Expression of microRNA-99a-3p in Prostate Cancer Based on Bioinformatics Data and Meta-Analysis of a Literature Review of 965 Cases

Hai-biao Yan, Yu Zhang, Jie-mei Cen, Xiao Wang, Bin-liang Gan, Jia-cheng Huang, Jia-yi Li, Qian-hui Song, Sheng-hua Li, Gang Chen

Background: microRNAs (miRNAs) have a role as biomarkers in human cancer. The aim of this study was to use bioinformatics data, and review of cases identified from the literature, to investigate the role of microRNA-99a-3p (miR-99a-3p) in prostate cancer, including the identification of its target genes and signaling pathways.

Material/Methods: Meta-analysis from a literature review included 965 cases of prostate cancer. Bioinformatics databases interrogated for miR-99a-3p in prostate cancer included The Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO), and ArrayExpress. Twelve computational predictive algorithms were developed to integrate miR-99a-3p target gene prediction data. Bioinformatics analysis data from Gene Ontology (GO), the Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein-protein interaction (PPI) network analysis were used investigate the possible pathways and target genes for miR-99a-3p in prostate cancer.

Results: TCGA data showed that miR-99a was down-regulated in prostate cancer when compared with normal prostate tissue. Receiver-operating characteristic (ROC) curve area under the curve (AUC) for miR-99a-3p was 0.660 (95% CI, 0.587–0.732) or a moderate level of discriminations. Pathway analysis showed that miR-99a-3p was associated with the Wnt and vascular endothelial growth factor (VEGF) signaling pathways. The PPP3CA and HYOU1 genes, selected from the PPI network, were highly expressed in prostate cancer tissue compared with normal prostate tissue, and negatively correlated with the expression of miR-99a-3p.

Conclusions: In prostate cancer, miR-99a-3p expression was associated with the Wnt and VEGF signaling pathways, which might inhibit the expression of PPP3CA or HYOU1.

MeSH Keywords: Afferent Pathways • MicroRNAs • Prostatic Neoplasms

Abbreviations: TCGA – The Cancer Genome Atlas; GEO – Gene Expression Omnibus; GO – Gene Ontology; KEGG – Kyoto Encyclopedia of Genes and Genomes; PPI – protein-protein interaction; AUC – area under the curve; ROC – receiver-operating characteristic; miRNA – microRNA; FP – false positives; TP – true positives; TN – true negatives; FN – false negatives; BP – biological process; CC – cellular component; MF – molecular function; STRING – Search Tool for the Retrieval of Interacting Genes; SMD – standard mean deviation

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/908057
Background

Worldwide, prostate cancer is one of the most common causes of cancer death in the male population [1,2]. In recent years, surgical prostatectomy, radiotherapy, hormone therapy, and immunotherapy have been the major methods used to treat prostate cancer, but treatment options remain limited in advanced-stage disease [3–7]. High rates of metastasis and cancer-associated mortality worsen the prognoses of prostate cancer patients, especially for patients with advanced-stage prostate cancer [8–10]. It is important to continue to study the molecular mechanisms involved in prostate cancer, which may provide novel perspectives on the diagnosis and treatment of prostate cancer patients in the future.

There is increasing published evidence that has shown that small regulatory noncoding RNAs, such as microRNAs (miRNAs) have a role as biomarkers in human cancer, and are involved in RNA silencing and the down-regulation of gene expression [11–14]. Several studies have confirmed that miRNAs play important roles in the development and progression of many types of human cancers [1,15–18]. The miRNAs may promote tumor development and progression by combining with the 3'-untranslated region (3'-UTR) of target mRNAs [19,20]. Currently, several studies have shown the expression and potential role of microRNA-99a-3p (miR-99a-3p) in cancer [21–23]. For example, miR-99a-3p has been shown to be overexpressed in colorectal cancer and may predict chemotherapy response in patients with advanced colorectal cancer [22]. Also, miR-99a-3p has been shown to be down-regulated in endometrioid endometrial carcinoma [23]. Although investigators have described the involvement of miRNAs in the biological processes of cancers, including that of miR-99a-3p, little is known about the specific mechanisms involved in the interaction between miR-99a-3p and prostate cancer [24].

The aim of this study was to explore the role and mechanism of miR-99a-3p in prostate cancer based on bioinformatics analysis and using a meta-analysis of patient data from a literature review. This study extracted original data provided by The Cancer Genome Atlas (TCGA) database, and twelve miRNA prediction algorithms were utilized to predict the target genes of miR-99a-3p. Gene expression microarrays for prostate cancer were downloaded from the Gene Expression Omnibus (GEO) and ArrayExpress databases.

Material and Methods

Validation the expression of microRNA-99a-3p (miR-99a-3p) based on The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), and ArrayExpress

The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov) is a database of expression profiles for at least 30 kinds of cancers, including prostate cancer [25–27]. TCGA can be used to explore clinicopathological parameters associated with patients with cancer, which were used in this study [28,29]. In the current study, the RNA sequencing (RNA-Seq) data for prostate cancer patients, which were taken from the Illumina MiSeq RNA-Seq platform, contained 498 prostate cancer cases and 52 normal prostate cases, up to 1st November 2017. The expression data for microRNA-99a-3p (miR-99a-3p) were shown in reads per million (RPM), and miR-99a-3p expression levels were normalized via the ‘R’ language package DESeq.

