa as well as the protein-protein interaction (PPI) network was constructed through STRING and Cytoscape software.

Results

Firstly, 4 DEMs (miR-19a-3p, miR-144-3p, miR-223-5p, and miR-483-3p) were found having significantly associated with overall survival in PCa. Based on the criteria with FDR < 0.05 and |log2FC| > 1, 33 genes were screened out as DEGs. Besides, the functional enrichment analysis revealed that these DEGs of 4 miRNAs may participate in cancer-related pathways like FoxO and PI3K-Akt signaling pathway. Lastly, the low expression of \textit{CD177} may be potentially associated with poor survival of patients in PCa.

Conclusion

This study systematically analyzed multiple PCa prognostic DEMs (miR-19a-3p, miR-144-3p, miR-223-5p, and miR-483-3p), and verified a novel DEG signature (\textit{CD177}) that can be used to effectively assess the prognosis of PCa patients.

Background

Prostate cancer (PCa) is the most common urological cancer among men, ranking as fifth in mortality on male in the world (1, 2). There are about 1.3 million new cases of prostate cancer, and near 0.36 million men will die from PCa each year (3). PCa is still incurable despite having powerful technical support and advancement of therapeutic strategies (4). Patients with prostate cancer exhibit a poor prognosis, and the
total 5-year survival rate is just about 28% (5, 6). Many factors may be contributed to the poor prognosis of PCa, such as late diagnosis, drug resistance, and distant metastasis. So far, the methods of prognostic evaluation are still mainly relied on the clinical TNM staging system and conventional histopathological examinations(7, 8), which are hard to accurately evaluate the prognosis of patients in PCa. Therefore, exploration of novel, specific, and efficient prognostic biomarkers for PCa is extremely urgent.

MicroRNAs (miRNAs), a class of internal non-coding small RNAs, can post-transcriptionally participate in the regulation of gene expressions (9). Since the miRNA lin-4 was firstly discovered in Caenorhabditis elegans in 1993 (10), more than 1,400 human miRNAs have been identified. These miRNAs can regulate approximately 30% of human transcripts through suppressing gene expressions or mRNA degradation (11), and many of them are closely related to human malignancy (12). The increasing studies have shown that miRNAs are involved in diverse biological processes, such as cell proliferation, cellular differentiation, angiogenesis, invasion, and cancer development (13). As is well-known, miRNAs can affect tumor biological function through regulating the expression of target genes. For instance, miR-296 can significantly inhibit the proliferation and invasion of PCa cells through partially reducing the expression of HMGA1 gene (14). Gan et al. also demonstrated that miR-16-5p enhanced the radio sensitivity of PCa cells, possibly due to regulate the cyclin D1 / cyclin E1 / pRb / E2F1 pathway to cause cell cycle stagnation in the G0 / G1 phase (15). These findings suggest that quite a few miRNAs, which are differentially expressed, may be used as diagnostic or prognostic biomarkers or therapeutic targets for PCa.

Although some previous studies have indicated the relationship of numerous miRNAs with the prognosis of PCa (16–18), no study systematically illuminated the relationship between differentially expressed miRNAs (DEM) of PCa and prognosis. Therefore, the data of large sample size with abundant clinical information are essential for the prognosis of PCa. The Cancer Genome Atlas (TCGA) database has accumulated multiple sets of clinical data with various types of cancer patients, which has the great significance for PCa prognosis research.

In this study, we screened out the DEMs between PCa tissues and normal tissues through TCGA database. In combination with the survival curve, the DEMs associated with prognosis were further identified. Both differentially expressed genes (DEGs) and the target genes of DEMs were also predicted using multiple miRNA databases, and their potential functions were deeply analyzed through Gene Ontology (GO), Kyoto Gene and Genomic Encyclopedia (KEGG). Those identified microRNAs can be used as the novel and potential prognostic biomarkers that can further improve the clinical prognosis of PCa.

Materials And Methods

Identification of DEMs and DEGs

The data of miRNA and mRNA expressions in PCa were downloaded from TCGA database (https://portal.gdc.cancer.gov/). The inclusion criteria included the followings: (a) the cases with RNA expression and associated clinic data, (b) the cases with complete prognostic data. Ultimately, the total
of 131 DEMs and 524 DEGs were taken into account. Volcano plots and hierarchical clustering heatmaps of the RNA-seq derived from the TCGA database were drawn using R software. Cytoscape software was used to show the regulatory mechanism network of these DEMs and DEGs.

**Survival analysis of DEMs and DEGs**

Survival package of R software was employed to perform an overall survival (OS) analysis of the DEMs and DEGs in patients with PCa, respectively.

