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Original Article

Analysis of Differential Expression of microRNAs and Their Target Genes in Prostate Cancer: A Bioinformatics Study on Microarray Gene Expression Data

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Early diagnosis of prostate cancer (PCa) as the second most common cancer in men is not associated with precise and specific results. Thus, alternate methods with high specificity and sensitivity are needed for accurate and timely detection of PCa. MicroRNAs regulate the molecular pathways involved in cancer by targeting multiple genes. The aberrant expression of the microRNAs has been reported in different cancer types including PCa. In this bioinformatics study, we studied differential expression profiles of microRNAs and their target genes in four PCa gene expression omnibus (GEO) databases. PCa diagnostic biomarker candidates were investigated using bioinformatics tools for analysis of gene expression data, microRNA target prediction, pathway and GO annotation, as well as ROC curves. The results of this study revealed significant changes in the expression of 14 microRNAs and 40 relevant target genes, which ultimately composed four combination panels (miR- 375+96+663/ miR- 133b+143- 3p + 205/ *C2ORF72 + ENTPD5 + GLYAT11/LAMB3 + NTNG2+TSLP*) as candidate biomarkers capable to distinguish between PCa tumor samples and normal prostate tissue samples. These biomarkers may be suggested for a more accurate early diagnosis of PCa patients along with current diagnostic tests.

Key words: Prostate cancer, differential expression, microRNA, gene, biomarker, bioinformatics

Cancer is a complex disease with unknown precise cause, and is considered as a health problem worldwide (1, 2). Prostate cancer (PCa) or prostate adenocarcinoma (PRAD) is a common malignancy in men which displays clinical heterogeneous manifestations ranging from latent to aggressive form (3). Available screening tests for PCa including prostate specific antigen (PSA) and *digital rectal* exam (DRE) have shown low specificity and sensitivity. Thus, alternative tests with higher accuracy are needed for timely diagnosis of PCa (4). Also, because end- stage PCa is resistant to available cancer treatments, novel small molecules either as diagnostic biomarkers or

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therapeutic targets are widely desired (4, 5). PCa displays heterogeneous genetic variations and gene expression profiles. Several molecular signaling pathways including androgen receptor (AR), Wnt and PI3 kinase contribute to the pathogenesis of the disease (6, 7). Dysregulated expression of the key genes in these signaling pathways has been reported in PCa (8). MicroRNAs (miRNAs, miRs) comprise a group of endogenous small non-coding RNAs (ncRNAs) that play a regulatory role in the post-transcriptional level in multiple biological processes such as cell cycle, apoptosis, and angiogenesis (9). Moreover, their regulatory effects have been reported in the molecular signaling pathways involved in the pathogenesis of PCa (10, 11). Given the changes in the expression profile of microRNAs in the clinical samples of patients with cancer compared to normal individuals, it has been posited that the molecular signature of the expression profile of microRNAs can conduce to the diagnosis or determining the prognosis of cancer (12). A bunch of microRNAs (oncomiRs) with effects on tumor suppressor genes have been reported to be up regulated in cancer. Another microRNAs group known as tumor suppository with regulatory effects on oncogenes show decreased expression in cancerous tissues (13). By binding to the 3′ UTR region of target mRNAs, microRNAs regulate the expression of their target genes through degrading or inhibiting the translation of the target mRNA (14). Since the discovery of microRNAs, there have been numerous on-line web resources that provide experimental data on microRNAs studies such as sequencing, expression variations in a variety of cancers and KEGG biological pathways. Computational study in addition to omics analysis can be useful for investigating novel diagnostic or prognostic biomarkers or drug targets. Extensive bioinformatics resources are also available for assessment of microRNAs and their target genes. These websites use computational methods and multiple algorithms for the prediction of microRNAs effects on biological processes (15). Investigating microRNAs/mRNAs in cancers can help better fathom the factors and signaling pathways involved in the pathogenesis of cancers, and introduce new candidate molecules for therapeutic purposes (16). Recently, several studies have been carried out to examine the differential expression of protein coding genes and microRNAs using bioinformatics methods in various cancers (17-20). In a bioinformatics study, Cao et al. reported a five-gene panel as potential diagnostic biomarkers and therapeutic targets in gastric cancer (18). In another study, Hu et al. introduced miR-17 as a diagnostic biomarker in gastric cancer using open access databases and bioinformatics tools (21). Considering the need for accurate and timely diagnostic biomarkers for PCa as well as the possibility of merging the results of several bioinformatics studies, we investigated the differential expression profiles of microRNAs and their target genes through available microarray databases.

Material and methods

Microarray data collection

In this study, the microRNA expression dataset, GSE21036 (22) (99 primary tumors, 14 metastases, 28 normal tissues and 1 cancer cell line) and three mRNA expression datasets GSE69223 (23) (30 match malignant and non- malignant prostate tissue), GSE104749 (24) (8 prostate tissue specimens) and GSE46602 (25) (50 prostate tissue samples) were used from the gene expression omnibus (GEO) database (https: //www. ncbi. nlm.nih. gov/gds/). Data for metastatic samples and cell line were excluded from GSE21036 so that only PCa patients' samples and their relevant normal samples were used for further analysis. More details of the datasets used in the present study are shown in Table 1.

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Table 1. Datasets used to investigating of differential expression genes and microRNAs**.**

GEO Accession Dataset	microRNAs/ mRNAs	Number of tumors	Number of controls (normal/BPH)	Contributors (References)	Platforms	Year
GSE46602	mRNAs	36	14	Mortense et al.(25)	Affymetrix Human Genome U133 Plus 2.0 Array (GPL570)	2015
GSE69223	mRNAs	15	15	Meller et al. (23)	Affymetrix Human Genome U133 Plus 2.0 Array (GPL570)	2015
GSE104749	mRNAs	4	4	Shan et al.(24)	Affymetrix Human Genome U133 Plus 2.0 Array (GPL570)	2017
GSE21036	microRNAs	99	28	Taylor et al.(22)	Agilent-019118 Human miRNA Microarray 2.0 G4470B (GPL8227)	2010

Microarray data pre-processing and evaluation of differential expression microRNAs/ genes

Differential expression analysis of microRNA (DEM)/gene (DEG) on tumor and control samples was performed using Limma package in R 3.5.0 statistical program.

Since four different microarray datasets were used in this analysis, we employed the ComBat function in SVA (surrogate variable analysis) package to remove batch effects (early merging) (26). Statistically, significant expression changes were selected by applying adj p values (Benjamini – Hochberg method) (27) of less than 0.05, and $|\log_2 FC| \geq 1$. The gene targets of deregulated microRNAs were searched in the predicted target module-miRwalk 2.0 databases [24]. Intersection of miRWalk 2.0 candidate target genes and deregulated microRNAs were considered for further analysis. Visualization of DEM/DEG of the samples was performed with heat map plots based on average linkage clustering method and Euclidean distance method on heatmapper web server (http:// www.heatmapper.ca/) (28).

Annotation of the functional and pathway analysis of microRNAs /mRNAs

DIANA TOOLS- miRpath v.3 (http://snf-515788.vm.okeanos.grnet.gr/) was used for Kyoto encyclopedia of genes and genomes (KEGG) molecular pathway and gene ontology (GO) analysis of deregulated microRNAs (29). For the functional classification of the gene targets of dysregulated microRNAs, we used PANTHER (protein analysis through evolutionary relationships) classification system tools /gene list analysis (http://www.pantherdb.org/) (30).

