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Original Article

Association of microRNA-23b-3p down-regulation with progression and survival in pancreatic duct adenocarcinoma and its prospective function via bioinformatics analysis

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Abstract: Background: MicroRNAs (miRNAs) have been reported in various human malignancies, which play important roles in tumorigenesis and progression, acting as oncogenes or tumor suppressors. However, the role of miR-23b-3p in pancreatic duct adenocarcinoma (PDAC) is to be elucidated. The goal of this study was to explore the association of miR-23b-3p expression with clinic features in PDAC formalin-fixed, paraffin embedded (FFPE) tissues and survival in PDAC patients and to investigate the prospective function of miR-23b-3p via bioinformatics analysis. Methods: The expression of miR-23b-3p was detected in 57 PDAC and 25 adjacent normal pancreatic tissues (ANT), also in five PDAC cell lines and an immortal pancreatic epithelium cell line HPDE6c-7 by qRT-PCR. The relationship between miR-23b-3p level and clinicopathological parameters including survival of PDAC patients was analyzed with Spearman correlation, Kaplan-Meier method and Cox proportional hazards mode, respectively. In addition, the validated target genes of miR-23b-3p gathered from DIANA-TarBase v7.0 and miRTarBase 6.0 were assessed with Gene Ontology (GO) and KEGG pathway enrichment analyses. Results: The relative level of miR-23b-3p was significantly lower in PDAC compared to ANT (P=0.0053). Remarkably lesser expression of miR-23b-3p was also found in capan-1, aspc1 and panc-1 PDAC cells, compared with HPDE6c-7 cell line, respectively (all P<0.05). Furthermore, miR-23b-3p expression level was significantly correlated with tumor size (r=0.341, P=0.001), depth of invasion (r=0.264 P=0.048) and tumor stage (r=0.281, P=0.034). Kaplan-Meier analysis displayed that patients with lower miR-23b-3p expression presented a poorer disease specific survival (DSS) (P=0.036). Additionally, multivariate analysis revealed that down-expression of miR-23b-3p, as well as the histological grade and tumor stage, was an independent predictor of DSS of PDAC. A total of 173 validated targets were collected and they had enriched GO terms in different pathways. KEGG enrichment analysis revealed that the validated targets of miR-23b-3p were significantly enriched in some well-known oncogenic pathways of malignancies, including the pathway of "Pancreatic cancer" with the genes of E2F1, RAF1, JAK1, RB1, BCL2L1, CHUK and RAD51. Conclusion: MiR-23b-3p might become as a potential indicator related to progress and prognosis via targeting various key pathways in PDAC.

Keywords: Pancreatic duct adenocarcinoma (PDAC), MiR-23b-3p, disease specific survival (DSS), prognosis, real time RT-qPCR

Introduction

Pancreatic cancer (PC) is considered as a highly aggressive malignancy with poor prognosis and affects approximately 50,000 patients per year [1-3]. In the 10 leading cancer types for the estimated deaths, pancreatic cancer ranks both the fourth place in males and females in United States in 2016 [4]. The incidence and morbidity of PC have also been increased in China [5]. The overall 5-year survival rate for PC is the lowest of all cancers with the ratio of around 7%, due to inadequate diagnostic approaches, disease aggressiveness and absence of efficient targeted therapies [1-3]. More than 90% of PCs are pancreatic duct adenocarcinoma (PDAC) [1-3]. Patients with PADC have extremely unfavorable prognosis. Thus, to im-

Table 1. Relationship between miR-23b-3p Expression and Clinicopathological Features of PDAC

Characteristics	Number Of case	miR-23b-3p expression				*P* value
		High (n=20)	%	Low (n=37)	%	
Age (years)	57	58.75±2.210		59.24±1.348		0.841
Gender						
Male	35	10	50.00%	25	59.46%	0.194
Female	22	10	50.00%	12	40.54%	
Tumor size						
<4 cm	24	13	65.00%	11	29.73%	0.01
≥4 cm	33	7	35.00%	26	70.27%	
Location						
Pancreatic head	38	14	70.00%	24	64.86%	0.695
Pancreatic tail	19	6	30.00%	13	35.14%	
Histologic grade						
Well	12	3	15.00%	9	24.32%	0.672
Moderately	33	13	65.00%	20	54.50%	
Poorly/others	12	4	20.00%	8	21.18%	
Depth of invasion						
T1, T2	14	8	40.00%	6	16.23%	0.048
T3, T4	43	12	60.00%	31	83.87%	
Lymphatic metastasis						
Absent	30	12	60.00%	18	48.65%	0.417
Present	27	8	40.00%	19	51.35%	
venous invasion						
Absent	36	15	75%	21	56.76%	0.173
Present	21	5	25%	16	43.24%	
Nervous invasion						
Absent	42	17	85.00%	25	67.57%	0.154
Present	15	3	15.00%	12	32.43%	
Distant metastasis						
Absent	46	17	85.00%	29	78.38%	0.549
Present	11	3	15.00%	8	21.62%	
Tumor stage						
I and II	29	14	70.00%	15	40.54%	0.034
III and IV	28	6	30.00%	22	59.46%	
CA199						
<37 μ/ml	15	4	20.00%	11	29.73%	0.426
>37 μ/ml	42	16	80.00%	26	70.27%	
CEA						
<5 ng/ml	32	9	45.00%	23	62.16%	0.213
>5 ng/ml	25	11	55.00%	14	37.74%	

prove the diagnosis, prevention and treatment for PDAC, we should discover the thorough mechanism on the development and progression of PDAC.

