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Screening the key microRNAs and transcription factors in prostate cancer based on microRNA functional synergistic relationships

Fan Feng, MM, Jitao Wu, MD, Zhenli Gao, MM, Shengqiang Yu, MD, Yuanshan Cui, MM*

Abstract

Prostate cancer (PC) is a common neoplasm, and metastatic PC remains incurable. The study aims to screen key microRNAs (miRNAs) and transcription factors (TFs) involved in PC.

The miRNA expression profile dataset (GSE45604) was downloaded from Gene Expression Omnibus database, including 50 PC and 10 normal specimens. Differentially expressed miRNAs (DEmiRNAs) were identified through limma package in R, and DEmiRNA–DEmiRNA co-regulation network was constructed based on the number of co-regulated target genes. Functional enrichment analysis of co-regulated target genes was performed using clusterProfiler package in R, and miRNA interactions sharing at least 1 functional term were used to construct a DEmiRNA–DEmiRNA functional synergistic network (MFSN). Based on Transcriptional Regulatory Element Database, cancer-related TFs which were co-regulated by DEmiRNAs were utilized to construct a DEmiRNA–TF regulation network.

A total of 66 DEmiRNAs were identified, including 7 up-regulated miRNAs with 18,642 target genes and 59 down-regulated miRNAs with 130,694 target genes. Then, the DEmiRNA–DEmiRNA co-regulation network was constructed, including 66 DEmiRNAs and 2024 co-regulation relationships. In MFSN, hsa-miR-1184, hsa-miR-1207-5p, and hsa-miR-24 had significant functional synergistic relationships. The DEmiRNA–TF network contained 6 up-regulated DEmiRNAs and 4 of them were highlighted, as hsa-miR-1184, hsa-miR-1207-5p, hsa-miR-182, and hsa-miR-183. In subnetwork of the 4 miRNAs, peroxisome proliferative activated receptor, alpha (PPARA) and cyclic AMP-responsive element modulator (CREM) were the critical regulated TFs.

Four up-regulated miRNAs (hsa-miR-1207-5p, hsa-miR-1184, hsa-miR-182, and hsa-miR-183) and 2 TFs (PPARA and CREM) were identified as key regulators in PC progression. The above 4 miRNAs might participate in PC progression by targeting PPARA and CREM.

Abbreviations: BP = biological process, COX2 = cyclooxygenase 2, CREM = cyclic AMP-responsive element modulator, DEmiRNA = differentially expressed miRNAs, DKK3 = dickkopf 3 homolog, FC = fold change, FDR = false discovery rate, GO = gene ontology, MFSN = DEmiRNA–DEmiRNA functional synergistic network, miRNAs = microRNAs, NFKB1 = nuclear factor of kappa light polypeptide, PC = prostate cancer, PPARA = peroxisome proliferative activated receptor, alpha, pT = pathological, RAS = p21 ras, SMAD4 = drosophila mothers against decapentaplegic family member 4, TF = transcription factor, TRED = Transcriptional Regulatory Element Database.

Keywords: differentially expressed microRNAs, functional enrichment analysis, functional synergistic network, prostate cancer, transcription factor

1. Introduction

Prostate cancer (PC) is a slowly developed malignancy among men that could become more aggressive in response to androgen blockade.[1] PC is the most prevalent nonskin cancer and the second leading cause of cancer-related deaths in men.[2] Although effective surgical and radiation treatments have been clinically utilized, metastatic PC remains incurable.[3] Better understanding of the molecular mechanism of PC is beneficial for development of novel therapeutic targets. Thus, several studies have been investigated to reveal the molecular mechanisms. For instance, frequent and large-scale genomic rearrangements have been identified in PC tissue.[4] Catechol-O-methyltransferase and glutathione reductase are less active in PC.[5] Sex-steroid hormone, androgens, and estrogens play regulatory roles in PC progression.[6] Testosterone is carcinogenic for rat prostate, as testosterone could be converted to reactive metabolites which adduct to DNA and potentially lead to mutations.[5] Estradiol and estrone are converted to estrogen semiquinones and estrogen quinones which adduct to DNA and redox cycling, producing reactive oxygen species and damaging DNA.[7] Therefore, androgen receptor, enzymes involved in androgens and estrogens metabolism, and DNA repair-related genes are critical for PC progression.[2,8–10]

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FF and JW are the co-first authors.

Conceived and designed the experiments: YC and FF; acquired and analyzed the data: FF, JW, and ZG; drafted the manuscript: SY; and approved the manuscript: YC.

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Department of Urological Surgery, Yantai Yuhuangding Hospital, Yantai, Shandong, China.

