Comparison of miRNA and gene expression profiles between metastatic and primary prostate cancer

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Abstract. The present study aimed to identify the regulatory mechanisms associated with the metastasis of prostate cancer (PC). The microRNA (miRNA/miR) microarray dataset GSE21036 and gene transcript dataset GSE21034 were downloaded from the Gene Expression Omnibus database. Following pre-processing, differentially expressed miRNAs (DEMs) and differentially expressed genes (DEGs) between samples from patients with primary prostate cancer (PPC) and metastatic prostate cancer (MPC) with $|\log_2$ fold change (FC)| >1 and a false discovery rate <0.05 were selected using the Linear Models for Microarray and RNA-seq Data 4 package of R. Next, a DEM-DEG regulatory network was constructed by downloading miRNA-DEG pairs from the miRNA.org database. Finally, functional annotation of each DEM-DEG module was performed using the Database for Annotation, Visualization and Integrated Discovery based on the Gene Ontology database. The upregulated miRNAs, including miR-144, miR-494 and miR-181a, exhibited a higher degree of connections compared with other nodes, including in the DEM-DEG regulatory network, and regulated a number of downregulated DEGs. According to the functional annotation of the DEM-DEG modules, miR-144 and its targeted DEGs enriched the highest number of biological process terms (36 terms), followed by miR-494 (24 terms), miR-30d (18 terms), miR-181a (15 terms), hsa-miR-196a (8 terms), miR-708 (7 terms) and miR-486-5p (2 terms). Therefore, these miRNAs may serve roles in the metastasis of PC cells via downregulation of their corresponding target DEGs.

Introduction

Prostate cancer (PC) is the most common cancer in men and the second leading cause of cancer-associated mortality in USA (1). PC is challenging to treat due to its high metastasis rate (2); despite considerable advances in the treatment of PC, metastatic prostate cancer (MPC) remains incurable (3). The molecular mechanisms underlying the metastasis of PC cells remain largely unknown and require further study.

MicroRNAs (miRNAs/miRs) are a group of non-coding RNAs of 17-27 nucleotides in length that regulate gene expression by binding to the 3'untranslated regions of messenger RNAs (mRNAs) (4). miRNAs have been demonstrated to serve important roles in a number of cellular processes as post-transcriptional regulators, in addition to roles in cancer development and progression (5,6). Dysregulation of miRNAs has been demonstrated to contribute to tumorigenesis by stimulating proliferation, angiogenesis and invasion (7,8). Previous studies have investigated miRNA expression profiles in primary prostate cancer (PPC) or MPC and several miRNAs have been suggested as diagnostic markers for PC (9,10). However, the molecular mechanisms underlying the roles of miRNAs and their target differentially expressed genes (DEGs) in PC metastasis remain unclear.

Based on 218 prostate tumor samples (181 primaries and 37 metastases), Taylor *et al* (11) conducted an integrated analysis (including concordant assessment of DNA copy number, mRNA expression and focused exon resequencing), and revealed that nuclear receptor coactivator *NCOA2* functions as an oncogene in ~11% of PC tumors, and *FOXP*, *RYBP* and *SHQ1* serve as potential cooperative tumor suppressors in human PC. Using the same microarray datasets, the aim of the present study was to identify miRNAs and DEGs that are associated with the metastasis of PC cells by screening miRNAs and genes that are differentially expressed between MPC and PPC samples, with the objective to further understand the molecular mechanisms of MPC.

Materials and methods

Source of microarray data. The raw microarray datasets GSE21036 and GSE21034 were downloaded from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) database. The miRNA expression dataset GSE21036 was collected from 141 patients with PC treated by radical prostatectomy, including 14 metastatic samples, 99 primary non-metastatic tumor samples and 28 normal adjacent benign prostate samples. The annotation platform was the Agilent-019118 Human miRNA Microarray 2.0 G4470B (miRNA ID version)

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Table I. Microarray datasets used from the Gene Expression Omnibus database, and the proportions of metastatic, primary and normal samples in each dataset.

Microarray dataset	Metastatic	Primary	Normal	Total
miRNA (GSE21036)	14	99	28	141
Transcript (GSE21034)	19	131	29	179
miRNA and transcript (common to GSE21036 and GSE21034)	13	98	28	139

miRNA, microRNA.