MicroRNAs (miRNAs) have 18-25 nucleotide RNA molecules with high conservation. When miRNAs bind loosely to complementary sequences in the 3'-untranslated region of target mRNA, it can repress translation or induce mRNA cleavage, hence to regulate the stability or translational efficiency of complementary target mRNA [6-9]. Moreover, some studies have proved that miRNAs were closely associ-

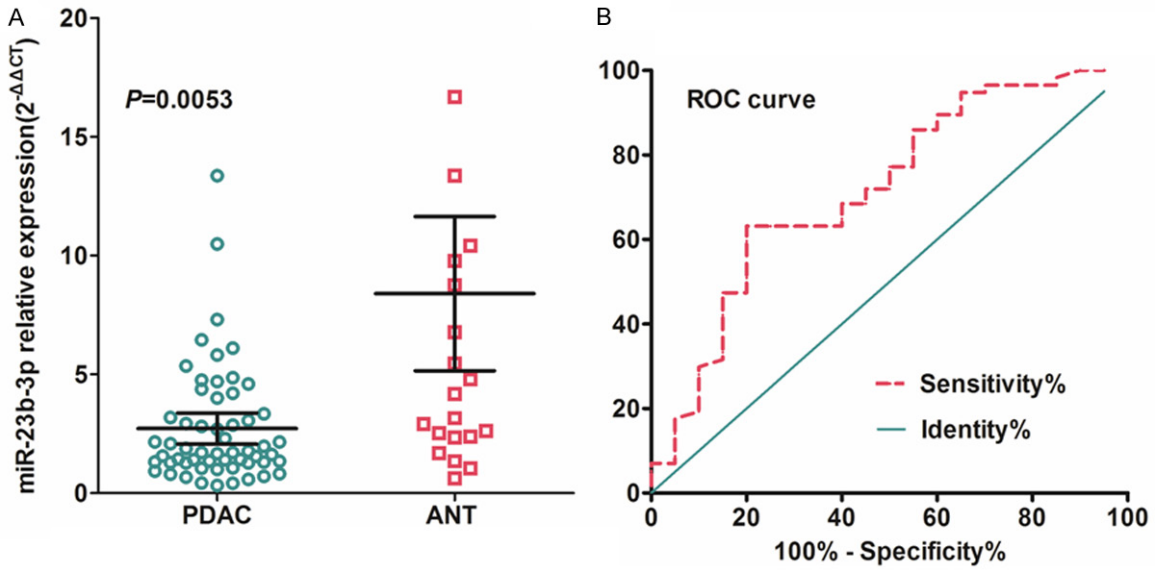

Figure 1. Expression of miR-23b-3p in pancreatic duct adenocarcinoma (PDAC) tissues. A: Relative level of miR-23b-3p in PDAC and adjacent non-tumor (ANT) pancreatic tissues. B: ROC curve.

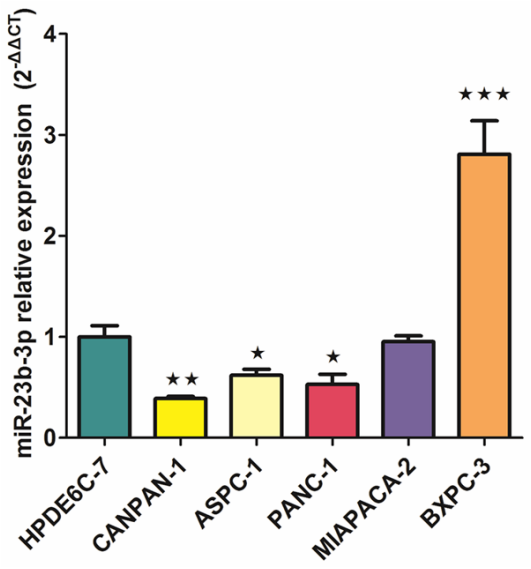

Figure 2. Expression of miR-23b-3p in pancreatic cancer (PC) cells. Relative level of miR-23b-3p in PC and normal pancreatic cells (HPDE6C-7). ★P<0.05, ★★P<0.01, ★★★P<0.001.

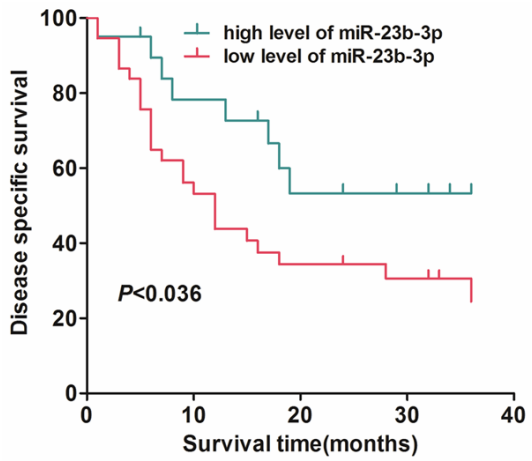

Figure 3. Relationship between expression of miR-23b-3p and disease specific survival (DSS) in pancreatic duct adenocarcinoma (PDAC). The prognostic value of miR-23b-3p in PDAC was evaluated by Kaplan-Meier method.

ated with PDAC tumorigenesis. A few miRNAs involved in PDAC biology have been reported that they can affect tumor growth, metastatic potential, and chemosensitivity [10-12]. MiR-23b-3p is miRNA that can modulate various normal processes such as embryogenesis, cell behavior and cell motility [13], and differentiation [14]. Besides, miR-23b-3p was found to be upregulated in uterine cancer [15], breast cancer [16], renal cancer [17], melanoma [18] and down-regulated in prostate cancer [18], colon cancer [19], bladder cancer [11], and cervical cancer [20]. Although previous studies have shown that miR-23b-3p could regulate autophagy associated with radio-resistance of PC cells [21, 22], there is no study on the role of miR-23b-3p in PDAC. Hence, our study intent was to detect miR-23b-3p level and explore the relationship between miR-23b-3p and clinicopathological factors including patient survival in PDAC.