Receiver operating characteristic (ROC) curve analysis

To assess the predictive value of the deregulated microRNAs and applicable target genes for distinguishing between tumor and non-tumor samples, expression fold-change data were utilized in receiver operating characteristic (ROC) curve analysis by MedCalc software (variant 12.1.4.0). A logistic regression model was specified for a combined evaluation of the microRNAs and their predicted target genes (31). For this purpose,

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modified variables were constructed via the coefficient of logistic model; these variables were finally conducted in the construction of ROC curves. Area under curve (AUC), sensitivity, and specificity parameters were used to compare the predictive values of microRNAs and their target genes.

Results

DEM and DEG determination

Overall, DEM analysis of 127 tumor samples compared to relevant normal groups by R program showed significantly deregulated expression of 14 microRNAs. Our results revealed increased expression levels for hsa-miR-96-5p, hsa-miR-148a-3p, hsa-miR-153-3p, hsa-miR-183-5p, hsa-miR-663a, hsa-miR-375-3p, and decreased expression levels for hsa-miR-221-3p, hsa-miR-143-3p, hsa-miR-145-5p, hsa-miR-133b, hsa-miR-143-5p, hsa-miR-222-3p, hsa- miR-886-3p, and hsa-miR-205-5p (Table 2). The miRWalk algorithm predicted 2957 putative target genes for the upregulated microRNAs, among them 22 genes were shared with the results of the R analysis (Table 3). Also, for downregulated microRNAs, miRWalk algorithm determined 4241 putative target genes of which 18 genes were also shared with the R analysis results (Table 4). The results for clustering of the upregulated microRNAs identified two distinct groups. One cluster contained miR-148a and miR-375, which have a significant regulatory role in endocytosis and central carbon metabolism in cancer. Also, the other cluster contained miR-96, miR-663, miR-153, and miR-183 that regulate the pathways of the Hippo signaling, adherence junction, prostate cancer, transcriptional dysregulation in cancer, regulation of actin cytoskeleton, and ECM receptor interaction. The clustering of downregulated microRNAs showed three distinct groups; a cluster containing miR-145, which plays a role in the pathways of proteoglycans in cancer, adherence junction, focal adhesion, pathways in cancer, signaling pathways regulating pluripotency of stem cells, transcriptional dysregulation in cancer, and TGF-beta signaling pathway. The other cluster contained miR-222, miR-886, and miR-143-5p which regulate cell adhesion molecules (CAMs),

Table 2. Differential expression miRNAs from R analysis results for tumor samples compared with normal samples.

microRNA ID	Accession number	Log_2FC	Regulation	P.Value	adj.P.Val
hsa-miR-96-5p	MIMAT0000095	1.445869	Up	3.96E-15	1.42E-12
hsa-miR-148a-3p	MIMAT0000243	1.08223	Up	1.14E-12	1.57E-10
hsa-miR-153-3p	MIMAT0000439	1.124276	Up	7.72E-12	7.32E-10
hsa-miR-183-5p	MIMAT0000261	1.127885	Up	1.16E-11	1.02E-09
hsa-miR-663a	MIMAT0003326	1.039093	Up	4.35E-10	2.68E-08
hsa-miR-375-3p	MIMAT0000728	1.110174	Up	4.76E-08	1.57E-06
hsa-miR-221-3p	MIMAT0000278	-1.22942	Down	4.09E-14	9.50E-12
hsa-miR-143-3p	MIMAT0000435	-1.40632	Down	3.17E-17	6.00E-14
hsa-miR-145-5p	MIMAT0000437	-1.36381	Down	1.14E-14	3.47E-12
hsa-miR-133b	MIMAT0000770	-1.16986	Down	6.54E-13	1.15E-10
hsa-miR-143-5p	MIMAT0004599	-1.09946	Down	1.32E-12	2.03E-10
hsa-miR-222-3p	MIMAT0000279	-1.21943	Down	2.82E-12	3.12E-10
hsa-miR-886-3p	MIMAT0004906	-1.02351	Down	2.12E-09	1.11E-07
hsa-miR-205-5p	MIMAT0000266	-1.77151	Down	2.78E-05	0.00035971

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Table 3. Selected down regulated target genes for up regulated miRs.

Gene symbol	Full name	Log_2FC	P.Value	adj.P.Val	Targeted by
SLC14A1	solute carrier family 14 member 1	-3.0688	4.23E-19	1.93E-15	miR-153-3p
CYP3A5	cytochrome P450 family 3 subfamily A member 5	-1.70664	3.74E-18	1.22E-14	miR-183-5p
ACOX2	acyl-CoA oxidase 2	-1.09276	2.40E-15	1.77E-12	miR-153-3p
NTNG2	netrin G2	-1.73367	4.16E-15	2.72E-12	miR-96-5p
FRMD6	FERM domain containing 6	-1.13602	2.44E-14	1.14E-11	miR-183-5p
FAM83B	family with sequence similarity 83 member B	-1.06322	5.21E-14	2.01E-11	miR-96-5p
LAMB3	laminin subunit beta 3	-1.83415	1.43E-13	4.47E-11	miR-663a
CPA6	carboxypeptidase A6	-1.17438	1.61E-13	4.84E-11	miR-183-5p
GSTM2	glutathione S-transferase mu 2	-1.47078	2.74E-13	7.64E-11	miR-96-5p, miR-663a
ACSF2	acyl-CoA synthetase family member 2	-1.03856	9.58E-12	1.27E-09	miR-96-5p
TSLP	thymic stromal lymphopoietin	-1.2069	3.31E-11	3.36E-09	miR-148a-3p
PTGS1	prostaglandin-endoperoxide synthase 1	-1.14004	8.02E-11	6.92E-09	miR-148a-3p
EFS	embryonal Fyn-associated substrate	-1.0616	1.15E-10	9.37E-09	miR-96-5p, miR-183-5p
ANO5	anoctamin 5	-1.10704	1.89E-09	9.21E-08	miR-96-5p
PRIMA1	proline rich membrane anchor 1	-1.06743	2.46E-09	1.15E-07	miR-96-5p
NTN4	netrin 4	-1.07622	1.06E-08	3.96E-07	miR-96-5p, miR-183-5p
GABRE	gamma-aminobutyric acid type A receptor epsilon subunit	-1.36916	1.12E-08	4.13E-07	miR-96-5p
FOXQ1	forkhead box Q1	-1.4297	1.81E-08	6.24E-07	miR-96-5p
ARMCX1	armadillo repeat containing X-linked 1	-1.18953	4.90E-08	1.44E-06	miR-96-5p
PDK4	pyruvate dehydrogenase kinase 4	-1.07171	1.95E-07	4.55E-06	miR-148a-3p
LRCH2	leucine rich repeats and calponin homology domain containing 2	-1.02514	1.01E-06	1.80E-05	miR-96-5p
CD177	CD177 molecule	-2.05064	1.11E-05	0.00013116	miR-148a-3p

spliceosome, glycolysis/gluconeogenesis, mRNA surveillance pathway, ubiquitin mediated proteolysis, and cell cycle. Finally, another cluster which contained miR-133b, miR-221, miR-205, and miR-143-3p regulates the pathways of fatty acid biosynthesis/ metabolism / elongation, nucleotide excision repair, RNA transport, estrogen signaling pathway, viral carcinogenesis, and cell cycle (Fig. 1A, 1B). Similar pattern was also observed in the DEG clustering heatmap plot. The clustering results of upregulated genes showed three distinct groups; the cluster containing *AGR2*, *ENTPD5*, and *MYO6* which regulates purine/ pyrimidine metabolism; the cluster containing *GJB1*, *EFCAB4A*, *TFF3* and *ARHGEF26* that is involved in the bacterial invasion of epithelial cells, and the cluster which contained other remaining genes that contribute to the pathways of glycolysis/gluconeogenesis, the metabolic pathways of the chemical carcinogenesis, calcium signaling pathway, and drug metabolism - cytochrome. Also, the clustering results for downregulated genes showed two distinct groups; a group including *FRMD6*, *GSTM2*, *NTN4*, *NTNG2*, *SLC14A1* which are involved in the pathways of the Hippo signaling pathway, CAMs, and another group including the genes which play a role in the 0eroxisome proliferator-activated receptors (PPARs) signaling pathway, cytokine-cytokine receptor interaction, JA-STAT signaling pathway, ECM receptor

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interaction, PI3K-Akt signaling pathway, focal adhesion, metabolic pathways, platelet activation, primary bile acid biosynthesis, and pathways in cancer (Fig. 1C, 1D).