(Agilent Technologies, Inc., Santa Clara, CA, USA). The GSE21034 transcript dataset was collected form 179 samples, including 29 normal samples, 131 primary samples and 19 metastatic samples. The annotation platform was the Affymetrix Human Exon 1.0 ST Array (Affymetrix, Inc., Santa Clara, CA, USA). As illustrated in Table I, 139 samples from datasets of GSE21036 and GSE21034 were overlapped and used for the following analyses.

Microarray data pre-processing. Using the AgiMicroRna Bioconductor library of R (http://bioconductor.org/help/search/index.html?q=AgiMicroRna), the GSE21036 raw data were subject to pre-processing as previously described (12), including removing the probes with a low detection rate (failure in >75% samples), background adjustment and quantile normalization by the Robust Multiarray Averaging (RMA) method (13). The GSE21034 gene transcript data had been pre-processed (by background adjustment and quantile normalization using the RMA method) prior to being downloaded, and were subsequently subjected to $\log_2$-transformation.

Differential expression analysis of miRNA and genes. The differential expression analyses of miRNAs and gene transcripts between patients with MPC and patients with PPC were performed using the Linear Models for Microarray and RNA-Seq Data 4 package of R (14). miRNAs or genes with $|\log_2$ fold change (FC)$|$ >1 and a false discovery rate (FDR) value <0.05 were selected as differentially expressed miRNAs (DEMs) or DEGs. The FDR value was obtained by adjusting the raw P-values with the Benjamini and Hochberg method (15).

Construction of a DEM-DEG regulatory network. First, miRNA-gene pairs predicted by the miRanda method were downloaded from the miRNA.org database (16). Then, miRNA-gene interaction pairs of upregulated DEM to downregulated DEG, and downregulated DEM to upregulated DEG were screened to construct a DEM-DEG regulatory network. Cytoscape (version 3.2.0; http://www.cytoscape.org/release_notes_3_2_0.html) was used to visualize the resulting network (17).

Functional annotation of DEM-DEG modules. In the constructed DEM-DEG network, a miRNA and its target genes were defined as a module. For the DEGs in each module, functional annotation analysis was performed using the Database for Annotation, Visualization and Integrated Discovery online tool based on the Gene Ontology database (P<0.01) (18).

Table II. Overview of differentially expressed microRNAs and genes.

Differential expression	Upregulated	Downregulated	Total
DEM	25	48	73
DEG	22	191	213

DEM, differentially expressed miRNA; DEG, differentially expressed gene.

Results

Differentially expressed miRNA and genes between patients with MPC and PPC. Based on the analytical threshold, DEMs/DEGs between MPC and PPC were screened out. The numbers of DEMs/DEGs between patients with MPC and those with PPC are presented in Table II, in addition to the numbers of up- and downregulated DEMs/DEGs. The number of downregulated DEMs/DEGs was markedly greater compared with the number of upregulated DEM/DEGs identified between patients with MPC and PPC. DEGs with $|\log_2$ FC$|$ >2 are presented in Table III.

DEM-DEG regulatory network. The resulting DEM-DEG regulatory network is illustrated in Fig. 1. The average connection degree of DEM was 12.4 (523/43), and that of DEG was 3.2 (523/166) (Table IV). Compared with that of the downregulated miRNAs, upregulated miRNAs had a higher degree of connection, that is, the latter regulate more downregulated DEGs, including miR-144, miR-494 and miR-181a. Certain DEGs were regulated by several miRNAs, including glutamate ionotropic receptor NMDA type subunit 3A (*GRIN3A*), topoisomerase II alpha (*TOP2A*) and caldesmon 1 (*CALD1*). GRIN3A and TOP2A were upregulated by 14 and 13 downregulated miRNAs, respectively, while *CALD1* was downregulated by 8 upregulated DEMs.

Functional annotation of DEM-DEG modules. Seven modules were obtained (P<0.01), and their enriched biological processes (BPs) are presented in Fig. 2. DEGs regulated

Table III. Differentially expressed genes with $|\log_2 FC| > 2$ between metastatic and primary prostate cancer samples.