Table 2. Univariate Analysis of Clinicopathological Factors for Disease-specific Survival in PDAC

Variable	PC (N)	HR (Hazard ratio)	95% CI	*P* value
Age (years)	57			
<58	26	1		
≥58	31	0.977	0.495-1.929	0.948
Gender				
Male	35	1		
Female	22	1.56	0.793-3.069	0.198
Tumor size				
<4 cm	25	1		
≥4 cm	32	1.665	0.823-3.369	0.156
Location				
PC head	38	1		
PC tail	19	0.82	0.390-1.721	0.6
Histologic grade				
Well	12			
Moderately	33	1		
Poorly/others	12	1.974	1.161-3.357	0.012
Depth of invasion				
T1, T2	14	1		
T3, T4	43	2	0.891-5.239	0.088
Lymphatic metastasis				
Absent	30	1		
Present	27	1.382	0.702-2.721	0.349
venous invasion				
Absent	36	1		
Present	21	1.307	0.659-2.594	0.444
Nervous invasion				
Absent	42	1		
Present	15	1.292	0.616-2.711	0.498
Distant metastasis				
Absent	46	1		
Present	11	1.334	0.600-2.963	0.48
Tumor stage				
I and II	29	1		0.005
III and IV	28	2.76	1.351-5.641	
CA199				
<37 u/ml	16	1		0.808
≥37 u/ml	41	0.91	0.424-1.953	
CEA				
<5 ng/ml	30	1		
≥5 ng/ml	27	1.101	0.561-2,161	0.799
miR-23b-3p				
Low	37	1		
High	20	0.804	0.658-0.982	0.033

Each single miRNA has ability to target many gene transcripts concurrently according to the rules of perfectly or imperfectly complementary miRNA, and these target interactions have prompted some miRNA-target predicting tools. However, off-targets effects could not be ignored. Several databases provide lists of confirmed miRNA and its target via experiment, which push on the progress of miRNA research greatly [23]. Am-ong these databases, two well-known ones are miRTarBase (http://miRTarBase.mbc.nctu.edu.tw/) [24] and DIANA-TarBase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index/) [25]. Thus, in the current study, after investigating the clinical role of miR-23b-3p in PDAC tissues, we further performed a bioinformatics analysis on the possible target genes and pathways of miR-23b-3p in PDAC.

Materials and methods

Patients and tissue samples

From January 2010 to November 2011, we selected 57 PADC FFPE tissues and 25 ANT FFPE tissues with PADC diagnostic standard (confirmed by Gang Chen, Dan-Ming Wei and Dian-Zhong Luo), which were obtained from primary surgical resection of PADC at the First Affiliated Hospital of Guangxi Medical University (GMC) in China. General clinicopathological factors were collected, such as age, gender, clinical stage, grade, venous invasion, nervous invasion, status of lymphatic metastasis, distant metastasis, tumor node metastasis (TNM) stage [26], the serum level of carbohydrate antigen 19-9 (CA19-9) and carcinoma embryonic antigen (CEA). All patients follow-up after surgery were performed half a year till November 30, 2013. DSS was collected, which was defined as the survival time between the surgery and death. The study achieved permission from the Research Ethics Committee of the First Affiliated Hospital of GMC. All participating patients were informed to sign the consents.

Table 3. Multivariate Analysis of Clinicopathological Factors for Disease-specific Survival in PDAC

Variable	HR	95% CI	*P* value
Histologic grade	2.198	1.285-3.818	0.005
Depth of invasion	1.036	0.300-3.572	0.956
Tumor stage	3.601	1.604-8.8085	0.002
miR-23b-3p	0.831	0.702-0.983	0.031

Cell lines

The BxPc-3, AsPC-1, CAPAN-1, and HPDE6C-7 cell lines were cultured in supplemented 1640 (JiBICO, China) with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin; PANC-1 and MiAPANC-2 cell lines were maintained routinely in Dulbecco's modified Eagle's medium with 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin (JiBICO, China), which were cultured at 37°C with 5% CO_2 atmosphere. All cell lines were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Wuhan, China).

Real-time RT-qPCR

RNA preparation, real-time reverse transcription (RT) and quantitative PCR were performed as previously reported [27]. Total RNAs were extracted from FFPE tissues by using mirRNeasy FFPE Kit (217504, QIAGEN, Germany) and Cell RNA by using an RNA isolation kit (Trizol Reagent, Invitrogen, USA), performed according to the manufacturer protocol. RT and qPCR kits (PrimeScript™ miRNA qPCR Starter kits, Takara) were applied to measure expression of miR-23b-3p. The RT reactions were incubated at 37°C for 60 min, at 85°C for 5s with a miRNA PrimeScript RT Enzyme Mix in 20 μl qPCR system: 1 μl diluted RT products, 12.5 μl 2 × SYBR Premix Ex Taq II (Roche, Switzerland), 1 μl forward and reverse primers and 10.5 μl nuclease-free water were mixed in a final volume of 25 μl. Internal control was U6. Primers list were as follows: miR-23b-3p (reverse), 5'GATCACATTGCCAGGGATTACC3'; Forward primer of miR-23b-3p and primers for U6 (NR_002752) were provided by Takara (PrimeScript™ miRNA qPCR Starter kits). QPCR reactions conditions were set as: 95°C for 30 s, 95°C for 15 s and 53°C for 1 min for 40 cycles. Relative mRNA expression of miR-23b-3p was calculated with the comparative threshold cycle (CT) method as ($2^{-\Delta\Delta CT}$).

Candidate target genes of miR-23b-3p

In this study, human miR-23b-3p target gene data come from DIANA-TarBase v7.0 and miRTarBase 6.0. DIANA-TarBase is a database which comprises experimentally supported miRNA targets via manually curated collection. MiRTarBase also provides experimentally validated miRNA-target interactions (MTIs) by reporter assay, western blot, qPCR, microarray and next-generation sequencing experiments.

GO and pathway enrichment analysis for validated genes of miR-23b-3p

Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/home.jsp) were used for GO enrichment analysis. We also performed pathway enrichment analysis using pathway data obtained from the FTP service of KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/). Furthermore, we used MSigDB (http://software.broadinstitute.org/gsea/msigdb/index.jsp) databases to assess the pathways of these target genes of miR-23b-3p. Molecular Signatures Molecular Tag Database (MSigDB) built by GSEA (Broad Institute, Cambridge, MA, USA) was applied for enrichment analyses.