The relationship between miR-99a-3p and clinicopathological parameters in prostate cancer cases was further analyzed based on the expression data in TCGA database using a Student’s t-test. The receiver-operating characteristic (ROC) curve was utilized to assess the value of miR-99a-3p levels in discriminating between prostate cancer patients and normal controls. The chip datasets from the Gene Expression Omnibus (GEO) were also searched (http://www.ncbi.nlm.nih.gov/geo/), and the public database of microarray gene expression, ArrayExpress was used (http://www.ebi.ac.uk/arrayexpress/).

For database searches, the following keywords were used: prostate OR prostate gland OR prostate cancer AND miR or miRNA OR microRNA or miR or miRNA OR microRNA. The expression data for miR-99a-3p were extracted from GEO and ArrayExpress databases.

A literature review of miR-99a-3p and prostate cancer

The prostate cancer-related miR-99a-3p microarray data in GEO and ArrayExpress and the RNA-seq data in TCGA were all downloaded. Also, this study included a literature search for for publications related to miR-99a-3p in prostate cancer in twelve online databases: PubMed, Google Scholar, Web of Science, EMBASE, Ovid, Wiley Online Library, LILACS, Science Direct, Cochrane Central Register of Controlled Trials, Chinese CNKI, Wan Fang, Chong Qing VIP, and on the China Biology Medicine disc. The retrieval date was October 30, 2017. The retrieval was performed and checked by two people (Bin-liang Gan and Jie-mei Cen). A group discussion was organized if there were any disagreements. The number of false positives (FP), true positives (TP), true negatives (TN) and false negatives (FN) were extracted.
The potential target genes of miR-99a-3p

In the present study, twelve online target prediction algorithms were selected to predict the target genes of miR-99a-3p. These twelve algorithms were used were miRWalk (http://zmf.umm.uni-heidelberg.de/), miRmap (http://mirmap.ezlab.org/), miRecords (http://c1.accurascience.com/miRecords/), DIANA-mT (http://diana.imis.athena-innovation.gr/), miRanda (http://www.microrna.org), miRDB (http://www.mirdb.org/), RNAhybrid (http://bibiserv2.ccb.jhu.edu/rna22.html), PICTAR4 (http://diana.imis.athena-innovation.gr/), PICTARS (http://diana.imis.athena-innovation.gr/), PITA (http://genie.weizmann.ac.il/), RNA22 (http://cbcsrv.watson.ibm.com/rna22.html), and TargetScan (http://www.

Table 1. Differential expression of miR-99a and clinicopathological parameters in prostate cancer based on TCGA.

| Clinicopathological features | N   | miR-99a expression | T      | P value |
|------------------------------|-----|--------------------|--------|---------|
| Tissues                      |     |                    |        |         |
| Normal prostate              | 52  | 13.685±0.722       | -4.304 | <0.001  |
| Prostate cancer              | 498 | 13.216±0.955       |        |         |
| Age                          |     |                    |        |         |
| <60                          | 201 | 13.215±0.976       | 0.103  | 0.917   |
| ≥60                          | 203 | 13.205±0.938       |        |         |
| Race                         |     |                    |        |         |
| White                        | 146 | 13.275±1.003       |        |         |
| Black                        | 7   | 13.120±0.792       | F=0.276| 0.759   |
| Asian                        | 2   | 12.826±0.649       |        |         |
| Stage                        |     |                    |        |         |
| I+II                         | 187 | 13.265±1.016       | 0.153  | 0.347   |
| III+IV                       | 300 | 13.182±0.918       |        |         |
| T (tumor)                    |     |                    |        |         |
| T1+T2                        | 348 | 13.219±0.904       | 0.190  | 0.349   |
| T3+T4                        | 55  | 13.091±1.183       |        |         |
| N (node)                     |     |                    |        |         |
| Yes                          | 78  | 13.415±0.953       | -2.277 | 0.023   |
| No                           | 344 | 13.14±0.965        |        |         |
| M (metastasis)               |     |                    |        |         |
| Yes                          | 3   | 12.936±1.278       | 0.506  | 0.613   |
| No                           | 454 | 13.220±0.968       |        |         |

Figure 1. Clinical significance of microRNA-99a-3p (miR-99a-3p) in prostate cancer based on The Cancer Genome Atlas (TCGA) database. (A) Differential expression of microRNA-99a-3p (miR-99a-3p) between prostate cancer tissue and non-cancerous prostate tissue. (B) Differential expression of miR-99a-3p in cases with lymph node metastasis compared with those without lymph node metastasis. (C) The receiver-operating characteristic (ROC) curve for miR-99a-3p in prostate cancer.
Table 2. Characteristics of microarray datasets included in the study.

