Investigation of the molecular mechanisms underlying metastasis in prostate cancer by gene expression profiling

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Abstract. The present study aimed to screen potential genes associated with metastatic prostate cancer (PCa), in order to improve the understanding of the mechanisms underlying PCa metastasis. The GSE3325 microarray dataset, which was downloaded from the Gene Expression Omnibus database, consists of seven clinically localized PCa samples, six hormone-refractory metastatic PCa samples and six benign prostate tissue samples. The Linear Models for Microarray Data package was used to identify differentially-expressed genes (DEGs) and a hierarchical cluster analysis for DEGs was performed with the pheatmap package. Furthermore, potential functions for the DEGs were predicted by a functional enrichment analysis. Subsequently, microRNAs (miRNAs) potentially involved in the regulation of PCa metastasis were identified by WebGestalt software, and the miRNA-DEG regulatory network was visualized using Cytoscape. In addition, a pathway enrichment analysis for DEGs in the regulatory network was performed. A total of 306 and 2,073 genes were differentially expressed in the clinically localized PCa and the metastatic PCa groups, respectively, as compared with the benign prostate group, of which 174 were differentially expressed in both groups. A number of the DEGs, including *CAMK2D* and *SH3BP4*, were significantly enriched in the cell cycle, and others, such as *MAF*, were associated with the regulation of cell proliferation. Furthermore, some DEGs (*CAMK2D* and *PCDH17*) were observed to be regulated by miR-30, whereas others (*ADCY2*, *MAF*, *SH3BP4* and *PCDH17*) were modulated by miR-182. Additionally, *ADCY2* and *CAMK2D* were distinctly enriched in the calcium signaling pathway. The present study identified novel DEGs, including *ADCY2*, *CAMK2D*, *MAF*, *SH3BP4* and *PCDH17,* that may be involved in the metastasis of PCa.

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Introduction

Prostate cancer (PCa) is the most common cancer among European and American men, and accounts for 27% (233,000) of cancer incidences in men in the USA (1). It has a high mortality rate as a result of its high propensity for metastasis (2,3). PCa has been shown to preferentially metastasize to the bone marrow stroma of the axial skeleton (4); however, the precise mechanism underlying PCa metastasis is currently unclear. Therefore, the identification of specific metastasis biomarkers and novel diagnostic targets is required in order to improve the prognosis and treatment of the disease.

Previous studies have made considerable progress in identifying the key regulators in the PCa metastatic process. E-cadherin, which is attached to the actin cytoskeleton via intracellular catenin, has been implicated in the process of PCa metastasis; in primary PCa, reduced E-cadherin expression was associated with bone metastasis and a poor prognosis (5). In addition, the expression of the *DLC1* tumor-suppressor gene in metastatic PCa cells has been shown to upregulate the expression of E-cadherin, resulting in the suppression of highly metastatic PCa cell invasion by inhibiting the activity of RhoA-GTP and RhoC-GTP (6). The activation of Rho GTPases is dependent on the downstream Ras protein, which has a major influence on cell signaling (7). Members of the Rho GTPase family are involved in cancer cell motility by regulating actin dynamics and controlling morphological changes (8). A previous study demonstrated that the suppression of the farnesyl and geranyl-geranyl prenylation pathways markedly reduced the migration and motility of PCa cells by inhibiting Ras prenylation and concurrent Rho activation (9). Furthermore, activation of the phosphoinositide 3-kinase/protein kinase B (AKT) signaling pathway has been more frequently observed in resistant and metastatic PCa compared with primary PCa, and thus targeting this signaling pathway may improve the outcome of patients with aggressive PCa (10). Previous studies have reported various genes able to promote PCa tumorigenesis and metastasis, including *CCL2* (11), *SERPINB5* (12), *SRC* (13), *TMPRSS2-ERG* gene fusion and *PCA3* (14). In addition, microRNAs (miRNAs), which are considered to be important regulators of gene expression, have been associated with the development of metastatic PCa. For instance, miR-203 (15), miR-16 (16), miR-205 (17), miR-24 (18),

miR-29a (19) and miR-145 (16) have all been implicated in PCa metastasis.

