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The expression of miR-375 in prostate cancer: A study based on GEO, TCGA data and bioinformatics analysis

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ABSTRACT

Background: MiR-375, as a member of miRNA family, plays essential roles in prostate cancer (PC). We purpose to explore the expression and possible molecular mechanism of the miR-375 in PC using database analysis.

Methods: First, Student's *t*-test, overall and subgroup meta-analyses with 20 eligible datasets in the Gene Expression Omnibus (GEO) database were performed to explore the expression of miR-375 in PC. Then the results of meta-analyses were verified in The Cancer Genome Atlas (TCGA) database by Student's *t*-test and Paired *t*-test. The candidate genes of miR-375 were predicted by four platforms. Protein-protein interaction (PPI) networks, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to investigate the potential molecular mechanism of miR-375 in PC.

Results: The overall meta-analysis showed the expression of miR-375 was significantly up-regulated in PC groups compared with non-cancerous group (SMD; 0.71; 95% CI: 0.38–1.04). In addition, the meta-analyses by subgroup showed the expression of miR-375 in PC tissues was higher than that in healthy prostate tissues and adjacent non-cancerous tissues. The results of TCGA database verified the expression of miR-375 in PC tissues was obviously higher than that in adjacent non-cancerous tissues. Moreover, GO and KEGG analysis revealed that the latent target genes were mainly involved in protein binding function and ubiquitin mediated proteolysis. PPI analysis identified JAK2, EHMT1 and QKI as the hub genes (highly connected genes with high degree in PPI).

Conclusions: MiR-375 was up-regulated in PC tissues. Meanwhile, miR-375 may play an important role in aggressive PC by targeting its potential target genes.

1. Introduction

Prostate cancer (PC) is the second common malignancy cancer among males worldwide and the most frequently occurring cancer among males in developed countries [1]. In 2016, 1.4 million people suffered PC and 381,000 patients died of PC globally. According to a global burden of disease study, the global burden contributed by PC was 6.1 million disability adjusted life years in 2016, of which 91% came from years of life lost and 9% from years lived with disability [2]. Research data showed that the 5-year relative survival rate of localized PC patients was 100% but radically declined to 28% for patients who were diagnosed at an advanced stage [3]. Therefore, early detection and precise diagnosis of PC played an important role in their prognosis [4].

In the past decades, the risk factors about PC have undergone remarkable developments [5–8], including genetic factors, biomarkers, age, race/ethnicity, family history, smoking, fruits and alcohol consumption and so on [9–13]. Despite the serum prostate-specific antigen (PSA) test is the most common biomarker to diagnose PC [14], serum PSA remains difficult and insufficient to distinguish indolent or aggressive cancer stages [15]. Moreover, using serum PSA to diagnose PC may fluctuate because of infections, inflammation, or hyperplasia, which usually resulted in over-diagnosis and over-treatment [16,17]. Therefore, specific and sensitive predictive parameters for early PC

Abbreviations: PC, prostate cancer; BPH, benign prostatic hyperplasia; BPH T, benign prostatic hyperplasia tissues; PC T, prostate cancer tissues; HPT, healthy prostate tissues; PC C, prostate cancer cell lines; NCL, normal cell lines; ADJ T, adjacent non-cancerous tissues; BP, biological process; CC, cellular component; MF, molecular function

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screening are remained to be detected and warranted.

MicroRNAs (miRNAs) which are located in open chromatin, are a class of small, single-stranded, non-coding RNAs with a length of 18–25 nucleotides [18,19]. Many researches reported that miRNAs play an important role in growth, proliferation, differentiation and apoptosis of cancer cells, which indicated that miRNAs can be used as potential predictive parameters for the diagnosis and prognosis of PC [20,21].

MiR-375 (has-miR-375), which is located on the genetic regions of CRYBA2 and CCDC108 on 2q35, has been known as an islet-specific miRNA that could regulate insulin secretion [22]. MiR-375 can also be involved in several types of cancer by targeting important genes like SEC23 A, FGF2, IGF1R and PDK1 [23–26]. MiR-375 expression correlated with PC development, metastasis and other clinicopathological characteristics (cases parameters of clinical and pathological information including age, biochemical recurrence, lymph node metastasis, histological type, race, TNM stage and so on) were broadly reported [27–42]. Of which, some studies have demonstrated that, in PC samples, miR-375 expression was higher than that in normal prostate samples [30–41]. However, some other studies detected even opposite results, which founded that miR-375 was significantly down-regulated in PC patients [42]. Moreover, some negative signals for the relationship between miR-375 and PC were also reported [43]. Therefore, data published on expression of miR-375 for PC remains controversial and need a more deeper validation study.

To investigate the expression and potential biological processes of miR-375 in PC, we conducted a comprehensive and objective meta-analysis and subgroup meta-analyses to evaluate the latent diagnostic value of miR-375 expression in PC based on data obtained from Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database. Then, the data on miR-375 expression and clinicopathological characteristics in PC from The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/) was mined to verify the results of subgroup meta-analysis. Moreover, multiple bioinformatic analyses were also conducted to predicted the possible molecular mechanism of miR-375 in PC.

2. Materials and methods

2.1. Data extraction based on GEO database for meta-analyses

Datasets selection. We searched the microarray files of PC from the GEO database in NCBI. The following search terms were used in GEO database: (prostate OR prostatic) AND (cancer OR carcinoma OR tumor OR neoplas* OR malignan* OR adenocarcinoma). We selected “Homo sapiens” in the “Top Organisms” and “Non-coding RNA profiling by array” in the “Study type”. A total of 105 datasets were identified in initial search.

Inclusion criteria: (1) Each dataset included PC sample group (PC tissues and PC cell lines) and control sample group (healthy prostate tissues, adjacent non-cancerous tissues, normal cell lines and benign prostatic hyperplasia (BPH) tissues); (2) The expression of miR-375 for the samples were available for both groups; (3) At least two samples were included in each group.

Exclusion criteria: (1) Duplicate datasets which were parts of other datasets; (2) Only lncRNA expression data, no miR-375 expression data; (3) Datasets lack of PC sample group; (4) Datasets lack of control sample group.

The ultimate search identified a total of 20 datasets for meta-analysis after removing 85 records (Fig. 1).