| First author (publication year) | Country | Data source | Test method/Platform | Cancer group | Normal controls | Mean1 ±SD1 | Mean0 ±SD0 |
|---------------------------------|---------|-------------|----------------------|--------------|----------------|-----------|-----------|
| Taylor B. et al. (2010)         | USA     | GEO: GSE21036 | Agilent GPL8227      | 113          | 28             | 3.778±0.648 | 4.518±0.33 |
| Wach S. et al. (2010)           | Germany | GEO: GSE23022 | Affymetrix GPL8786   | 20           | 20             | 0.921±0.177 | 0.909±0.228 |
| Mattila H. et al. (2010)        | Finland | GEO: GSE24201 | Agilent GPL7731      | 14           | 15             | 15.684±5.269 | 16.757±8.92 |
| Keller A. et al. (2011)         | Germany | GEO: GSE31568 | Febit GPL9040        | 23           | 70             | 49.266±49.74 | 42.421±34.322 |
| Zhong W. et al. (2012)          | China   | GEO: GSE34932 | Agilent GPL11487     | 9            | 7              | 1.773±0.766  | 1.406±0.293  |
| Lin P.C. et al. (2012)          | USA     | GEO: GSE36802 | Affymetrix GPL8786   | 21           | 21             | 6.197±1.993  | 6.788±0.952  |
| Jalava S.E. et al. (2012)       | Finland | ArrayExpress: E-MTAB-408 | Agilent A-MEXP-1663 | 28           | 26             | 3.138±0.792  | 1.811±1.371  |
| TCGA (2017)                     | USA     | TCGA         | NR                   | 498          | 52             | 13.216±0.955 | 13.685±0.722 |

Mean1 ±SD1 – prostate cancer tissues; Mean0 ±SD0 – non-tumor tissues.

Figure 2. Differential expression of microRNA-99a-3p (miR-99a-3p) in prostate cancer based on the Gene Expression Omnibus (GEO) and ArrayExpress datasets. (A) GSE21036. (B) GSE24201. (C) GSE36802. (D) GSE23022. (E) GSE31568. (F) GSE34932. (G) E-MTAB-408.
A Venn diagram tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) was chosen for the identification of candidate genes. Genes that were concurrently predicted by more than two target prediction algorithms were selected for further analysis.

Also, the gene expression microarrays for prostate cancer were downloaded from GEO using the following keywords: miR-99a-3p OR miRNA-99a-3p OR microRNA-99a-3p AND prostate OR prostatic gland OR prostat* AND cancer OR carcinoma OR tumor OR neoplas* OR malignant OR adenocarcinoma. Then, the genes that were differentially expressed in prostate cancer were selected.

Figure 3. The procedures involved in the meta-analysis of the literature review.

Figure 4. The expression conditions of microRNA-99a-3p (miR-99a-3p) in prostate cancer tissue compared with normal prostate tissue. (A) Forest plot of the datasets evaluating microRNA-99a-3p (miR-99a-3p) expression in prostate cancer patients compared with normal control groups (fix-effects model). (B) Forest plot of datasets evaluating miR-99a-3p expression between prostate cancer and normal control groups (random-effects model). (C) Sensitivity analysis to exclude the main studies one at a time. (D) Funnel plot of datasets, indicating that no publication bias was found in the analysis.
The meta-analysis of the literature review of published studies on microRNA-99a-3p (miR-99a-3p) and prostate cancer included 965 cases of prostate cancer. (A) The summary receiver-operating characteristic (SROC) curve, which represents the performance of the association of miR-99a-3p with prostate cancer, based on data from a meta-analysis. (B) The pooled sensitivity and specificity of the included studies. The diagnostic DLR is the ratio of the likelihood of the observed test result in patients with prostate cancer vs. a population without prostate cancer. (C) The pooled positive diagnostic likelihood ratio (DLR) and negative DLR values of the included studies. The diagnostic DLR is the ratio of the likelihood of the observed test result in patients with prostate cancer vs. a population without prostate cancer. (D) The pooled diagnostic score and diagnostic odds ratio (OR) of the included studies. (E) The publication bias: 1/root (ESS) refers to the inverse root of the effective sample size. Each circle represents an included study.
To further explore the potential functions and pathways associated with miR-99a-3p, bioinformatics analysis, including Gene Ontology (GO), the Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein-protein interaction (PPI) network analysis, were applied to study the underlying functions, networks, and pathways of the genes [30–32]. The DAVID Bioinformatics Tool (https://david.ncifcrf.gov/ Version 6.7) was utilized to perform the GO and KEGG analysis [33–35]. Biological process (BP), cellular component (CC) and molecular function (MF) data were exported from GO. Cytoscape (Version 3.0) (http://cytoscape.org) was used to create the functional network between miR-99a-3p and the potential genes.

The interaction pairs of the potential genes were surveyed by using the Search Tool for the Retrieval of Interacting Genes (STRING) Version 9.0 (http://string-db.org) [34,36,37]. A STRING database has been constructed to provide a global perspective for as many species as possible, including humans. The known and predicted relationships were integrated and scored, and a combined score of >0.4 was recognized in the construction of the PPI network.

**Identification of miR-99a-transcription factors (TFs)**

The CircuitsDB (http://biocluster.di.unito.it/circuits) database is a web-server used to research miRNA transcription factor (TF) regulatory circuits in humans and mice [38]. The TFs were extracted from the CircuitsDB database, and a regulatory network was constructed between miR-99a-3p and these TFs using Cytoscape software (Version 3.0).