Varambally *et al* (20) performed an integrative genomic and proteomic analysis of benign prostate and metastatic PCa; they reported 48-64% concordance between protein and transcript levels and demonstrated that proteomic alterations between metastatic and clinically localized PCa, which map concordantly to gene transcripts, can serve as predictors of clinical outcome in PCa as well as other solid tumors. However, to the best of our knowledge, the potential miRNAs involved in metastatic PCa, and the interactions of differentially-expressed genes (DEGs) targeted by miRNAs, have yet to be investigated. Therefore, the present study aimed to further elucidate the molecular mechanisms underlying the metastasis of PCa by analyzing the microarray data of benign prostate, clinically localized and metastatic PCa deposited by Varambally *et al* (20) in the Gene Expression Omnibus (GEO) database. Initially a hierarchical cluster analysis for DEGs was performed, followed by a Gene Ontology (GO) functional enrichment analysis. Furthermore, potential miRNAs in metastatic PCa were identified and a miRNA-DEG regulatory network was constructed. Finally, a pathway enrichment analysis for DEGs in the regulatory network was performed. The results of this bioinformatics analysis may shed light on the molecular mechanisms underlying the metastasis of PCa and provide novel diagnostic biomarkers.

Materials and methods

Affymetrix microarray data. The GSE3325 gene expression profile data (20) was downloaded from the GEO (http://www.ncbi.nlm.nih.gov/geo/) and was based on the GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array platform. A total of 19 human prostate tissue samples were available for further analysis, including seven clinically localized PCa samples, six hormone-refractory metastatic PCa samples and six benign prostate tissue samples.

CEL and probe annotation files were downloaded from GEO, and the gene expression data for all samples were preprocessed via Robust Multichip Averaging background correction, quantile normalization and probe summarization (21) in the affy software package (version 1.34.0; http://bioconductor.org/packages/release/bioc/html/affy.html), as described previously (22).

DEGs screening. The Linear Models for Microarray Data package of R (https://bioconductor.org/packages/release/bioc/html/limma.html) was used to identify genes that were differentially expressed in the primary PCa and metastatic PCa groups, as compared with the benign prostate group, as described previously (23). The raw P-value was adjusted according to the false discovery rate (FDR) using the Benjamin and Hochberg method (24). Only genes with a cut-off criteria of $|\log_2 \text{fold change}| > 1$ and FDR<0.01 were considered to be differentially expressed.

Hierarchical cluster analysis for DEGs. Hierarchical clustering is a common method used to determine clusters of similar data points in a multidimensional space (25). The pheatmap package (version 1.0.2; https://cran.r-project.org/web/packages/pheatmap/index.html)was used to perform hierarchical clustering of the DEGs via joint between-within distances, as described previously (26). Expression values from multiple clones or probe sets mapping to the same Unigene Cluster ID were averaged.

GO functional enrichment analysis for DEGs. The Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/) provides a comprehensive set of novel and powerful tools for assigning biological meaning to a set of genes (27). FDR<0.05 was used as the cut-off criterion for GO functional enrichment analysis by DAVID.

Integrated miRNA-DEG regulatory network construction. The common miRNAs in Gene set B, as predicted by the databases of miRecords (http://c1.accurascience.com/miRecords/), TarBase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index) and TargetScan (http://www.targetscan.org/), were selected using WEB-based GEne SeT AnaLysis Toolkit software (update 2013; http://bioinfo.vanderbilt.edu/webgestalt/), and P<0.05 was used as the cut-off criterion. Subsequently, the Search Tool for the Retrieval of Interacting Genes (http://string-db.org/) was used to analyze the interactions between the DEGs targeted by miRNAs by calculating their combined score; a score of >0.4 was set as the cut-off criterion. Finally, the integrated miRNA-DEG regulatory network was visualized using Cytoscape (http://cytoscape.org/).

Pathway enrichment analysis for DEGs in the regulatory network. Pathway enrichment analysis was conducted as described previously (28) to identify significant metabolic pathways for the DEGs. P<0.05 was used as the cut-off criterion for the Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis using DAVID.

Results

Identification of DEGs. Based on the cut-off criteria, 2,727 DEGs were identified for the clinically localized PCa and metastatic PCa groups, of which 306 were differentially expressed in the clinically localized PCa group only (Gene set A). A total of 2,073 genes were differentially expressed in the metastatic PCa group only (Gene set B) and 174 genes were differentially expressed in both groups (Gene set C; Fig. 1), as compared with the benign prostate group.