KEGG pathway and GO annotation for DEM/ DEG

The results of the mirPath v.3 analysis on the upregulated microRNAs revealed that they are involved in steroid biosynthesis, viral carcinogenesis, proteoglycan in cancer, oocyte meiosis, p53 signaling, cell cycle, FoxO signaling, and hepatitis B KEGG molecular pathways. Two microRNAs are also involved in PCa by targeting 22 genes (*P-value:* 0.000887). The analysis of GO on the upregulated microRNAs showed their contribution in some cellular compositions (CC) such as nucleoplasm, cytosol, organelle, protein complex, microtubule organizing center, focal adhesion, some molecular functions (MF) such as enzyme binding, ion binding, protein binding transcription factor activity and cytoskeletal protein binding, some biological processes (BP) like cell death, cell cycle, response to stress , immune system process, protein complex assembly, gene expression, epidermal growth factor receptor signaling pathway, and chromatin organization. The assessment of KEGG pathways for the down regulated microRNAs by the mirPath v.3 showed

Table 4. Selected up regulated target genes for down regulated miRs.

Gene symbol	Full name	Log_2FC	P.Value	adj.P.Val	Targeted by
GJB1	gap junction protein beta 1	1.413366	8.17E-15	4.91E-12	miR-143-5p
SIM2	SIM bHLH transcription factor 2	1.242935	8.37E-15	4.91E-12	miR-143-5p, miR-205-5p
C2ORF72	chromosome 2 open reading frame 72	1.000061	4.31E-14	1.83E-11	miR-143-3p
EFCAB4A	EF-hand calcium binding domain 4A	1.126401	5.66E-14	2.07E-11	miR-143-3p, miR-205-5p
MS4A8	membrane spanning 4-domains A8	1.823825	8.17E-14	2.83E-11	miR-133b
STX19	syntaxin 19	1.367034	1.34E-13	4.25E-11	miR-143-3p
GLYATL1	glycine-N-acyltransferase like 1	1.625489	7.33E-12	1.04E-09	miR-205-5p
ARHGEF26	Rho guanine nucleotide exchange factor 26	1.218229	1.11E-11	1.44E-09	miR-205-5p
ENTPD5	ectonucleoside triphosphate diphosphohydrolase 5	1.000572	1.88E-11	2.17E-09	miR-143-5p
LUZP2	leucine zipper protein 2	2.07617	2.23E-10	1.60E-08	miR-145-5p
ALDH3B2	aldehyde dehydrogenase 3 family member B2	1.270465	8.46E-10	4.79E-08	miR-143-5p
MYO6	myosin VI	1.195531	2.50E-08	8.13E-07	miR-145-5p, miR-143-3p
CTHRC1	collagen triple helix repeat containing 1	1.636065	3.76E-08	1.16E-06	miR-133b
SAMD5	sterile alpha motif domain containing 5	1.026208	3.97E-06	5.60E-05	miR-133b, miR-143-3p
AGR2	anterior gradient 2, protein disulphide isomerase family member	1.547001	4.04E-06	5.68E-05	miR-143-3p
PCDHGA4	protocadherin gamma subfamily A, 4	1.209697	6.29E-06	8.22E-05	miR-133b
TFF3	trefoil factor 3	1.28993	8.73E-06	0.00010802	miR-143-3p
CHRM3	cholinergic receptor muscarinic 3	1.018549	1.43E-05	0.00016284	miR-205-5p

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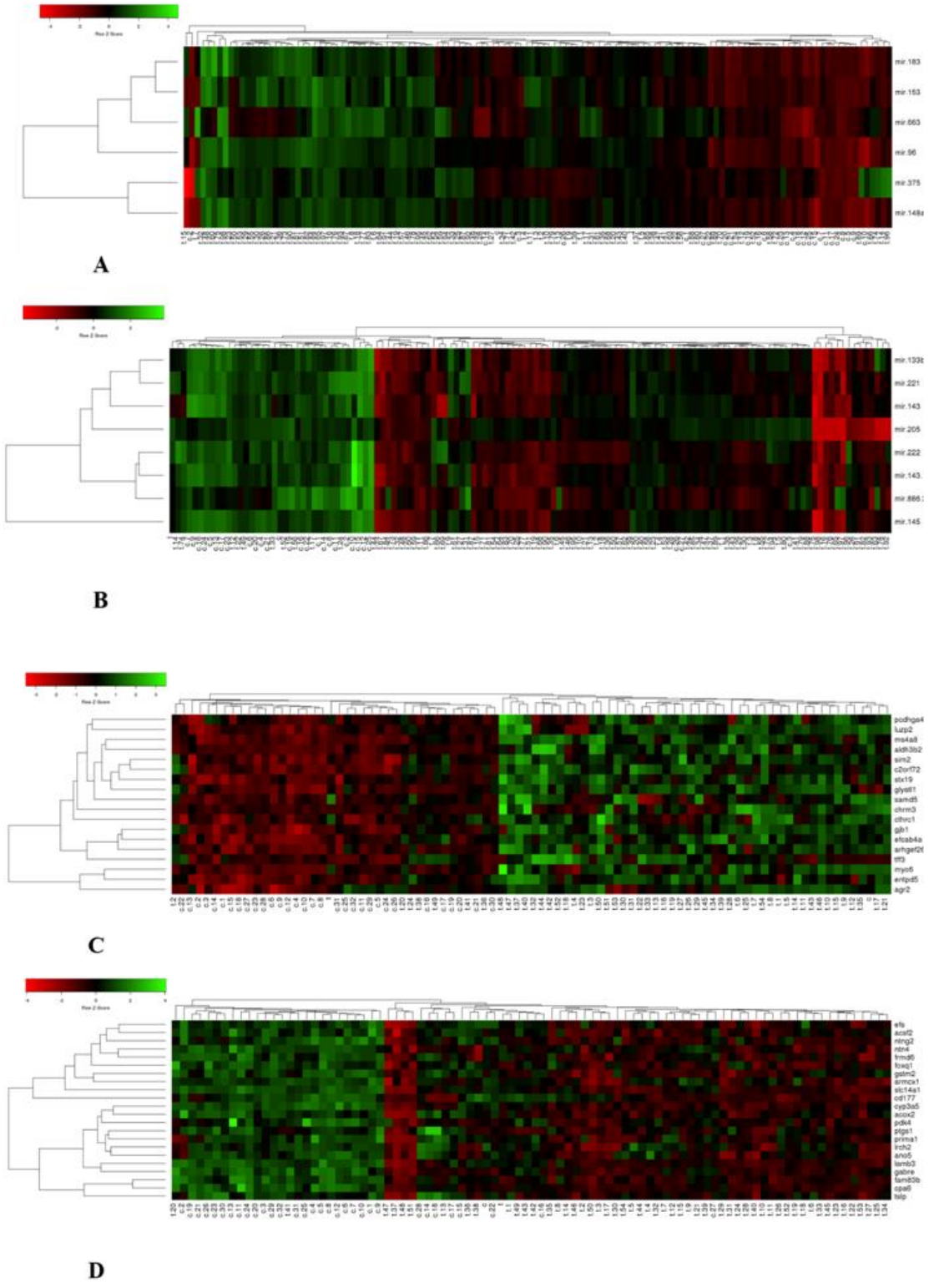

Fig. 1. The heatmap plots of the deregulated microRNAs in GSE21036 dataset and deregulated genes for integrative analysis of GSE46602, GSE69223, and GSE104749 datasets. A: The heat map plot of the six up regulated microRNAs; B: The heat map of the eight down regulated microRNAs. Each row illustrates the desired microRNAs and each column shows samples; C: The heat map of the up regulated genes; D: The heat map of the down regulated genes. Each row illustrates the desired genes and each column shows samples.