**Statistical analysis**

Statistical analysis was performed using SPSS version 22.0 (SPSS, IBM, Chicago, IL, USA). A student's t-test was carried to compare the expression of miR-99a-3p in prostate cancer and normal prostate tissue. A receiver-operating characteristic (ROC) curve was created to differentiate prostate cancer from normal prostate tissues via miR-99a-3p expression. Spearman's rank correlation coefficient was used to determine the relationship between miR-99a-3p and potentially associated genes. P<0.05 (two-sided) was considered to indicate statistical significance.
**Results**

The expression of microRNA-99a-3p (miR-99a-3p) based on The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), and ArrayExpress

The original data from The Cancer Genome Atlas (TCGA) was further investigated and showed that miR-99a-3p was significantly down-regulated in prostate cancer when compared with non-cancerous prostate tissues (P<0.001) (Figure 1A). The relationship between miR-99a-3p and the clinicopathological parameters of prostate cancer showed that high expression levels of miR-99a-3p were positively related to lymph node metastasis (P=0.023) (Figure 1B; Table 1). Also, the receiver-operating characteristic (ROC) curve showed that the area under the curve (AUC) for miR-99a was 0.660 (95% CI 0.587–0.732) (P<0.001) for prostate cancer patients, which reflected the moderate level of the ability of miR-99a-3p to discriminate between prostate cancer patients and normal controls (Figure 1C). The correlations between the expression of miR-99a-3p and other clinical parameters in prostate cancer were explored, but no positive associations were identified from the TCGA data.

Also, seven clip datasets (GSE21036, GSE23022, GSE24201, GSE31568, GSE34932, GSE36802, and E-MTAB-408) were chosen based on GEO and ArrayExpress. The detailed characteristics of the microarray datasets included in the study are shown in Table 2. A Student’s t-test was applied to compare miR-99a-3p expression in prostate cancer with normal prostate tissues. The down-regulated expression of miR-99a-3p in prostate cancer was found in GSE21036, GSE24201, and GSE36802, whereas GSE23022, GSE31568, GSE34932, and E-MTAB-408 showed upregulation of expression of miR-99a-3p in prostate cancer (Figure 2A–2G).

Analysis from the literature review of the association between miR-99a-3p and prostate cancer: The identification of 965 cases

The literature review identified 965 cases from three centers (six datasets from GEO, one dataset from ArrayExpress, and the original data from TCGA). The procedure involved in the analysis is shown in Figure 3. For miR-99a-3p expression in the prostate cancer group, compared with the normal group, a fixed-effect model was first used to calculate the standard

### Table 3. Top-six enrichment GO terms (BP, CC, and MF) of the target genes of miR-99a-3p.

| ID       | Term                                                                 | Ontology | Count | Fold enrichment | P     |
|----------|----------------------------------------------------------------------|----------|-------|-----------------|-------|
| GO: 0045944 | Positive regulation of transcription from RNA polymerase II promoter | BP       | 15    | 1.82098163      | 0.034475 |
| GO: 0006468 | Protein phosphorylation                                             | BP       | 9     | 2.35053919      | 0.037316 |
| GO: 0030335 | Positive regulation of cell migration                              | BP       | 7     | 4.530681468     | 0.004461 |
| GO: 0038095 | Fc-epsilon receptor signaling pathway                              | BP       | 6     | 4.014343772     | 0.016754 |
| GO: 0071300 | Cellular response to retinoic acid                                 | BP       | 4     | 6.80526849      | 0.020687 |
| GO: 0034976 | Response to endoplasmic reticulum stress                           | BP       | 4     | 6.35158924      | 0.024768 |
| GO: 0005634 | Nucleus                                                            | CC       | 63    | 1.49312914      | 0.000222 |
| GO: 0016020 | Membrane                                                           | CC       | 29    | 1.691728553     | 0.005267 |
| GO: 0005654 | Nucleoplasm                                                        | CC       | 32    | 1.475149749     | 0.021198 |
| GO: 0000932 | Cytoplasmic mRNA processing body                                   | CC       | 4     | 6.581437342     | 0.022627 |
| GO: 0042470 | Melanosome                                                         | CC       | 4     | 5.082694185     | 0.043657 |
| GO: 0005829 | Cytosol                                                            | CC       | 35    | 1.355001806     | 0.046229 |
| GO: 0030371 | Translation repressor activity                                     | MF       | 3     | 31.75109718     | 0.003779 |
| GO: 0005524 | ATP binding                                                        | MF       | 23    | 1.791087533     | 0.008073 |
| GO: 0005515 | Protein binding                                                    | MF       | 88    | 1.166194728     | 0.02601  |
| GO: 0031624 | Ubiquitin conjugating enzyme binding                               | MF       | 3     | 11.26651835     | 0.02859  |
| GO: 0015276 | Ligand-gated ion channel activity                                  | MF       | 3     | 9.701724138     | 0.003768 |
| GO: 0008270 | Zinc ion binding                                                  | MF       | 17    | 1.693029704     | 0.041044 |
Table 4. Top-ten KEGG pathway enrichment analysis of the target genes of miR-99a-3p.

| KEGG ID  | KEGG term                        | Count | Fold Enrichment | P        |
|----------|----------------------------------|-------|-----------------|----------|
| hsa05166 | HTLV-I infection                 | 10    | 4.152644231     | 0.000528 |
| hsa04662 | B cell receptor signaling pathway | 5     | 7.703455964     | 0.003688 |
| hsa04022 | cGMP-PKG signaling pathway       | 7     | 4.482854495     | 0.004163 |
| hsa04310 | Wnt signaling pathway            | 6     | 4.622073579     | 0.00877  |
| hsa04931 | Insulin resistance               | 5     | 4.921652422     | 0.013068 |
| hsa04922 | Glucagon signaling pathway       | 5     | 5.369075369     | 0.017494 |
| hsa04370 | VEGF signaling pathway           | 4     | 6.970996217     | 0.018625 |
| hsa04722 | Neurotrophin signaling pathway   | 5     | 4.429487179     | 0.024709 |
| hsa04360 | Axon guidance                    | 5     | 4.185342217     | 0.029641 |
| hsa04261 | Adrenergic signaling in cardiomycocytes | 7  | 3.640674394  | 0.045801 |

mean deviation (SMD). The combined SMD reached -0.26 (-0.44, -0.09), with high heterogeneity (I²=86.8%) (P<0.05), indicating the down-regulation of expression of miR-99a-3p in prostate cancer cases (Figure 4A).