**Target gene prediction of DEMs**

Three bioinformatics tools, including TargetScan (http://www.targetscan.org), miRDB (http://mirdb.org/miRDB), and miRanda (http://www.microrna.org) were used to forecast the potential target genes related to prognostic DEMs. Furthermore, Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was also used to further confirm these target genes for bioinformatics credibility.

**Function analysis**

The function annotation of GO consisting 3 main offtakes such as biological process (BP), cellular component (CC), and molecular function (MF) as well as the KEGG signaling pathway play the crucial role in this step. R software was used to perform gene functional enrichment analysis. Importantly, the GO and KEGG enrichment pathways with P values less than 0.05 are considered as significant categories. Furthermore, the protein-protein interaction (PPI) network was constructed using STRING (http://www.string-db.org) and Cytoscape software, and the target genes with a combined score of more than 0.4 were selected. Cytoscape software was used to produce the interaction network of these genes.

**Statistical analysis**

All statistics were performed using R software. In OS between groups, Kaplan-Meier curves were drawn, and log-rank was used to verify the significant difference. Univariate and multivariate Cox proportional hazard regression analysis was performed to obtain the correlation between risk score and OS P < 0.05 or False Discovery Rate (FDR) adjusted P < 0.05 was considered statistically significant in all analyses.

**Results**

**Identification of prognostic related DEMs**

The miRNA-seq data of 499 prostate cancer tissue samples and 52 non-tumor samples were downloaded from TCGA database. Based on the criteria for FDR ≤ 0.05 and [log2FC] ≥ 1, the total of 131 DEMs ultimately were extracted. The hierarchical cluster heatmaps and volcano plots of 131 DEMs are shown in Figure 1. To explore the correlation between the prognostic implication of PCa and miRNA expressions, the clinical data of 495 PCa patients were downloaded from TCGA database. Subsequently, according to
the Kaplan-Meier survival analysis, four miRNAs including miR-19a-3p, miR-144-3p, miR-223-5p, and miR-483-3p associated with OS of PCa patients were screened out (Figure 2).

**Prediction and identification of target genes**

In order to investigate the potential target genes of miR-19a-3p, miR-144-3p, miR-223-5p, and miR-483-3p, three bioinformatic databases, such as TargetScan, miRanda, and miRDB were employed. According to Venn diagram, there are the total of 1703 qualified target genes were selected, including miR-19a-3p targeted 713 genes, miR-144-3p targeted 521 genes, miR-223-5p targeted 420 genes, and miR-483-3p targeted 49 genes (Figure 3).

The mRNA-seq data of 489 prostate cancer and 51 non-tumor tissue samples were downloaded from TCGA database. The differential expression values of 524 DEGs were extracted with the criteria of FDR \(< 0.05\) and \([\log_2 FC]\) \(> 1\). Integrating these 524 DEGs and 1703 potential target genes of those four DEMs, 41 qualified DEGs ultimately were identified (Figure 4).

According to the relationship of miRNA and its target mRNA, which actually be negatively correlated (19), we removed 9 target genes (**AMACR, ATP1B1, ERG, ETV1, FN1, FOXF1, ITPR1, TGM3**), and screened out 33 target genes as the final DEGs (Figure 5). And the network of 4 DEMs and their target genes showed that **MBNL2, TMEM47, GJA1, CFL2, DKK3, FBXO32, SDC1, PDE5A, SGK1, JAZF1, FLNC, RHOB, SYNM, SLMAP, CALD1, ANO1, DNAJB1, CCND2**, and **SYNPO2** were down-regulated while miR-19a-3p miRNA was up-regulated. Meanwhile, **HSPA1A, TEAD1, DDIT4, KLF5, ACTC1, MBNL2, TMEM47, GJA1, PALLD, SGCB, CD177**, and **NEURL1B** were down-regulated while miR-223-5p miRNA was up-regulated. In addition, **RCAN2** was down-regulated while miR-483-3p miRNA was up-regulated as well as **TRIB1, CAMKK2, SLC4A4**, and **TBX1** were up-regulated while miR-144-3p miRNA was down-regulated.

**Function enrichment analysis of DEGs**

The GO term function enrichment and the KEGG pathway enrichment analyses were performed to study the function connections among the genes related to miR-144-3p, miR-223-5p, miR-483-3p, and miR-19a-3p.

The top enriched GO term showed that the main BP of these 33 DEGs was muscle cell differentiation and development, and epithelial cell development. CC of these 33 DEGs was contractile fiber, myofibril and cell-substrate adheren junctions, while the MF of these was dramatically concentrated in actin binding and cell adhesion molecule binding. Additionally, the KEGG pathway analysis indicated that the final DEGs were significantly enriched in pathways involved in FoxO and PI3K-Akt signaling pathways (Figure 6).