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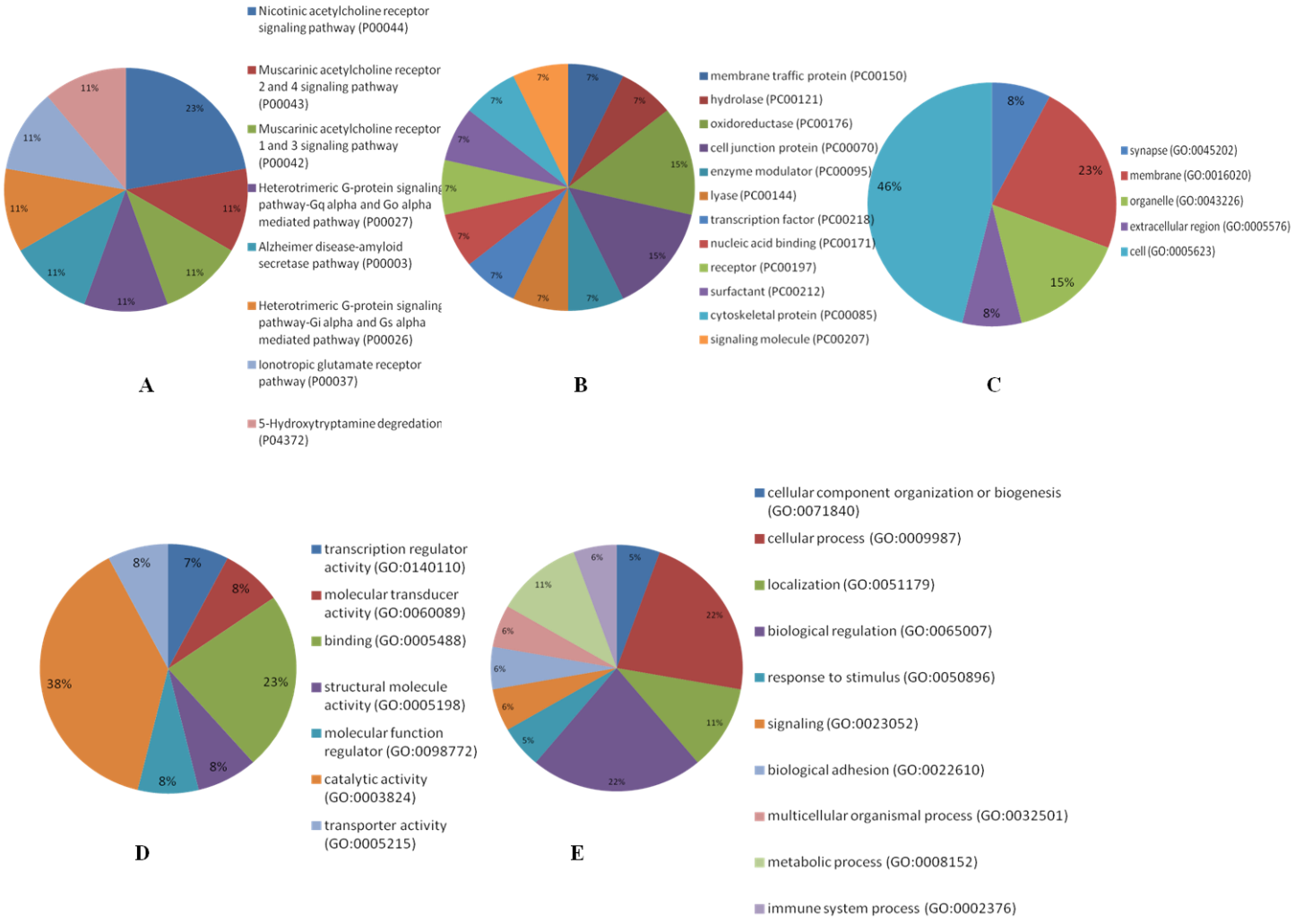

Fig. 2. Pie chart for functional classification of the up regulated genes by the panther. A: pathway analysis; B: protein class; C: cellular components; D: molecular function and E: biological processes based on GO-slim.

that they are linked to some molecular pathways such as p53 signaling pathway, estrogen signaling pathway, viral carcinogenesis, Hippo signaling pathway, and lysine degradation. The pathways in cancer for microRNAs by targeting 36 genes was also reported (*P-value:* 0.0010524). In addition, GO analysis for evaluation of the downregulated microRNAs revealed that some of them are assigned in intersectional category for CC, MF and BP and are linked to cytosol, protein complex, organelle, enzyme binding, ion binding, biological process, gene expression, and response to stress. Ultimately, the pathway and GO-slim analysis for either up or down regulated target genes was performed by using PANTHER software. The results of these assessments are shown in Figs. 2 and 3.

DEM and DEG discriminate PCa tumor samples

The expression ratios of significant DEM and DEG in the tumor samples were converted to ROC/AUC data, which determined the specificity and sensitivity of each microRNA/target gene assay for distinguishing tumor samples from control samples. While individual assays were capable of discrimination between tumor and non- tumor samples with acceptable accuracy, four

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combination panels showed higher AUC, sensitivity and specificity (Figs. 4, 5, and 6). Regarding the upregulated microRNAs, a combination panel [miR-375 + miR-96 + miR-663] with AUC = 0.962, sensitivity = 89.9, specificity = 92.86, and 95% CI: 0.913 to 0.988 showed the best results. Out of the down regulated microRNAs, a combination panel consisted of miR-133b+ miR-143-3p+ miR-205 with AUC = 0.994, sensitivity = 97.98, specificity = 96.43, 95% CI: 0.961 to 1.000 had significantly superior accuracy. Also, among the genes with upregulated expression, the combined expression panel of *C2ORF72* + *CTHRC1*+ *ENTPD5*+ *GLYATL1* with AUC =0.986, sensitivity = 96.36, specificity = 93.94, and 95% CI: 0.933 to 0.999 displayed remarkable accuracy. Finally, the combination panel of *LAMB3*+ *NTNG2* + *TSLP* with AUC = 0.986, sensitivity = 98.18, specificity = 93.94, and CI %95 = 0.934 to 0.999 95% had better results than individual assay in the downregulated genes.