Data extraction. For each dataset, we obtained the following information: (1) microarray files; (2) clinical files; (3) samples definition, number of PC samples and control samples. Of the 20 datasets, 3 datasets have two groups of different source control samples (GSE60371, GSE59156, GSE34932). To ensure a comprehensive analysis, we divided each of the 3 datasets into two sub-datasets with the same PC samples but different control samples, for example recorded as

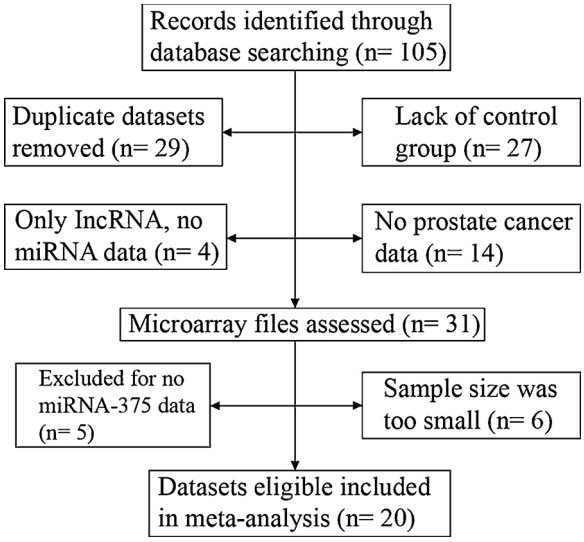

Fig. 1. Flow diagram of GEO datasets selection process.

“GSE60371-1”and “GSE60371-2”. As a result, 20 datasets were obtained (Table 1). Of which, 17 datasets were already log_2-transformed (transformation of the ratio is the logarithm base 2). We log_2-transformed the expression data of other 3 datasets (GSE17312, GSE45604, GSE54010) for further analyses.

2.2. Data extraction based on TCGA database for validation

TCGA database, which is a collaboration between the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI), was also searched to obtain data of miR-375 expression and clinical information with PC for validation. After carefully search and download data of miR-375 expression and clinical information, 223 cases were excluded. As miRNA expression data was not searched in 217 case of FM program and 6 cases of TCGA program, ultimately, 494 cases of TCGA program were obtained. Of which, 442 cases only had PC tissues and 52 cases had paired tissues samples (PC tissues VS. adjacent non-cancerous tissues). Moreover, data of miR-375 expression and 7 clinicopathological characteristics data including tissues type, age, biochemical recurrence, lymph node metastasis, histological type, race, TNM stage were also extracted. Log_2-transformation was performed with the expression of miR-375 for further analyses.

3. Statistical analysis

First, for each GEO dataset, Student’s *t*-test was used to analyze the significance of miR-375 expression difference between PC samples and control samples. Then for overall meta-analysis of the association between the expression of miR-375 and PC, standard mean deviation (SMD) and 95% CI was calculated to evaluate the miR-375 expression level in PC by the forest plot of all the 20 GEO datasets. If the upper limit of 95% CI of SMD < 0, it means that the expression of miR-375 was lower in cancer samples than that in non-cancerous samples. In contrast, if the lower limit of 95% CI of SMD > 0, the expression of miR-375 was considered to be higher in cancer samples than that in non-cancerous samples. Chi-square test and I^2 statistic were used to present the heterogeneity of meta-analysis. If $I^2 > 50\%$ and P value < 0.05, random-effects model was selected. Otherwise, the fixed-effects model was selected. Meta-analyses by subgroup was applied to explore the association between miR-375 expression and PC with different source control samples which may be a confounding factor of miR-375 expression. Funnel graph was generated to evaluated publication bias. Begg’s and Egger’s test were adopted as the indices of publication.

For the TCGA dataset, expression data were presented as the mean

Table 1
Overview of the 20 Datasets Selected from GEO.

Datasets	Country	Year	Total number	PC number	Control number	PC definition	Control definition	Manufacturer
GSE54010	South Korea	2014	8	6	2	PC tissues	BPH tissues	Agilent Technologies
GSE46738	Brazil	2013	57	53	4	PC tissues	BPH tissues	Affymetrix
GSE49298	Turkey	2013	8	4	4	PC tissues	BPH tissues	Agilent Technologies
GSE18671	Germany	2011	20	14	6	PC tissues	BPH tissues	Laboratory for microarray applications, IZKF, University of Wuerzburg
GSE59156	Finland	2016	42	24	18	PC tissues	15 BPH tissues + 3 healthy prostate tissues	Agilent Technologies
GSE34932	China	2012	16	8	8	PC tissues	3 BPH tissues + 5 adjacent noncancerous tissues	Agilent Technologies
GSE45604	Spain	2013	60	50	10	PC tissues	healthy prostate tissues	Affymetrix
GSE36802	USA	2013	42	21	21	PC tissues	healthy prostate tissues	Affymetrix
GSE24201	Finland	2011	29	14	15	PC tissues	healthy prostate tissues	Agilent Technologies
GSE16512	USA	2009	13	6	7	PC tissues	healthy prostate tissues	CombiMatrix Corporation
GSE31568	Germany	2011	93	23	70	PC tissues	healthy prostate tissues	febit biomed
GSE60371	Italy	2017	81	56	25	PC tissues	21 healthy prostate tissues + 4 normal cell lines	Agilent Technologies
GSE23022	Germany	2010	40	20	20	PC tissues	adjacent noncancerous tissues	Affymetrix
GSE21036	USA	2010	141	113	28	PC tissues	adjacent noncancerous tissues	Agilent Technologies
GSE17321	USA	2015	27	11	16	PC tissues	adjacent noncancerous tissues	Exiqon A/S
GSE8126	USA	2008	76	60	16	PC tissues	adjacent noncancerous tissues	Microarray Shared Resource, Comprehensive Cancer Center, The Ohio State University (OSU-CCC)
GSE76260	Italy	2015	64	32	32	PC tissues	adjacent noncancerous tissues	Illumina Inc
GSE64318	USA	2015	54	27	27	PC tissues	adjacent noncancerous tissues	Agilent Technologies
GSE40026	Japan	2013	5	3	2	PC cell lines	normal cell lines	TORAY Industries
GSE17317	Germany	2012	12	9	3	PC cell lines	normal cell lines	CombiMatrix

BPH: benign prostatic hyperplasia.

and SD. The differences between miR-375 expression and the 7 clinicopathological characteristics were also analyzed. Random design studies and paired design studies were estimated by Student's *t*-test and Paired *t*-test. For comparisons more than two groups, one-way analysis of variance (ANOVA) was applied.