Gene transcript	$\log_2$ FC value	Adjusted P-value	Gene transcript	$\log_2$ FC value	Adjusted P-value
TAGLN	-2.4964	1.1987×10^{-31}	*SORBS1*	-2.24347	1.4898×10^{-23}
SLC22A3	-2.0225	5.9268×10^{-10}	*SLC14A1*	-2.0417	4.5910×10^{-12}
SERPINA3	-2.1640	1.0787×10^{-08}	*PI15*	-2.5879	1.0048×10^{-13}
PGM5	-2.1181	5.0371×10^{-23}	*PDE5A*	-2.1878	5.7525×10^{-19}
PCP4	-2.3182	1.0747×10^{-24}	*MYLK*	-2.5515	1.9944×10^{-29}
MYH11	-3.7948	1.5295×10^{-42}	*MYBPC1*	-2.3552	1.8570×10^{-12}
MSMB	-3.1696	1.6542×10^{-15}	*LTF*	-2.5535	4.2645×10^{-07}
IGJ	-2.0615	2.2689×10^{-07}	*PAM3B*	-2.1011	5.5298×10^{-11}
DDP4	-2.4582	1.0125×10^{-12}	*CSRP1*	-2.0686	7.1619×10^{-22}
CNN1	-2.7962	1.4001×10^{-35}	*CHRDL1*	-2.2994	1.2660×10^{-22}
AZGP1	-2.1882	2.8966×10^{-18}	*ACTG2*	-3.2887	3.1041×10^{-33}

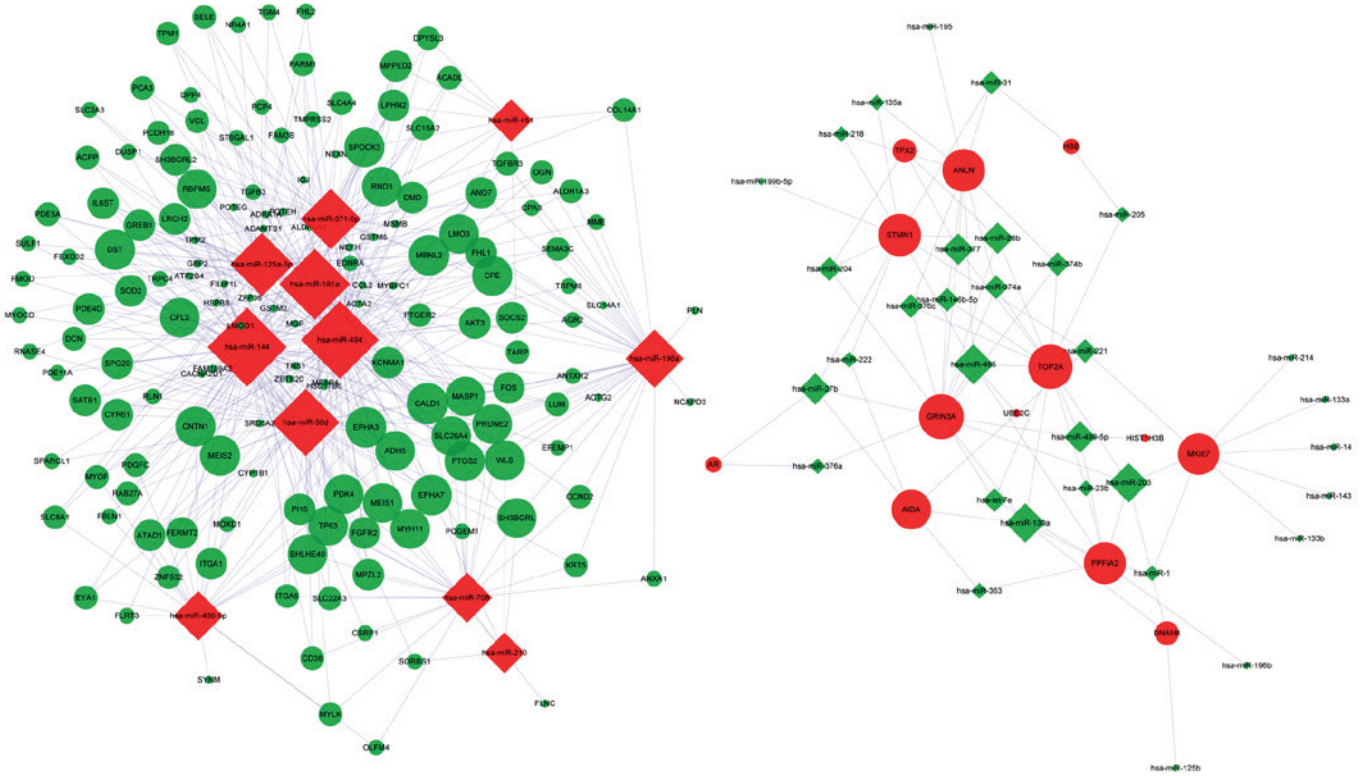

Figure 1. The DEM-DEG regulatory network. The rhombi represent upregulated DEMs; the circles represent DEGs. The green color indicates downregulation of a miRNA or gene; the red color indicates upregulation of a miRNA or gene. DEM, differentially expressed miRNA; DEG, differentially expressed gene; hsa, *Homo sapiens*; miR/miRNA, microRNA.

by miR-144 enriched the most BP terms (36), followed by miR-494 (24), miR-30d (18), miR-181a (15), miR-196a (8), miR-708 (7) and miR-486-5p (2). All these miRNAs were upregulated.