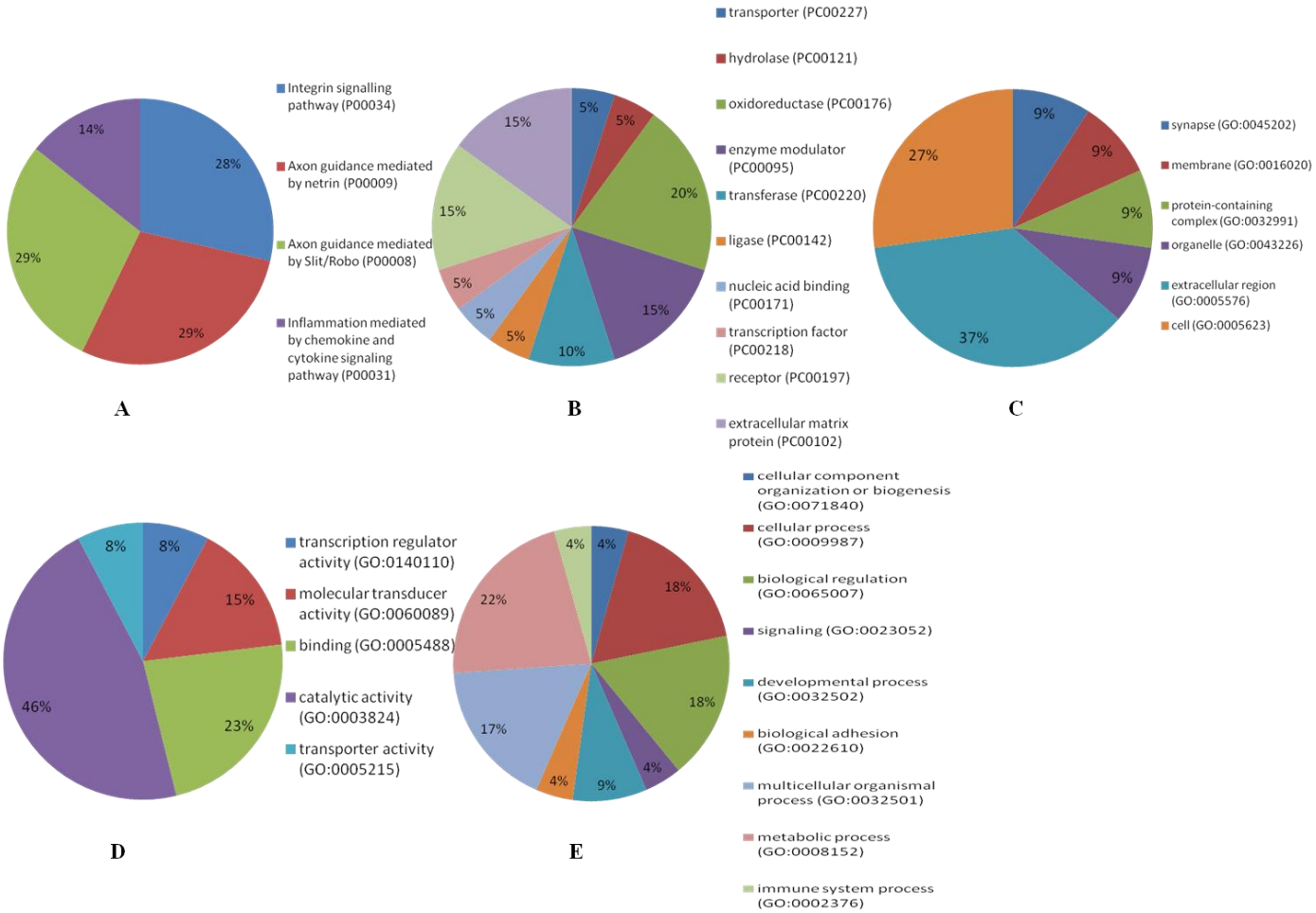

Fig. 3. Pie chart for functional classification of the down regulated genes by the panther. A: pathway analysis; B: protein class; C: cellular components; D: molecular function and E: biological processes based on GO-slim

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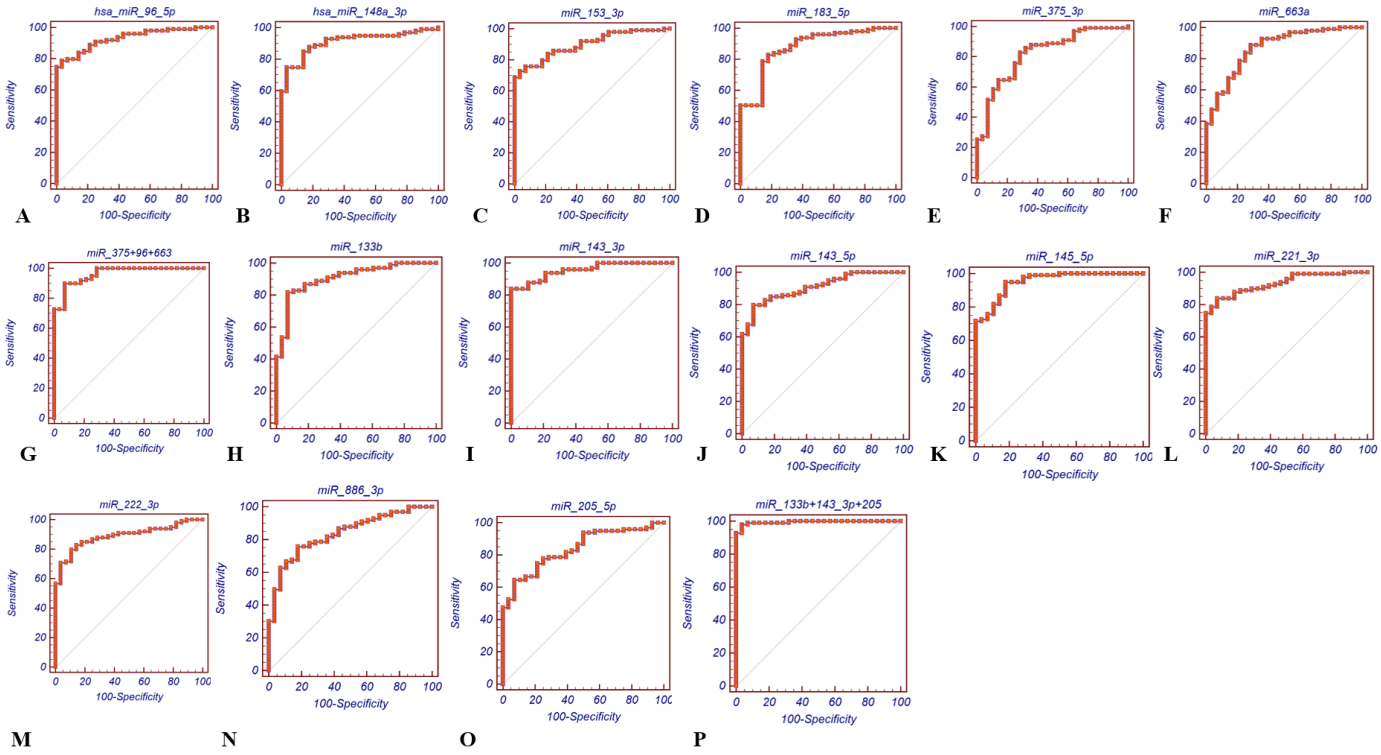

microRNAs	AUC	Sensitivity	Specificity	95% CI	Significance level P (Area=0.5)
miR_96_5p	0.929	78.79	96.43	0.869 to 0.967	<0.0001
miR_148a_3p	0.909	74.75	96.43	0.846 to 0.953	<0.0001
miR_153_3p	0.898	72.73	96.43	0.832 to 0.945	<0.0001
miR_183_5p	0.877	82.83	82.14	0.807 to 0.929	<0.0001
miR_375_3p	0.822	82.83	71.43	0.744 to 0.884	<0.0001
miR_663a	0.868	88.89	71.43	0.796 to 0.921	<0.0001
miR-375+96+663	0.962	89.9	92.86	0.913 to 0.988	<0.0001
miR-133b	0.906	81.82	92.86	0.841 to 0.950	<0.0001
miR-143-3p	0.955	83.84	100	0.903 to 0.984	<0.0001
miR-143-5p	0.907	79.8	92.86	0.843 to 0.952	<0.0001
miR-145-5p	0.952	94.95	82.14	0.900 to 0.982	<0.0001
miR-221-3p	0.931	83.84	92.86	0.872 to 0.968	<0.0001
miR-222-3p	0.889	79.8	89.29	0.821 to 0.937	<0.0001
miR-886-3p	0.835	75.76	82.14	0.759 to 0.895	<0.0001
miR-205-5p	0.838	64.65	92.86	0.762 to 0.897	<0.0001
miR-133b+143-3p+205	0.994	97.98	96.43	0.961 to 1.000	<0.0001

Fig. 4. Receiver operating characteristic (ROC) curves of deregulated microRNAs expression profiles in tumor and control samples. A-G show the ROC curves as well as sensitivity and specificity of up regulated microRNAs and microRNAs combination assays; H-P show the ROC curves as well as sensitivity and specificity of down regulated microRNAs and microRNAs combination assays.

