Microarray analysis of circular RNA expression profiles associated with gemcitabine resistance in pancreatic cancer cells

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Abstract. Pancreatic cancer (PC) is one of the most malignant tumors of the digestive system due to its rapid progression, metastasis and resistance to chemotherapy. Gemcitabine (GEM) chemotherapy is the first-choice treatment for advanced PC. However, the effect of GEM-based chemotherapy on PC is limited due to the development of chemoresistance, and the molecular mechanisms underlying this resistance have yet to be investigated. Circular RNAs (circRNAs), which can function as microRNA sponges, have been found to be involved in the development of several types of cancer. However, research on circRNAs in PC drug resistance is limited. In the present study, the GEM-resistant PC cell line, SW1990/GZ, was successfully established by treating parental SW1990 cells *in vitro* with increasing concentrations of GEM in culture medium intermittently for 10 months. By analyzing the expression profiles of circRNAs in microarray between SW1990/GZ and parental SW1990 cells, we identified 26 upregulated and 55 downregulated circRNAs (fold change ≥2 and P<0.05) among 12,866 detected circRNAs in SW1990/GZ compared with SW1990 cells. Furthermore, the changes in the expression of six representative circRNAs was validated by reverse transcription-quantitative PCR. In addition, Kyoto Encyclopedia of Genes and Genomes pathway analysis and Gene Ontology analysis were performed. These analyses revealed that the dysregulated circRNAs regulated several cancer-related pathways, such as the mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) signaling pathways, and may be involved in the biological process of the regulation of chemoresistance, including nucleic acid metabolic process and cellular response to stress. The present study undertook a comprehensive expression analysis and revealed the functional profiles of differentially expressed circRNAs associated with GEM-resistance in PC, thereby indicating the possible participation of these dysregulated circRNAs in the development of chemoresistance and providing novel potential therapeutic targets for PC.

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Introduction

Pancreatic cancer (PC) is one of the most lethal human malignancies, with an overall five-year survival rate of <5% (1). The high mortality rate associated with PC can be largely attributed to its highly aggressive nature, wherein local invasion and remote metastasis may occur during the early stages of carcinogenesis (2). Thus, the majority of patients diagnosed with PC cannot undergo surgery and chemotherapy is thus the main treatment option. At present, gemcitabine (GEM) is the first-line drug used in the treatment of PC. However, its therapeutic efficacy is far from satisfactory due to the inherent chemoresistance of PC (3). A previous study revealed that only 23.8% of GEM-treated patients received therapeutic benefits in their early stages of treatment (4). However, the majority of these patients faced therapeutic failure due to the obtained chemoresistance against GEM. Thus, a better understanding of the molecular mechanisms underlying the development of GEM chemoresistance is necessary to develop novel-targeted therapies to 'flip the switch' from drug resistance to susceptibility in PC.

Recently, a set of non-coding RNAs (ncRNAs), including microRNAs (miRNAs or miRs) and long non-coding RNAs (lncRNAs), have been found to be involved in PC pathogenesis. Circular RNAs (circRNAs) are a special class of endogenously expressed non-coding RNAs, which are featured with a covalently closed loop structure without a 5' to 3' polarity and polyadenylated tail (5). circRNAs are highly conserved in mammals and are mainly expressed in a cell type-specific or developmental stage-specific manner, indicating their involvement in various physiological and pathological processes (6-8). Currently, studies have confirmed that circRNAs contain conserved miRNA binding sites and function as miRNA sponges to modulate the expression of target genes. To date, the dysregulation of circRNAs has been reported in a set

of human diseases, particularly in cancer development and progression (9-16). A recent study demonstrated that clusters of circRNAs were aberrantly expressed in PC compared with normal samples (17); however, the specific roles of circRNAs in PC pathogenesis remain unknown.

In the present study, to explore the roles of circRNAs in the development of chemoresistance in PC, we first developed a GEM-resistant PC cell line (SW1990/GZ) by exposing SW1990 PC cells to gradient concentrations of GEM. We then performed microarray analysis of SW1990/GZ cells, along with their parental control cells. Our results demonstrated several differentially expressed circRNAs that may be involved in the transformation of the GEM resistance of PC and may provide potential molecular biomarkers or therapeutic targets for PC in the future.

Materials and methods

Establishment of GEM-resistant cell line, SW1990/GZ. The PC cell line, SW1990, was purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). The GEM-resistant cell line, SW1990/GZ, was established by repeated subcultures in the presence of stepwise increases in GEM concentrations (Tocris Bioscience, Ellisville, MO, USA) during the growth of SW1990 cells. First, SW1990 cells were cultured in RPMI-1640 (HyClone Laboratories/GE Healthcare, Chicago, IL, USA) containing 10% fetal bovine serum (Gibco/Thermo Fisher Scientific, Inc., Waltham, MA, USA) and various concentrations of GEM. Subsequently, cell death was observed, and the median lethal dose of SW1990 cells was set to 0.07 μg/ml. Subsequently, the SW1990 cells were cultured in medium containing GEM at a concentration of 0.1 μg/ml. Following incubation at 37˚C for 48 h, the culture and dead cells were replaced with fresh drug-free medium. The remaining cells could then grow and probably enter the logarithmic phase of cell growth. They were passaged twice and then re-cultured in medium containing GEM at a concentration of 0.1 μg/ml. The medium was then replaced with culture medium containing GEM at a concentration of 0.4 μg/ml, and the cells were cultured with the aforementioned cycle progress, according to four-fold increase in the drug concentration. Finally, the cells were cultured in a medium with a drug concentration of 400 μg/ml. Therefore, the filter viable cells produced a stable resistance to high concentrations of GEM. After 10 months, we had successfully acquired a stable GEM-resistant cell line designated as SW1990/GZ.