Discussion

In the present study, DEMs and DEGs between patients with PPC and MPC may function as biomarkers for the occurrence of MPC. Furthermore, miRNAs and genes (particularly those with large differential expression values) involved in the seven resulting DEM-DEG regulatory modules may serve significant roles in the metastasis of PC cells.

The target DEGs of miR-144 were enriched in the most BP terms, implying that this integrated analysis of multidimensional data miRNA and its targeted DEGs may perform important roles in the occurrence of MPC. miR-144 has been reported to improve the growth of HeLa cells (19), suggesting its role in tumor cell proliferation. Zhang *et al* (20) have proposed that miR-144 promotes the malignant progression of nasopharyngeal carcinoma cells by targeting the tumor-suppressor gene phosphatase and tensin homolog; however, its role in PC

Table IV. Nodes and regulation pair statistics of the DEM-DEG regulatory network.

	Nodes			
Regulation	DEM	DEG	Edges	Total
Up	11	13	DEM.up-DEG.down	442
Down	32	153	DEM.down-DEG.up	81

DEM, differentially expressed miRNA; DEG, differentially expressed gene; up, upregulated; down, downregulated.

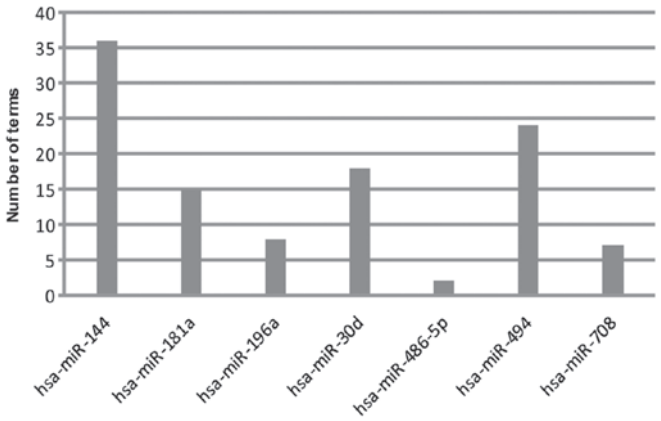

Figure 2. The number of enriched biological process terms of each differentially expressed miRNA-differentially expressed gene module, as analyzed using the Database for Annotation, Visualization and Integrated Discovery online tool. hsa, *Homo sapiens*; miR, microRNA.

has not been reported thus far, to the best of our knowledge. In the present study, numerous DEGs were observed to be downregulated by miR-144. Among them, myosin heavy chain 11 (*MYH11*), solute carrier family 22 member 3 (*SLC22A3*) and dipeptidyl peptidase 4 (*DPP4*) exhibited large $|\log_2 FC|$ values. *MYH11* (also known as *SMMHC*) encodes the smooth muscle myosin heavy chain, which serves a key role in smooth muscle contraction (21). Its downregulation has been reported by Lin *et al* (22) in proliferating smooth muscle cells of human prostate tissue. *SLC22A3* is one of three similar cation transporter genes located in a cluster on chromosome 6 and has previously been suggested to be associated with PC pathogenesis (23). *DPP4* encodes a serine exopeptidase that has been implicated in cell-extracellular matrix interactions and bioactive peptide/cytokine/growth factor metabolism (24,25). *DPP4* activity is elevated in PC and adjacent benign hyperplastic glands (26). Taken together, miR-144 may serve roles in the metastasis of PC cells by downregulating a number of target genes, including *MYH11, SLC22A3* and *DPP4*.