Hierarchical cluster analysis. An unsupervised hierarchical cluster analysis of the data revealed that the DEGs could be used to accurately classify prostate samples as benign, clinically localized prostate cancer or metastatic disease (Fig. 2).

GO functional enrichment analysis for Gene sets A, B and C. In Gene set A, *DLX2*, *DLX1*, *HOXD10* and *HOXD11* DEGs were associated with proximal/distal pattern formation (FDR=3.55E-04), whereas *RBP4*, *PDE3B* and *PPARG* were implicated in the response to insulin (FDR=7.8400), homeostatic processes (FDR=9.6200), chemical homeostasis (FDR=0.0019) and responses to peptide hormones (FDR=0.0023) and organic substances (FDR=0.0029) (Table I).

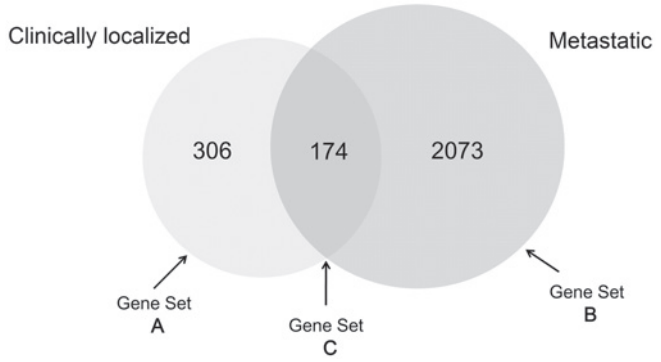

Figure 1. Venn diagram for the differentially expressed genes in the clinically localized and metastatic prostate cancer groups. Gene set A represents the genes only differentially expressed in the clinically localized prostate cancer group; Gene set B represents the genes only differentially expressed in the metastatic prostate cancer group; Gene set C represents the genes differentially expressed in both groups.

In Gene set B, the DEGs were predominantly associated with the cell cycle: *PRC1*, *ZAK*, *PTTG1*, *TGFB2*, *CDCA8*, *CDC6* and *CENPF* were associated with the cell cycle process (FDR=5.2300); *PRC1*, *PTTG1*, *CCNE1*, *CDCA2* and *CDC6* were involved in cell division (FDR=4.6100); and *HRAS*, *CD38*, *IL6ST*, *PDGFA*, *TP63*, *MAF* and *TGFB3* were associated with the regulation of cell proliferation (FDR=0.0012) (Table I).

In Gene set C, the DEGs were also predominantly associated with the cell cycle. *DLGAP5*, *SGOL1*, *NUSAP1*, *PBK*, *BIRC5* and *CCNB1* were associated with the cell cycle process (FDR=3.3000), M phase (FDR=2.7400), mitosis (FDR=4.5000) and organelle fission (FDR=6.3000), whereas *SH3BP4*, *KIF2C*, *CCNB2*, *CENPA* and *CAMK2D* were associated with the cell cycle only (FDR=1.1800) (Table I).

Analysis of the miRNA-DEG regulatory network. A total of 10 miRNAs were identified in Gene set B, including miR-374, miR-128, miR-182, miR-30, miR-302c and miR-524. Notably, miR-30 targeted the majority of the DEGs (11 DEGs, including *CAMK2D*, *PCDH17*, *EDNRB*, *KCNJ3* and *SOX4*), and miR-182 targeted seven DEGs, including *EDNRB*, *MAF*, *ADCY2*, *PCDH17*, *RET*, *SH3BP4* and *BCL11A* (Table II).

The miRNA-DEG regulatory network in Fig. 3 contained 10 miRNAs and 43 corresponding DEGs. *ADCY2* was regulated by miR-128, miR-34B and miR-182; *EDNRB* was regulated by miR-30, miR-182 and miR-302C; *CAMK2D* was regulated by miR-30; *PCDH17* was modulated by miR-217, miR-30, miR-182 and miR-524; *SH3BP4* was modulated by miR-182; and *MAF* interacted with miR-182, miR-302c and *BCL11A*.

Pathway enrichment analysis for the DEGs in the regulatory network. The DEGs in the regulatory network were enriched in two pathways, including the calcium signaling pathway (*EDNRB*, *ADCY2* and *CAMK2D*), and thyroid cancer (*RET* and *MYC*; Table III).

Discussion

The present study identified 306 and 2,073 genes that were differentially expressed in the clinically localized PCa group and the metastatic PCa group, respectively, as compared with the benign prostate group. Of these, 174 genes were differentially expressed in both the clinically localized PCa and metastatic PCa groups.