Drug sensitivity assay. Cells ($3x10^3$ cells/well) were seeded in 96-well plates. After 12 h, the cells were exposed to increasing concentrations of GEM (0, 25, 50, 100, 150, 200, 250, 300, 350 and 400 μg/ml) and incubated at 37˚C for 72 h to determine the IC_{50} value using Cell Counting Kit-8 assay (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) to evaluate the sensitivity to GEM.

RNA extraction, purification and array hybridization. Total RNA was extracted from three samples of SW1990/GZ and SW1990 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated with Rnase R (Epicentre, Madison, WI, USA) to remove linear RNA. Subsequently, enriched circRNA samples were amplified and transcribed into fluorescent cRNA by a random priming method (Arraystar Super RNA Labeling kit; Arraystar, Inc., Rockville, MD, USA). The labeled cRNAs were purified by the RNeasy mini kit (Qiagen GmbH, Hilden, Germany). The concentration and specific activity of the labeled cRNAs (pmol Cy3/μg cRNA) were determined by NanoDrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA). Labeled cRNA (1 μl each) was fragmented by adding 5 μl of 10X blocking agent and 1 μl of 25X fragmentation buffer, and the mixture was then heated at 60˚C for 30 min. Subsequently, the labeled cRNA was diluted with 25 μl of 2X hybridization buffer. Finally, the labeled cRNA was hybridized using Human 8x15 K circRNA Array (Arraystar). The hybridized arrays were washed, fixed and scanned using the Agilent DNA Microarray Scanner G2505C (Agilent Technologies, Inc., Santa Clara, CA, USA).

Microarray data analysis. Agilent Feature Extraction software (version 11.0.1.1; Agilent Technologies) was used to analyze scanned images for raw data extraction. Quintile normalization and subsequent data processing were performed using the R software package R version 3.1.2 (Agilent Technologies), and low intensity filtering was performed. In the comparison of the cricRNA profiles of two groups using Student's t-test, the differences with a fold change (FC) of ≥2 and $P<0.05$ were considered statistically significant.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the cell samples using TRIzol reagent (Invitrogen/Thermo Fisher Scientific, Inc., Waltham, MA, USA). Briefly, cDNA was synthesized from 1 μg of total RNA with SuperScript III Reverse Transcriptase (Invitrogen Japan) samples. Quantitative PCR (qPCR) was performed using a SYBR PrimeScript RT-PCR kit (Takara, Kyoto, Japan) on a Rotor-Gene 6000 real-time genetic analyzer (Corbett Life Science, Mortlake, Australia). Primer sequences for candidate genes are listed in Table I. The PCR program was initiated for 10 min at 95˚C before 40 thermal cycles, each at 10 sec at 95˚C and 1 min at 60˚C to collect fluorescent signals. The relative quantification of circRNA expression levels was determined by taking the average of the GAPDH-normalized $2^{-\Delta\Delta Cq}$ values (18).

GO and pathway analyses. The potential functions of the parental genes of differential circRNAs were analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/). The parental gene function was then predicted by GO functional annotation in terms of biological process (BP), cellular component (CC) and molecular function (MF). The results of the GO analysis are presented in a scatter plot, and the related pathways of the parental genes of differential circRNAs were analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/).

miRNA prediction. The circRNA-miRNA interaction was predicted by using Arraystar home-made miRNA target prediction software (Arraystar), which is based on the TargetScan (http://www.targetscan.org/vert_72/) and

Table I. The list of primers (F, forward and R, reverse) used in this study.

circRNA	Primer sequence
circ_101543	F: 5'-AAAAAGCACAGGCAGTTACTCA-3'
	R: 5'-CATTCCAGTAGGCGCTAAGA-3'
circ_000926	F: 5'-TTGTGCTTTCTGGAGGGTCT-3'
	R: 5'-GCACAAATAAACCCCACATTTT-3'
circ_003251	F: 5'-TATTATTCCCCCAGCTGCTC-3'
	R: 5'-CTGCTGCAACAGAAACCTGA-3'
circ_004077	F: 5'-AAGATCCCGGATGACATGAG-3'
	R: 5'-GAGTCTTGGGAGGGTTGTCA-3'
circ_101672	F: 5'-GGTTCTGCACCATCTTCAGG-3'
	R: 5'-TGGTGGTGGTCTTGTAGTCG-3'
circ_102747	F: 5'-GTATCCTGGCCTGCCATC-3'
	R: 5'-TTGCCTCATCACCAACCA-3'
GAPDH	F:5'-CATGAGAAGTATGACAACAGCCT-3'
	R: 5'-AGTCCTTCCACGATACCAAAGT-3'

F, forward; R, reverse.

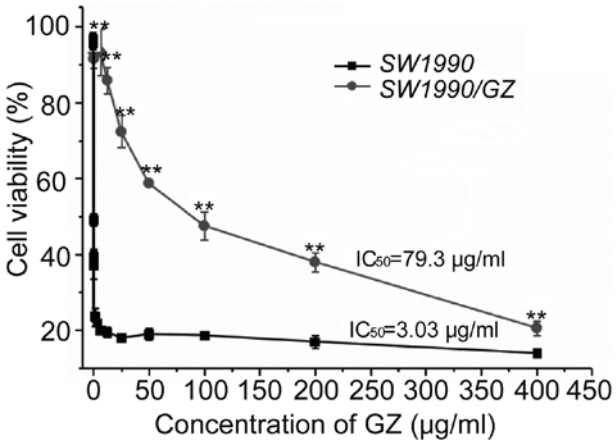

Figure 1. Cell viability of SW1990 and SW1990/GZ cells exposed to GEM. SW1990 and SW1990/GZ cells were treated with various concentrations of GEM (0, 25, 50, 100, 150, 200, 250, 300, 350 and 400 μg/l) for 72 h. Cell viability was determined by CCK-8 assay. Data shown are the mean ± SEM. Each experiment was performed in triplicate. **P<0.01 vs. SW1990 cells. GEM, gemcitabine.

miRanda (http://www.microrna.org/) prediction algorithm. The differentially expressed circRNAs in all the comparisons were annotated in detail with the circRNA/miRNA interaction information.