All the statistical analyses were performed with Stata12.0 (Stata Corporation, College Station, TX, USA) and SPSS 23.0 (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered as a statistical significance.

3.1. Prediction of miR-375 target genes

The most related genes were gathered using 4 prediction databases: TargetScan, miRWalk, miRDB and starBase. The frequency of each gene predicted by the 4 prediction databases was counted. Only those predicted by at least two prediction databases were screened out as target genes for further study.

3.2. Bioinformatics analysis of miR-375 target genes

Potential miR-375 target genes were uploaded to DAVID (the Database for Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov/)) to perform GO and KEGG pathway enrichment analysis. The protein-protein interaction (PPI) network was constructed with the STRING database (https://string-db.org/) and visualized using Cytoscape [44]. A combined score > 0.4 was selected to determine significant interactions among candidate genes. Degree was considered to indicate importance of genes in the PPI network [45,46].

4. Results

4.1. Separate- and meta- analysis of the association between miR-375 expression and PC based on GEO datasets

In total, 20 eligible microarray datasets which included miR-375 expression detected from both PC samples and control samples, were collected in this study. The separate analysis of the association between miR-375 expression and PC for each GEO dataset were presented in Table 2. Of which, significant statistical differences were detected in 10 databases. The expression of miR-375 extracted from 554 PC samples and 334 control samples was applied in overall meta-analysis. As there was high heterogeneity ($I^2 = 73.9\%$; $P = 0.000$), the random-effects model was selected. Fig. 2 showed the overall meta-analysis of the 20 datasets that expression of miR-375 was significantly up-regulated in PC groups than that in non-cancerous groups (SMD; 0.71; 95% CI: 0.38–1.04). No publication bias was detected in these studies as indicated by Begg's funnel plot ($P = 1.000$) and Egger's tests ($P = 0.853$) (Fig. 3).

6 datasets reported the level of miR-375 expression in 109 PC tissue samples and 34 BPH tissue samples. As high heterogeneity was detected ($I^2 = 77.1\%$; $P = 0.001$), random-effects model analysis showed that the SMD was 0.29 (95% CI: -0.66-1.24) for the subgroup meta-analysis which indicated no statistical difference between the expression of miR-375 in PC tissue samples and BPH tissue samples (Fig. 4).

3 datasets reported the level of miR-375 expression in 68 PC cell line samples and 9 normal cell line samples. As high heterogeneity was detected ($I^2 = 80.7\%$; $P = 0.006$), random-effects model analysis showed that the SMD was 1.49 (95% CI: -0.41-3.39) for the subgroup meta-analysis which demonstrated no statistical difference between the expression of miR-375 in PC cell lines and normal cell lines (Fig. 4).

7 datasets reported the level of miR-375 expression in 194 PC tissue samples and 147 healthy prostate tissue samples. As high heterogeneity was detected ($I^2 = 77.5\%$; $P = 0.000$), random-effects model analysis showed that the SMD was 0.61 (95% CI: 0.05–1.18) for the subgroup meta-analysis which demonstrated that expression of miR-375 in the PC tissues is higher than that in the healthy prostate tissues (Fig. 4).

7 datasets reported the level of miR-375 expression in 271 PC tissue samples and 144 adjacent non-cancerous tissue samples. As high heterogeneity was detected ($I^2 = 68.4\%$; $P = 0.004$), random-effects model analysis showed that the SMD was 0.83 (95% CI: 0.40–1.25) for the subgroup meta-analysis which demonstrated that miR-375 expression in the PC tissues is higher than that in the adjacent non-cancerous tissues (Fig. 4).

Table 2
Separate Analyses of the Association between miR-375 and PC for Each GEO Datasets.

Datasets	Subgroups	Patients			Control			*t*	*P*
		Mean	SD	*n*	Mean	SD	*n*		
GSE54010	PC T VS BPH	8.2343	2.2284	6	10.0487	4.9294	2	0.5040	0.6940
GSE46738	PC T VS BPH	10.2878	0.9875	53	6.8133	3.8888	4	1.7830	0.1720
GSE49298	PC T VS BPH	5.6578	1.7269	4	6.7357	1.7783	4	−0.8700	0.4180
GSE18671	PC T VS BPH	6.9156	0.3984	14	6.8895	0.6002	6	0.1150	0.9090
GSE59156-1	PC T VS BPH	7.7231	0.3527	24	7.8540	0.4205	15	−1.0470	0.3020
GSE59156-2	PC T VS HPT	7.7231	0.3527	24	7.6734	0.2842	3	0.2330	0.8170
GSE59156	24 PC T VS 15 BPH + 3 HPT	7.7231	0.3527	24	7.8200	0.4000	18	0.8660	0.3920
GSE34932-1	PC T VS BPH	8.8150	1.4217	8	8.2500	0.7475	3	0.6410	0.5380
GSE34932-2	PC T VS ADJ T	8.8150	1.4217	8	9.7200	0.8040	5	−1.2870	0.2250
GSE34932	8 PC T VS 3 BPH + 5 ADJ T	8.8150	1.4217	8	9.1700	1.0530	8	−0.5660	0.5810
GSE45604	PC T VS HPT	11.4086	0.6158	50	9.6492	1.7774	10	−3.0930	0.0120
GSE36802	PC T VS HPT	10.9184	0.6145	21	9.9438	0.7181	21	4.7260	0.0000
GSE24201	PC T VS HPT	−0.3591	3.5676	14	−1.3168	0.5625	15	1.0270	0.3130
GSE16512	PC T VS HPT	0.0780	0.4616	6	0.0111	0.2785	7	0.4040	0.6910
GSE31568	PC T VS HPT	23.7734	36.3091	23	23.8883	29.0157	70	−0.0150	0.9880
GSE60371-1	PC T VS HPT	9.3035	1.0421	56	8.9228	0.4505	21	2.2330	0.0290
GSE60371-2	PC C VS NCL	9.3035	1.0421	56	6.0332	0.0118	4	23.4640	0.000
GSE60371	56 PC T VS 21 HPT + 4 NCL	9.3035	1.0421	56	8.4600	1.1570	25	−3.2510	0.0020
GSE23022	PC T VS ADJ T	4.7323	1.2889	20	2.7690	1.4367	20	4.5490	0.0000
GSE21036	PC T VS ADJ T	11.6899	1.3463	113	10.6835	0.9096	28	3.7440	0.0000
GSE17321	PC T VS ADJ T	9.4085	0.7208	11	8.2838	0.2523	16	−4.9700	0.0000
GSE8126	PC T VS ADJ T	12.0723	1.1169	60	11.2528	1.5668	16	2.3840	0.0200
GSE76260	PC T VS ADJ T	14.2710	0.1789	32	13.8996	0.5651	32	3.5450	0.0010
GSE64318	PC T VS ADJ T	8.7086	0.7570	27	8.1300	1.7680	27	1.5580	0.1260
GSE40026	PC C VS NCL	3.0955	2.5717	3	1.6463	0.3703	2	0.7520	0.5070
GSE17317	PC C VS NCL	9.7664	0.2387	9	9.6332	0.0958	3	0.9180	0.3800