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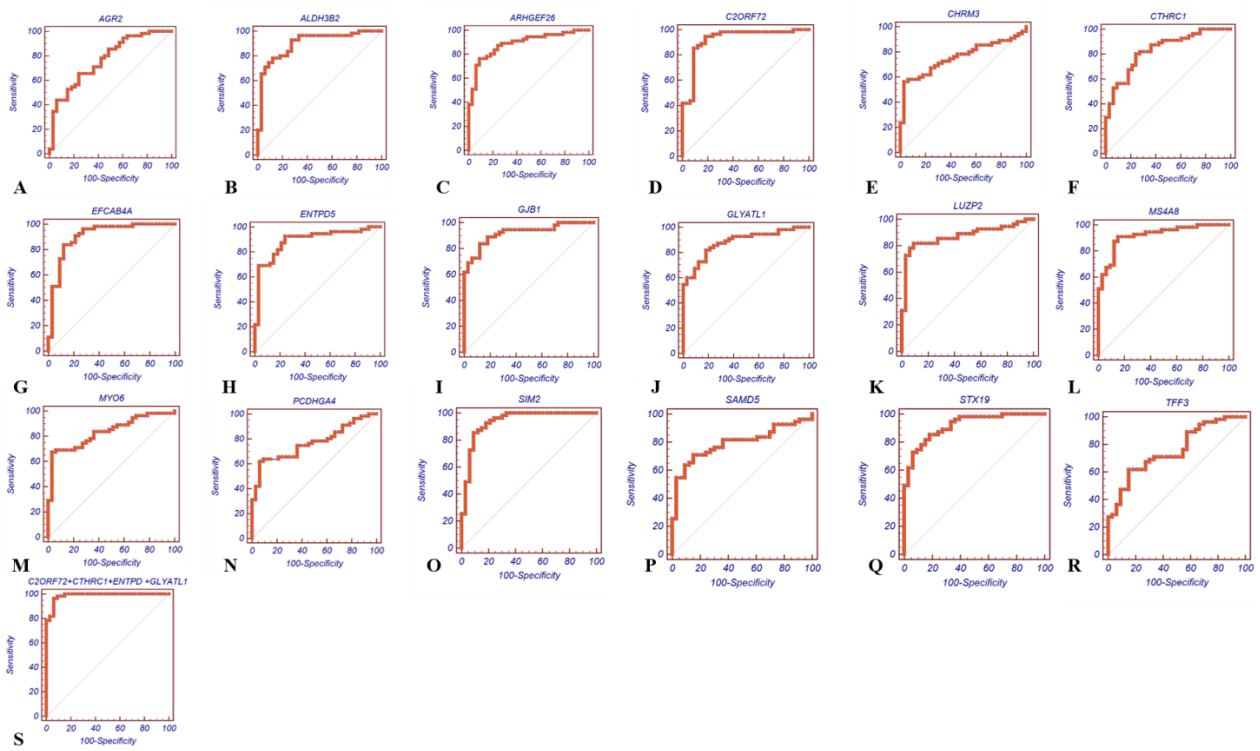

Genes	AUC	Sensitivity	Specificity	95% CI	Significance level P (Area=0.5)
AGR2	0.764	65.45	75.76	0.661 to 0.848	<0.0001
ALDH3B3	0.898	78.18	87.88	0.814 to 0.952	<0.0001
ARHGEF26	0.886	76.36	90.91	0.800 to 0.944	<0.0001
C2ORF72	0.92	94.55	81.82	0.843 to 0.967	<0.0001
CHRM3	0.756	56.36	96.97	0.653 to 0.842	<0.0001
CTHRC1	0.832	80	75.76	0.737 to 0.903	<0.0001
EFCAB4A	0.907	83.64	87.88	0.826 to 0.958	<0.0001
ENTPD5	0.89	92.73	75.76	0.805 to 0.947	<0.0001
GJB1	0.919	83.64	87.88	0.841 to 0.966	<0.0001
GLYATL1	0.882	81.82	81.82	0.796 to 0.941	<0.0001
LUZP2	0.873	81.82	90.91	0.785 to 0.934	<0.0001
MS4A8	0.919	90.91	84.85	0.841 to 0.966	<0.0001
MYO6	0.833	67.27	96.97	0.738 to 0.904	<0.0001
PCDHGA4	0.765	61.82	93.94	0.663 to 0.849	<0.0001
SIM2	0.936	85.45	90.91	0.862 to 0.977	<0.0001
SAMD5	0.793	70.91	84.85	0.693 to 0.872	<0.0001
STX19	0.917	85.45	81.82	0.839 to 0.965	<0.0001
TFF3	0.755	61.82	84.85	0.652 to 0.841	<0.0001
C2ORF72+CTHRC1+ENTPD +GLYATL1	0.986	96.36	93.94	0.933 to 0.999	<0.0001

Fig. 5. Receiver operating characteristic (ROC) curves of up regulated genes expression profiles in tumor and control samples. A-S show the ROC curves as well as sensitivity and specificity of up regulated genes and genes combination assays.

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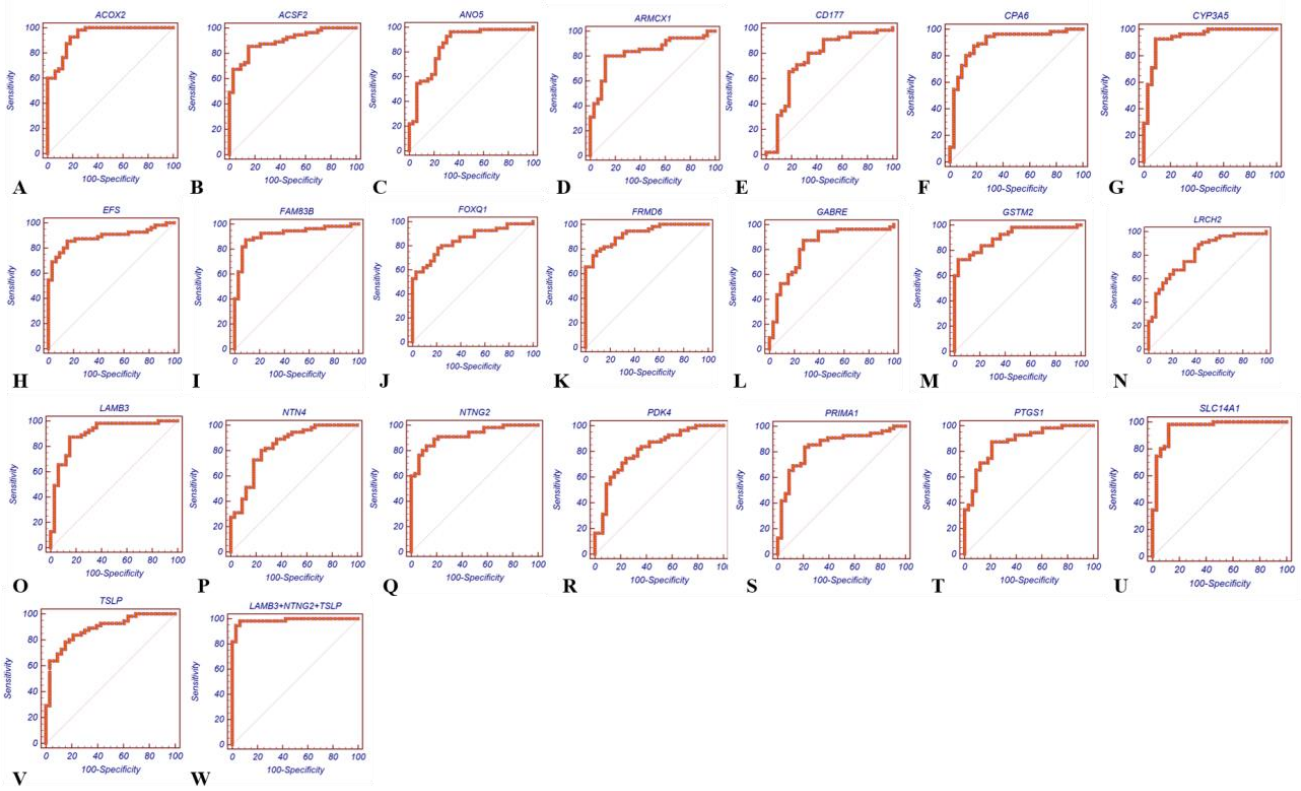