CircRNA-miRNA-target gene network. To further elucidate the associations between circRNAs and miRNAs, potential circRNA-miRNA-target gene interaction analysis was conducted by Cytoscape (version 3.6.1; http://www.cytoscape.org/). The size of each node represents the number of putative miRNA functionally connected to each circRNA.

Statistical analysis. Statistical analysis was conducted using SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). The Student's t test was used for comparisons between two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Drug resistance index of SW1990/GZ. The GEM resistance of the SW1990/GZ cell line was identified by determining its IC_{50} value against the parental SWl990 cell line. The IC_{50} value of GEM for the SW1990/GZ cells was 79.3±5.31 μg/ml, which was 26.2-fold higher than that of the parental cell line, SWl990 (3.03±0.27 μg/ml), indicating that the drug resistance index of SWl990/GZ cells relative to the parental SW1990 cells was 26.2 (Fig. 1). The GEM-resistant cell line, SW1990/GZ, was thus successfully established.

Overview of circRNA profiles. The expression of human circRNAs was screened in the SW1990 and SW1990/GZ cell samples using a microarray platform. Hierarchical clustering and box plot visualization revealed a distinguishable circRNA expression pattern between SW1990 and SW1990/GZ cells (Fig. 2A and B). In total, 81 circRNAs were differentially expressed (fold change ≥2.0 and P<0.05) between the SW1990/GZ and SW1990 cells. Among these, 26 circRNAs were upregulated and 55 circRNAs were downregulated >2-fold in SW1990/GZ cells. The 10 circRNAs with the most signficant increased and decreased in expression in the SW1990/GZ cells compared to the SW1990 cells are displayed in Fig. 2A and Table II. Significantly, the expression levels of circRNA_101672, circRNA_004077 and circRNA_003251 were upregulated in SW1990/GZ by 3.47-, 2.82- and 2.81-fold, respectively. Furthermore, circRNA_101543, circRNA_102747 and circRNA_000926 were downregulated by 3.94-, 3.88- and 3.51-fold, respectively. We also analyzed the chromosome distribution of these deregulated circRNAs. The differentially expressed circRNAs with statistically significance between SW1990/GZ and SW1990 cells were identified by volcano plot filtering (Fig. 2C). As displayed in Fig. 2D, each chromosome had circRNA locations, while chromosomes 1, 2, 4, 5, 12 and 17 had considerably more circRNA locations than the other chromosomes, indicating a stronger association with GEM resistance in PC. The top two upregulated circRNAs, circRNA_101672 and circRNA_004077, were both located on chromosome 16, which may be the most important circRNA in GEM resistance in PC. Among the deregulated circRNAs, there were 72 exonic, 1 antisense, 3 intronic and 5 sense overlapping (Fig. 2E).

Validation of the microarray data by RT-qPCR. To validate the microarray data, three upregulated and three downregulated circRNAs were selected as representatives for further validation by RT-qPCR. According to the data shown in Fig. 3, four of the six tested circRNAs yielded results quite similar to those of the microarray. These well-validated circRNAs included two upregulated circRNAs (circRNA_101672 and circRNA_003251) and two downregulated circRNAs (circRNA_101543 and circRNA_102747). Although the other