PC T: PC tissues HPT: healthy prostate tissues.
PC C: PC cell lines NCL: normal cell lines.
ADJ T: adjacent non-cancerous tissues BPH T: BPH tissues.

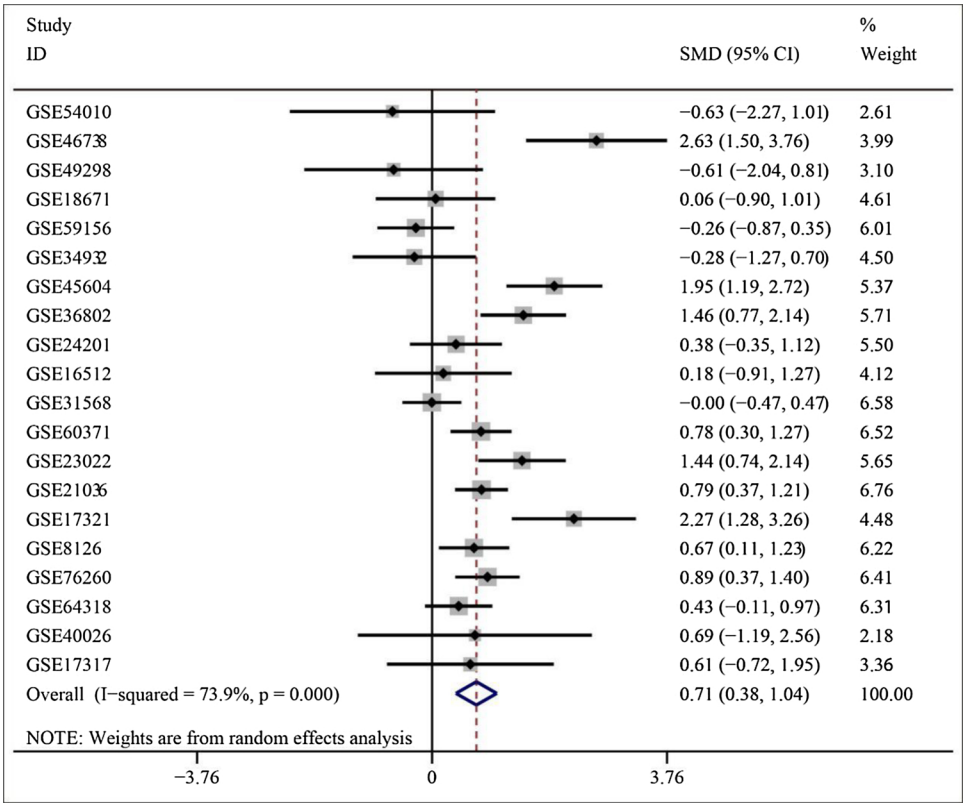

Fig. 2. Overall meta-analysis of the miR-375 expression between PC groups and non- cancerous groups in 20 datasets. Total SMD = 0.71 (95% CI: 0.38–1.04).

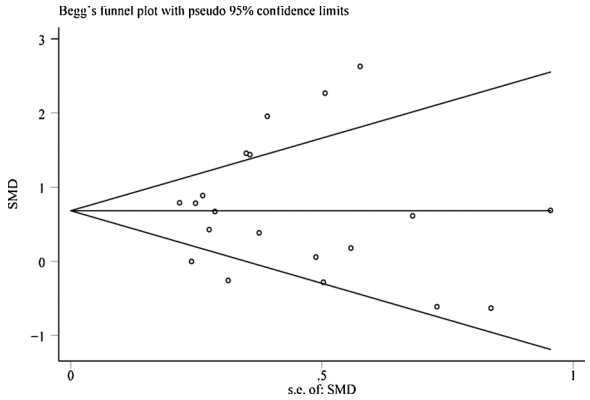

Fig. 3. Funnel plot of combined SMD for study included in overall meta-analysis. Each point represents a single dataset.

4.2. Basic characteristics and comparison of miR-375 expression of TCGA data for validation

The data on 494 PC cases (494 cancer tissues and 52 adjacent non-cancerous tissues) with miR-375 expression was extracted from TCGA database. Paired *t*-test revealed that the miR-375 expression in PC tissues was significantly up-regulated compared with paired adjacent non-cancerous tissues (52 PC tissues and paired adjacent non-cancerous tissues for the same cases) ($P = 0.00$, Table 3 and Fig. 5(a)). Student's *t*-test revealed that the miR-375 expression in PC tissues was significantly up-regulated compared with unpaired adjacent non-cancerous tissues (PC tissues for 442 cases and adjacent non-cancerous tissues for other 52 cases) ($P = 0.00$, Table 3 and Fig. 5(b)). Overall, the results demonstrated that the expression of miR-375 in PC tissues was higher than that in adjacent non-cancerous tissues, which was consistent with the result of subgroup meta-analysis by GEO datasets.

For the relationship between the expression of miR-375 and clinicopathological characteristics in 494 PC cases, only significant difference in histological types was detected ($P = 0.016$) (Table 3).

4.3. Identification of miR-375 candidate genes in PC

A total of 2101 miR-375 related genes were predicted by 4 databases separately. After applying the criteria that target genes were predicted by at least two databases, a total of 128 miR-375 candidate genes were screened out and subjected to GO enrichment analysis and KEGG pathway analysis.

