Identifying microRNA-mRNA regulatory network in gemcitabine-resistant cells derived from human pancreatic cancer cells

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Abstract Pancreatic cancer is unresectable in over 80 % of patients owing to difficulty in early diagnosis. Chemotherapy is the most frequently adopted therapy for advanced pancreatic cancer. The development of drug resistance to gemcitabine (GEM), which is always used in standard chemotherapy, often results in therapeutic failure. However, the molecular mechanisms underlying the gemcitabine resistance remain unclear. Therefore, we sought to explore the microRNA-mRNA network that is associated with the development of gemcitabine resistance and to identify molecular targets for overcoming the gemcitabine resistance. By exposing SW1990 pancreatic cancer cells to long-term gemcitabine with increasing concentrations, we established a gemcitabine-resistant cell line (SW1990/GEM) with a high IC50 (the concentration needed for 50 % growth inhibition, 847.23 μM). The mRNA and microRNA expression profiles of SW1990 cells and SW1990/GEM cells were determined using RNA-seq analysis. By comparing the results in control SW1990 cells, 507 upregulated genes and 550 downregulated genes in SW1990/GEM cells were identified as differentially expressed genes correlated with gemcitabine sensitivity. Gene ontology (GO) analysis showed that the differentially expressed genes were related to diverse biological processes. The upregulated genes were mainly associated with drug response and apoptosis, and the downregulated genes were correlated with cell cycle progression and RNA splicing. Concurrently, the differentially expressed microRNAs, which are the important player in drug resistance development, were also examined in SW1990/GEM cells, and 56 differential microRNAs were identified. Additionally, the expression profiles of selected genes and microRNAs were confirmed by using Q-PCR assays. Furthermore, combining the differentially expressed microRNAs and mRNAs as well as the predicted targets for these microRNAs, a core microRNA-mRNA regulatory network was constructed, which included hub microRNAs, such as hsa-miR-643, hsa-miR-4644, hsa-miR-4650-5p, hsa-miR-4455, hsa-miR-1261, and hsa-miR-3676. The predicted targets of these hub microRNAs in the microRNA-mRNA network were also observed in the identified differential genes. As a result, a differential gene and microRNA expression pattern was constructed in gemcitabine-resistant pancreatic cancer cells. Therefore, these data may be useful for the detection and treatment of drug resistance in pancreatic cancer patients, and the microRNA-mRNA network-based analysis is expected to be more effective and provides deep insights into the molecular mechanism of drug resistance.

Keywords Pancreatic cancer · Gemcitabine resistance · RNA-seq · Differential genes and microRNAs · MicroRNA-mRNA network

Introduction

Pancreatic cancer is among the most lethal cancers because of extensive local invasion, early metastasis, and bad prognosis with a 5-year survival rate of <4 % [1, 2]. To date, the only curative treatment for pancreatic cancer is surgery, while only
<20 % of patients are suitable for radical resection at the time of diagnosis [3]. Chemotherapy is the most frequently adopted therapy for advanced pancreatic cancer, but the occurrence of drug resistance reduces its effectiveness and is thought to be the primary reason for the failures of anti-cancer therapy [4, 5]. Elucidation of the molecular mechanisms underlying drug resistance in pancreatic cancer is largely needed to improve the therapeutic effect of chemotherapy.

Gemcitabine (GEM), a deoxycytidine analog related to cytarabine, is a potent anti-tumor reagent with a broad spectrum in the treatment of solid tumors, such as pancreatic, bladder, and non-small cell lung cancers. Moreover, the promising activity of gemcitabine against head and neck, breast, and ovarian carcinomas has also been reported [6]. Although gemcitabine is a well-tolerated drug for the treatment of symptomatic pancreatic cancers, the efficacy rates remain at only 20–30 % because of patient resistance to gemcitabine. Many pancreatic cancer patients show initial sensitivity to gemcitabine therapy followed by the rapid acquisition of resistance to gemcitabine treatment, which leads to poor patient outcomes [7]. Therefore, investigating the mechanisms of gemcitabine resistance is critical for the development of superior therapies in pancreatic cancer.