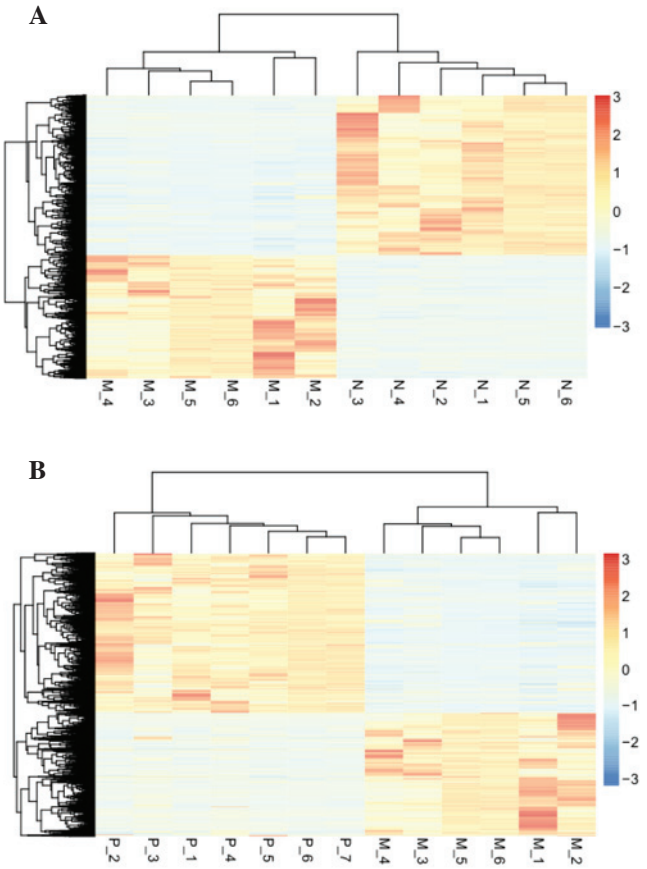

Figure 2. Hierarchical cluster analysis for the genes differentially expressed between the (A) metastatic prostate cancer and the benign prostate groups, and (B) the metastatic and clinically localized prostate cancer groups. Each row represents a single gene; each column represents a tissue sample. M represents the metastatic prostate cancer group; N represents the benign prostate group; P represents the clinically localized prostate cancer group. The gradual color change from orange to blue represents the changing process from upregulation to downregulation.

ADCY2, which encodes adenylate cyclase 2, and *CAMK2D*, which encodes calcium/calmodulin-dependent protein kinase II δ (29,30), were shown to be enriched in the calcium signaling pathway. Metastasis is the predominant cause of mortality in patients with PCa, and Ca^{2+} is a crucial regulator of cell migration (31). Elevated intracellular concentrations of Ca^{2+} may facilitate the metastasis of PCa by triggering the activation of the Akt signaling pathway and promoting PCa cell (PC3) attachment (32). *CAMK2D* encodes components of the Wnt/β-catenin-signaling pathway, the inhibition of which delays metastatic PCa cell cycle progression and proliferation (33). In the present study, *CAMK2D* was associated with the cell cycle, which is known to be a critical event in tumor growth and metastasis (34). Furthermore, *CAMK2D* was observed to be regulated by miR-30. As a tumor suppressor, miR-30 has been shown to be downregulated by oncogenic signals, such as hepatocyte growth factor and epidermal growth factor, in PCa samples (35), and overexpression of miR-30 in PCa cells was

Table I. Enriched terms for Gene sets A, B and C.