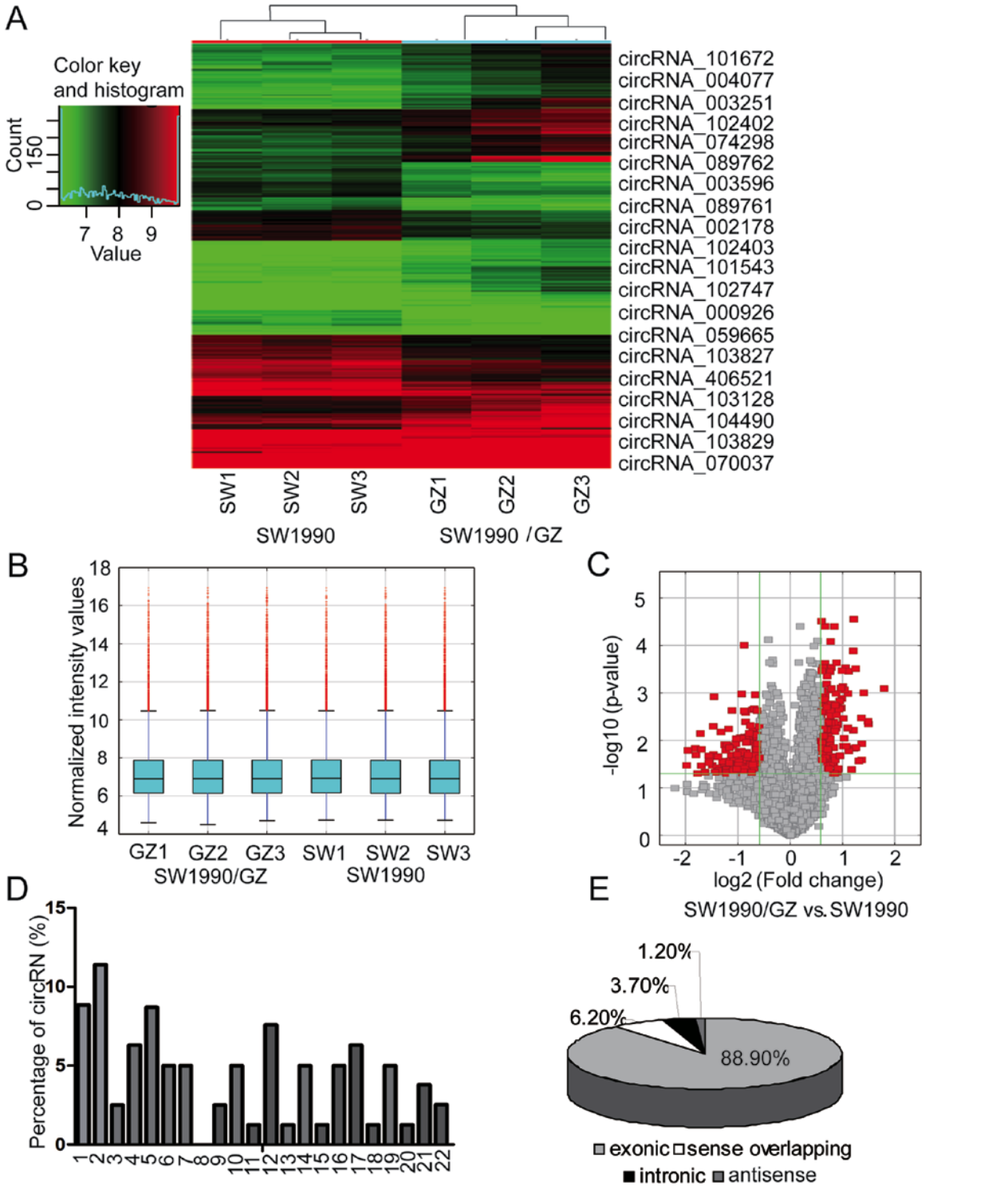

Figure 2. circRNA expression profile comparison between the SW1990/GZ and SW1990 cells. (A) Hierarchical clustering demonstrated a distinguishable circRNA expression profiling among SW1990/GZ and SW1990 cells. The top 10 most increased and decreased circRNAs in SW1990/GZ compared to SW1990 are shown. (B) Box plots demonstrating the distributions of a dataset for the circRNA profiles. After normalization, the distributions were nearly the same. (C) Volcano plot of the differentially expressed circRNAs. The x- and y-axes show the log2 fold change and negative log10 P-value, respectively. The red points in the plot represent the differentially expressed circRNAs with statistical significance. (D) Chromosomal locations of differentially expressed circRNAs. The x-axis denotes the ordinal of the chromosome, and the y-axis denotes the percentage of circRNAs that were expressed differently between SW1990/GZ and parental SW1990 cells (fold change >2) (E). Types of variably expressed circRNAs based on their genomic proximity to the neighboring gene.

two circRNAs were not well repeated, the direction of change was similar to that noted in the microarray data. This result indicated that most of the circRNAs identified by microarray were reliable.

GO and pathway analysis of the parental genes of circRNAs. Differentially regulated circRNAs and their parental genes were further analyzed by GO analysis to speculate circRNA potential functions on three different aspects: BP, CC and

Table II. Top dysregulated circRNAs in GEM-resistant PC cells.

circRNA	Gene symbol	Type	Chrom	P-value	FC (abs)
Upregulated circRNAs					
circ_101672	RAB40C	Exonic	chr16	0.000815496	3.4745157
circ_004077	VAT1L	Exonic	chr16	0.003990298	2.8248131
circ_003251	WNK1	Exonic	chr12	0.004520208	2.8156161
circ_102402	DAZAP1	Exonic	chr19	0.000995122	2.646115
circ_074298	HARS	Exonic	chr5	0.011756552	2.5846195
circ_089762	JA760602	Exonic	chrM	0.025705535	2.5812949
circ_003596	COL5A1	Exonic	chr9	0.003354102	2.5655559
circ_089761	JA760602	Exonic	chrM	0.027054021	2.4890659
circ_002178	RPPH1	Sense overlapping	chr14	0.014113033	2.3939466
circ_102403	DAZAP1	Exonic	chr19	0.000309541	2.3735941
Downregulated circRNAs					
circRNA_101543	VPS13C	Exonic	chr15	0.033434975	3.9419461
circRNA_102747	ACTR2	Exonic	chr2	0.016163204	3.8810596
circRNA_000926	ACTR2	Sense overlapping	chr2	0.014583979	3.5123931
circRNA_059665	ABHD12	Exonic	chr20	0.020206272	3.4890827
circRNA_103827	HMGCS1	Exonic	chr5	0.049403727	3.4092977
circRNA_406521	UGT8	Sense overlapping	chr4	0.007149841	3.283491
circRNA_103128	DYRK1A	Exonic	chr21	0.034078531	3.2488121
circRNA_104490	MKLN1	Exonic	chr7	0.015401059	3.2463403
circRNA_103829	HMGCS1	Exonic	chr5	0.046511499	3.0960301
circRNA_070037	NUP54	Exonic	chr4	0.021180458	2.938704

GEM, gemcitabine; PC, pancreatic cancer.

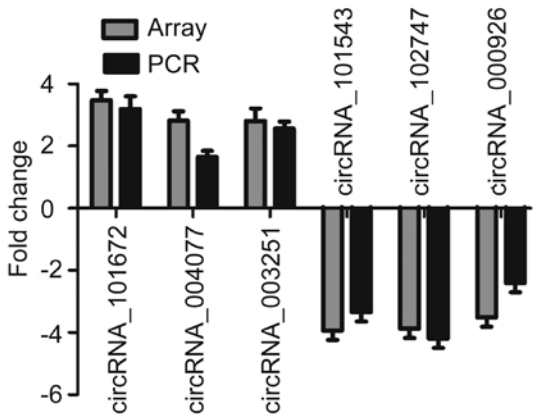

Figure 3. Validation of microarray data by RT-qPCR. Six differentially expressed circRNAs were validated by RT-qPCR. The y-axis column in the chart denotes the median fold changes.