Gemcitabine resistance is mainly related to both genetic and epigenetic alterations, which are associated with gemcitabine transport and metabolism, such as nucleoside transporter-1 (hENT1), an important element in gemcitabine uptake, and deoxycytidine kinase and ribonucleoside reductases M1/M2 involved in gemcitabine metabolism [6, 8, 9]. Dysregulated expression of genes related to cell survival and apoptosis have been revealed, such as the S100 family member S100A4, HMGA1, and the tyrosine kinases FAK and c-Src [10–13]. To fully understand the cellular response in gemcitabine resistance, a large number of studies have performed large-scale screening analysis, such as microarray and mass spectrometry analysis, to identify gemcitabine resistance-related genes or proteins, and many altered genes or proteins have been found [14–16]. Since the characteristics of pancreatic cancer can vary between individuals, the predictive value of the reported factors is still controversial [14], and the precise molecular mechanism of gemcitabine resistance in pancreatic cancer cells still remains unclear.

MicroRNAs are endogenously expressed small non-coding RNAs that are evolutionarily conserved and function as regulators of gene expression in a variety of biological processes [17]. The role of microRNAs in the development of drug resistance has been elucidated in a variety of malignancies [18]. Numerous microRNAs, such as microRNA-21, miRNA-205, microRNA-200, microRNA-29a, and let-7a, were revealed to be involved in the gemcitabine resistance for pancreatic cancer cells [19–23], while only Ohuchida et al. performed microRNA array to screen differential microRNAs in gemcitabine-resistant pancreatic cancer cells [24]. However, rare microRNAs are overlapped with the previously reported functional microRNAs in gemcitabine resistance.

Based on the wide acceptance, microRNAs and their target genes are inversely regulated in a specific condition [25]. Integration of predicted microRNA-target regulations with both microRNA and mRNA expression makes it possible to identify the functional candidates of microRNA-mRNA pairs related to gemcitabine resistance. More importantly, increasing studies highlight the success of the strategy of biological network analyses to investigate microRNA-mediated regulation [26, 27]. However, gemcitabine resistance-associated microRNA-mRNA regulatory network has not been investigated previously. Therefore, network-based analyses are expected to be more effective and provide deep insights into the molecular mechanism of gemcitabine resistance development.

Here, we established a gemcitabine-resistant pancreatic cancer cell line and conducted an extensive mRNA profiling and microRNA-profiling study on parental and gemcitabine-resistant pancreatic cancer cells. The differential mRNAs and microRNAs were identified, the microRNA-target regulation information was integrated, and a microRNA-mRNA regulatory network associated with gemcitabine resistance development in pancreatic cancer cells was constructed.

Materials and methods

Cell line generation, cell culture, and cytotoxicity analysis

The SW1990 pancreatic cancer cell line was purchased from ATCC (Manassas, VA, USA). The gemcitabine-resistant subline (SW1990/GEM) was generated by continuously culturing the gemcitabine-sensitive parental cell line (SW1990) in medium containing incrementally increasing concentrations of gemcitabine. Cells were cultured in PRMI1640 (GIBCO, Carlsbad, CA, USA) supplemented with 10 % FCS, 100 units/ml penicillin, and 100 units/ml streptomycin at 37 °C and 5 % CO2. To avoid the influence of drugs, established SW1990/GEM cells were cultured in gemcitabine-free media for at least 1 month before cytotoxicity analysis. For in vitro cytotoxicity analysis, cells were seeded into flat-bottomed 96-well plates and cultured for 24 hours. Gemcitabine with indicated concentrations was added to each well and cells were continuously cultured for 24 hours and released in gemcitabine-free media for 24 hours before being subjected to an 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma, Dorset, UK) assay.

RNA extraction

RNA was isolated from SW1990 and SW1990/GEM cells that were grown to 80–90 % confluency before RNA was harvested.
Total RNA was extracted using Trizol reagents (Invitrogen, CA, USA) according to the manufacturer’s instructions. The RNA sample was purified with an RNeasy Mini Column (Qiagen, CA, USA) and the RNA quality was assessed by 1.0% agarose gel electrophoresis. Total RNA were subjected to RNA-seq analysis of mRNA and microRNA expression profile (Novel Bioinformatics, Shanghai, China).

Quantitative real-time PCR (Q-PCR) analysis

cDNA was synthesized using random primers with Super-Script III (Invitrogen, CA, USA) according to the manufacturer’s protocol. Q-PCR was performed using Taqman Universal PCR Master Mix. Human glyceraldehyde 3-phosphate dehydrogenase (hGAPDH) was used as a control. Primer sequences are available upon request. Expression of microRNAs was analyzed using the TaqMan MicroRNA Assays, TaqMan MicroRNA RT Kit, and Taqman Universal PCR Master Mix without UNG Amperase (Applied Biosystems, Foster City, CA) on an Applied Biosystems 7500 Fast Real-Time PCR System. The Applied Biosystems 7500 Fast software was used to analyze the Ct values of different microRNAs normalized to an endogenous control (U6).