A random-effect model was used, and the combined SMD reached -0.06 (-0.58, 0.45), with heterogeneity of >50% (Figure 4B). To investigate whether a certain study had contributed to this heterogeneity, a sensitivity analysis was applied, and this sensitivity analysis showed that the pooled SMD was stable (Figure 4C). Also, no significant publication bias was found (Figure 4D).

The diagnostic analysis demonstrated that the area under the curve (AUC) of the summary receiver-operating characteristic (SROC) curve was 0.62 (0.57–0.66) (Figure 5A), with a sensitivity and specificity of 0.38 (95% CI, 0.12–0.73) and 0.80 (95% CI, 0.38–0.96), respectively (Figure 5B). Also, the results confirmed the AUC (0.660) of the original data from TCGA, which indicated the moderate value of miR-99a-3p in predicting prostate cancer. The negative and positive diagnostic likelihood ratio (DLR) values were 0.77 (0.49–1.22) and 1.89 (0.57–6.27), respectively (Figure 5C). A DLR value of 1.89 suggested that patients with prostate cancer had an approximately 1.89-fold greater chance of being miR-99a-3p assay-positive. The diagnostic score and odds ratio (OR) were 0.89 (~0.65–2.43) and 2.44 (0.52–11.39), respectively (Figure 5D). No significant publication bias was found (P=0.44) (Figure 5E).

Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

Based on the twelve target prediction algorithms, 1,062 genes were predicted by more than two prediction algorithms. Furthermore, only one gene dataset (GSE85614) was found to relate to miR-99a-3p in prostate cancer. A total of 5,270 differentially expressed genes were selected. Also, 156 genes were found to overlap in both prediction algorithms and the GEO database (5,270). These 156 genes were used for the GO and pathway analysis. The GO analysis showed that the strongly enriched terms were protein phosphorylation, translation repressor activity, and protein binding (Figure 6; Table 3). The KEGG pathway analysis showed that miR-99a-3p was associated with different pathways, the Wnt and vascular endothelial growth factor (VEGF) signaling.
pathways (Figure 7; Table 4). When analyzed together, the GO and KEGG pathway items indicated that miR-99a-3p might be involved in biological mechanisms of prostate cancer.

A network of these 156 genes was constructed (Figure 8). The relationships between miR-99a-3p and the differentially expressed genes were observed from this network. A protein-protein interaction (PPI) network was constructed using STRING online, and a total of 72 PPI pairs with combined scores >0.4 were noted (Figure 9). Also, PPP2CA, LYN, TRRAP, PPP3CA, PIK3CD, and HYOU1 were found to have higher degrees of association (>5) according to the PPI network. Also, investigation of the expression levels of these six genes and their correlations with miR-99a-3p was based on TCGA, which showed that only the protein phosphatase 3 catalytic subunit alpha (PPP3CA) gene and the hypoxia upregulated protein 1 (HYOU1) gene were more highly expressed in prostate cancer tissue when compared with normal prostate tissue (P<0.01) (Figure 10A, 10B). However, there was a negative correlation between expression of miR-99a-3p and both PPP3CA (r=–0.049, P=0.276) (Figure 10C) and HYOU1 (r=–0.058, P=0.195) (Figure 10D).

The AUC values of PPP3CA and HYOU1 were 0.776 (95% CI, 0.721–0.832) (P<0.001) (Figure 10E) and 0.668 (95% CI, 0.604–0.732) (P<0.001) (Figure 10F), respectively. The Human Protein Atlas (HPA) database (http://www.proteinatlas.org/) was used to clarify the protein levels of PPP3CA and HYOU1.

Figure 8. Network analysis between microRNA-99a-3p (miR-99a-3p) and the target genes.
Based on the results described above, it might be possible to hypothesize that miR-99a-3p may influence the expression of the PPP3CA or HYOU1 genes that encode proteins involved in prostate cancer. Figure 12 shows the regulatory network of miR-99a-3p and transcription factors, constructed using Cytoscape, an open source bioinformatics software platform to visualize molecular networks and their integrating and interaction with gene expression profiles (www.cytoscape.org).

**Discussion**

The initial aim of this study was to investigate the expression levels of microRNA-99a-3p (miR-99a-3p) in prostate cancer based on The Cancer Genome Atlas (TCGA), and the results showed that miR-99a-3p was down-regulated in prostate cancer compared with normal prostate tissue. The receiver operating characteristic (ROC) curve showed that miR-99a-3p might have a moderate ability to discriminate between prostate cancer patients and normal controls.