Category	Term	No. of genes	FDR	Genes
Gene set A	GO:0009954~proximal/distal pattern formation	5	3.5500	*DLX2, DLX1, GREM1, HOXD10, HOXD11*
	GO:0032868~response to insulin stimulus	8	7.8400	*RBP4, EIF4EBP1, FADS1, PPARG, PDE3B, STXBP4, GAL, VLDLR*
	GO:0042592~homeostatic process	24	9.6200	*RBP4, SLC12A2, PPARG, F2RL1, PRDX4, PDE3B, CACNG2, ITPR3, PPARGC1A, MUC6...*
	GO:0001501~skeletal system development	14	0.0011	*RBP4, HOXD10, HOXD11, MSX2, DLX2, DLX1, COL9A2, BCL2, CLEC3A, NAB1...*
	GO:0048878~chemical homeostasis	18	0.0019	*RBP4, F2RL1, NOX1, PPARG, PDE3B, PPARGC1A, PRKCB, CCL11, MALL, ATP7B...*
	GO:0021877~forebrain neuron fate commitment	3	0.0022	*DLX2, DLX1, LHX6*
	GO:0043434~response to peptide hormone stimulus	9	0.0023	*RBP4, EIF4EBP1, FADS1, BCL2, PPARG, PDE3B, STXBP4, GAL, VLDLR*
	GO:0010033~response to organic substance	22	0.0029	*RBP4, ADCY1, GNRH1, FADS1, LOC646626, PPARG, PTGS1, PDE3B, COLEC12, STXBP4...*
	GO:0009725~response to hormone stimulus	14	0.0038	*RBP4, ADCY1, GNRH1, FADS1, PTGS1, PPARG, PDE3B, STXBP4, GAL, EIF4EBP1...*
	GO:0034637~cellular carbohydrate biosynthetic process	6	60.0038	*RBP4, ISYNA1, UAP1, GNE, PPARGC1A, ACN9*
Gene set B	GO:0022402~cell cycle process	102	5.2300	*PRC1, ZAK, AIF1, BTRC, CDCA8, CDC6, CENPF, PTTG1, AURKB, TGFB2...*
	GO:0051726~regulation of cell cycle	68	1.1100	E2F2, *PTGS2, ZAK, FAM175A, PKMYT1, PDCD4, PTEN, GTSE1, TGFB2, MYC...*
	GO:0007049~cell cycle	128	1.8200	*ZAK, PRC1, AIF1, BTRC, PKMYT1, RBM7, AURKA, AURKB, PTTG1, TGFB2...*
	GO:0051301~cell division	61	4.6100	*PRC1, PTTG1, CCNE1, CDCA2, CDC6, CABLES2, CDCA5, CCNA2, ASPM, CDK1...*
	GO:0022403~cell cycle phase	78	4.7700	*E2F1, PRC1, PKMYT1, RBM7, AURKA, AURKB, PTTG1, GTSE1, CCNE1, CDCA8...*
	GO:0010035~response to inorganic substance	47	6.4600	*CAV1, GCLC, PTGS2, PDGFA, SNCA, TPM1, PTEN, KCNMB1, FOS, GSN...*
	GO:0007346~regulation of mitotic cell cycle	38	0.0011	*CAV2, HOXA13, PML, PKMYT1, ASNS, ANLN, ZNF655, RCC1, SCRIB, MYC...*
	GO:0042127~regulation of cell proliferation	126	0.0012	*HRAS, CD38, IL6ST, PDGFA, TP63, MAF, TGFB3, STRN, PNP, TGFB2...*

Table I. Continued.

Category	Term	No. of genes	FDR	Genes
	GO:0030030~cell projection organization	70	0.0015	*CAV2, HOXA13, PML, PKMYT1, ANLN, ZNF655, RCC1, SCRIB, GTSE1, MYC*...
	GO:0000278~mitotic cell cycle	70	0.0018	*E2F1, PRC1, BTRC, PKMYT1, AURKA, AURKB, PTTG1, GTSE1, CCNE1, NDE1*...
Gene set C	GO:0022402~cell cycle process	21	3.3000	*MKI67, DLGAP5, SGOL1, NUSAP1, BIRC5, PBK, CDKN3, CCNB1, CENPA, CAMK2D*...
	GO:0022403~cell cycle phase	18	3.5700	*MKI67, DLGAP5, SGOL1, NUSAP1, TTK, BIRC5, PBK, CCNB1, CAMK2D, ID4*...
	GO:0000278~mitotic cell cycle	17	4.0700	*DLGAP5, SGOL1, NUSAP1, TTK, BIRC5, PBK, CDKN3, CCNB1, CENPA, CAMK2D*...
	GO:0000279~M phase	15	2.7400	*MKI67, DLGAP5, SGOL1, NUSAP1, TTK, BIRC5, PBK, UBE2C, CCNB1, KIF2C*...
	GO:0007049~cell cycle	22	1.1800	*DLGAP5, SGOL1, NUSAP1, BIRC5, PBK, CCNB1, SH3BP4, KIF2C, CENPA, CAMK2D*...
	GO:0000280~nuclear division	11	4.5000	*CCNB1, KIF2C, CCNB2, DLGAP5, SGOL1, NUSAP1, BIRC5, PBK, UBE2C, ERCC6L*...
	GO:0007067~mitosis	11	4.5000	*CCNB1, KIF2C, CCNB2, DLGAP5, SGOL1, NUSAP1, BIRC5, PBK, UBE2C, ERCC6L*...
	GO:0000087~M phase of mitotic cell cycle	11	5.2300	*CCNB1, KIF2C, CCNB2, DLGAP5, SGOL1, NUSAP1, BIRC5, PBK, UBE2C, ERCC6L*...
	GO:0048285~organelle fission	11	6.3000	*CCNB1, KIF2C, CCNB2, DLGAP5, SGOL1, NUSAP1, BIRC5, PBK, UBE2C, ERCC6L*...
	GO:0007346~regulation of mitotic cell cycle	9	9.6800	*DLGAP5, CAMK2D, NUSAP1, TTK, BIRC5, AFAP1L2, GAS1, UBE2C, MYC*