Western blot analysis

Western blot analysis was conducted as previously reported [25]. The following primary antibodies were used: anti-MDR-1 (1:400, Santa Cruz Biotechnology) and anti-GAPDH (1:8000, Abcam).

Data analysis

Quality control The raw sequencing data are evaluated by FAST-QC including quality distribution of nucleotides, position specific sequencing quality, GC content, the proportion of PCR duplication, kmer frequency, etc.

Mapping We used TopHat for RNA-seq alignment. TopHat is a fast splice junction mapper for RNA-seq reads. After aligning the reads to the reference genome based on Bowtie, the mapped reads are assembled using MAQ to identify the possible splicing junctions, while the unmapped reads may be divided into small segments to allow them to align to the reference genome and define splice junctions by seed extension procedure.

Dif-gene-finder We applied Limma algorithm [28] to filter the differentially expressed genes, after the significant analysis and FDR analysis [29] under the following criteria: (i) fold change >2 or <0.5, (ii) FDR <0.05.

Target analysis We utilized Targetscan and miRnada as the tools for predicting microRNA target on the differentially expressed microRNA.

Go analysis Gene ontology (GO) analysis was performed to facilitate elucidating the biological implications of unique genes in the significant or representative profiles of the target gene of the differentially expressed microRNA in the experiment [30]. We downloaded the GO annotations from NCBI (http://www.ncbi.nlm.nih.gov/), UniProt (http://www.uniprot.org/), and the Gene Ontology (http://www.geneontology.org/). Fisher’s exact test was applied to identify the significant GO categories and FDR was used to correct the p values.

Pathway analysis Pathway analysis was used to find out the significant pathway of the differential genes according to KEGG database. We turn to the Fisher’s exact test to select the significant pathway, and the threshold of significance was defined by p value and FDR [31].

MicroRNA-mRNA-network The relationship of the microRNAs and mRNAs were counted by their differential expression values and according to the interactions of microRNA and genes in Sanger MicroRNA database to build the MicroRNA-Gene-Network. The adjacency matrix of microRNA and genes A = [ai,j] is made by the attribute relationships among genes and microRNA, and ai,j represents the relationship weight of gene i and microRNA j. In the MicroRNA-mRNA-Network, the circle represents gene, and the shape of square represents microRNA, and their relationship was represented by one edge. The center of the network was represented by degree. Degree means the contribution of one microRNA to the genes around or the contribution of one gene to the microRNAs around. The key microRNA and gene in the network always have the biggest degrees.

Statistical analysis

Statistical analysis was performed by two-way ANOVA using GraphPad Prism software (San Diego, CA) and statistical significance is indicated (*p<0.05).

Results

Establishment of gemcitabine-resistant pancreatic cancer cells (SW1990/GEM)

The pancreatic cancer cell line SW1990 was used to create gemcitabine-resistant sub-lines (SW1990/GEM) by exposure to incrementally increasing concentrations of gemcitabine. SW1990/GEM sub-line was established after 7 months of gemcitabine induction (final gemcitabine concentration,
1 mM). The cell morphology of SW1990 cells co-cultured with 2, 5, 10, 20, 50, and 100 μM was different from parental SW1990 cells (Fig. 1a). After more than 1 month in gemcitabine-free culture, the cytotoxicity of gemcitabine to the parental and gemcitabine-resistant lines was determined by MTT assay. The resistance index of both cell lines was calculated (IC50 of SW1990, 1.14 μM; IC50 of SW1990/GEM, 847.23 μM) and compared with SW1990 cells, the gemcitabine-resistant sub-lines tolerate a dramatically higher concentration (743-fold, \( p < 0.05 \)) of gemcitabine (Fig. 1b). The cell cycle of parental and gemcitabine-resistant pancreatic cell lines was examined by flow cytometry. The proportion of SW1990/GEM cells in S phase (DNA synthesis) was relatively lower than that of parental cell line (Fig. 1c). Correspondingly, the cell proliferation rate of gemcitabine-resistant cells was also lower than SW1990 cells (Fig. 1d). In addition, the protein level of a transmembrane protein called MDR-1, which is extensively involved in the development of multidrug resistance [32], was determined by Western blot analysis. As expected, the protein level of MDR-1 was significantly upregulated by increasing concentrations of gemcitabine treatment (Fig. 1e). Overall, we established a gemcitabine-resistant pancreatic cancer sub-cell line for the following study.