Statistical analysis

To assess the significance of differences between groups, the Student test, one ANOVA test, or χ^2 test were performed on SPSS20.0 software (Chicago, USA). Spearman correlation, Receiver operator characteristic curve (ROC), Kaplan-Meier method with the log-rank test and Cox proportional hazards mode were used for analyzing clinic relationship, diagnostic value, survival analysis and prognosis, respectively. *P* values less than 0.05 were considered statistically significant.

Results

Expression of miR-23b-3p in PADC tissues

QPCR results showed that miR-23b-3p mRNA level was significantly lower in PADC compared with ANT tissues (*P*=0.0053, **Table 1**; **Figure 1A**). Additionally, ROC curve results indicated that miR-23b-3p mRNA level might have diagnostic value in PDAC. The calculated area under curve (AUC) of miR-23b-3p was 0.7162 (95% CI 0.5809-0.8515, *P*=0.0042). The cut-off value for miR-23b-3p was 2.888. Diagnostic sensitiv-

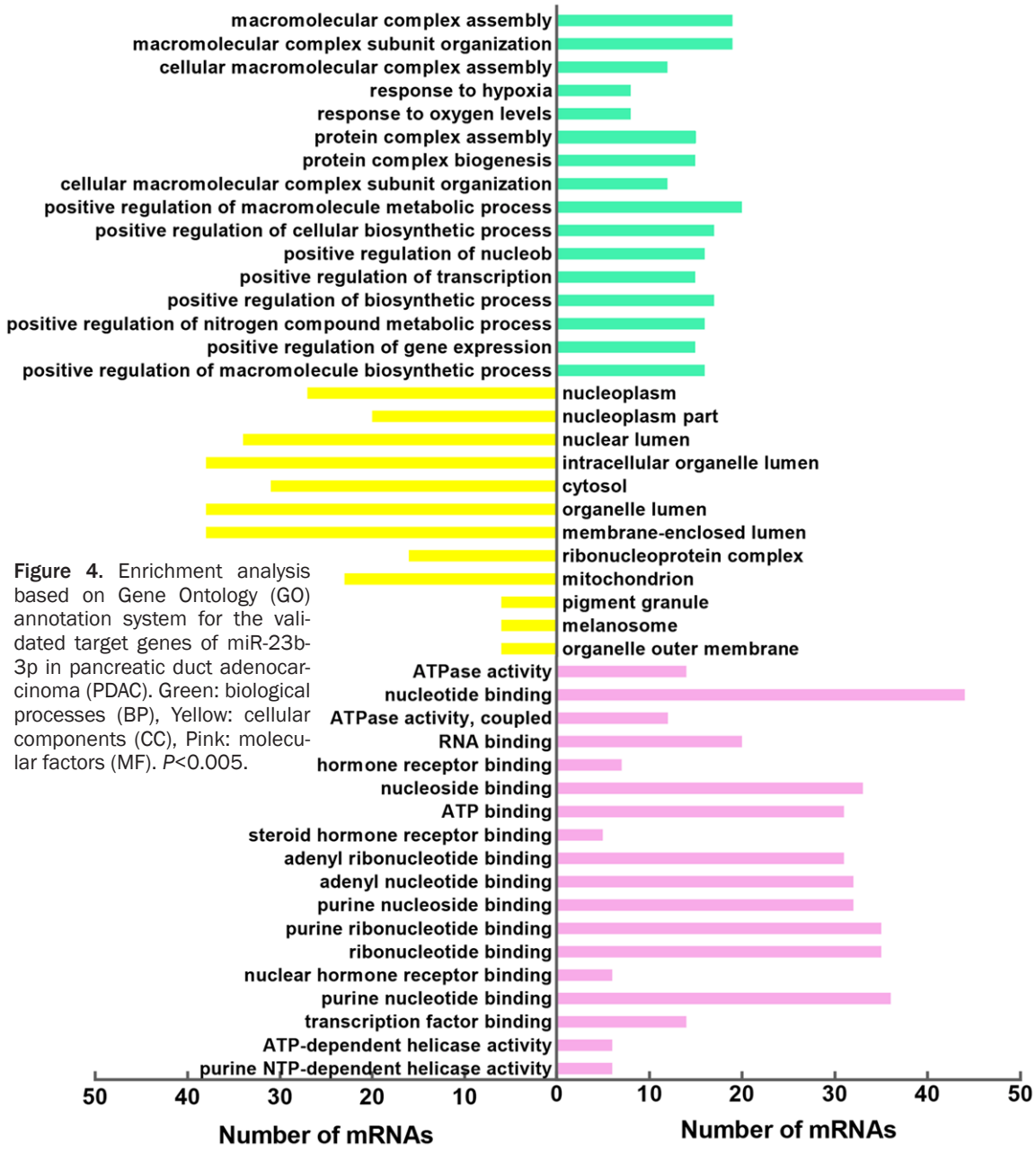

Figure 4. Enrichment analysis based on Gene Ontology (GO) annotation system for the validated target genes of miR-23b-3p in pancreatic duct adenocarcinoma (PDAC). Green: biological processes (BP), Yellow: cellular components (CC), Pink: molecular factors (MF). $P<0.005$.

ity and specificity were 68.4% and 60%, respectively (**Figure 1B**).

Expression of miR-23b-3p in PC cell lines and HPDE6C-7 cells

In vitro, qPCR results showed that miR-23b-3p mRNA level in PDAC cell lines and HPDE6C-7 cell lines were not uniform. The lower expression of miR-23b-3p was detected in capan-1, aspc1 and panc-1 cells than that in HPDE6C-7 cells (q=4.922, q=3.066, q=3.8, respectively, all $P<0.05$). At the same time, the higher miR-23b-3p mRNA level was found in bxpc3 cell than that in HPDE6C-7 (q=14.6, $P<0.001$). The miR-23b-3p mRNA levels between miapaca-2 and HPDE6C-7 cells were no significantly different (q=0.3792, $P>0.05$, **Figure 2**).