Furthermore, to explore the interactions of the final DEGs, the PPI network was constructed and showed 20 DEGs mostly related to 4 DEMs, including **TBX1, SLMAP, CAMKK2, PALLD, TMEM47, DKK3, SGCB, SGK1, GJA1, DDIT4, FBXO32, SYNM, ANO1, CALD1, SYNPO2, FLNC, ACTC1, CFL2, DNAJB1, and HSPA1A** (Supplementary Figure 1).
Survival analysis of DEGs

The Kaplan-Meier survival analysis was performed to reveal the relationship between DEMs and OS of PCa patients, and the result showed that only the low expression of CD177 gene is associated with poor survival of patients with PCa (P < 0.05) (Figure 7).

Discussion

With the increasing researches on PCa, there is a great progress in understanding and treatments of this malignancy. However, the effective treatment for PCa has not been found due to the controversial screening signature for prostate cancer (20). Meanwhile, there are no reliable prognostic markers to discriminate indolent tumors from those likely to cause aggressive metastatic diseases (21). Thus, it is necessary to understand the relevant mechanisms of PCa and explore reliable prognostic biomarkers of PCa.

There are accumulating studies had demonstrated that miRNAs can build complex networks of gene regulation and participate in multiple biological pathways, which can affect the pathogenesis of cancer (22, 23). In tumorigenesis, miRNA is a double-edged sword, which acts either as oncogenes or tumor suppressors depending on the tumor type, cellular context, clinical-stage, genetic background, and even therapeutic regimen (24). A previous study documented that miR-451a expression was down-regulated, while PSMB8 expression was up-regulated in PCa cell lines, that inhibits cell invasion and promotes PCa cell apoptosis to further inhibit cell proliferation as well as colony formation (25). However, miR-340-5p was highly expressed in esophageal squamous cells of carcinoma (ESCC) tissue, which exhibits a negative correlation with PIK3C3 expression to inhibit the cell proliferation (26). A large number of researchers have tried to explore the relationship of malignant progress of PCa with miRNA, while there are the limited findings about the comprehensive analysis of miRNAs in PCa clinical significance to date. To reveal the relationship of miRNAs with prognosis of PCa, several miRNAs were screened out and identified. This study demonstrated that miRNAs, playing crucial role in the prognosis of PCa patients, can be used as the potential prognostic biomarkers of PCa patients for further developing individualized treatments.

In this study, we investigated RNA-seq datasets from the TCGA database of prostate cancer cohort to analyze DEMs in PCa. The total of 499 PCa tissue samples and 52 non-tumor samples were valid on the DEM screening. Ultimately, 131 DEMs were identified, and of which four miRNAs such as miR-19a-3p, miR-144-3p, miR-223-5p, and miR-483-3p were associated with prognosis of PCa as the independent prognostic signatures in line with the Kaplan-Meier survival analysis, while the role of these DEMs in the progress of PCa is diverse depending on cancer types, which may act as oncogenes or tumor suppressors. MiR-19a-3p, as a key tumor-related miRNA of the miR-17-92 cluster (27), has been reported down-regulation in many types of cancers, including breast cancer (28), hepatocellular carcinoma (29), and glioma (30). MiR-19a-3p has been found high expression in primary PCa cell 22RV1, but was significantly decreased in bone metastatic PCa cell lines, including PC-3, C4-2B, and VCaP. There are
accumulating evidences shown that miR-19a-3p can inhibit the invasion and migration of PCa cells through targeting downstream effectors \textit{SMAD2} and \textit{SMAD4} of TGF-\(\beta\) signaling, resulting in inactivation of TGF-\(\beta\) signaling (31). In addition, miR-144-3p has involved in the formation and development of multiple malignancies, and may act as a tumor suppressor through down-regulating CEP55 to induce cell apoptosis in PCa as well as plays a crucial role in PCa tumor growth and cell proliferation via regulating G1-S phase transition (32). MiR-483-3p is recognized as onco-miR involved in the formation and development of multiple malignancies. A previous study reported that miR-483-3p was overexpressed in neuroblastoma. Recently, miR-483-3p was found up-regulation in lymphoblastoid cell lines of Finnish PCa families, and miR-483-3p could act as the antiapoptotic role through interactions with the 3’UTR of PUMA mRNA to inhibit the antiapoptotic factors BCL3 and BCLXL (33). In addition, miR-223-5p also appeared as an important miRNA associated with tumor invasiveness and metastasis in multiple tumor types, including breast cancer, gastric cancer, and hepatocellular carcinoma, which might act as oncogenes or tumor suppressors to influence the biological behaviors of cancer through several pathways. MiR-223-5p was also remarkably down-regulated in non-small cell lung cancer (NSCLC) and \textit{E2F8} as a functional target of miR-223-5p, and the overexpression of miR-223-5p could significantly inhibit the expression of \textit{E2F8}, leading to suppress the proliferation, the migration, and invasion of NSCLC cells (34). However, there is still no report showed that miR-223-5p could influence the prognosis of PCa.