The regulator miR-494 may also serve an important role in the metastasis of PC. *MYH11* was the only target gene with $|\log_2 FC| >2$ among the downregulated DEGs of miR-494 in the present study. In a previous study, using multiple experimental methods, Shen *et al* (27) demonstrated that miRNA-494-3p could suppress the proliferation, invasion and migration of PC by downregulating C-X-C motif chemokine receptor 4 (*CXCR4*), which was overexpressed in PC. This is consistent with the upregulation of this miRNA observed in the present study, although differential expression of *CXCR4* was not observed.

miR-30d downregulated a number of genes in the present study. Kobayashi *et al* (28) observed significantly higher expression levels of miR-30d in three PC cell lines compared with those in two normal prostate cell lines using miRNA microarray and quantitative polymerase chain reaction analysis (28). Furthermore, the authors suggested that miR-30d mediated its effects in PC by downregulating suppressor of cytokine signaling 1. In the present study, among the target DEGs of miR-30d, sorbin and SH3 domain containing 1 (*SORBS1*), phosphodiesterase-5 (*PDE5*) and myosin light chain kinase (*MYLK*) exhibited large $|\log_2 FC|$ values. *SORBS1* encodes a Cbl-associated protein involved in the formation of actin stress fibers and focal adhesions (29). The observed downregulation of *SORBS1* in the present study was consistent with that reported by Vanaja *et al* (30) in PC tissues. *PDE5* encodes an enzyme that hydrolyzes the 3',5'-phosphodiester bond in the second messenger molecule cyclic guanosine monophosphate (GMP) to form 5'-GMP (31). Its expression has been observed in the smooth muscles of the prostate, and *PDE5* inhibitors (such as sildenafil) are able to relax the prostate (32). Therefore, the upregulation of *PDE5* is suggested to be disadvantageous for patients with PC. *MYLK* has been demonstrated to be necessary for the invasiveness of MPC cells (33). However, *PDE5* and *MYLK* were observed to be downregulated in the present study; therefore, further studies are required to elucidate the roles of *PDE5* and *MYLK* in PC metastasis.

In addition to their observed downregulation by miR-144 *MYH11* and *SLC22A3* were downregulated by miR-181a, which was upregulated in the present study. miR-181a has been reported in previous studies to induce apoptosis in a number of cancer types by downregulating the apoptosis regulator B-cell lymphoma 2 (11,34), and it has been observed to mediate bufalin-induced apoptosis in PC-3 PC cells by Zhai *et al* (35). However, the implications of upregulation of miR-181a in MPC samples remains unclear. Additionally, Su *et al* (36) reported that the downregulation of miR-30d and miR-181a in prostate tumors cooperatively suppresses the expression of glucose-regulated protein, 78 kDa (*GRP78*), a major endoplasmic reticulum chaperone and signaling regulator that is typically overexpressed in cancer (37). Differential expression of *GRP78* was not observed in the present study; however, *MYH11* was revealed to be another common target of miR-30d and miR-181a, indicating that miR-30d and miR-181a may cooperate to regulate the metastasis of PC cells.

A previous study suggested that miR-196a regulates homeobox (*Hox*) gene expression during vertebrate embryogenesis (38). In addition, a correlation between aberrant HoxC8 expression and a malignant phenotype in human PC has been reported (39,40). Therefore, it can be inferred that miR-196a may serve a role in the occurrence of MPC. In the present study, one of the target genes of miR-196a, actin, gamma 2 (*ACTG2*), exhibited $|\log_2 FC| >2$, in agreement with the significant downregulation in metastatic and primary tumor samples observed by Chandran *et al* (41). The findings from the present study further suggest that miR-196a may serve a role in the metastasis of PC cells by downregulating *ACTG2*.

Regarding miR-708 and miR-486-5p, no DEGs with a $|\log_2 FC| > 2$ were observed among their target genes in the present study, implying that these two miRNAs do not perform crucial roles in the occurrence of MPC. According to Watahiki *et al* (10), miR-708 exhibited a >5-fold decrease in an MPC cell line following comparative analysis of miRNAs libraries between MPC and PPC cell lines. This was not consistent with the upregulation of miR-708 observed in the present study. Therefore, the expression changes and role of miR-708 in MPC require further investigation. Additionally, Watahiki *et al* (10) also reported that elevated miR-486 level enhanced the invasiveness of MPC cells, which is consistent with its upregulation in the present study.

In conclusion, the significantly upregulated miR-144, miR-494, miR-30d, miR-181a, miR-196a, miR-708 and miR-486-5p screened in the present study may participate in the metastasis of PC cells via the downregulation of their corresponding target DEGs, particularly those with large $|\log_2 FC|$ values, including *MYH11, SLC22A3*, *DPP4*, *SORBS1*, *PDE5*, *MYLK* and *ACTG2*. The effects on these target DEGs require further experimental verification. A number of these DEMs or DEGs have been associated with the occurrence of PC; however, the molecular mechanisms underlying their roles in the occurrence of MPC remain unclear and require further investigation.

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