Genes	AUC	Sensitivity	Specificity	95% CI	Significance level P (Area=0.5)
ACOX2	0.939	92.73	81.82	0.867 to 0.979	<0.0001
ACSF2	0.896	85.45	84.85	0.813 to 0.951	<0.0001
ANO5	0.853	96.36	66.67	0.762 to 0.920	<0.0001
ARMCX1	0.835	80	87.88	0.740 to 0.905	<0.0001
CD177	0.754	65.45	81.82	0.650 to 0.839	<0.0001
CPA6	0.897	87.27	81.82	0.814 to 0.952	<0.0001
CYP3A5	0.938	92.73	90.91	0.865 to 0.978	<0.0001
EFS	0.885	85.45	84.85	0.800 to 0.943	<0.0001
FAM83B	0.918	87.27	90.91	0.840 to 0.966	<0.0001
FOXQ1	0.847	78.18	78.79	0.755 to 0.915	<0.0001
FRMD6	0.922	78.18	90.91	0.845 to 0.968	<0.0001
GABRE	0.823	87.27	72.73	0.727 to 0.896	<0.0001
GSTM2	0.906	72.73	96.97	0.825 to 0.958	<0.0001
LRCH2	0.806	89.09	57.58	0.708 to 0.883	<0.0001
LAMB3	0.899	87.27	84.85	0.816 to 0.953	<0.0001
NTN4	0.831	80	75.76	0.737 to 0.903	<0.0001
NTNG2	0.918	83.64	87.88	0.840 to 0.966	<0.0001
PDK4	0.807	74.55	75.76	0.709 to 0.883	<0.0001
PRIMA1	0.843	83.64	78.79	0.750 to 0.912	<0.0001
PTGS1	0.872	87.27	78.79	0.783 to 0.933	<0.0001
SLC14A1	0.955	98.18	87.88	0.888 to 0.988	<0.0001
TSLP	0.883	78.18	84.85	0.796 to 0.941	<0.0001
LAMB3+NTNG2+TSLP	0.986	98.18	93.94	0.934 to 0.999	<0.0001

Fig. 6. Receiver operating characteristic (ROC) curves of down regulated genes expression profiles in tumor and control samples. A-W show the ROC curves as well as sensitivity and specificity of down regulated genes and genes combination assays.

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Discussion

Deeper understanding of the molecular pathways involved in the pathogenesis of PCa may lead to new diagnostic biomarkers (32). MicroRNAs have a regulatory role in the biological processes as they silence their target genes at post-transcriptional or translational levels (33). MicroRNAs further regulate the molecular pathways involved in cancers, including PCa, by targeting multiple genes (34, 35). On the other hand, the aberrant expression of microRNAs in cancers has been widely reported. Therefore, the examination of microRNAs/their targets expression profiles can be conducive to identifying diagnostic cancer biomarkers (36). Bioinformatics tools, along with molecular biology techniques, are useful for comprehensive transcriptome studies and investigation of the molecular pathways involved in cancer (37). In this study, we used systems biology tools and high-*throughput microarray* datasets to investigate changes in the expression of microRNAs in 99 PCa tissue samples compared to normal *prostate* tissue samples. Further studied was the expression profile of the target genes through analyzing the integrative data *obtained from three independent microarray experiments*. Overall, our study results showed significant changes in the expression of 14 microRNAs and 40 relevant target genes, which ultimately introduced four combination panels as candidate biomarkers that can distinguish PCa tumor samples against normal prostate tissue samples. Interestingly, the deregulation of the highlighted microRNAs (miR-96, miR-375, mir-663, miR-133b, miR-143, and miR-205) in PCa was reported in previous studies using qRT-PCR or northern blot assays (38-43). Mihelich et al. reported the upregulation of miR-96 expression (a member of the miR-183 family) in PCa tissue samples (43). In line with the results of the previous studies, our results showed that miR-96, targeting different genes such as *FOXO1*and *NTNG2* in the FOXO signaling pathway, androgen receptor signaling, apoptosis, and adhesion junction plays regulatory roles in the pathogenesis of PCa. Jiao et al., in a study on 127 PCa patients, indicated that miR-663 expression in tumor tissues was higher than normal samples, and associated with Gleason score and disease stage (38). MiR-663a, which was in the same cluster with miR-96 in our analysis, also has a pivotal role in targeting various genes such as *LAMB3*, involved in molecular PCa pathogenesis via the PI3K-Akt signaling pathway, ECM receptor interaction, and focal adhesion. In the same way, Szczyrba et al. conducted a 26-sample study using qRT-PCR technique, which showed an increase in the expression of miR-375 in 81% of tumor samples compared with normal specimens (39). The results of our study with regard to miR-375 were similar to those reported by Szczyrba et al. Although there were no changes in the expression of its target genes, the analysis of the miRpath3.0 showed that p53, FOXO, and Hippo signaling pathway are regulated by miR-375. Clape′ et al. reported the reduced expression of miR-143 in 25 PCa tissue samples, and suggested that it could be considered as a drug target (41). Verdoodt et al. studied 86 formalin fixed paraffin-embedded (FFPE) tissue samples obtained by primary PCa prostatectomy. Using qRT-PCR, they observed that the down- regulated expression of miR-205 was inversely related to tumor size in 76 samples (40). Our study results showed similar expression changes with regard to miR-143 and miR-205 in tumor samples compared to non- tumor counterparts. The results further revealed that miR-143-3p can be involved in the pathogenesis of PCa by targeting *C2ORF72*, *MYO6*, *TFF3*, *AGR2*, and *STX19*. Furthermore, miR-205 plays a similar role in targeting genes such as *GLYAT11*, *SIM2*, *CHRM3*, *EFCAB4A*, and *ARHGEF26*. Few studies on miR-133b as a member of the family of myomiRs (muscle specific microRNAs) in PCa tissues have been published so far. Using qRT-PCR method Guze et al. reported lower expressions of

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miR-133b in 23 prostate tumor secretion samples compared with 25 BPH secretion samples (44, 45). As a result of our study, expression levels of miR-133b in tumor samples were reduced compared to non-tumor samples. Also, miR-133b contributes to the pathogenesis and progression of PCa by targeting the *CTHRC1*, *SAMD5*, and *PCDHGA4* genes involved in biological processes such as biological adhesion and immune system process.

In conclusion, our bioinformatics study indicated four combination panels (i.e., miR- 375+ 96+ 663/ miR- 133b+ 143- 3p+ 205/ *C2ORF72*+ *ENTPD5*+ *GLYAT11*/ *LAMB3*+ *NTNG2*+ *TSLP*) that can discriminate prostate tumor tissue samples from normal specimens. These biomarkers may help with early diagnosis of PCa along with currently available diagnostic tests. However, since the aim of our study was to investigate the data from *high-throughput microarray* data, the validation of the biomarker panels suggested by this study needs more investigation *in future* studies.