4.4. Function analysis of miR-375 candidate genes in PC

Fig. 6 and Table 4 showed the top 9 records of the GO enrichment analysis and KEGG pathway analysis results. The GO enrichment analysis comprised three categories: biological process (BP), cellular component (CC) and molecular function (MF). In terms of BP, the target genes were mainly involved in 32 GO terms ($P < 0.05$), such as regulation of transcription from RNA polymerase II promoter, regulation of transcription and DNA-templated (Fig. 6(a) and Table 4). Based on CC, they were mainly enriched in 9 GO items ($P < 0.05$), such as nucleus, transcription factor complex and cytoplasm (Fig. 6(b) and Table 4). Regarding MF, they were mainly related to 28 GO terms ($P < 0.05$),

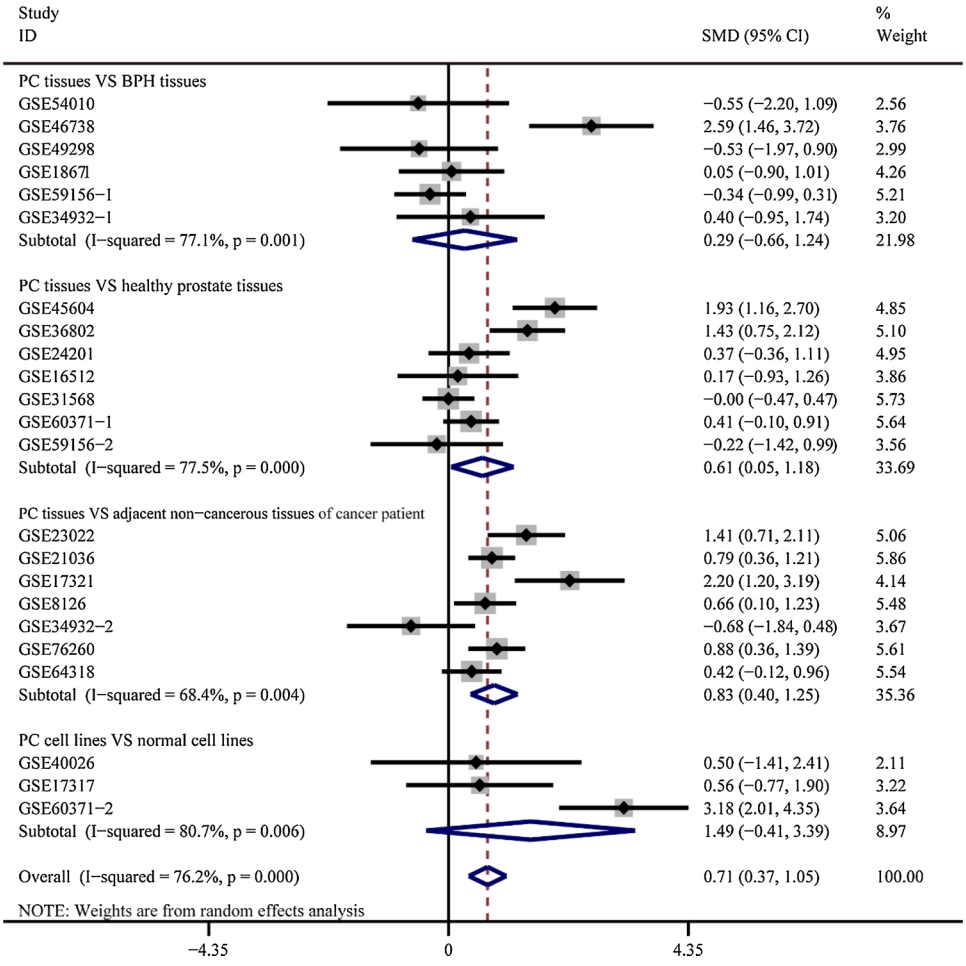

Fig. 4. Subgroup meta-analyses of the miR-375 expression between PC samples and non-cancerous samples.

Table 3
Association between the Expression of miR-375 and Clinicopathological Characteristics in PC Tissues Analyzed by Data from the TCGA.

Clinicopathological characteristics		*n*	miRNA-375 relevant expression		
			Mean ± SD	*t*	*P*
Tissue	PC (paired cases)	52	16.29182 ± 0.6777	−9.408	0.000
	Adjacent non-cancerous tissues	52	13.40565 ± 2.2536		
	PC (unpaired cases)	442	16.50934 ± 0.8522	−9.849	0.000
	Adjacent non-cancerous tissues	52	13.40565 ± 2.2536		
Age(years)	≤61	249	16.46668 ± 0.8616	−0.507	0.612
	> 61	245	16.50498 ± 0.8153		
Biochemical recurrence	Yes	58	16.43640 ± 0.9497	0.506	0.613
	No	369	16.49587 ± 0.8117		
Lymph node metastasis	Yes	79	16.59963 ± 0.7742	−1.192	0.234
	No	325	16.47228 ± 0.8695		
Histological types	Prostate adenocarcinoma acinar type	479	16.46955 ± 0.8373	2.428	0.016
	Other subtypes	15	17.00061 ± 0.7210		
Race	White	146	16.24806 ± 0.8238	1.023*	0.362
	Asian	2	15.88699 ± 0.8024		
	Black	7	16.65021 ± 0.6265		
TNM stage	I/II	148	16.54663 ± 0.8076	0.83	0.407
	III/IV	297	16.47623 ± 0.8607		

Student's *t*-test or paired *t*-test was used for difference comparison between two groups.
* One-way analysis of variance (ANOVA) test was performed on difference comparison among three groups.

such as protein binding, poly(A) RNA binding and transcription factor activity (Fig. 6(c) and Table 4). As shown in Fig. 6(d) and Table 4, 4 KEGG pathway were enriched by the candidate genes, including thyroid hormone signaling pathway, ubiquitin mediated proteolysis, mTOR signaling pathway and notch signaling pathway ($P < 0.05$). All the results of GO enrichment analysis and KEGG pathway analysis were shown in Supplemental file.

A total of 128 candidate genes were submitted to STRING database. As shown in Fig. 7, three top genes (JAK2, EHMT1, QKI) with highest degree were chosen as the hub genes in PC, with the degree of 7, 7, 6.