Differential gene expression between parental and gemcitabine-resistant pancreatic cancer cells

To investigate the mechanisms underlying the development of gemcitabine resistance, the RNA-seq analysis was conducted to compare the expression profile of SW1990 and SW1990/GEM cells. Among the over 30,000 tested transcripts, the expression levels of 507 genes were upregulated and 550 genes were downregulated (fold change \( \geq 2, p < 0.05 \)) in SW1990/GEM cells (Fig. 2a, Table S1) when compared with SW1990 cells. Moreover, the 60 top-ranking altered genes between the gemcitabine-resistant sub-line and parental cell line were subjected to an unsupervised hierarchical cluster analysis (Fig. 2b). It revealed several clusters of genes with similar gene expression patterns in SW1990 and SW1990/GEM cells, and the refined genes from clusters could successfully discriminate the parental and
gemcitabine-resistant pancreatic cancer cells, possibly based on their sensitivity to gemcitabine (Fig. 2b). Among the listed genes in Fig. 2b, multiple upregulated genes (such as PSCA, LY6D, VGF, PDGFA, DUSP5, TRIM21, RRM1, etc.) and downregulated genes (such as CXCL5, ASCL2, PDSS1, RRS1, GPAT CH4, etc.) were involved in drug response, anti-oxidant activity, apoptosis, and cell cycle, which are the most possible cellular response in drug resistance development.

To annotate the differentially expressed genes related to gemcitabine resistance, gene ontology (GO) and pathway analyses were performed for the upregulated and downregulated genes in Fig. 2a, respectively. The 1057 annotated genes exhibited a wide range of functions. The 507 upregulated genes were mainly related to response to drug, endoplasmic reticulum (ER) unfolding, and apoptotic processes, and the GO terms of 550 downregulated genes included gene expression, RNA splicing, and cell cycle-related process (Fig. 2c). The pathway analysis showed that the upregulated genes were associated with lysosome, focal adhesion, and MAPK signaling pathway and that the declined genes were involved in spliceosome, ribosome biogenesis, RNA transport, and cell cycle (Fig. 2d). Combining the results in GO and pathway analysis, we demonstrate that during the development of gemcitabine resistance, SW1990 cells produce a series of cellular responses to drug treatment such as the activation of drug responsive genes, apoptosis, and ER stress. Concurrently, the cellular processes related to gene expression,
DNA synthesis, and cell cycle progression are inhibited by gemcitabine induction. It is consistent with the characteristics of SW1990/GEM cells, which showed lower cell proliferation rate and lower percentage of S phase cells (Fig. 1b, c).

Next, quantitative real-time PCR (Q-PCR) was performed to validate the relative gene expression of the 20 selected genes that were our interest in our further study from the list in Fig. 2b. As shown in Fig. 2e, the mRNA expression levels of 10 upregulated and 10 downregulated genes as measured by Q-PCR were highly comparable to the RNA-seq results. Comparing with the parental SW1990 cells, the expression of PSCA, LY6D, KLK7, FIBCD1, and SERPINE1 was most upregulated in SW1990/GEM cells, and in contrast, CXCL5, BCRN1, SFXN2, ASCL2, and PDSS1 were the significantly downregulated genes in gemcitabine-resistant cells. Because of the detection sensitivity of both methods, the fold changes in Q-PCR analysis were not exactly matched with the results observed in RNA-seq data, but there was a highly significant correlation between the two groups of data ($r=0.894; p=0.036$).