Relationship between miR-23b-3p expression and clinicopathological factors in PDAC

According to the $2^{-\Delta\Delta CT}$ mean value of miR-23b-3p mRNA level, 57 PDAC tissues were categorized as low expression group (n=37) or high miR-23b-3p (n=20) expression group. Lower expression of miR-23b-3p presented inversely in the groups of larger tumor size, later tumor

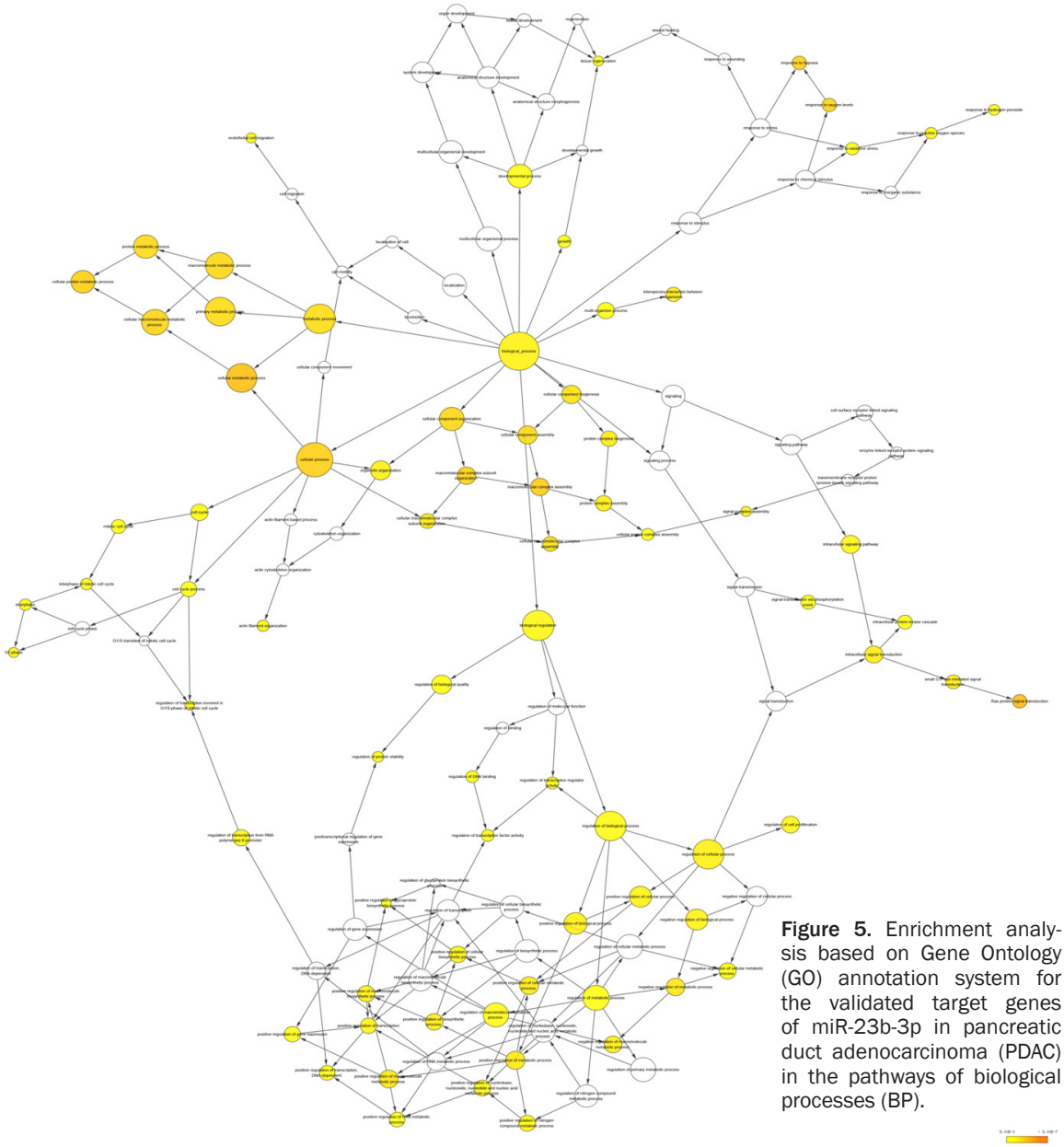

Figure 5. Enrichment analysis based on Gene Ontology (GO) annotation system for the validated target genes of miR-23b-3p in pancreatic duct adenocarcinoma (PDAC) in the pathways of biological processes (BP).

stage and deeper invasion than that in the different sub-groups (all $P<0.05$, **Table 1**). Further correlation analyzed found that miR-23b-3p expression level was closely correlated with tumor size ($r=0.341$, $P=0.01$), depth of invasion ($r=0.264$, $P=0.048$) and tumor stage ($r=0.281$, $P=0.034$). However, there was no relationship between miR-23b-3p expression and other clinicopathological factors (all $P>0.05$, **Table 1**).

Correlation between miR-23b-3p expression and prognosis of PDAC patients

Kaplan-Meier analysis discovered that patients in low miR-23b-3p expression group had a significantly poorer prognosis than those in high expression group ($P=0.036$) as presented in **Figure 3**, which indicated that miR-23b-3p may be a suppressing factor for survival. Univariate analysis of DSS showed that the miR-23b-3p mRNA level was a prognostic indicators ($P=0.033$), as well as tumor histologic grade ($P=0.012$) and tumor stage ($P=0.005$). Other clinicopathological features excluding above three factors were not statistically significant as prognostic indicators (all $P>0.05$, **Table 2**). In multivariate analysis, factors of variables with a value $P<0.1$, such as miR-23b-3p mRNA level, tumor histologic grade, tumor stage and depth