** Correspondence: Yuanshan Cui, Department of Urological Surgery, Yantai Yuhuangding Hospital, Yuhuangding Dong Road 20, Yantai, Shandong 264000, China (e-mail: fengurology@126.com).*

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In addition to hormone-related metabolites and genes, microRNAs (miRNAs) also play critical roles in PC progression. MiRNAs are small regulatory RNAs which post-transcriptionally repress the expression of target genes by binding to mRNA sequences and promoting mRNA degradation.[11] Widespread down-regulation of miR-125b, miR-145, and let-7c has been found in human PC tissue.[11] Moreover, it is reported that the target genes of these down-regulated miRNAs are up-regulated in PC tissue, including RAS (p21 ras), E2F transcription factor 3, B-cell lymphoma 2, and myeloid cell leukemia-1.[11] Therefore, screening abnormally expressed miRNAs should be highlighted in the understanding of PC mechanism. Moreover, miRNAs are highly related to the transcription factors (TFs) for the control of gene expressions. The TFs regulate gene expressions at transcriptional level, while the miRNAs at post-transcriptional level.[12] Interestingly, they could be regulated by each other: TF could modulate miRNA-mediated expression via certain pathways; and miRNAs could also regulate the TF-mediated issues to inactivate the target genes.[12] In PC, it is found the miRNAs coordinate with TFs to regulate genes in some transcriptional pathways.[13] However, the direct regulation between miRNAs and TFs are rarely reported. Therefore, we focused on the correlations between miRNAs and TFs in PC progression. In this study, differentially expressed miRNAs (DEmiRNAs) between PC and normal specimens were identified by using bioinformatics tools, and DEmiRNA–DEmiRNA co-regulation network was constructed. Then, DEmiRNA–DEmiRNA functional synergistic network (MFSN) was built based on gene ontology (GO) biological process (BP) functional categories that were enriched by the target genes of DEmiRNAs. Following that, a DEmiRNA–TF regulation network was constructed among the cancer-related TFs and their corresponding DEmiRNAs, and key DEmiRNAs and TFs were identified by analyzing these networks. This study might help researchers to understand the mechanism of PC at molecular level.

2. Materials and methods

2.1. Microarray data

The miRNAs expression profile dataset GSE45604[14] was downloaded from Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). The data derived from 60 specimens (10 normal persons and 50 PC patients before treatment) were available based on platform GPL14613 ([miRNA-2_0] Affymetrix Multispecies miRNA-2_0 Array, Affymetrix Inc, Santa Clara, CA). Based on Casanova's description, before tissue collection, the written informed consent was obtained from patients enrolled in the dataset of GSE45604. Additionally, this study was approved by the institutional ethics committee.[14]

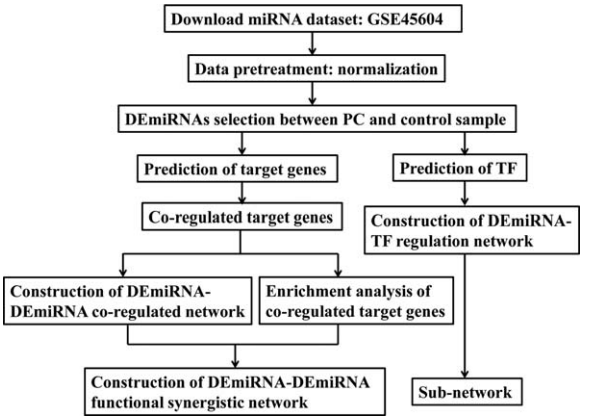

Figure 1. Flow chart of the analyses in this study. PC = prostate cancer.

2.2. Data preprocessing

According to the annotation file of GPL14613, probe identifiers were converted into miRNA symbols. During this conversion, a probe was removed if it mapped to multiple miRNAs, while if multiple probes corresponded to the same miRNA, their values were averaged to calculate the miRNA expression. Furthermore, miRNAs expression values were normalized through median method.

2.3. DEmiRNAs screening

To screen the DEmiRNAs between normal and PC specimens, the Linear Models for Microarray Analysis (limma) package in R (available at http://www.bioconductor.org/packages/release/bioc/html/limma.html, version: 3.22.1) was utilized, which is the most popular method for differential expression analysis.[15,16] In the significance analysis, P value was adjusted as false discovery rate (FDR) by Benjamini and Hochberg method.[17] Only the miRNAs met with the criteria of $|\log_2$ fold change (FC)$| > 1$ and FDR < 0.05 were defined as DEmiRNAs.

2.4. DEmiRNA–target gene prediction

The target genes of DEmiRNAs were explored from 4 databases, including TargetScan (http://www.targetscan.org/),[18] PicTar (http://pictar.mdc-berlin.de/),[19] miRbase (http://www.mirbase.org/),[20] and DIANA-microT (http://diana.cslab.ece.ntua.gr/),[21] predicting potential DEmiRNA–target interactions.

2.5. Construction of DEmiRNA–DEmiRNA co-regulation network

DEmiRNAs co-regulating same target gene were identified, based on which a DEmiRNA–DEmiRNA co-regulation network was

Table 1

Details of 50 prostate cancer patients.

Gleason score	No. of specimen	pT stage	No. of specimen	TMPRSS2-ERG status	No. of specimen
≤6	15	≤pT2	35	Negative	15
7	25	≥pT3	14	Positive	26
8–10	10				

pT stage = pathological stage, TMPRSS2-ERG status = the specific TMPRSS2 and ERG rearrangement at 21q22.