MF. The top 10 enrichment GO entries for upregulated and downregulated circRNAs are displayed in Fig. 4. We found that the upregulated circRNAs were mainly enriched for GO terms related to nucleic acid metabolic process involved in BP, poly(A) RNA binding linked with MF, and nucleoplasm in CC. For the downregulated circRNAs, the most significantly enriched GO terms in BP, MF, and CC were peptidyl-threonine phosphorylation, enzyme binding and intracellular organelle, respectively. Furthermore, KEGG pathway analysis was performed. The upregulated circRNAs were involved in 11 pathways, while the downregulated circRNAs were involved in 37 pathways. The predominant pathways are displayed in Fig. 5. The top three enriched pathways for upregulated circRNAs were cell cycle, ubiquitin-mediated proteolysis and the cGMP-PKG signaling pathway. The top three enriched pathways for downregulated circRNAs were pancreatic cancer, EGFR tyrosine kinase inhibitor resistance and the mitogen-activated protein kinase (MAPK) signaling pathway. Among these enriched pathways, the MAPK and mammalian target of rapamycin (mTOR) signaling pathways have been previously reported to be involved in the chemoresistance of PC (19,20). As shown in Fig. 5C, the potential target genes of downregulated circRNAs incuding ATF2, BRAF, DUSP16, MAPK8, NFATC3, RAF1, TAB2, TAOK1 are directly associated with the MAPK signaling pathway.

Prediction for circRNA-miRNA interaction and circRNA-miRNA-target gene network. Given that circRNAs can function as sponges or inhibitors of their interacting miRNAs to regulate gene expression, circRNA-miRNA interaction was predicted with Arraystar homemade miRNA target prediction software based on TargetScan and miRanda. A total of 378 mature miRNAs were predicted to have docking sites in the differentially expressed circRNAs. Therefore, they can interact with the circRNAs. The circRNA-miRNA-target gene

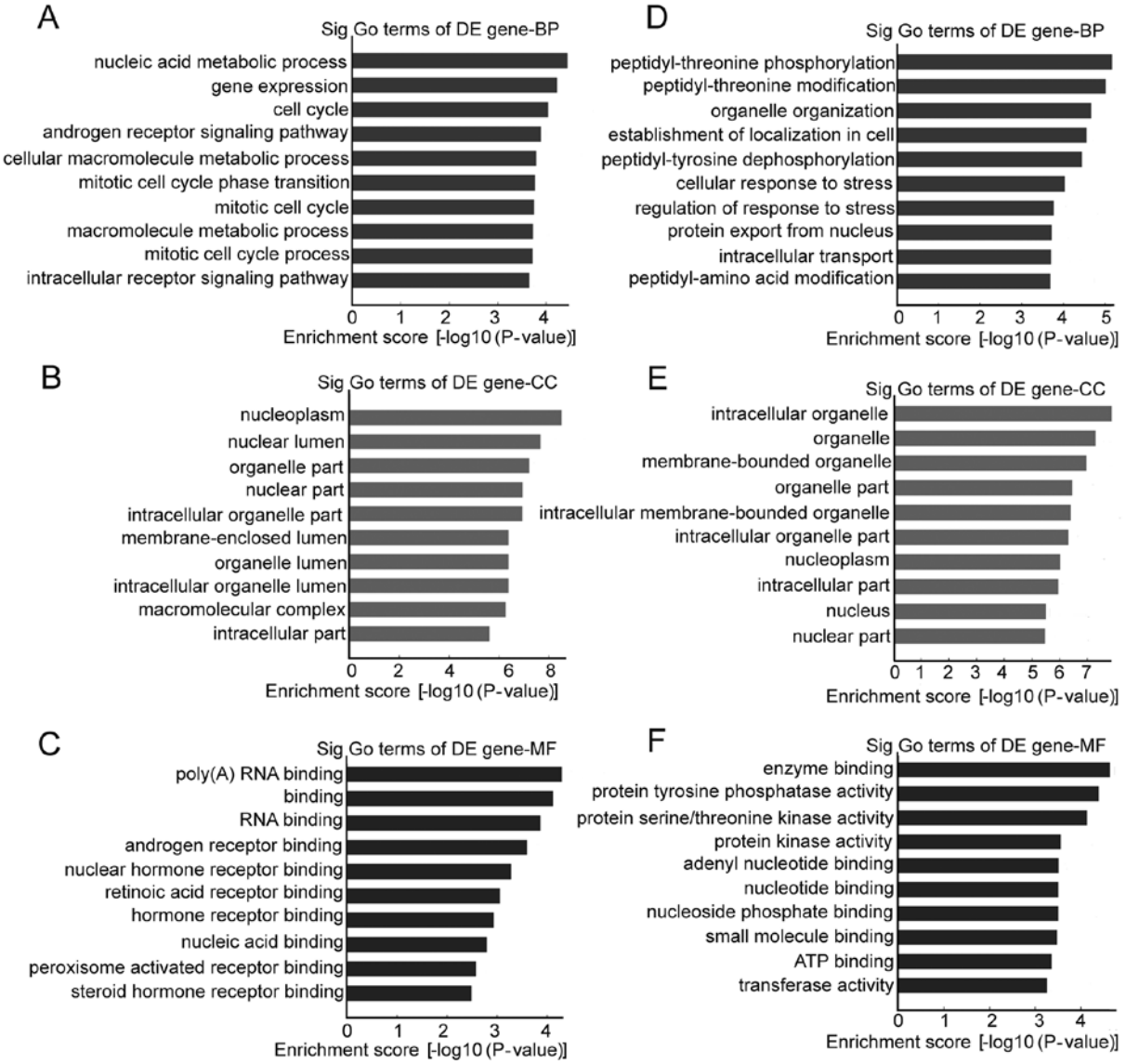

Figure 4. Gene ontology (GO) analysis of the parental genes of the dysregulated circRNAs. The GO analysis includes three categories: biological process (BP), cellular component (CC) and molecular function (MF). (A-C) GO enrichment corresponds to the upregulated circRNAs. (D-F) GO enrichment corresponds to the downregulated circRNAs. The P-value represents the significance of GO term enrichment in the dysregulated circRNA list. P-value ≤0.05 was assigned to indicate the significance.

interacting network of the top 10 upregulated circRNAs was established (Fig. 6). circRNA_101672 was annotated in detail using the circRNA-miRNA interaction information (Fig. 7).