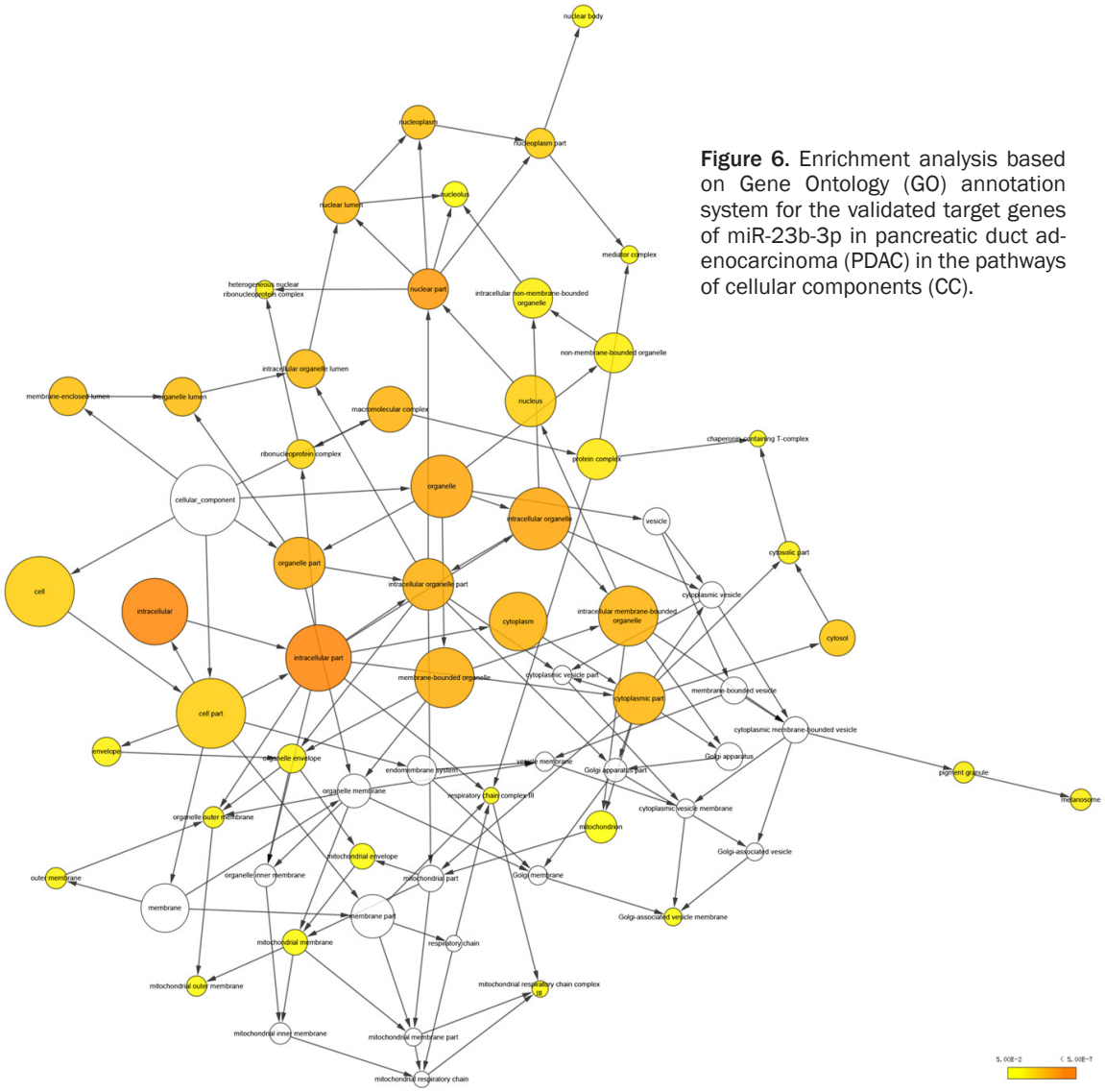

Figure 6. Enrichment analysis based on Gene Ontology (GO) annotation system for the validated target genes of miR-23b-3p in pancreatic duct adenocarcinoma (PDAC) in the pathways of cellular components (CC).

of invasion were selected into analysis of Cox proportional hazards mode (**Table 2**). Multivariate Cox analysis indicated that miR-23b-3p mRNA level (HR=0.831, 95% CI: 0.732-0.983, *P*=0.031) was an independent prognostic indicator for PDAC patients. Moreover, tumor histological grade (HR=2.198, 95% CI: 1.285-3.818, *P*=0.005) and tumor stage (HR=3.601, 95% CI: 1.604-8.8085, *P*=0.002) were also independent prognostic indicators in patients with PDAC (*P*<0.05, **Table 3**).

Enrichment analysis of validated targets of miR-23b-3p

A total of 173 validated targets were collected from DIANA-TarBase v7.0 and miRTarBase 6.0. Afterwards, the enrichment analysis was performed on the basis of GO annotation system. As shown in **Figures 4**-**7**, the validated targets for miR-23b-3p had enriched GO terms related to macromolecular complex assembly, macromolecular complex subunit organization, cellular macromolecular complex assembly, etc, for biological processes (BP); nucleoplasm, nucleoplasm part, and nuclear lumen, etc, for cellular components (CC); and ATPase activity, nucleotide binding, and ATPase activity, etc, for molecular factors (MF). More interestingly, KEGG enrichment analysis revealed that the validated targets of miR-23b-3p were significantly enriched in some well-known oncogenic pathways for malignancies, such as Pathways in cancer, Chronic myeloid leukemia, Prostate cancer,