5. Discussion

In this study, we systemically retrieved previous GEO microarrays data for meta-analyses to retrospectively verify the relationship between expression of miR-375 and PC, with deeper validation based on TCGA data. Additionally, we discovered promising target genes and possible molecular mechanisms for miR-375 that were involved in the regulation of biological processes in PC by GO enrichment analysis and KEGG pathway analysis. To the best of our knowledge, this is the first study to investigate the role of miR-375 in PC combined with the TCGA and GEO datasets, which offered significant amounts of data.

By the overall meta-analysis, we found that miR-375 expression in PC samples was significantly up-regulated than that in control samples, which is inconsistent with the findings of Kachakova et al and McDonald et al. The discrepancies between these studies may due to different methods used for analysis and underestimated treatments of the patients. Kachakova and coworkers explained plausible reasons for the downregulation of miR-375 with the possible interference arising from diabetes, allergic conditions and inflammations. Additionally, the subgroup meta-analyses based on different control source showed miRNA-375 was significantly up-regulated in aggressive PC tissues than that in healthy prostate tissues and adjacent noncancerous tissues. The results of validation by TCGA database consist with the observation of adjacent non-cancerous tissues control group. Thus, the results of meta-analyses implied that determination of miR-375 expression has potential diagnostic value to distinguish PC patients from healthy control, which could act as a reference for other conventional diagnosis biomarker and exploration of novel diagnosis biomarker. Larger scale prospective researches are required in future to further verify its diagnostic effect. If it could be verified in a large-scale research, miR-375 might be useful as a novel screening tool for clinical practice of PC.

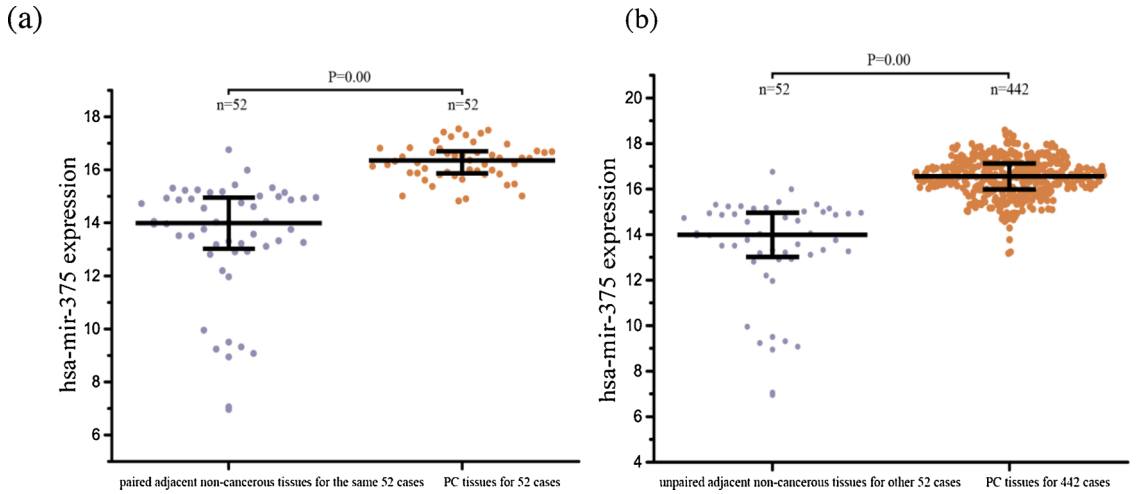

Fig. 5. MiR-375 expression in adjacent non-cancerous tissues and PC tissues obtained from TCGA. (a): PC tissues and paired adjacent non-cancerous tissues for the same cases (a total of 52 cases); (b): PC tissues for 442 cases and unpaired adjacent non-cancerous tissues for other 52 cases.

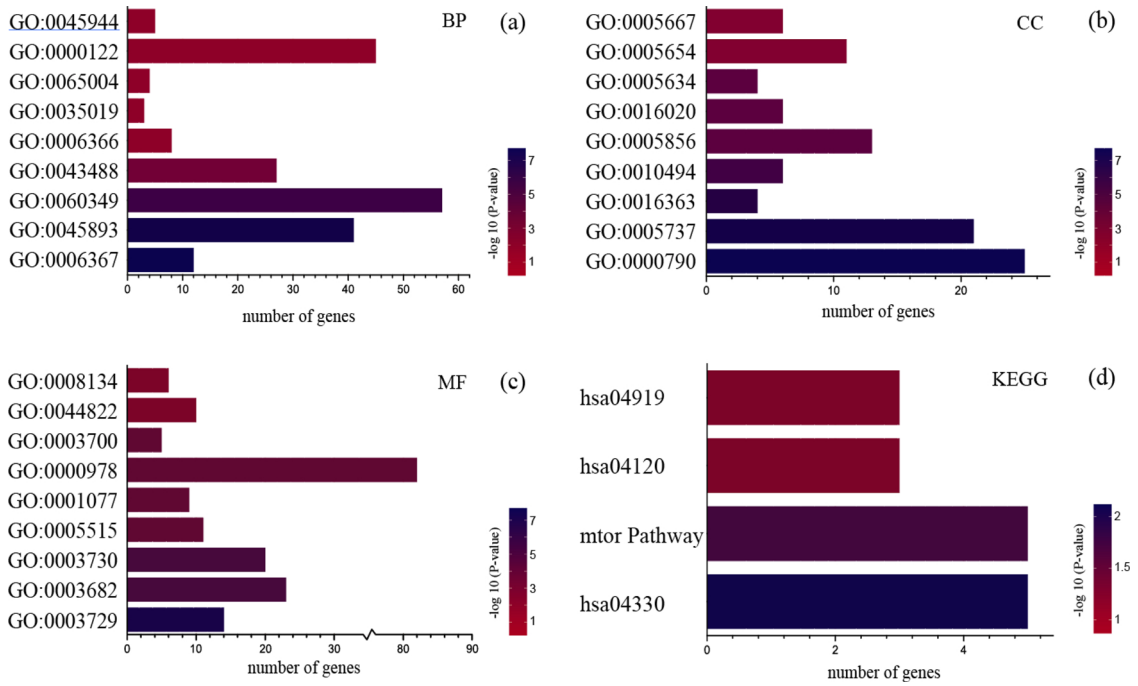

Fig. 6. Part of the bar chart for enriched GO and KEGG items ($P < 0.05$), only the 9 top GO terms were showed. (a) BP: biological process; (b) CC: cellular component; (c) MF: molecular function; (d) KEGG pathways.