Table 2

Differentially expressed miRNAs between prostate cancer and normal samples.

	miRNA name	Log_2 fold change	*P* value	Adjusted *P* value
Up-regulated	hsa-mir-200c	10.84792	2.33E−05	0.001988
	hsa-mir-375	10.81401	1.28E−05	0.001637
	hsa-mir-182	8.281506	0.000589	0.017193
	hsa-mir-1207-5p	7.494128	0.002539	0.047191
	hsa-mir-183	5.409361	0.001699	0.034443
	hsa-mir-1238	1.957193	0.000988	0.023634
	hsa-mir-1184	1.786823	0.000521	0.015575
Down-regulated	hsa-mir-4328	−1.08906	0.002736	0.049572
	hsa-mir-376a	−1.12815	0.000434	0.014123
	hsa-mir-199a-1	−1.14579	0.00074	0.020579
	hsa-mir-3200-3p	−1.32088	0.002358	0.044767
	hsa-mir-875-3p	−1.38307	0.001384	0.03123
	hsa-mir-454	−1.43013	0.001491	0.031847
	hsa-mir-509-3-5p	−1.65189	0.001868	0.036162
	hsa-mir-24	−1.87598	5.54E−05	0.003679
	hsa-mir-543	−1.88618	0.000308	0.010836
	hsa-mir-362-3p	−1.91579	0.001437	0.031824
	hsa-mir-30c-1	−1.95483	0.001489	0.031847
	hsa-mir-485-3p	−1.96353	0.000639	0.01821
	hsa-mir-154	−2.01832	0.001292	0.029713
	hsa-mir-4288	−2.1899	5.97E−05	0.003757
	hsa-mir-887	−2.20111	0.000954	0.023287
	hsa-mir-381	−2.25863	0.000923	0.022996
	hsa-mir-452	−2.40566	0.002565	0.047191
	hsa-mir-490-5p	−2.43741	0.000494	0.015555
	hsa-mir-34c-3p	−2.49891	0.00013	0.007419
	hsa-mir-204	−2.55147	0.000181	0.009337
	hsa-mir-24-2	−2.6042	0.000206	0.009764
	hsa-mir-181a	−3.04219	0.000901	0.022918
	hsa-mir-455-5p	−3.41187	0.000217	0.009764
	hsa-mir-424	−3.49982	0.001253	0.029379
	hsa-mir-503	−3.70824	0.000437	0.014123
	hsa-mir-338-5p	−3.8787	0.000231	0.009876
	hsa-mir-505	−3.928	0.000367	0.012528
	hsa-mir-181d	−4.00368	0.000187	0.009337
	hsa-mir-224	−4.42522	1.37E−05	0.001637
	hsa-mir-628-3p	−4.63882	1.65E−05	0.001798
	hsa-mir-133b	−4.80346	1.90E−05	0.001892
	hsa-mir-421	−4.84186	3.52E−06	0.000602
	hsa-mir-1271	−5.28039	4.67E−05	0.003283
	hsa-mir-422a	−6.14312	1.45E−07	8.65E−05
	hsa-mir-187	−6.24756	1.44E−06	0.000321
	hsa-mir-378c	−6.39558	9.35E−06	0.001398
	hsa-mir-31	−6.4792	0.001608	0.033369
	hsa-mir-378	−6.73812	0.000163	0.008855
	hsa-mir-139-5p	−6.74784	0.000302	0.010836
	hsa-mir-133a	−7.05564	1.61E−06	0.000321
	hsa-mir-1	−7.14977	0.00022	0.009764
	hsa-mir-181b	−7.16942	0.000772	0.020654
	hsa-mir-28-3p	−7.22595	0.000262	0.010777
	hsa-mir-100	−7.44023	0.000777	0.020654
	hsa-mir-99b	−7.47102	0.001618	0.033369
	hsa-mir-152	−7.81912	2.15E−05	0.001982
	hsa-mir-28-5p	−8.02489	4.40E−05	0.003283
	hsa-mir-30c	−8.74333	0.000284	0.010818
	hsa-mir-455-3p	−8.97619	0.000795	0.020681
	hsa-mir-205	−9.14687	0.001875	0.036162
	hsa-mir-27b	−9.15993	7.27E−07	0.000217
	hsa-let-7c	−9.61812	0.00051	0.015575
	hsa-mir-222	−9.63879	6.73E−07	0.000217
	hsa-mir-143	−10.1044	8.01E−05	0.004791
	hsa-mir-23b	−10.2596	3.30E−05	0.002633
	hsa-mir-221	−10.2908	1.20E−10	1.44E−07
	hsa-mir-199a-3p	−10.3638	0.000289	0.010818
	hsa-mir-125a-5p	−10.5363	0.001784	0.035561
	hsa-mir-145	−10.6103	0.00027	0.010777

constructed and visualized through Cytoscape software (available at http://www.cytoscape.org/, version: 3.2.0).[22]

2.6. Construction of MFSN

GO enrichment analysis is a common approach for functional annotation.[23] If co-regulated target genes of 2 (or more) DEmiRNAs were significantly enriched in 1 (or more) GO BP functional term, it is defined that functional synergistic relationships exist between the corresponding DEmiRNAs. To construct an MFSN, DEmiRNA interactions in DEmiRNA–DEmiRNA co-regulation network were ranked according to the numbers of their co-regulated target genes. Thereafter, the target genes co-regulated by the top 30 DEmiRNA pairs were put through GO BP functional enrichment analysis by clusterProfiler package in R (available at http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html, version: 2.0.0),[24] and the criteria for significant BP terms were $P < 0.05$ and $q < 0.05$. Moreover, based on functional enrichment results, DEmiRNA interactions sharing 1 (or more) GO BP functional term were utilized to construct MSFN, which was further visualized through Cytoscape software.