In order to investigate the potential target genes of miR-19a-3p, miR-144-3p, miR-223-5p, and miR-483-3p, three bioinformatic tools, such as TargetScan, miRanda, and miRDB, were used and analyzed. We also performed functional enrichment analysis, and constructed the PPI network. The gene of \textit{CD177} was identified ultimately, which was associated with prognosis of PCa as the independent prognostic signature in line with the Kaplan-Meier survival analysis. \textit{CD177} gene is a glycosphatidylinositol-linked extracellular membrane-bound protein that belongs to the Ly-6 family, and also is referred to as NB1, HNA-2a, or PRV1 (35). Previous studies have documented that the expression of \textit{CD177} gene is closely related to PCa, and the reduction of \textit{CD177} gene expression is correlated with good prognosis in PCa. There is an evidence shown that \textit{CD177} gene-deficiency is associated with the increase in \(\beta\)-catenin signaling, which may cat as a regulator of epithelial proliferation to participate the progress of PCa (36).

The advantage of this study is that we performed a comprehensive analysis of miRNA data, providing an effective statistical approach for exploration the role of DEMs in PCa, whereas, there are also several limitations in present study. Firstly, this study is just based on miRNA-seq data from TCGA, and multiple databases should be investigated to increase the sample size. Secondly, several DEMs were identified to be associated with PCa, but there were no functional experiments conducted to reveal biological functions and potential mechanism of these DEMs.

In conclusion, our study discovered that four DEMs (miR-19a-3p, miR-144-3p, miR-223-5p, and miR-483-3p) can affect the prognosis of PCa, and miR-223-5p mainly affects the prognosis of PCa via regulating the expression level of \textit{CD177} gene. These findings could be employed as potential biomarkers of PCa prognosis or treatment targets of PCa in the future.
Abbreviations

biological process, **CC**: cellular component, **DEGs**: differentially expressed genes, **DEM**: differentially expressed miRNAs, **FDR**: false discovery rate, **GO**: Gene Ontology, **KEGG**: Kyoto Gene and Genomic Encyclopedia, **MF**: molecular function, **miRNA**: microRNA, **OS**: overall survival, **PCa**: prostate cancer, **PPI**: protein-protein interaction, **TCGA**: The Cancer Genome Atlas

Declarations

**Ethics approval and consent to participate**

Consent for participation for all patients was obtained through The Cancer Genome Atlas Project.

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no conflict of interest.

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**Authors’ Contributions**

CC, and XC contributed to the conception and design. CC, XC, XG and QZ contributed to the development of the methodology. CC, XC, LY, and ML contributed to the acquisition of the data. CC, XC, and XD contributed to the analysis and interpretation of data. CC, XC, ML, LX, and XG contributed to the writing, review, and/or revision of the manuscript. CC, XC and QZ contributed to the study supervision. All authors read and approved the final manuscript.

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Figures
Figure 1

The correlation between CD177 and overall survival. Kaplan–Meier curves of different level of CD177.
Figure 2

Gene functional enrichment of DEGs. BP (A, B), CC (C, D), MF (E, F) and KEGG pathway analyses (G, H). The red circles represent up-regulation and the blue represent down-regulation.

Figure 3

Regulatory network of the predicted genes and their target miRNAs. The red triangles represent up-regulated DEMs, while the green represent down-regulated. The blue circles indicate down-regulated DEGs, and the purple represent up-regulated.
Figure 4

Differentially expressed genes. (A) Heatmap of the differentially expressed genes (DEGs) between PCa and normal tissues samples from TCGA dataset. (B) Volcano map of DEGs. Red dots representing significantly up-regulated DEGs, green dots representing significantly down-regulated DEGs. (C) Expression patterns of 41 DEGs.
Figure 5

Target gene from prediction of differentially expressed microRNAs. The overlaps indicated the numbers of genes predicted by TargetScan, miRanda, miRDB (A: miR-19a-3p; B: miR-144-3p; C: miR-223-5p; D: miR-483-3p).
Figure 6

The correlation between four DEMs and overall survival. Kaplan–Meier curves of different levels of (A) miR-19a-3p, (B) miR-144-3p (C) miR-223-5p, and (D) miR-483-3p.
Figure 7

Differentially expressed microRNAs. (A) Heatmap of the differentially expressed microRNAs (DEM) between PCa and normal tissues samples from TCGA dataset. (B) Volcano map of DEMs. Red dots representing significantly up-regulated DEMs, green dots representing significantly down-regulated DEMs.

Supplementary Files

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- supplementaryfig1.pdf