



Data in Brief

Microarray expression profile of circular RNAs in human pancreatic ductal adenocarcinoma



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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) remains a common and deadly cancer. Despite numerous efforts, no reliable biomarker is available for daily clinical practice. Circular RNAs (circRNAs) are an abundant, stable and conserved class of RNA molecules that exhibit tissue/developmental-stage-specific expression (Salzman et al., 2012; Jeck et al., 2013; Memczak et al., 2013). CircRNAs play a crucial role in disease, especially in cancer, and provide new potential diagnostic and therapeutic targets for disease (Hansen et al., 2013; Qu et al., 2015). This research was designed to explore the expression profile of circRNAs in PDAC to serve as new diagnosis and treatment strategies for PDAC. Microarray and sample annotation data were deposited in Gene Expression Omnibus (GEO) under accession number [GSE69362](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69362).

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Specifications

Organism/cell line/tissue	<i>Homo sapiens</i> /pancreatic ductal adenocarcinoma and adjacent normal tissues
Sex	Male and female
Sequencer or array type	Arraystar Human CircRNA Array (8 × 15K, Arraystar)
Data format	Raw and analyzed
Experimental factors	Tumor vs. corresponding normal tissues from 4 pancreatic ductal adenocarcinoma patients
Experimental features	Microarray expression profile analysis of circular RNAs in human pancreatic ductal adenocarcinoma
Consent	All patients provided written informed consent
Sample source location	Xi'an, China

1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69362>

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2. Experimental design, materials and methods

2.1. Tissue samples

The Ethics Review Board of Xijing Hospital (No: XJYYLL-2015075) approved the study. Tissue samples were prospectively collected from patients undergoing operation at the Department of Hepatobiliary Surgery at the Xijing Hospital. Tumor and adjacent normal pancreatic tissue samples were snap-frozen in liquid nitrogen immediately after resection and stored at $-130\text{ }^{\circ}\text{C}$ until use. Histology of the tissue specimen was confirmed by two uropathologists.

2.2. RNA preparation

Total RNA was isolated from 4 PDAC samples and paired adjacent normal tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Total RNA from each specimen was quantified and quality was verified using NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA). Additionally, RNA integrity was assessed by electrophoresis on a denaturing agarose gel.

2.3. Labeling and hybridization

Sample labeling and array hybridization were performed according to the manufacturer's protocol (Arraystar, Rockville, Maryland, USA). In short, circRNAs were treated with Ribonuclease R (RNase R) to

remove linear RNAs according to the manufacturer's protocol (Epicenter, Madison, WI, USA). Then, each sample was amplified and transcribed into fluorescent cRNA utilizing a random priming method with a Super RNA Labeling Kit (Arraystar). The labeled cRNAs were purified using an RNeasy Mini Kit (Qiagen, Hilden, Germany). The concentration and specific activity of the labeled cRNAs (pmol Cy3/ μg cRNA) were measured using NanoDrop ND-1000. Then, 1 μg of each labeled cRNA was fragmented by adding 5 μl 10 \times Blocking Agent and 1 μl of 25 \times Fragmentation Buffer. The mixture was heated at 60 $^{\circ}\text{C}$ for 30 min, and 25 μl 2 \times Hybridization buffer was added to dilute the labeled cRNA. Then, 50 μl of the hybridization solution was dispensed into the gasket slide, which was assembled with Human Circular RNA Array slides. The slides were incubated for 17 h at 65 $^{\circ}\text{C}$ in an Agilent Hybridization Oven. The hybridized arrays were washed, fixed and scanned using an Agilent G2505C Scanner.

2.4. Microarray and quality control

Scanned images were imported into Agilent Feature Extraction software (version 11.0.1.1) for raw data extraction. Quantile normalization

of raw data and subsequent data processing were performed using the R software package (R version 3.1.2). After quantile normalization of the raw data, low intensity filtering was performed, and circRNAs with at least 2 out of 8 samples that had flags in "P" or "M" ("All Targets Value") were retained for further analyses. The log₂-ratio was used for quantile normalization. CircRNAs differentially expressed between the two groups were conveniently estimated by fold-change filtering and Student's t-test. CircRNAs exhibiting fold changes ≥ 2.0 and p-values ≤ 0.05 were selected as significantly differentially expressed circRNAs.

The experiment workflow is listed in Fig. 1.

3. Discussion

Large amounts of circRNAs are recently discovered and represent a new special class of endogenous noncoding RNA. Recent researches have revealed that circRNAs are an abundant, stable, diverse and conserved class of RNA molecules [1–3]. Moreover, circRNAs can function as miRNA sponges or regulate parent gene expression to affect disease