Gene set A represents the genes only differentially expressed in the clinically localized prostate cancer group; Gene set B represents the genes only differentially expressed in the metastatic prostate cancer group; Gene set C represents the genes differentially expressed in both groups. FDR, false discovery rate.

Table II. Enriched microRNAs in Gene set B.

microRNA	P-value	Count	Genes targeted by microRNA
hsa_TATTATA, MIR-374	2.1100	10	*RORB*, *HOMER1*, *KIF20A*, *SYBU*, *DACH1*, *GATA3*, *ARHGAP28*, *AFAP1L2*, *SOX4*, *SYT1*
hsa_CACTGTG, MIR-128	0.0003	9	*RORB, FBLN2, ADCY2, ACOT11, INSM1, SYT1, FOXQ1, MME, BCL11A*
hsa_ATGCAGT, MIR-217	0.0003	6	*STX1A, MAF, PCDH17, DACH1, EZH2, BCL11A*
hsa_TGTTTAC, MIR-30	0.0005	11	*SOBP, CAMK2D, COL13A1, SLC36A1, PCDH17, AFAP1L2, EDNRB, KCNJ3, SOX4, MATR3*…..…
hsa_ACAACTT, MIR-382	0.0032	4	*NDRG2, SYT1, MATR3, DACH1*
hsa_ACTGCCT, MIR-34B	0.0032	6	*INSM1, SOX4, MYC, ADCY2, PIEZO2, JAKMIP1*
hsa_TTGCCAA, MIR-182	0.0036	7	*EDNRB, MAF, ADCY2, PCDH17, RET, SH3BP4, BCL11A*
hsa_CTTTGTA, MIR-524	0.0036	8	*SOBP, CTHRC1, PCDH17, ECT2, ID4, RCAN2, HOXD13, SOX4*
hsa_ATGTTAA, MIR-302C	0.0038	6	*EDNRB, SALL3, MAF, MATR3, DACH1, BCL11A*
hsa_TGCACTT, MIR-519	0.0038	8	*SOBP, RORB, SYBU, NETO2, SOX4, SYT1, APCDD1, JAKMIP1*

Count represents the number of differentially-expressed genes targeted by microRNA. Gene set B represents the genes only differentially expressed in the metastatic prostate cancer group.

Table III. Enriched pathways for the differentially-expressed genes in the regulatory network.

Term	Description	Count	P-value	Genes
hsa04020	Calcium signaling pathway	3	0.02231	*EDNRB, ADCY2, CAMK2D*
hsa05216	Thyroid cancer	2	0.03927	*RET, MYC*