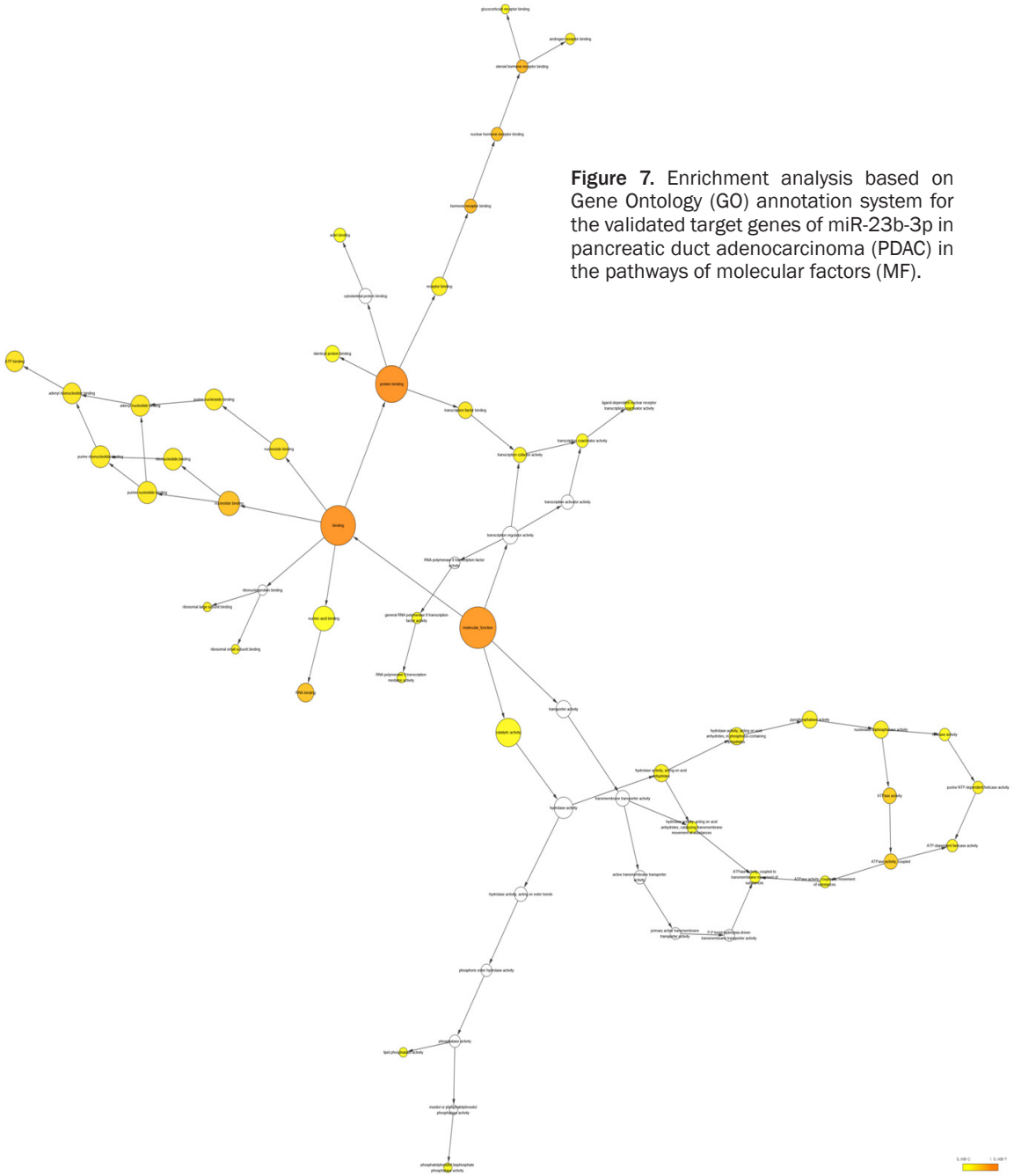

Figure 7. Enrichment analysis based on Gene Ontology (GO) annotation system for the validated target genes of miR-23b-3p in pancreatic duct adenocarcinoma (PDAC) in the pathways of molecular factors (MF).

Pancreatic cancer and Focal adhesion, etc (**Figure 8**). In the pathway of "Pancreatic cancer", the verified targets genes of miR-23b-5p were E2F1, RAF1, JAK1, RB1, BCL2L1, CHUK and RAD51. Furthermore, Molecular Signatures Molecular Tag Database (MSigDB) built by GSEA (Broad Institute, Cambridge, MA, USA) was also applied for enrichment analyses. Using the gene expression profile graph from the Global Cancer Map, we identified the expression of significantly altered differential genes among the miR-23b-3p target genes, as well as their relative tendencies of expression in the defined functional groups (**Figure 9**).

Discussion

PC is a devastating disease with extraordinarily poor prognosis. Discovery of miRNAs has provided a new opportunity to study a prominent

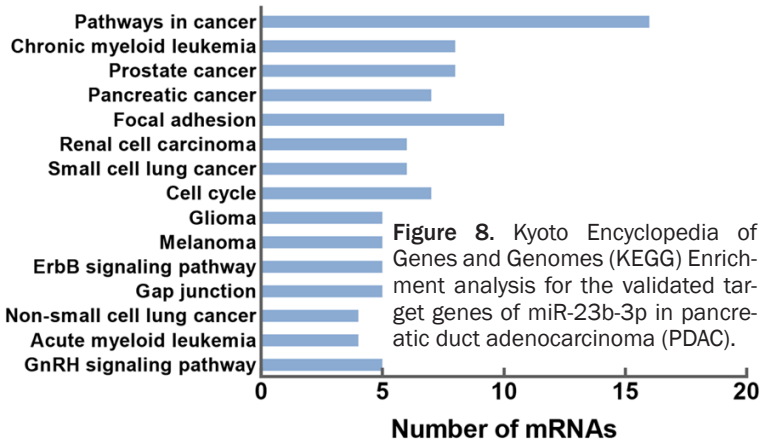

Figure 8. Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment analysis for the validated target genes of miR-23b-3p in pancreatic duct adenocarcinoma (PDAC).

class of gene regulators in cancers, including PDAC. Moreover, some miRNAs have been proved to be related to cell proliferation, invasion, and poor survival of PC, however, it remains unclear for researchers that the precise mechanisms involved in controlling the above processes. All key questions are to guide further investigation including how these miRNA become dysregulated and what their targets are. As a result, discovering the mechanisms and functions of miRNAs linked with clinic has generated great interests in PC.

MiR-23b-3p was first reported to regulate neuronal differentiation and development by targeting the Hairy/enhancer of Split protein 1 or Hes1 (a bHLH1 transcription factor involved in Notch signaling) in mouse P19 cells [28]. Increasing miR-23b-3p levels could induce the expression of pro-inflammatory cytokines like IL-10 in bone marrow derived dendritic cells and promote differentiation of regulatory T cell [29]. MiR-23b-3p also plays an important part in the initiation and progression of various cancers. However, no report of miR-23b-3p in PDAC has been available. Herein, we provided the first evidence that miR-23b-3p might play a critical role in pancreatic tumorigenesis and progression.