Discussion

GEM is the most efficient mono-drug therapy for treating PC. Thus, it is recommended as first-line therapeutic option for advanced PC patients. However, acquired GEM resistance contributes to treatment failure in a substantial number of PC patients. Thus, deciphering the mechanisms underlying GEM resistance is essential to overcome the problem. circRNAs were recently identified as novel functional endogenous ncRNAs, which can function as miRNA sponges, thereby interfering with the post-transcriptional actions of miRNAs as suppressors of the target genes (21). Accumulating evidence has indicated that circRNAs are involved in a number of human diseases, particularly in carcinomas, including hepatoma, neuroglioma, bladder carcinoma and breast cancer (22-25). Hence, circRNAs may serve as novel diagnostic and therapeutic strategies in human diseases. However, the changes in the expression of circRNAs and the related functional significance in PC chemoresistance has been rarely reported.

In the present study, we first generated a GEM-resistant PC cell line, SW1990/GZ, by stepwise selection and then analyzed the circRNA expression profiles between the SW1990/GZ and parental SW1990 cells with high-throughput circRNA microarrays to investigate the mechanisms of acquired GEM resistance. We observed that 26 circRNAs were upregulated, and 55 circRNAs were downregulated by >2-fold in the SW1990/GZ cells compared with the SW1990 cells. circRNA_101672, circRNA_004077 and circRNA_003251 were upregulated with top magnitudes. Conversely, circRNA_101543, circRNA_102747 and circRNA_000926 were downregulated with top magnitudes. The expression

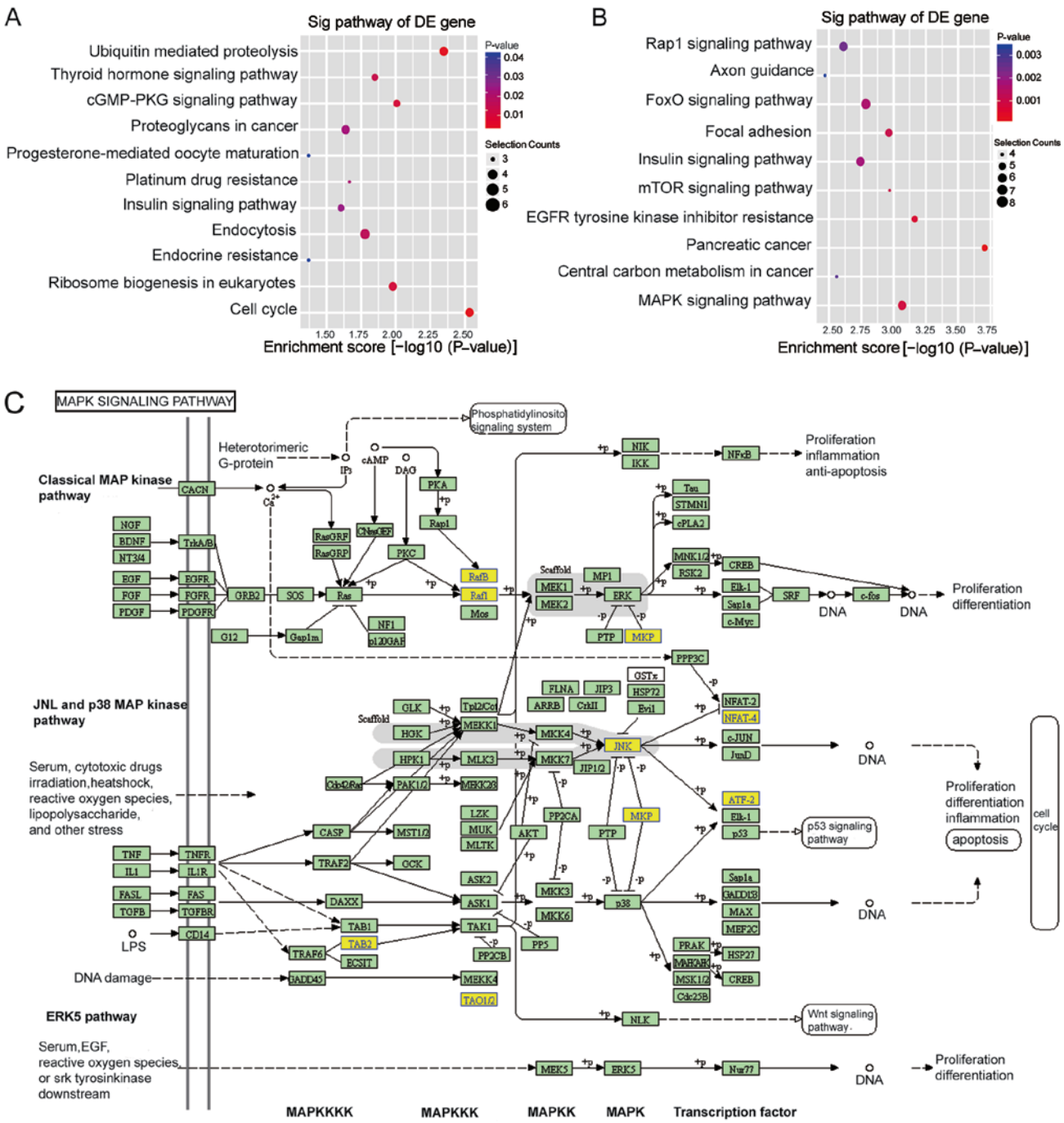

Figure 5. KEGG pathway analyses of the differential circRNA parental genes. The pathways correspond to (A) the upregulated circRNAs and (B) the downregulated circRNAs. The results are presented in a scatter plot. Enrichement factor refers to the ratio of the number of genes located in the pathway entry and the total number of genes in the pathway entry. (C) MAPK signaling pathway. Nodes marked in yellow are associated with target gene enrichment on MAPK signaling pathway.