Table 4
Results of part GO and KEGG enrichment analyses.

Category	Term	Count	P-Value	FDR
GOTERM- Biological processes				
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	25	7.98E-08	1.25E-04
GO:0000122	negative regulation of transcription from RNA polymerase II promoter	21	1.53E-07	2.39E-04
GO:0065004	protein-DNA complex assembly	4	3.43E-06	0.005363
GO:0035019	somatic stem cell population maintenance	6	9.66E-05	0.150775
GO:0006366	transcription from RNA polymerase II promoter	13	2.84E-04	0.442972
GO:0043488	regulation of mRNA stability	6	8.27E-04	1.283752
GO:0060349	bone morphogenesis	4	8.96E-04	1.390669
GO:0045893	positive regulation of transcription, DNA-templated	11	0.0036284	5.517646
GO:0006367	transcription initiation from RNA polymerase II promoter	6	0.0045461	6.867392
GOTERM- Cellular components				
GO:0005667	transcription factor complex	12	5.51E-08	6.63E-05
GO:0005654	nucleoplasm	41	4.94E-07	5.95E-04
GO:0005634	nucleus	57	4.73E-05	0.056898
GO:0016020	membrane	27	0.0018074	2.153745
GO:0005856	cytoskeleton	8	0.0109721	12.43489
GO:0010494	cytoplasmic stress granule	3	0.023051	24.47342
GO:0016363	nuclear matrix	4	0.0256809	26.88446
GO:0005737	cytoplasm	45	0.0300391	30.7251
GO:0000790	nuclear chromatin	5	0.0378227	37.1274
GOTERM-Molecular function				
GO:0008134	transcription factor binding	14	1.05E-07	1.39E-04
GO:0044822	poly(A) RNA binding	23	1.16E-05	0.015425
GO:0003700	transcription factor activity, sequence-specific DNA binding	20	4.11E-05	0.054512
GO:0000978	RNA polymerase II core promoter proximal region sequence-specific DNA binding	11	2.08E-04	0.27564
GO:0001077	transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	9	2.68E-04	0.354852
GO:0005515	protein binding	82	2.70E-04	0.357358
GO:0003730	mRNA 3'-UTR binding	5	3.89E-04	0.514968
GO:0003682	chromatin binding	10	0.0018016	2.362465
GO:0003729	mRNA binding	6	0.0019149	2.509259
KEGG PATHWAY				
hsa04919	Thyroid hormone signaling pathway	5	0.0090294	9.56017
hsa04120	Ubiquitin mediated proteolysis	5	0.0168376	17.14853
mtor Pathway	mTOR Signaling Pathway	3	0.0392027	33.89204
hsa04330	Notch signaling pathway	3	0.0468641	41.24155

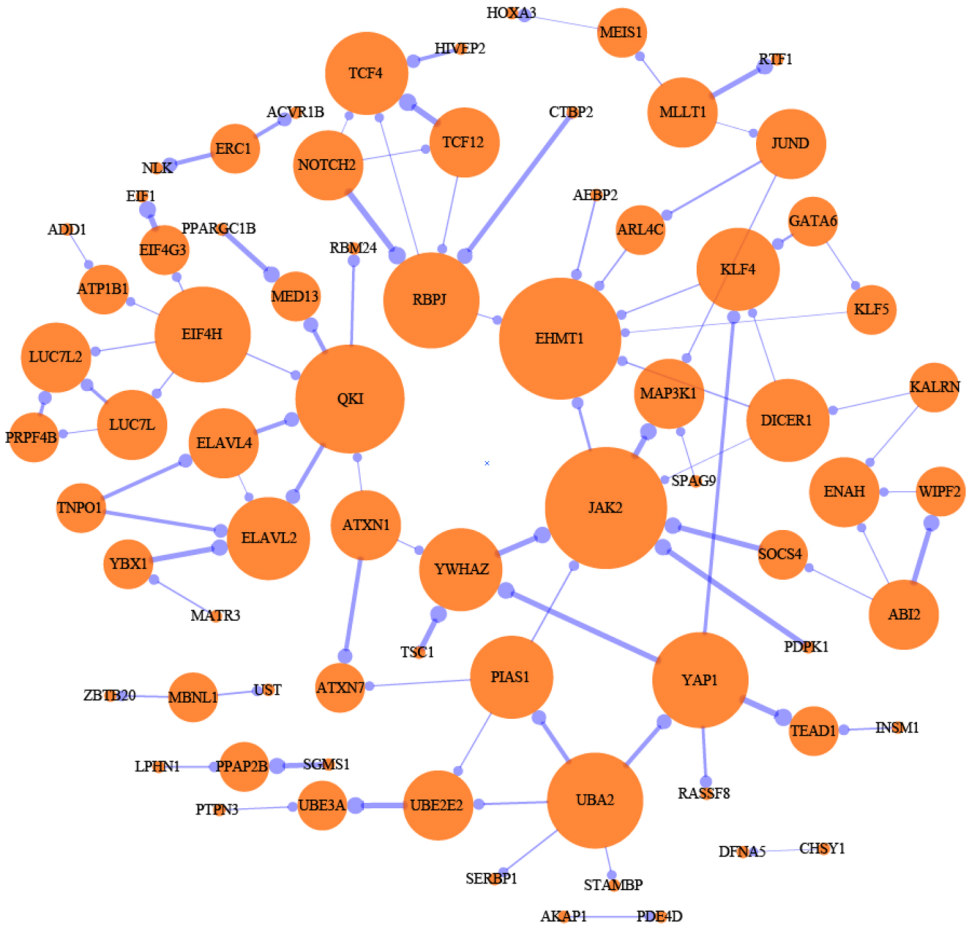

Fig. 7. Protein-protein interaction network of target genes. The thickness of the edge represented the size of combined score. The size of nodes represented the degree.