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Conflict of interest

None declared.

References

1. Wishart DS. Is Cancer a Genetic Disease or a Metabolic Disease? EBioMedicine 2015;2:478-9.

2. Global Burden of Disease Cancer C, Fitzmaurice C, Allen C, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncol 2017;3:524-48.

3. Helsmoortel H, Everaert C, Lumen N, et al. Detecting long non-coding RNA biomarkers in prostate cancer liquid biopsies: Hype or hope? Noncoding RNA Res 2018;3:64-74.

4. Alford AV, Brito JM, Yadav KK, et al. The Use of Biomarkers in Prostate Cancer Screening and Treatment. Rev Urol 2017;19:221-34.

5. Martin SK, Vaughan TB, Atkinson T, et al. Emerging biomarkers of prostate cancer (Review). Oncol Rep 2012;28:409-17.

6. Fraser M, Berlin A, Bristow RG, et al. Genomic, pathological, and clinical heterogeneity as drivers of personalized medicine in prostate cancer. Urol Oncol 2015;33:85-94.

7. Shtivelman E, Beer TM, Evans CP. Molecular pathways and targets in prostate cancer. Oncotarget 2014;5:7217-59.

8. Hornberg JJ, Bruggeman FJ, Westerhoff HV, et al. Cancer: a Systems Biology disease. Biosystems 2006;83:81-90.

9. Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics 2010;11:537-61.

10. Takayama KI, Misawa A, Inoue S. Significance of microRNAs in Androgen Signaling and Prostate Cancer Progression. Cancers (Basel) 2017;9.

11. Jackson BL, Grabowska A, Ratan HL. MicroRNA in prostate cancer: functional importance and potential as circulating biomarkers. BMC Cancer 2014;14:930.

12. van't Veer LJ, Bernards R. Enabling personalized cancer medicine through analysis of gene-expression patterns. Nature 2008;452:564-70.

13. Oliveto S, Mancino M, Manfrini N, et al. Role of microRNAs in translation regulation and cancer. World J Biol Chem 2017;8:45-56.

14. Mulrane L, McGee SF, Gallagher WM, et al. miRNA dysregulation in breast cancer. Cancer Res 2013;73:6554-62.

15. Cesario A, Marcus F. Research and Clinical Applications. Cancer Systems Biology, Bioinformatics and Medicine. 1 ed: Springer Netherlands; 2011.

16. Li X, Gill R, Cooper NG, et al. Modeling microRNA-mRNA interactions using PLS regression in human colon cancer. BMC Med Genomics 2011;4:44.

17. Yang X, Zhu S, Li L, et al. Identification of differentially expressed genes and signaling pathways in ovarian cancer by integrated bioinformatics analysis. Onco Targets Ther 2018;11:1457-74.

18. Cao L, Chen Y, Zhang M, et al. Identification of hub genes and potential molecular mechanisms in gastric cancer by integrated bioinformatics analysis. PeerJ 2018;6:e5180.

19. Izadi F. Differential Connectivity in Colorectal Cancer Gene Expression Network. Iran Biomed J 2019;23:34-46.

[DOI: 10.22088/IJMCM.BUMS.8.2.103]

20. Guo K, Liang Z, Li F, et al. Comparison of miRNA and gene expression profiles between metastatic and primary prostate cancer. Oncol Lett 2017;14:6085-90.

21. Hu G, Lv Q, Yan J, et al. MicroRNA-17 as a promising diagnostic biomarker of gastric cancer: An investigation combining TCGA, GEO, meta-analysis, and bioinformatics. FEBS Open Bio 2018;8:1508-23.

22. Taylor BS, Schultz N, Hieronymus H, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11-22.

23. Meller S, Meyer HA, Bethan B, et al. Integration of tissue metabolomics, transcriptomics and immunohistochemistry reveals ERG- and gleason score-specific metabolomic alterations in prostate cancer. Oncotarget 2016;7:1421-38.

24. Shan M, Xia Q, Yan D, et al. Molecular analyses of prostate tumors for diagnosis of malignancy on fine-needle aspiration biopsies. Oncotarget 2017;8:104761-71.

25. Mortensen MM, Hoyer S, Lynnerup AS, et al. Expression profiling of prostate cancer tissue delineates genes associated with recurrence after prostatectomy. Sci Rep 2015;5:16018.

26. Leek JT, Johnson WE, Parker HS, et al. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 2012;28:882-3.

27. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 1995;57:289-300.

28. Babicki S, Arndt D, Marcu A, et al. Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res 2016;44:W147-53.

29. Vlachos IS, Zagganas K, Paraskevopoulou MD, et al. DIANA-miRPath v3.0: deciphering microRNA function with experimental support. Nucleic Acids Res 2015;43:W460-6.

30. Mi H, Muruganujan A, Thomas PD. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res 2013;41:D377-86.

31. Carter JV, Pan J, Rai SN, et al. ROC-ing along: Evaluation and interpretation of receiver operating characteristic curves. Surgery 2016;159:1638-45.

32. Chang L, Ni J, Beretov J, et al. Identification of protein biomarkers and signaling pathways associated with prostate cancer radioresistance using label-free LC-MS/MS proteomic approach. Sci Rep 2017;7:41834.

33. Kreth S, Hubner M, Hinske LC. MicroRNAs as Clinical Biomarkers and Therapeutic Tools in Perioperative Medicine. Anesth Analg 2018;126:670-81.

34. Kumar B, Lupold SE. MicroRNA expression and function in prostate cancer: a review of current knowledge and opportunities for discovery. Asian J Androl 2016;18:559-67.

35. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 2014;20:460-9.

36. Palmero EI, de Campos SG, Campos M, et al. Mechanisms and role of microRNA deregulation in cancer onset and progression. Genet Mol Biol 2011;34:363-70.

37. Likic VA, McConville MJ, Lithgow T, et al. Systems biology: the next frontier for bioinformatics. Adv Bioinformatics 2010:268925.

38. Jiao L, Deng Z, Xu C, et al. miR-663 induces castration-resistant prostate cancer transformation and predicts clinical recurrence. J Cell Physiol 2014;229:834-44.

39. Szczyrba J, Loprich E, Wach S, et al. The microRNA profile of prostate carcinoma obtained by deep sequencing. Mol Cancer Res 2010;8:529-38.

40. Verdoodt B, Neid M, Vogt M, et al. MicroRNA-205, a novel regulator of the anti-apoptotic protein Bcl2, is downregulated in prostate cancer. Int J Oncol 2013;43:307-14.

41. Clape C, Fritz V, Henriquet C, et al. miR-143 interferes with ERK5 signaling, and abrogates prostate cancer progression in mice. PLoS One 2009;4:e7542.

42. Li X, Wan X, Chen H, et al. Identification of miR-133b and RB1CC1 as independent predictors for biochemical recurrence and potential therapeutic targets for prostate cancer. Clin Cancer Res 2014;20:2312-25.

43. Mihelich BL, Khramtsova EA, Arva N, et al. miR-183-96-182 cluster is overexpressed in prostate tissue and regulates zinc homeostasis in prostate cells. J Biol Chem 2011;286:44503-11.

44. Li D, Xia L, Chen M, et al. miR-133b, a particular member of myomiRs, coming into playing its unique pathological role in human cancer. Oncotarget 2017;8:50193-208.

45. Guzel E, Karatas OF, Semercioz A, et al. Identification of microRNAs differentially expressed in prostatic secretions of patients with prostate cancer. Int J Cancer 2015;136:875-9.

[DOI: 10.22088/IJMCM.BUMS.8.2.103]