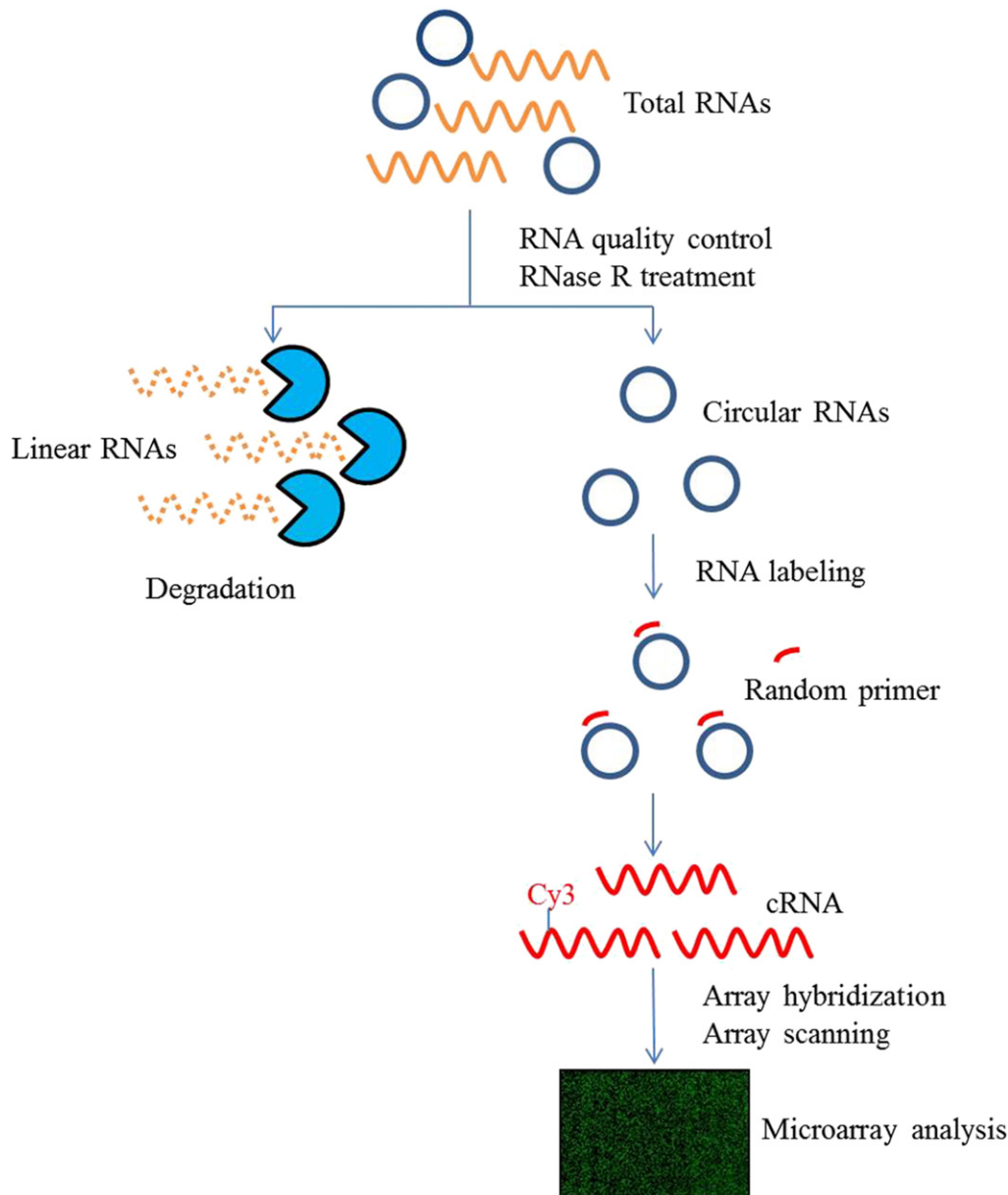


Fig. 1. Experiment workflow of microarray expression profile of circular RNAs.

initiation and progression [4–7]. These studies indicate that circRNAs have great potential to become diagnostic or predictive biomarkers of disease and provide new insights into the treatment of diseases. In this study, we have explored the expression profile of circRNAs in 4 PDAC samples and paired adjacent normal tissues. We have also revealed that the circRNA expression signatures of PDAC are dysregulated. The identification of novel differentially expressed circRNAs is a crucial step towards better understanding of PDAC. These findings indicate that circRNAs can be involved in the initiation and progression of PDAC.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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References

- [1] J. Salzman, C. Gawad, P.L. Wang, N. Lacayo, P.O. Brown, Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS ONE* 7 (2012) e30733.
- [2] W.R. Jeck, J.A. Sorrentino, K. Wang, M.K. Slevin, C.E. Burd, J. Liu, et al., Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 19 (2013) 141–157.
- [3] S. Memczak, M. Jens, A. Elefsinioti, F. Torti, J. Krueger, A. Rybak, et al., Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495 (2013) 333–338.
- [4] T.B. Hansen, J. Kjems, C.K. Damgaard, Circular RNA and miR-7 in cancer. *Cancer Res.* 73 (2013) 5609–5612.
- [5] S. Qu, X. Yang, X. Li, J. Wang, Y. Gao, R. Shang, et al., Circular RNA: A new star of non-coding RNAs. *Cancer Lett.* 365 (2015) 141–148.
- [6] T.B. Hansen, T.I. Jensen, B.H. Clausen, J.B. Bramsen, B. Finsen, C.K. Damgaard, et al., Natural RNA circles function as efficient microRNA sponges. *Nature* 495 (2013) 384–388.
- [7] Z. Li, C. Huang, C. Bao, L. Chen, M. Lin, X. Wang, et al., Exon–intron circular RNAs regulate transcription in the nucleus. *Nat. Struct. Mol. Biol.* 22 (2015) 256–264.