However, in the subgroup meta-analyses, no statistical significance was detected in the normal cell lines control group and BPH tissues control group. For normal cell lines control group, it is obvious that cells in culture represent an artificial and simplified system. Unlike the situation *in vivo*, a tumor is a complex consisting of interacting cells and specific micro environment. With times of subcultures increased, *in vitro* experiment is a possible source of heterogeneity in cells mutation [47–49], thus, the expression of miR-375 cells *in vitro* may be different with cells *in vivo*. BPH is an imbalance of cell growth and death resulting in hyperplasia of glandular-epithelial and stromal/muscle tissue in the prostate [50]. It has been shown that uncontrolled cell proliferation is the primary feature of cancer [51]. Many studies revealed that miR-375 promote PC cell proliferation and invasion [52,53], such as Szczyrba et al. found that miR-375 could inhibit expression of Sec23 A protein to stimulate PC cell proliferation [52]. We guess that BPH cell hyperplasia may also be stimulated by the up-regulation of miR-375. But up to now, no studies have reported up-expression of miR-375 in BPH tissues compared with normal tissues. Thus, experiments could be designed and performed to elucidate the expression and role of miR-375 in the occurrence and development of BPH. According to subgroup meta-analyses results. it is crucial to select the control group samples to study the expression of miR-375 in PC. The healthy prostate tissues and adjacent noncancerous tissues may be better than the normal cell lines control group and BPH tissues control group. Moreover, no significant differences have been observed between miR-375 expression and part of clinicopathologic characteristics in TCGA data, including age, biochemical recurrence, lymph node metastasis, race and TNM stage. Thus, further exploration should be conducted to validate the correlation between miR-375-3p expression and clinicopathologic characteristics.

Recently, it has been suggested that miR-375 targets several genes to have a pivotal role in multiple types of cancer. In hepatocellular carcinoma, miR-375 could inhibit the expression of ARG-1, and suppressed liver cancer cell growth [54]. Hudson et al. demonstrated that overexpression of miR-375 remarkably suppressed consequent proliferation by down-regulating YAP1 in medullary thyroid cancer [55]. It was reported that expression of PDK1 or 14-3-3zeta was decreased by miR-375, which could reduce the viability of gastric carcinoma cells and induce apoptosis [56]. MiR-375 has been reported to suppress tumor in human cancers, however it acted as an oncogene in some cancers. Yoda et al. found that miR-375 was consistently overexpressed in non-small-cell lung cancer and targeted CLDN1, which promoted invasion and metastasis [57]. Pinheiro et al. found that CCND2 was induced by miR-375, and contribute to PC initiation and progression [58]. Wang et al. reported overexpression of miR-375 that targeted YAP1 caused cell growth inhibition and cell apoptosis [59]. Thus, the molecular mechanism that is related to miR-375 overexpression in PC has not yet been fully elucidated.

In this study, we performed GO and KEGG pathway analysis to investigate the potential biological meaning behind these candidate genes. GO term enrichment analysis showed that 82 genes were enriched in protein binding function. It has been reported that the binding of proteins can stimulate proliferation of cells [60]. NOL8, the binding protein of beta-catenin, can enhance the interaction between beta-catenin and TCF4, and activated beta-catenin/TCF signaling, which facilitated the growth, migration and colony formation of PC cells [61]. KEGG pathway analysis indicated that 5 candidate genes were significantly enriched in ubiquitin mediated proteolysis. Nandi et al.

reported that ubiquitin-proteasome system can selectively degrade cyclins and p53 to participate in generation of tumor [62]. Therefore, it is meaningful to further explore the possible relationships and mechanisms between the target genes and PC.

In the present study, three hub genes were finally screed out: JAK2, EHMT1 and QKI, which were 3 possible related target genes of miR-375. PC is frequently associated with the expression of IL-6 [63]. IL-6 participated in oncogenic effects through activating the Janus tyrosine family kinase-signal transducer and activator of transcription (JAK/STAT3) signaling pathway [64]. JAK2-STAT5a/b signaling has been showed that promotes metastatic progression of PC by inducing EMT and stem cell properties in PC cells [65]. Talati et al. found that STAT3 drove cancerization processes with possible underlying mechanisms such as proliferation, suppression of apoptosis, AR activity, and phenotypic plasticity governing EMT and cancer stem cell maintenance [66,67]. Consequently, JAK2 may also play an important role in the development of PC.

EHMT1 encode a histone methyltransferase that methylate the lysine-9 position of histone H3, which represses transcription [68,69]. Han et al. reported that promoter of MT1h is heavily methylated, and EHMT1 was activated by MT1h to carry out tumor suppression in PC [70]. Thus, the potential effect of EHMT1 in the occurrence of PC should not be overlooked.

QKI is an RNA-binding protein with three isoforms, QKI-5, QKI-6 and QKI-7 [71]. QKI can increase expression of androgen receptor (AR) by heat shock protein 90, of which AR plays important role in the development and progression of PC [72,73]. Zhao et al. reported that down-regulation of QKI-5 was frequently associated with the prostate cancer Gleason score, poor differentiation, degree of invasion, lymph node metastasis, TNM grading, and poor survival [74]. These results elucidated that the QKI expression could not be ignored as a latent factor in the occurrence and prognosis of PC patients.

However, certain limitations exist in the present study. At first, the expression of miR-375 was significantly different in histological types. However, based on the meta-analysis of GEO datasets we did not perform subgroup meta-analysis based on histological type. This may cause a potential heterogeneity for the meta-analysis results. Thus, subgroup meta-analyses based on large-scale clinical trials are required to further verify the relationship between miR-375 expression and different histological PC type. At second, the present study utilized public database to process secondary data mining and online prediction databases to conduct bioinformatic analyses, which lacks of clinical verification absolutely focusing on the mechanism and expression of miR-375 involved in PC. Thus, function experiments are needed to design to validate whether or not the genes mentioned above are targets genes of miR-375, which we will study in the future.

In summary, miR-375 expression clearly increased in the aggressive prostate tumor tissues compared with healthy prostate tissues and adjacent noncancerous tissues, which suggested miR-375 has potential diagnosis value to distinguish the PC from healthy controls. In addition, expression of miR-375 was related to different histological types. Moreover, bioinformatics analyses revealed possible molecular mechanism and target genes of miR-375 in PC. Further studies are needed on the regulated-mechanisms of miR-375 in PC.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.03.004.

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