2.7. Construction of DEmiRNA–TF regulation network and subnetwork

Comprising of eukaryotic TFs, TFs target genes, and regulatory binding sites, the TRANSFAC database (http://www.gene-regulation.com/index2) has been established and well developed.[25] According to TRANSFAC database and Transcriptional Regulatory Element Database (TRED, https://cb.utdallas.edu/cgi-bin/TRED/tred.cgi?process=home),[26] cancer-related TFs were screened out to build DEmiRNA–TF regulation network,

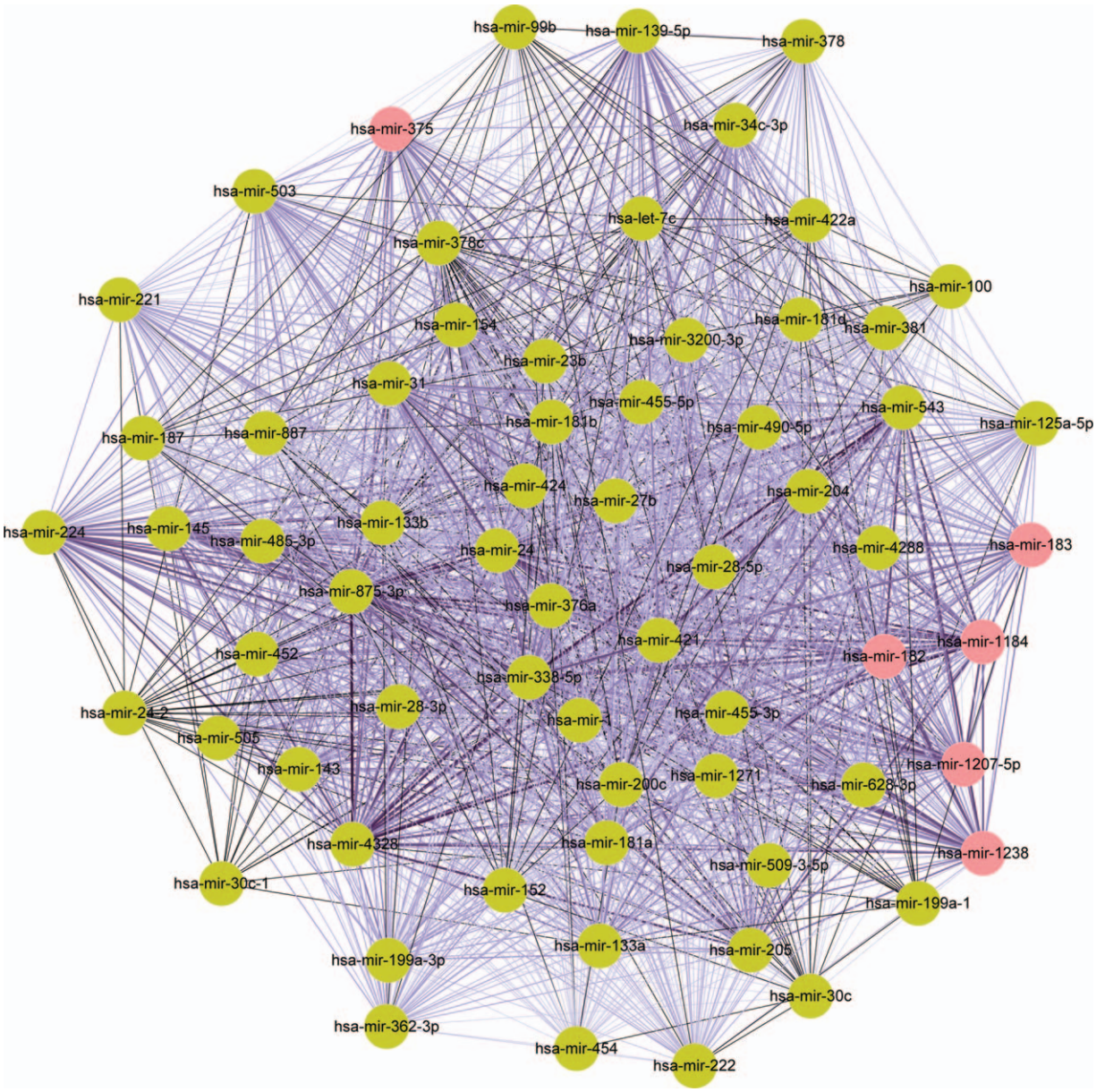

Figure 2. DEmiRNA–DEmiRNA co-regulation network. Edges represent the target genes co-regulated by a pair of miRNAs. Edge thickness represents the number of co-regulated target genes. Red nodes represent up-regulated DEmiRNAs, and yellow nodes represent down-regulated DEmiRNAs.

which was further visualized by Cytoscape software. Then, the node degrees of TFs in this network were analyzed, and subnetworks were mined.

3. Results

The process of analyses in our study is presented in Fig. 1.

3.1. Data preprocessing, DEmiRNAs screening, and DEmiRNA–target gene interaction constructing

The clinical details of 50 PC patients in the dataset of GSE45604 were listed in Table 1. After data preprocessing, expression information of 1196 miRNAs corresponding to 20,643 probes was available. Under the predefined criteria for DEmiRNAs and target genes screening ($|\log_2 FC| > 1$, FDR < 0.05), 66 significant DEmiRNAs were identified between PC patients and normal controls, including 7 up-regulated miRNAs with 18,642 target genes and 59 down-regulated miRNAs with 130,694 target genes. The 66 DEmiRNAs are listed in Table 2.