The present study was the first include a meta-analysis evaluating the expression and diagnostic value of miR-99a-3p in prostate cancer. As a result, the standard mean deviation (SMD) was
Figure 10. Clinical significance of the expression of the PPP3CA and HYOU1 genes in prostate cancer, based on The Cancer Genome Atlas (TCGA) database. (A) The differential expression of PPP3CA in tissue containing prostate cancer compared with normal, non-cancerous prostate tissue. (B) The differential expression of HYOU1 in tissue containing prostate cancer compared with normal, non-cancerous prostate tissue. (C) The negative correlation between PPP3CA and miR-99a-3p. (D) The negative correlation between HYOU1 and miR-99a-3p. (E) The receiver-operating characteristic (ROC) curve for PPP3CA in prostate cancer. (F) The ROC curve for HYOU1 in prostate cancer.
-0.26 (−0.44, −0.09) and this meta-analysis verified the down-regulated expression of miR-99a-3p in prostate cancer. In the diagnostic meta-analysis, 965 cases from the Gene Expression Omnibus (GEO), TCGA, and ArrayExpress were included, and the results were utilized to assess the ability of miR-99a-3p to detect prostate cancer. However, this meta-analysis had several limitations. A high level of heterogeneity was unavoidable, partly because blinding was present in only three included databases (GEO, ArrayExpress, and TCGA). Also, the different expression trends for miR-99a-3p in GEO and ArrayExpress may also have contributed to the high heterogeneity.

To further explore the potential functions and pathways associated with miR-99a-3p, bioinformatics analysis was applied to investigate the underlying functions, pathways, and networks of the genes. The Gene Ontology (GO) terms of protein phosphorylation, translation repressor activity, and protein binding were found to be highly enriched. Also, miR-99a-3p might be associated with the Wnt and vascular endothelial growth factor (VEGF) signaling pathways.

Protein-protein interaction (PPI) network analysis was used to investigate the most likely target genes for miR-99a-3p in

Figure 11. The immunohistochemical staining for protein phosphatase 3 catalytic subunit alpha (PPP3CA) and the hypoxia upregulated protein 1 (HYOU1) protein in prostate cancer tissues. (A, B) Prostate cancer tissues show positive immunostaining of cancer cells with antibody HPA012778 to protein phosphatase 3 catalytic subunit alpha (PPP3CA). (C, D) Prostate cancer tissues show positive immunostaining of cancer cells with antibody HPA049296 to the hypoxia upregulated protein 1 (HYOU1) protein.
For example, miR-99a might inhibit cell proliferation through targeting TNFAIP8 in osteosarcoma cells [40]. In breast cancer, miR-99a might inhibit aggressive tumor phenotypes via regulating HOXA1 [41]. However, miR-99a has been shown to promote cell proliferation via targeting FGFR3 in ovarian cancer cells [42].

From the findings of the present study, it might be possible to hypothesize that miR-99a-3p has a role in prostate cancer by targeting the PPP3CA or HYOU1 genes. As previously reported, PPP3CA is a target of miR-145 and thus be involved in caspase-dependent and caspase-independent cell death in urothelial cancer cells [43]. Also, PPP3CA has been shown to have a role in breast cancer, lung cancer, and Wilson's disease [44-46]. The HYOU1 gene has been found to be upregulated in nasopharyngeal carcinoma and may act as a molecular biomarker for the progression and prognosis of this type of cancer [47]. Also, the expression of the HYOU1 gene is associated with lymph node involvement in colorectal cancer [48]. However, the findings of the present study are preliminary, and the roles of the PPP3CA and HYOU1 genes in prostate cancer should be confirmed by both further functional and clinical studies.

Conclusions

The findings of this study showed that in patients with prostate cancer, expression of microRNA-99a-3p (miR-99a-3p) was associated with the Wnt and vascular endothelial growth factor (VEGF) signaling pathways and inhibited the expression of the protein phosphatase 3 catalytic subunit alpha (PPP3CA) gene and the hypoxia upregulated protein 1 (HYOU1) gene. The Wnt signaling pathway has been previously reported to be associated with metastasis, proliferation, apoptosis, and the cell cycle in prostate cancer [49–51]. In previously published studies, the VEGF signaling pathway has been shown to be associated with tumor angiogenesis and tumor progression [52–55]. Therefore, from the findings of this study, miR-99a-3p might have a role in prostate cancer by targeting the PPP3CA or HYOU1 and contributing to the Wnt and VEGF signaling pathways. However, these preliminary findings require further molecular and functional studies and large-scale controlled clinical studies to determine the role of microRNAs, including miR-99a-3p, in prostate cancer.

Acknowledgments

The authors thank The Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO), and ArrayExpress for providing the data.

Conflict of interest

None.
References:

1. Cheerla N, Gevaert O: MicroRNA based pan-cancer diagnosis and treatment recommendation. BMC Bioinformatics, 2017; 18: 32
2. Sekhon K, Bucay N, Majid S et al: MicroRNAs and epithelial-mesenchymal transition in prostate cancer. Oncotarget, 2016; 7: 67597–611
3. Janiczek M, Syrzycki L, Kasperska A et al: Immunotherapy as a promising treatment for prostate cancer: A systematic review. J Immunol Res, 2017; 2017: 4861570
4. Wadosky KM, Koekchepour S: Molecular mechanisms underlying resistance to androgen deprivation therapy in prostate cancer. Oncotarget, 2016; 7: 64447–70
5. Zhou P, Ma L, Zhou J et al: miR-17-92 plays an oncogenic role and conveys chemosensitivity to cisplatin in human prostate cancer cells. Int J Urol, 2016; 48: 1737–48
6. Liu J, Chen Z, Wang T et al: Influence of four radioisotopes in PET/CT on diagnostic accuracy for prostate cancer: a bivariate random-effects meta-analysis. Cell Physiol Biochem, 2016; 39: 467–80
7. Storm M, Sheng X, Arnoldussen S, Yaatzigou F: Prostate cancer and the unfolded protein response. Oncotarget, 2016; 7: 54051–66
8. Park SH, Keller ET, Shiozawa Y: Bone marrow microenvironment as a regulator and therapeutic target for prostate cancer bone metastasis. Calcif Tissue Int, 2018; 102(2): 152–62
9. Deng G, Zheng X, Jiang P et al: Notch1 suppresses prostate cancer cell invasion via the metastasis-associated 1-KISS-1 metastasis-suppressor pathway. Oncol Lett, 2017; 14: 4477–82
10. Mao Y, Xu X, Wang X et al: Is angiotensin-converting enzyme inhibitors/angiotensin receptor blockers therapy protective against prostate cancer? Oncotarget, 2016; 7: 7655–73
11. Heinemann FM, Jindra PT, Bockmeyer CL et al: Glomerulocapillary miRNA response to HLA-I class I antibody in vitro and in vivo. Sci Reports, 2017; 7: 14554
12. Santos PRB, Coutinho-Camilo CM, Soares FA et al: MicroRNAs expression pattern related to mast cell activation and angiogenesis in paraffin-embedded salivary gland tumors. Pathol Pract Res, 2017; 213(12): 1470–76
13. Gao Q, Yao X, Zheng J: MiR-323 inhibits prostate cancer cell proliferation through adiponectin receptor. Cell Physiol Biochem, 2015; 36: 1401–98
14. Zhao S, Han J, Zheng L et al: MiRNA-203 regulates growth and metastasis of breast cancer. Cell Physiol Biochem, 2015; 37: 35–42
15. Chang HY, Ye SP, Pan SL et al: Overexpression of miR-194 reverses HMG2A-driven signatures in colorectal cancer. Theranostics, 2017; 7: 3889–900
16. Ghosh T, Varshney A, Kumar P et al: MicroRNA-874 mediated inhibition of the major G1/S phase cyclin, CCNE1 is lost in osteosarcomas. J Biol Chem, 2017; 292(52): 21264–81
17. Pang C, Liu M, Fang W et al: MiR-139-5p is increased in the peripheral blood of patients with prostate cancer. Cell Physiol Biochem, 2016; 39: 1111–17
18. Li J, Yang X, Guan H et al: Exosome-derived microRNAs contribute to prostate cancer chemoresistance. Int J Oncol, 2016; 49: 838–46
19. Sun D, Lee YS, Malhotra A et al: miR-99a family of microRNAs suppresses the expression of prostate-specific antigen and prostate cancer cell proliferation. Cancer Res, 2011; 71: 1313–24
20. Shen S, Lin Y, Yuan X et al: Biomarker microRNAs for diagnosis, prognosis and treatment of hepatocellular carcinoma: A functional survey and comparison. Sci Rep, 2016; 6: 38311
21. Ji W, Sun S, Su C: Targeting microRNAs in cancer gene therapy. Genes (Basel), 2017; 8(1): pii: E21
22. Molina-Pinoles S, Carenno A, Rivera F et al: MiR-107 and miR-99a-3p predict chemotherapy response in patients with advanced cervical cancer. BMC Cancer, 2014; 14: 656
23. Xiong H, Li Q, Liu S et al: Integrated microRNA and mRNA transcriptome sequencing reveals the potential roles of microRNAs in stage I endometrioid endometrial carcinoma. PLoS One, 2014; 9: e110163
24. Li D, Liu X, Lin L et al: MicroRNA-99a inhibits hepatocellular carcinoma growth and correlates with prognosis of patients with hepatocellular carcinoma. J Biol Chem, 2011; 286: 36677–85
25. Borns PN, Schmidt M, Choonkon G et al: IL-10 and integrin signaling pathways are associated with head and neck cancer progression. BMC Genomics, 2016; 17: 38
26. Li Y, Kang K, Krahn JM et al: A comprehensive genomic pan-cancer classification using The Cancer Genome Atlas gene expression data. BMC Genomics, 2017; 18: 508
27. Zeng JH, Xiong DD, Pang YY et al: Identification of molecular targets for esophageal carcinoma diagnosis using miRNA-seq and RNA-seq data from The Cancer Genome Atlas: A study of 187 cases. Oncotarget, 2017; 8: 35681–99
28. Ferreira MJ, Pires-Luis AS, Vieira-Coimbra M et al: SETDB2 and RIOX2 are differentially expressed among renal cell tumor subtypes, associating with prognosis and metastatization. Epigenetics, 2017; 12(12):1057–64
29. Zeng Y, Wang T, Liu Y et al: LncRNA PVT1 as an effective biomarker for cancer diagnosis and detection based on transcriptome data and meta-analysis. Oncotarget, 2017; 8: 75455–66
30. Mou T, Zhu D, Wei X et al: Identification and interaction analysis of key genes and microRNAs in hepatocellular carcinoma by bioinformatics analysis. World J Surg Oncol, 2017; 15: 63
31. Ge QM, Huang CM, Zhu XY et al: Differentially expressed miRNAs in sepsis-induced acute kidney injury target oxidative stress and mitochondrial dysfunction pathways. PLoS One, 2017; 12: e0173292
32. Wang X, Li Y, Xu G et al: Mechanism study of peptide GMP130 and its receptor CRP78 in modulating gastric cancer MDR by iTRAQ-based proteomic analysis. BMC Cancer, 2015; 15: 358
33. Liu D, Liu P, Cao L et al: Screening the key genes of hepatocellular adenoma via microarray analysis of DNA expression and methylation profiles. Oncol Lett, 2017; 14: 3975–80
34. Liao J, Wei B, Chen H et al: Bioinformatics investigation of therapeutic mechanisms of Xesaliotong capsule treating ischemic cerebrovascular rat model with comparative transcriptome analysis. Am J Transl Res, 2016; 8: 2438–49
35. Zhang Y, He RQ, Dang YW et al: Comprehensive analysis of the long non-coding RNA HOXA1-AS gene interaction regulatory network in NSCLC cells. Cancer Cell Int, 2016; 16: 89
36. Franceschini A, Szklarczyk D, Frankish S et al: STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res, 2013; 41: D808–15
37. Dai Y, Jiang JB, Wang YL et al: Functional and protein-protein interaction network analysis of colorectal cancer induced by ulcerative colitis. Mol Med Rep, 2015; 12: 4947–58
38. Friard Q, Re A, Taverna D et al: CircuItDB: a database of mixed microRNA/ transcription factor binding-forward regulatory circuits in human and mouse. BMC Bioinformatics, 2010; 11: 435
39. Rane JK, Erb HH, Nappo G et al: Inhibition of the glucocorticoid receptor results in an enhanced miR-99a/100-mediated radiation response in stem-like cells from human prostate cancers. Oncotarget, 2016; 7: 51965–80
40. Xing B, Ren C: Tumor-suppressive miR-99a inhibits cell proliferation via targeting of TNFAIP8 in osteosarcoma cells. Am J Transl Res, 2016; 8: 1082–90
41. Wang X, Li Y, Qi W et al: MicroRNA-99a inhibits tumor aggressiveness through regulating adiponectin receptor. Cell Physiol Biochem, 2015; 36: 1401–98
42. Jiang H, Qu L, Wang Y et al: microRNA-99a promotes proliferation targeting FGFR3 in colorectal cancer. Metallomics, 2013; 5: 532–40
43. Ostenfeld MS, Bramsen JB, Lamy P et al: miR-145 induces caspase-dependent and -independent cell death in urothelial cancer cell lines with targeting of an expression signature present in Ta bladder tumors. Oncogene, 2010; 29: 1073–84
44. Gabrovská PN, Smith RA, Haupt LM, Griffiths LR: Investigation of two Wnt signalling pathway single nucleotide polymorphisms in a breast cancer-affected Australian population. Twin Res Hum Genet, 2011; 14: 562–67
45. Lee BH, Kim JH, Kim JM et al: The early molecular processes underlying the neurological manifestations of an animal model of Wilson’s disease. Metallomics, 2015; 7: 532–40
46. Campbell ID, Alexandrov A, Kim IJ et al: Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. Nat Genet, 2016; 48: 607–16
47. Zhou Y, Liao Q, Li X et al: HYOU1, regulated by LPLUNC1, is up-regulated in nasopharyngeal carcinoma and associated with poor prognosis. J Cancer, 2016; 7: 367–76