**Analysis of microRNA expression patterns of gemcitabine-resistant sub-lines**

MicroRNAs are a kind of non-coding RNAs implicated in the development of drug resistance of malignancies [18]. Therefore, microRNAs might play essential roles to generate high tolerance to gemcitabine through modulating the expression of targeting genes. Given that the transcriptional levels of hundreds of genes were altered between SW1990 and SW1990/GEM cells, the expression change of a proportion of genes should be elicited by differentially expressed microRNAs, which were detected by microRNA expression profiling. Among the detected microRNAs, 627 differentially expressed genes were identified. However, the sequencing reads of the majority of microRNAs, which represent the abundance of microRNAs in these cells, were very low. To screen the differential microRNAs with relatively high abundance, the microRNAs with low reads (<100) in both SW1990 and SW1990/GEM cells were excluded. Consequently, compared with SW1990 cells, 28 microRNAs were upregulated and 28 microRNAs were decreased (fold change ≥2) in SW1990/GEM cells (Fig. 3a, Table S2). Then, the expression of some differential microRNAs was confirmed by Q-PCR assays. We found that miR-643, miR-1261, miR-483-5p, miR-371a-5p, and miR-373-3p were upregulated and that the expression of miR-4455, miR-3676, miR-4650, miR-4791, and miR-4644 was decreased in SW1990/GEM cells. Generally, the tendency of expression changes was consistent between microRNA-seq and Q-PCR results. Taken together, 56 differentially expressed microRNAs with relatively high...
abundance were identified in gemcitabine-resistant cells by using microRNA-seq analysis, and these differential microRNAs might be associated with the development of gemcitabine resistance in pancreatic cancer cells.

The microRNA-mRNA regulatory network in gemcitabine-resistant pancreatic cancer cells

It has been widely accepted that the network-based detection of the hub microRNA signatures leads to higher accuracy than non network-based methods. Therefore, we established the strategy described in Fig. 4a to identify the microRNA-mRNA network in the development of gemcitabine resistance in pancreatic cancer cells. Briefly, the differentially expresses microRNAs and mRNAs between SW1990 and SW1990/GEM cells were obtained from our RNA-seq analysis. By using TargetScan and miRnada software, differential microRNA targets were predicted. Combining the expression pattern of these predicted targets (Fig. 2a), we habilitated significant microRNA-mRNA pairs, which exhibited reverse expression patterns between parental and gemcitabine-resistant cells. Therefore, integrative analyses of the microRNA and gene expression data can reveal the mechanisms underlying the progression of gemcitabine resistance.

Hubs are a central part of a network, important for the progression of the biological system. Likewise, microRNAs regulating several targets might play more important roles in gemcitabine resistance. Thus, it is interesting to investigate hub microRNAs in gemcitabine resistance. Hubs are usually defined as the top 15 % of the nodes by degree, and six differentially expressed microRNAs were identified as hub microRNAs, including miR-643, miR-1261, miR-3676, miR-4650-5p, miR4644, and miR-4455 (Fig. 4b). We found that each of these six microRNAs regulates at least 11 targets (Table. S3), and miR-4644 is the one regulating the most genes (320 predicted targets). Moreover, we found that these microRNAs regulate many common target genes in a combinational manner (Fig. 4b). To dissect the core microRNA-mRNA network, which is most functionally associated with gemcitabine resistance, the functional enrichment of these predicted targets were analyzed, and the genes tightly correlated with drug response, anti-oxidant activity, apoptosis, and cell cycle were selected out to construct the core hub microRNA-mRNA network. More importantly, these microRNAs and their targets must exhibit reverse expression patterns in SW1990 and SW1990/GEM cells. As shown in Fig. 4c, the upregulated microRNAs in SW1990/GEM cells, including miR-643 and miR-1261, might inhibit the expression of negative regulators, such as HMGB1 and SRSF2, to promote the development of gemcitabine resistance. In contrast, the down-regulated microRNAs (miR-3676, miR-4650-5p, miR4644, and miR-4455) might release the inhibition of their target genes, such as JUNB, FKBP8, FURIN, and VASP, the expression of which was increased in SW1990/GEM cells. The activation of these microRNA targets may help to establish the gemcitabine resistance. Collectively, we have identified that these core microRNAs might behave as key regulators by negatively targeting genes associated with drug response, anti-oxidant activity, apoptosis, and cell cycle, playing extensive important roles in the development of gemcitabine resistance in pancreatic cancer cells.

Discussion

Pancreatic cancer is one of the most lethal malignancies with bad prognosis, and the development of drug resistance accounts for the most failures of chemotherapy. Gemcitabine is
a nucleoside analog, which exhibits strong activity against solid tumors. Gemcitabine alone or in combination with other anti-cancer drugs has become a most widely used regimen in pancreatic cancer. However, the resistance to gemcitabine in some patients leads to failure of chemotherapy. Thus, a better understanding of the mechanisms of gemcitabine resistance is critical to the development of superior combination therapies or the replacement of gemcitabine as the gold standard in pancreatic cancer. Although several studies have been conducted investigating the molecular mechanism underlying gemcitabine resistance [10–13], the results from different groups differed from each other and were not widely accepted. Therefore, a more reliable method was used in this study to improve our understanding of gene regulations on gemcitabine resistance progression based on the analysis of microRNA-mRNA network.