3.2. Construction of DEmiRNA–DEmiRNA co-regulation network

Based on the numbers of co-regulated target genes, a DEmiRNA–DEmiRNA co-regulation network was constructed, involving 66 nodes (DEmiRNAs), 2024 edges (co-regulation relationships), and 11,086 co-regulated target genes (Fig. 2). In this network, there were strong relationships between hsa-miR-875-3p, hsa-miR-4328, hsa-miR-24, and hsa-miR-31; hsa-miR-338-5p and hsa-miR-543; hsa-miR-24 and hsa-miR-1184; hsa-miR-24 and hsa-miR-1207-5p; etc. Especially, hsa-miR-24 was down-regulated, whereas hsa-miR-1184 and hsa-miR-1207-5p were up-regulated, which shared 1321 and 1230 target genes with hsa-miR-24, respectively. Additionally, hsa-mir-875-3p was tightly linked to hsa-mir-4328 in the network, and both of them were down-regulated (Fig. 2).

3.3. Construction of DEmiRNA–DEmiRNA functional synergistic network

After GO BP functional enrichment analysis with the aforementioned criteria ($P < 0.05$, $q < 0.05$), significant functional synergistic relationships were identified among the top 30 DEmiRNA interactions with most co-regulated target genes in Fig. 2. Then, MFSN was constructed (Fig. 3). In addition, 693 significant GO BP terms were enriched by the co-regulated target genes of hsa-miR-1207-5p and hsa-miR-24, and 862 significant BP terms were enriched by the co-regulated target genes of hsa-miR-1184 and hsa-miR-24. Apparently, hsa-miR-1184, hsa-miR-1207-5p, and hsa-miR-24 had significant functional synergistic relationships.

3.4. Construction of DEmiRNA–TF regulation network and subnetwork

Using the information in TRANSFAC database, 331 TFs were predicted to regulate the 11,086 co-regulated target genes. Further, according to the information of 36 cancer-related TF families in TRED database, 52 of the 331 TFs belonged to 28 cancer-related TF families (Table 3). Then, regulation network was constructed between the 52 cancer-related TFs and their corresponding DEmiRNAs (Fig. 4). Considering miRNAs often suppress gene or TF expressions by targeting them, increased miRNAs might have more important roles in cancer development. Therefore, we mainly focused on the up-regulated DEmiRNAs. Among the 7 identified DEmiRNAs, 6 were exhibited in this network. As hsa-miR-1207-5p and hsa-miR-1184 both had the co-regulation relationship with the

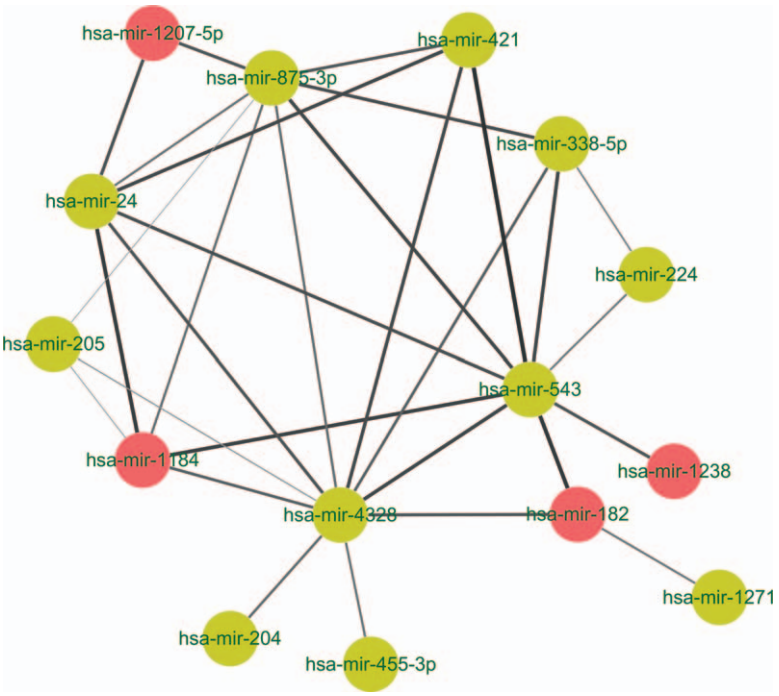

Figure 3. DEmiRNA–DEmiRNA functional synergistic network. Edge thickness represents the number of GO BP terms enriched by co-regulated target genes. Red nodes represent up-regulated DEmiRNAs, and yellow nodes represent down-regulated DEmiRNAs. BP = biological process, GO = gene ontology.

Table 3

The 52 cancer-related transcription factors regulated by differentially expressed miRNAs.