We firstly explored that relative expression level of miR-23b-3p was significantly lower in 57 PDAC tissues compared with 25 ANT by RT-qPCR. The same result was also measured in PDAC cell samples *in vitro*. The lower miR-23b-3p expression in capan-1, aspc1 and panc-1 cell lines was founded compared with HDPE-6C-7 cell line. The down-regulated expression of miR-23b-3p in tissues and cells of PDAC suggested that miR-23b-3p could act as a tumor suppressor in PDAC, similar to other tumors. At the same time, we also identified the diagnostic significance of miR-23b-3p expression in PDAC by applying the ROC curve. The calculated area of AUC was 0.7162, which suggests a potential diagnostic value of miR-23b-3p.

Next, Relationship between miR-23b-3p expression and clinicopathological parameters was further investigated. The results showed the inverse relationships between miR-23b-3p level and three clinicopathological parameters, such as tumor size, depth of invasion and tumor stage. Since these three clinicopathological parameters are indicators for the deterioration and progress in the cancer, miR-23b-3p might be a factor of tumor progression. However, this finding was derived from limited numbers of patients and needs to be supported in future through large-scale researches.

We also investigated the effect of miR-23b-3p expression on patient survival by using Kaplan-Meier analysis. Our outcome indicated that patient with lower miR-23b-3p expression had a poorer DSS than those of high miR-23b-3p expression in PDAC. Besides, univariate analysis result indicated that the miR-23b-3p mRNA level, tumor differentiation and tumor stage could be prognostic indicators. We went further to set up Cox proportional hazards mode and found that expression of miR-23b-3p as well as histologic grade and tumor stage was an independent predictor of DSS for PDAC. MiR-23b-3p not only can be used as a marker to help clarify the stage of a PDAC, but also a prognostic tool to assist the determination of disease outcomes.

The biological functions of miR-23b-3p depend on the identification of relevant target genes and signal-pathway. Ishteiwy et al [30] reported that ectopic expression of miR-23b-3p in prostate cancer cells increased levels of E-Cadherin (reflecting decreased epithelial-to-mesenchymal transition, EMT), reduced invasiveness, and decreased levels of Rac1 without effecting cell growth. Majid et al [31] also found that up-

Figure 9. Compendia expression profiles of miR-23b-3p in pancreatic duct adenocarcinoma (PDAC) adapted from Global Cancer Map. An expression dataset sorted by correlation with phenotype, the corresponding heat map and Gene family classification was present.

regulation miR-23b-3p mRNA level in prostate cells decreased migration, invasion, and the EMT as indicated by a decreasing vimentin and Snail and increasing E-Cadherin. Zhang et al [32] constructed a stable clones over-expressing miR-23b-3p on HCT116, and then found that miR-23b-3p overexpression inhibited cell migration and invasion, and promoted mesen-

chymal-to-epithelial epithelial. Shahana et al [11] demonstrated that Zeb1 was a direct target of miR-23b-3p in bladder cancer, Moreover, Wang et al [22] reported that overexpression of miR-23b-3p inhibited radiation induced autophagy in pancreatic cancer cells, and they also found that cancer cell presented radio-resistance via the regulation of its target protein autophagy related 12 (ATG12). Down-regulating miRNA-23b-3p expression induced apoptosis and reduced invasive capabilities in renal cell carcinoma (RCC) A-498 cell lines via directly targeting phosphatase and tensin homolog (PTEN) [17]. MiR-23b-3p could also affect expression of proteins involved in key signaling pathways such as, TGF-beta [33], Notch [34], and Akt/PI3K [35]. Thus, miR-23b-3p may target different genes in diverse diseases.

Since no target gene has been validated for miR-23b-3p in PDAC. We performed further bioinformatics analysis to predict the prospective target genes and signal-pathways of miR-23b-3p. Among the two softwares used in the current study, miRTarBase could provide 138 crosslinking and immunoprecipitation sequencing (CLIP-seq) data set from 21 independent studies, and then identify systematically Argonaute-miRNA-RNA interactions from above crosslinking and CLIP-seq data. Moreover, this database enriches in large amounts of resources including 4966 articles, 7439 strongly validated MTIs (using reporter assays or western blots) and 348,007 MTIs from CLIP-seq, which provides an effective outline of this exponential growth in the miRNA experimental data via integrating miRNA and gene expression profiles from The Cancer Genome Atlas (TCGA) [24]. Meanwhile, DIANA-TarBase has been an outstanding pioneer in cataloguing experimentally validated miRNA-gene interactions from published articles since 2006. DIANA-TarBase v7.0 indexes 9 to 250-fold more entries than any miRNA database and could offer hundreds of thousands of high quality validated miRNA-gene interactions via manually curated experiment and enhanced with detailed meta-data. There were more than half a million miRNA-gene interactions from published experiments on 356 different cell types from 24 species [25]. Eventually, 173 validated targets were gathered and enrichment pathway analyses showed that miR-23b-3p could be involved in a variety of key pathways, especially in some well-known oncogenic pathways, including the pathway of “Pancreatic cancer”. The following verified targets genes of miR-23b-5p were E2F1, RAF1, JAK1, RB1, BCL2L1, CHUK and RAD51 in the pathway of “Pancreatic cancer”. These genes could be preferable targets for further investigation in PDAC. In sum, specific target genes are controlled by miR-23b-3p and signal-pathways regulated by miR-23b-3p in PDAC still require to be validated by experiments.

In conclusion, the low miR-23b-3p expression was associated with progression and prognosis in PDAC. MiR-23b-3p can not only be used as a marker to clarify the stage of a PDAC, but also a prognostic tool to help determine disease outcomes. However, above results have to be validated in a large of samples. Further *in vitro* and i*n vivo* experiments are also required to explore the prospective functions and mechanisms of miR-23b-3p on the development, progression and prognosis of PDAC.

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Disclosure of conflict of interest

None.

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