This work is licensed under Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).
48. Slaby O, Sobkova K, Svoboda M et al: Significant overexpression of Hsp110 gene during colorectal cancer progression. Oncol Rep, 2009; 21: 1235–41
49. Wang L, Dehm SM, Hillman DW et al. A prospective genome-wide study of prostate cancer metastases reveals association of Wnt pathway activation and increased cell cycle proliferation with primary resistance to abiraterone acetate-prednisone. Ann Oncol, 2018; 29(2): 352–80
50. Shin S, Im HJ, Kwon YJ et al: Human steroid sulfatase induces Wnt/beta-catenin signaling and epithelial-mesenchymal transition by upregulating Twist1 and HIF-1alpha in human prostate and cervical cancer cells. Oncotarget, 2017; 8: 61604–17
51. Zheng Y, Trivedi T, Lin RC et al: Loss of the vitamin D receptor in human breast and prostate cancers strongly induces cell apoptosis through down-regulation of Wnt/beta-catenin signaling. Bone Res, 2017; 5: 17023
52. Liu L, Liang Z, Guo K, Wang H: Relationship between the expression of CD133, HIF-1alpha, VEGF and the proliferation and apoptosis in hypoxic human prostate cancer cells. Oncol Lett, 2017; 14: 4065–68
53. Zhang W, Shou WD, Xu YJ et al: Low-frequency ultrasound-induced VEGF suppression and synergy with dendritic cell-mediated anti-tumor immunity in murine prostate cancer cells in vivo. Sci Rep, 2017; 7: 5778
54. Alshaker H, Wang Q, Bohler T et al: Combination of RAD001 (everolimus) and docetaxel reduces prostate and breast cancer cell VEGF production and tumour vascularisation independently of sphingosine-kinase-1. Sci Rep, 2017; 7: 3493
55. Terzuoli E, Donnini S, Finetti F et al: Linking microsomal prostaglandin E Synthase-1/PGE-2 pathway with miR-15a and -186 expression: Novel mechanism of VEGF modulation in prostate cancer. Oncotarget, 2016; 7: 44350–64