Transcription factor	Family	Full name	Degree
ARNT	HIF	Hypoxia-inducible factor	14
ATF3	ATF	Activating transcription factor	5
ATF7	ATF	Activating transcription factor	20
BCL6	BCL	B-cell CLL/lymphoma	10
BRCA1	BRCA	Breast cancer susceptibility protein	1
BRCA2	BRCA	Breast cancer susceptibility protein	3
CREB1	CREB	cAMP responsive element binding protein	27
CREM	CREB	cAMP responsive element binding protein	49
E2F7	E2F	E2F transcription factor	15
EGR1	EGR	Early growth response protein	1
EGR2	EGR	Early growth response protein	6
ELK1	ELK	Member of ETS oncogene family	2
ELK4	ELK	Member of ETS oncogene family	2
ERG	ERG	ETS-related gene	28
ESR2	ER	Estrogen receptor	2
ETS1	ETS	ETS-domain transcription factor	18
FOSB	AP1	Activator protein 1	6
GLI1	GLI	Glioma-associated oncogene homolog	3
GLI3	GLI	Glioma-associated oncogene homolog	15
HIF1A	HIF	Hypoxia-inducible factor	7
LEF1	LEF	Lymphoid enhancing factor	4
MYBL1	MYB	Myeloblastosis oncogene	13
NFIA	NFI	Nuclear factor I; CCAAT-binding transcription factor	24
NFIB	NFI	Nuclear factor I; CCAAT-binding transcription factor	3
NFKB1	NFKB	Nuclear factor kappa B, reticuloendotheliosis oncogene	2
PAX2	PAX	Paired box gene	6
PAX3	PAX	Paired box gene	29
PAX6	PAX	Paired box gene	17
PAX7	PAX	Paired box gene	18
PGR	PR	Progesterone receptor	32
POU2F1	OCT	Octamer binding proteins	2
PPARA	PPAR	Peroxisome proliferator-activated receptor	26
PPARD	PPAR	Peroxisome proliferator-activated receptor	3
PPARG	PPAR	Peroxisome proliferator-activated receptor	3
RARA	RAR	Retinoic acid receptor	4
RARG	RAR	Retinoic acid receptor	7
RELA	NFKB	Nuclear factor kappa B, reticuloendotheliosis oncogene	8
SMAD1	SMAD	Mothers against decapentaplegic homolog	8
SMAD2	SMAD	Mothers against decapentaplegic homolog	28
SMAD3	SMAD	Mothers against decapentaplegic homolog	16
SMAD5	SMAD	Mothers against decapentaplegic homolog	19
SMAD6	SMAD	Mothers against decapentaplegic homolog	1
SMAD9	SMAD	Mothers against decapentaplegic homolog	22
SP1	SP	Sequence-specific transcription factor	14
SP3	SP	Sequence-specific transcription factor	17
STAT1	STAT	Signal transducer and activator of transcription	1
STAT2	STAT	Signal transducer and activator of transcription	9
STAT3	STAT	Signal transducer and activator of transcription	14
TP73	p53	P53 family	16
USF1	USF	Upstream stimulatory factor	6
USF2	USF	Upstream stimulatory factor	4
WT1	WT1	Wilms tumor 1 (zinc finger protein)	19

Degree represented the number of corresponding DEmiRNAs.

down-regulated hsa-miR-24 (Fig. 3); and hsa-miR-182 and hsa-miR-183 had been implicated in PC or other cancer types,[14,27,28] the 4 DEmiRNAs were further extracted to construct subnetwork (Fig. 5). It is found that the TFs of peroxisome proliferative activated receptor, alpha (PPARA) and cyclic AMP-responsive element modulator (CREM) were 2 critical nodes with the highest degree (=4), which indicated both of them were interplayed with the 4 up-regulated DEmiRNAs.

4. Discussion

PC is a prevalent cancer, whose occurrence and development is a complex process. Comprehensive analysis of miRNAs and TFs related to PC will help us to understand the molecular mechanism of PC and develop novel therapeutic targets for its treatment. In this study, we identified 66 significant DEmiRNAs between normal and PC specimens and their co-regulated cancer-related TFs through bioinformatics analysis. It