patterns of the above-mentioned circRNAs were then validated by RT-qPCR, which revealed a high consistency between the RT-qPCR results and microarray data. Although we only used the SW1990 cell line as a cellular model in the present study, we hypothesize that some of the deregulated circRNAs in SW1990/GZ cells compared to SW1990 cells may be important and common contributors in PC-acquired GEM resistance. Our next step will be to confirm the results in other PC cell lines in our future study.

circRNAs are primarily generated from exons or introns of their parental genes and involved in the parental gene expression regulation (26-28). Thus, we investigated the biological functions and potential mechanisms of circRNAs in GEM resistance based on the GO and KEGG pathway analyses. GO enrichment analysis revealed that deregulated circRNAs were involved in the regulation of some crucial biological processes, such as cellular response to stress and cell cycle, which were important during the development of chemoresistance. Markedly, among these enriched pathways, the MAPK and mTOR signaling pathways have been shown to contribute to GEM chemoresistance (29,30). Thus, some circRNAs may be involved in the GEM resistance of PC by regulating the above-mentioned signaling pathways.

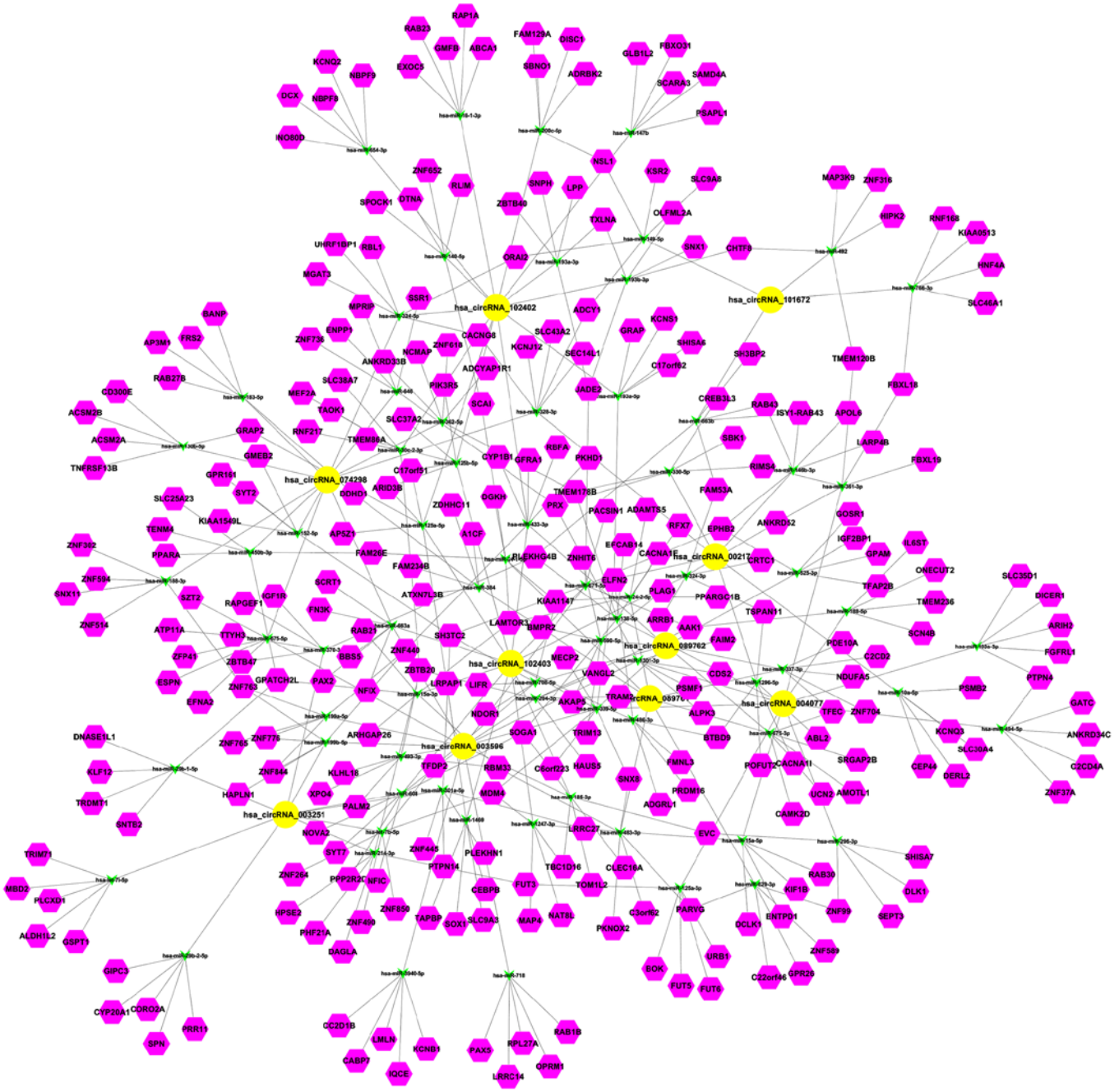

Figure 6. CircRNA-miRNA-target gene network analysis. Interactions between the top 10 upregulated circRNAs (shown by yellow nodes) and miRNAs and those between miRNAs and target genes in cancer signaling are shown in the map. The top 10 upregulated circRNAs are marked as yellow nodes. The pink nodes indicate the potetial target genes of the top 10 upregulated circRNAs.