Here, a widespread differential gene and microRNA expression profile was constructed in SW1990 cell line and an established gemcitabine-resistant sub-line (SW1990/GEM). By analyzing these differentially expressed genes and microRNAs, a paired microRNA and mRNA expression profiles and predicted microRNA-mRNA regulatory network, and signatures comprising hub microRNAs and their potential functional targets were established. The identified network might represent the regulations from microRNAs to mRNAs for underlying the development of drug resistance in pancreatic cancer cells.

At first, a SW1990/GEM sub-line was established and the characteristics were detected (Fig. 1). During the culture in GEM-containing medium, the cell morphology changed significantly, accompanying with cell apoptosis in the initial stage of establishing the gemcitabine-resistant cell line. The percentage of SW1990/GEM cells in S phase was reduced and cell proliferation rate is much lower than parental cells (Fig. 1c, d). It is consistent with the observation that several cell cycle-related genes were downregulated (Fig. 2c, d). Finally, a gemcitabine-resistant sub-line that is highly nonsensitive to gemcitabine was established, which could mimic the pancreatic cancer cells in gemcitabine-resistant patients.

Then, the mRNA and microRNA expression profile was analyzed by high-throughput RNA-seq technology. Although the comparison between parental and gemcitabine-resistant cell lines has been conducted by microarray analysis previously [15], whereas microarray results hardly reflects the abundance of gene expression, and microRNA regulation has not been considered in the previous study. In the present study, RNA-seq analysis and microRNA-mRNA network-based construction were performed, which generated more reliable results associated with gemcitabine resistance.

Among the differentially expressed genes, numerous altered genes were related to drug response, toxicity, apoptosis, and cell cycle (Fig. 2b–d). Combining the functional annotations and KEGG pathway analysis for differential genes in SW1990/GEM cells, we conclude that during the development of gemcitabine resistance, the pancreatic cancer cells produces cellular response to drug and apoptosis stimulation, and the cell cycle progression is suppressed concurrently. It demonstrates that these top-ranking differential genes (e.g., upregulated: PSCA, LY6D, KLK7, FIBCD1; downregulated: CXCL5, BCYRN1, SFXN2, ASCL2) might be strongly associated with the development of gemcitabine resistance. Moreover, the top-ranking altered genes were confirmed by Q-PCR analysis, which showed highly consistence with RNA-seq data. Intriguingly, many differential genes, such as PSCA, LY6D, KLK7, etc., were deregulated in pancreatic cancer patients or have reported to be involved in gemcitabine resistance [16, 33–35].

Furthermore, the differential microRNAs were also identified in the present study (Fig. 3). Among these differential microRNAs, several microRNAs, such as miR-210, miR-15b, miR-224, and miR-371, have been revealed to be involved in gemcitabine resistance or resistance to other kind of drugs [36–39]. It suggests that our screening of functional microRNAs related to gemcitabine resistance is reliable. Cellular response to drug stimulation is a complex process, and microRNA-target gene regulation is an essential kind of responsive manner. Thus, screening for microRNA-target with reverse expression patterns assists us to identify the potential microRNA and functional target genes in gemcitabine resistance. Bioinformatics analysis revealed 6 hub microRNAs (miR-643, miR-1261, miR-3676, miR-4650-5p, miR4644, and miR-4455) to compose the microRNA-mRNA network (Fig. 4b). Moreover, a core microRNA-mRNA network based on functional enrichment was also obtained (Fig. 4c). The corresponding targets for the 6 microRNAs, such as HMGB1, SRSF2, JUNB, FKBP8, FURIN, and VASP, are essential regulators in drug (gemcitabine) response, cell cycle, and apoptosis [40–44]. These data demonstrate that the core microRNA-mRNA partners might be key players in the development of gemcitabine resistance, which will be investigated further in our future study.

In summary, we not only identified the genes and microRNAs that might be the predictors and diagnosed valuable biomarkers of gemcitabine resistance, but also revealed a core functional microRNA-mRNA network in the context of gemcitabine resistance progression. This study shows that gemcitabine resistance of pancreatic cancer cells is correlated with drug response, apoptosis, and cell cycle disturbance. Further investigation of the selected microRNA-mRNA pairs will provide novel insights into the molecular mechanisms of drug resistance.

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Conflicts of interest The authors have declared no conflicts of interest.
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