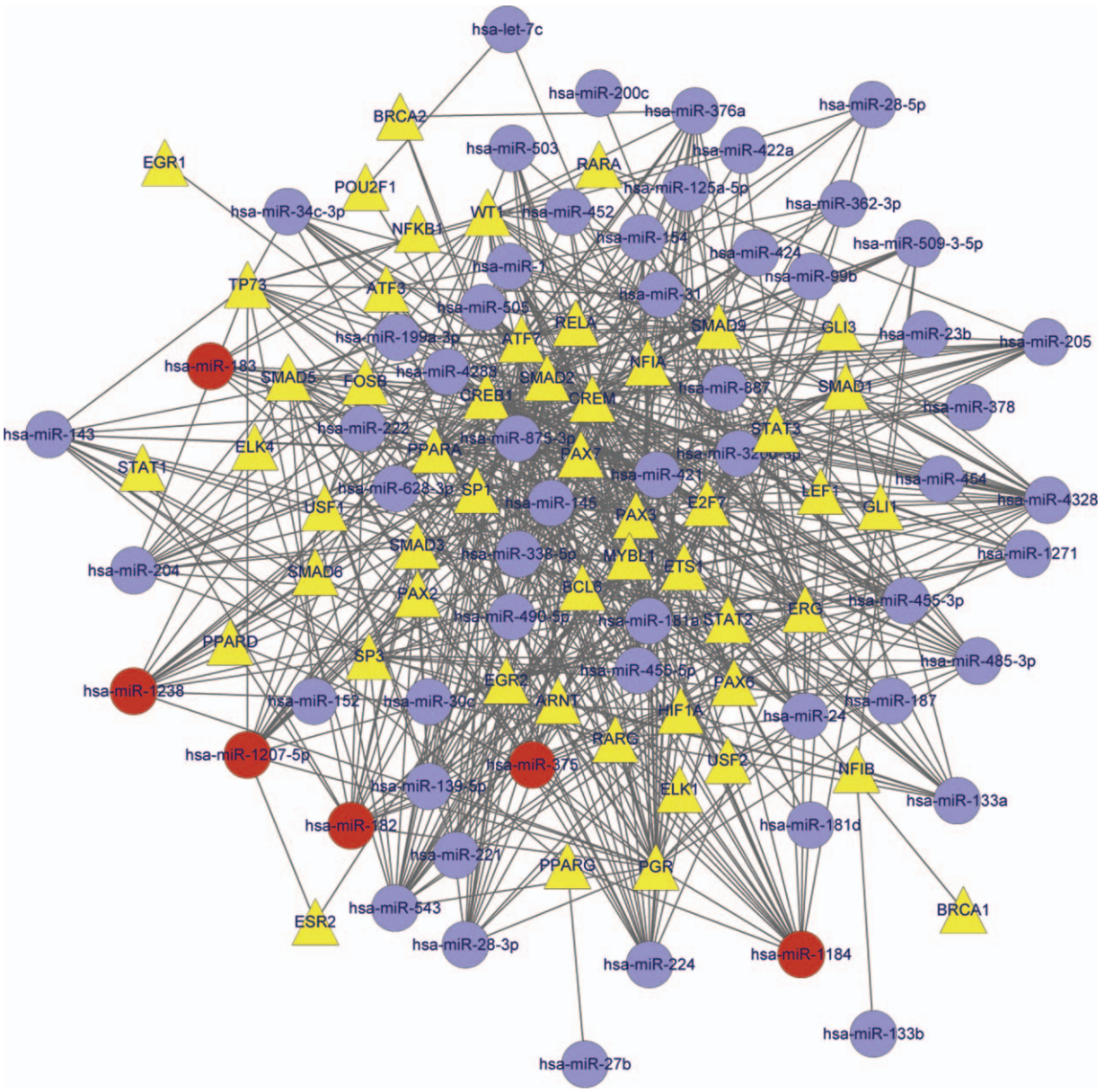

Figure 4. Regulation networks between 52 cancer-related TFs and the corresponding DEmiRNAs. Yellow triangles represent cancer-related TFs. Red nodes represent up-regulated DEmiRNAs. Purple nodes represent down-regulated DEmiRNAs. TFs = transcription factors.

was predicted that 4 up-regulated DEmiRNAs (hsa-miR-1207-5p, hsa-miR-1184, hsa-miR-182, and hsa-miR-183) and 2 of their co-regulated target TFs (PPARA and CREM) might participate in PC. In addition, 2 down-regulated DEmiRNAs, hsa-mir-875-3p and hsa-mir-4328 were also important for their strong relationships in the co-regulation network.

Among these DEmiRNAs, hsa-miR-1207-5p and hsa-miR-1184 shared more than 1200 target genes with hsa-miR-24, which enhances breast tumor metastasis and invasion by targeting PTPN9 and PTPRF, and promoting EGF signaling.[29] In addition, miRNA-24 is one of the most abundant miRNAs in cervical cancer cells, and it is reported to be up-regulated in solid stomach cancers.[29] It seems like hsa-miR-24 plays vital roles in various cancers. In PC, miR-24 is found to modulate apoptosis in the DU-145 cell lines, via targeting to the coding sequence region of fas associated factor 1.[30] Therefore, we speculated that it may be involved in PC progression. As large amount of target genes and GO BP terms were shared by hsa-miR-1184 and hsa-miR-1207-5p with hsa-miR-24, hsa-miR-1184 and hsa-miR-1207-5p might also play a role in the development of PC, and they might be used as potential targets for the treatment of PC. In addition, it is reported that hsa-miR-182-5p promotes cell proliferation, migration, and invasion by down regulating forkhead box F2, reversion-inducing cysteine-rich protein with Kazal motifs, and metastasis suppressor 1 in human PC, and the knock-down of miR-182-5p with its inhibitor significantly decreased prostate tumor growth.[31] Moreover, hsa-miR-183 is an oncogenic miRNA targeting dickkopf 3 homolog (DKK3) and drosophila mothers against decapentaplegic family member 4 (SMAD4) in PC.[27] The high expression of hsa-miR-183 was correlated with high pathological (pT) stage and short overall survival time of PC patients, and the knockdown of hsa-miR-183 significantly