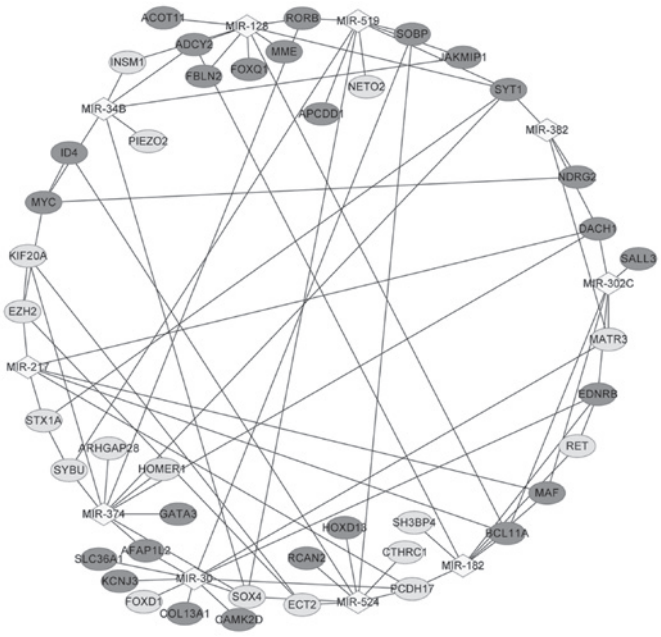

Figure 3. Regulatory network containing microRNAs and their corresponding differentially expressed genes for metastatic prostate cancer. Dark grey nodes represent upregulated genes; light grey nodes represent downregulated genes; and diamonds represent microRNAs.

reported to suppress the epithelial-to-mesenchymal transition and inhibit cell migration and invasion (36).

ADCY2 was observed to be modulated by miR-182. A previous study demonstrated that ectopic expression of miR-182 in PC3 significantly reduced protein expression levels of GNA13, GNA13-3'-untranslated region (UTR)-reporter activity and extracorporeal invasion of these cells (37). In addition, aberrant overexpression of miR-182 was shown to promote the proliferation, increase the invasion, facilitate the G1/S cell cycle transition and reduce early apoptosis of PC3 cells; and, miR-182 was able to suppress the expression of the *NDRG1* tumor suppressor gene by directly targeting the *NDRG1* 3'-UTR (38). Therefore, *CAMK2D* and *ADCY2* may be involved in the metastasis of PCa via calcium signaling and regulation by miR-30 and miR-182, respectively.

MAF, which was also modulated by miR-182 in the present study, was associated with the regulation of cell proliferation. MAF acts as a macrophage-activating factor and is generated from a precursor protein termed the Gc protein (39). Deglycosylation of the Gc protein prevents its conversion to MAF, inhibiting macrophage activation and resulting in immunosuppression (40). In a previous study, patients with metastatic PCa were administered Gc protein with MAF precursor activity (100 ng/week), and were shown to have serum activity

levels of Nagalase equivalent to those of healthy controls, thus suggesting that these patients were tumor-free (41). Furthermore, *MAF* expression has been associated with the receptor tyrosine kinase, platelet-derived growth factor receptor (PDGFR)-β status (42). In the miRNA-DEG regulatory network, *MAF* was also modulated by miR-302c, and it has been reported that miR-302c is downregulated in clinical PCa samples (43). In addition, *MAF* interacted with *BCL11A*, which was observed to be upregulated in PC3 holoclones (44). Therefore, *MAF* may have an important role in the metastasis of PCa by interacting with miR-182, miR-302c and *BCL11A*.

In the present study, the downregulated DEG *SH3BP4* was shown to be associated with the cell cycle and was also regulated by miR-182. *SH3BP4* encodes SH3-domain binding protein 4 (45). SH3 domains are found in a variety of proteins, including tyrosine kinases, such as Abl and Src, and are involved in endocytosis, intracellular sorting and the cell cycle (46). Another downregulated DEG *PCDH17,* which encodes protocadherin 17, was shown to interact with miR-182 and miR-30. *PCDH17* methylation is a common tumor-specific event in PCa and has been associated with a shorter biochemical recurrence-free survival rate and a reduced overall survival rate of patients with PCa following a radical prostatectomy (47). Therefore, *SH3BP4* and *PCDH17* may be responsible for the metastasis of PCa via their interactions with miR-182 and/or miR-30. Furthermore, miR-374 was significantly enriched in Gene set B. Previous studies have reported that miR-374 is markedly downregulated in PCa (48,49). Furthermore, miR-374b, which is a subtype of miR-374, has been shown to be downregulated in prostate fluid or serum samples from prostate cancer patients, and thus may serve as a PCa biomarker in clinical diagnosis (50).

In conclusion, the present study identified numerous important DEGs, including *ADCY2*, *CAMK2D*, *MAF*, *SH3BP4* and *PCDH17,* that may be involved in the metastasis of PCa. However, the results of the present study require validation by further experiments, and the molecular mechanisms underlying metastatic PCa require further investigation.

Acknowledgements

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