Increasing evidence has demonstrated that circRNAs can 'sponge up' miRNAs to promote the expression of miRNA target protein-coding genes (31,32). Given the important roles of miRNAs in the pathogenesis of PC, we hypothesized that some circRNAs may contribute to the GEM resistance of PC by interacting with miRNAs. Therefore, in this study, we performed *in silico* analyses to predict miRNAs targeted by these dysregulated circRNAs. For example, the upregulated circRNA with the largest fold change, circRNA_101672, potentially binds miR-492. A previous study indicated that miR-492 was involved in colon cancer chemoresistance via regulating the expression of CD147 (33). In addition, one of the top downregulated circRNAs, circ_102747, potentially binds miR-21. Hwang *et al* (34) and Dong *et al* (35) provided experimental evidence for the role of miR-21 in PC GEM resistance through modulation of apoptosis by directly regulating Bcl-2 and PTEN expression. In order to confirm whether these circRNAs are involved in PC chemoresistance, future studies, which will include the overexpression and knockdown of circRNA, their interaction with their potential targeted miRNAs and their involvement in GEM resistance in clinical PC samples are required.

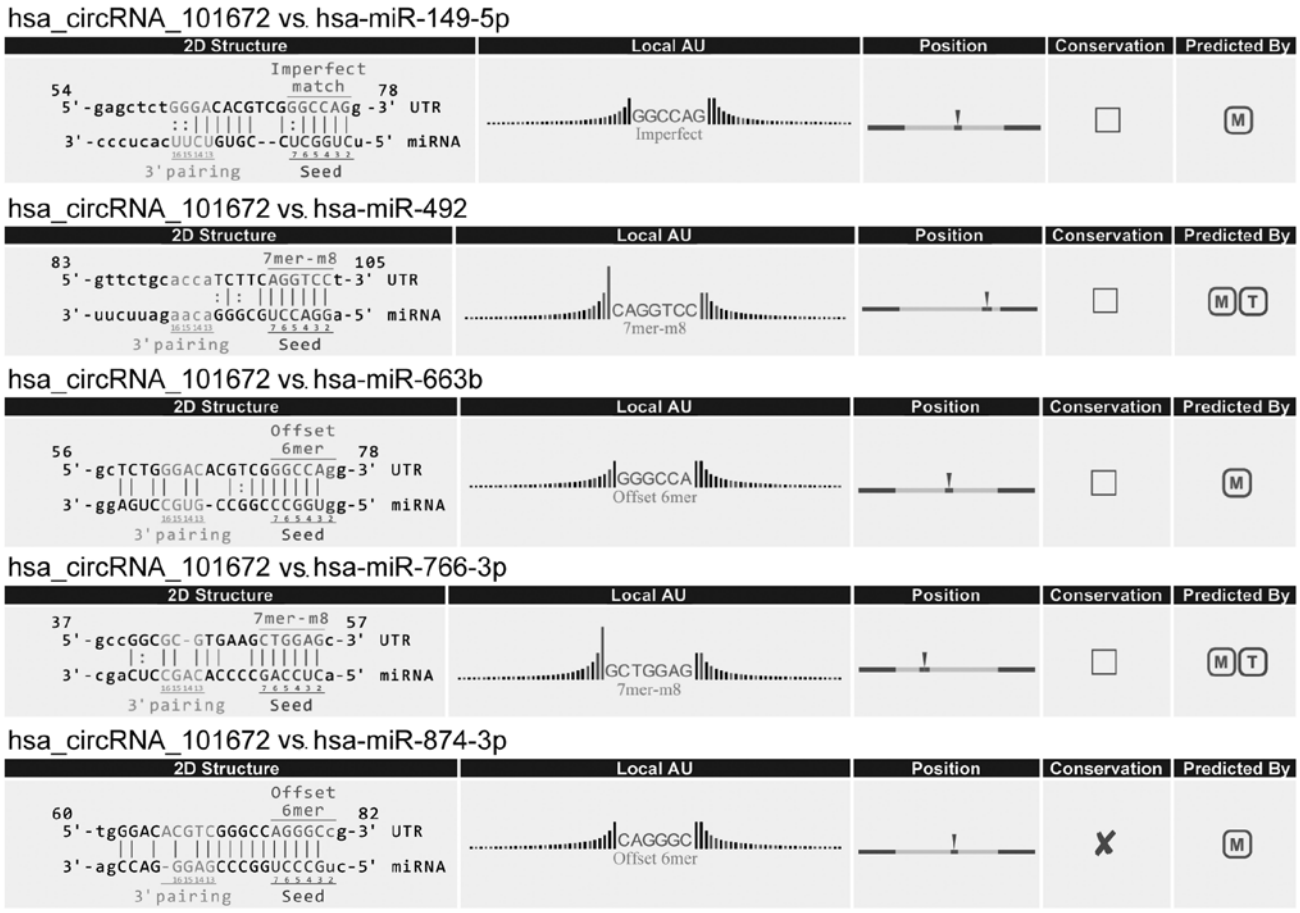

Figure 7. Detailed annotation for circRNA_101672-miRNA interaction. 7mer-m8: bases from number 2 to 8 match perfectly, and number 1 base is not A; offset 6mer: bases from number 3 to 8 match perfectly; imperfect match: imperfect base match was observed from number 2 to 7. M: circRNA-miRNA interaction predicted by miRanda; T: circRNA-miRNA interaction predicted by TargetScan.

In conclusion, the present study revealed that circRNAs were dysregulated in the GEM-resistant PC cell line compared with its parental cell line. For these dysregulated circRNAs, we conducted GO enrichment and pathway analyses for their parental genes, which indicated that circRNAs may play important roles in the development of GEM resistance. By predicting the circRNA-miRNA interaction, we found several dysregulated circRNA, i.e., circ_101672 and circ_102747, which can potentially bind some miRNAs involved in cancer chemoresistance. These could be essential molecular mechanisms underlying the function of circRNAs in the chemoresistance of PC. Since our results were only based on an *in vitro* cell line model, clinical sample and *in vivo* validation is warranted in the future. On the whole, our findings revealed the potential roles of circRNAs in PC chemoresistance and potential therapeutic targets for circRNAs in PC treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CX conceived and designed the study. CX and FD performed the research. CX wrote the manuscript. CX, YY and FD analyzed the data. All authors have read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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