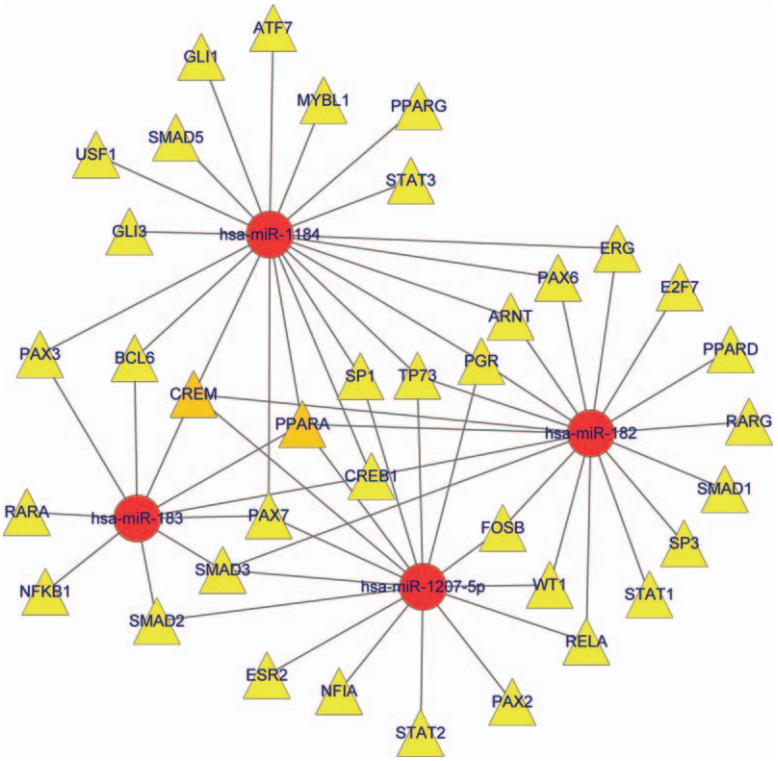

Figure 5. Regulation subnetworks between cancer-related TFs and the corresponding DEmiRNAs. Yellow triangles represent cancer-related TFs. Red nodes represent up-regulated DEmiRNAs. The 2 aggravated yellow triangles represent the TFs co-regulated by 4 DEmiRNAs. TFs = transcription factors.

decreased cell proliferation, motility, and prostate tumor growth.[27] In this study, hsa-miR-182 and hsa-miR-183 were significantly up-regulated in PC tissue, coinciding with previous studies. Therefore, these DEmiRNAs might be key regulators in PC progression, and they might be used as potential targets for the treatment of PC.

Regarding to the down-regulated DEmiRNAs, hsa-miR-875-5p is predicted to be associated with different PC tumor status.[32] Inconsistent with our findings in PC, in other cancer types such as intrahepatic cholangiocarcinoma and esophageal cancer, expression of hsa-miR-875-3p is increased in tumor groups compared with normal control.[33,34] This prompts us the down-regulation of hsa-miR-875-3p might be specific in PC, and could be used as a novel indicator for the disease progression. However, its detailed functions should be investigated by further studies. Currently, only a few studies reported the alteration of hsa-mir-4328 in cancers. In mucinous cystadenocarcinoma, hsa-mir-4328 is significantly decreased compared with the mucinous cystadenoma.[35] In addition, it is also down-regulated in keloid fibroblasts.[36] Nevertheless, dysregulation of this miRNA has not been reported in PC yet. Thus, based on our study, it might be inferred down-regulation of hsa-mir-4328 may be a novel biomarker for PC progression.

The DEmiRNA–TF regulation network displayed that PPARA and CREM were co-regulated by 4 up-regulated DEmiRNAs. CREM can bind to c-fos CRE and heterodimerize with CRE-binding protein, which could block cAMP induction and cAMP-induced apoptosis of germ cells.[27] In this study, CREM was found to be co-regulated by 4 DEmiRNAs, which might influence apoptosis and carcinogenesis in PC cells. However, no current studies have reported the regulation between this gene and the 4 miRNAs. PPARA is a peroxisome proliferator-activated receptor, which regulates key genes involved in fatty acid oxidation, extracellular lipid metabolism, hemostasis, and inflammation.[37] It is demonstrated that the up-regulation of PPARA and its downstream targets leads to increased lipogenesis, which slows cell growth and tumor progression.[38] In this study, the significant up-regulation of 4 DEmiRNAs might collectively decrease PPARA mRNA, promoting cell growth and tumor progression. However, these targeting relationships need to be further validated.

What is more, conflicts between DEmiRNAs co-regulating the same gene also exist. Nuclear factor of kappa light polypeptide (NFKB1) was presented in DEmiRNA–TF regulation subnetwork. NFKB1 is associated with colorectal cancer.[39] Constitutive NFKB activation was observed in 40% of colorectal cancer tissues.[40] In this study, NFKB1 was co-regulated by hsa-miR-183 and hsa-miR-338-5p, which were significantly up-regulated and down-regulated in PC tissue, respectively. Therefore, it is predicted that the balance between hsa-miR-183 and hsa-miR-338-5p might play a role in PC through co-regulating NFKB1. Moreover, cyclooxygenase 2 (COX2) is a key enzyme in the biosynthesis of prostaglandins, which promote inflammation.[41] Additionally, COX2 promotes cell proliferation and growth, and its overexpression is often found in tumor tissues.[42] The activation of P2Y2/Src/p38/COX2 pathway induces resistance to apoptosis in PC cells.[42] It is reported that miR-101 could inhibit COX2 expression post-transcriptionally, and exogenous miR-101 could effectively suppress PC cells growth, providing a new therapy for PC treatment.[43] In this study, COX2 is co-regulated by both up-regulated and down-regulated DEmiRNAs, like hsa-miR-490-5p, hsa-miR-509-3-5p, hsa-miR-143, hsa-miR-628-3p, hsa-miR-182, and hsa-miR-183, indicating that the balance between these DEmiRNAs might play a role in PC through co-regulating COX2.

Researches on miRNAs and TFs are fascinating for human to understand and recognize the mechanism of cancers and further develop new therapeutic strategies for cancers. The miRNAs and TFs found in our research might co-regulate PC and thus serve as new therapeutic targets for PC treatment. But more in vivo researches are needed, and we plan to validate the results of this study by using real-time PCR and dual-luciferase reporter assay in the future study. In addition, it will be useful to further analyze the dynamic changes of miRNA expression along the progression of PC using specimens from patients with different pT stage or Gleason score, and we plan to perform this analysis in the future.

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