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Differential MicroRNA Expression Profiles as Potential Biomarkers for Pancreatic Ductal Adenocarcinoma

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Abstract—Pancreatic ductal adenocarcinoma (PDAC) remains a clinical challenge due to its poor prognosis. Therefore, the early diagnosis of PDAC is extremely important for achieving a cure. MicroRNAs (miRNAs) could serve as a potential biomarker for the early detection and prognosis of PDAC. In this work we analyzed plasma samples from healthy persons and PDAC patients to assess differential miRNA expression profiles by next generation sequencing technology and bioinformatics analysis. In this way, 165 mature miRNAs were found to be significantly deregulated in the patient group, of which 75 and 90 mature miRNAs were up- and down-regulated compared with healthy individuals, respectively. Furthermore, 1029 novel miRNAs were identified. In conclusion, plasma miRNA expression profiles are different between healthy individuals and patients with PDAC. These data provide a possibility for use of miRNA as diagnostic and prognostic biomarkers of PDAC.

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Keywords: pancreatic ductal adenocarcinoma, plasma miRNA, expression profiling

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease with overall 5-year survival rate of less than 5% [1]. Due to its high metastasis rate and absence of early clinical symptoms, approximately 80% of patients are diagnosed at a late stage and lose the optimal surgical therapy time [2]. Therefore, novel approaches for early detection of PDAC are urgently required.

MicroRNAs (miRNAs), single-stranded RNAs with 19-25 nucleotides (nt) generated from endogenous hairpin-shaped transcripts, were discovered in 1993 [3]. MiRNAs do not encode protein but regulate approximately 50% of protein-coding genes [4]. These regulatory elements are first to be transcribed and then processed

Abbreviations: miRNA, microRNA; PDAC, pancreatic ductal adenocarcinoma.

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by Dicer and Drosha complexes into 21-23-nt mature miRNAs [5]. The miRNA is incorporated into the RNA-induced silencing complex (RISC) containing Dicer and other associated proteins [6]. RISC regulates posttranscriptional mRNA expression typically by binding to the 3′ untranslated region (3′-UTR) of the complementary mRNA sequence, preventing the recognition of cap by eIF4E and subsequent binding of translational factors [7]. Previous studies have demonstrated that miRNAs are not only highly correlated with tumorigenesis and progression [4, 8, 9], but also related to drug resistance, tumor metastasis, angiogenesis, cancer relapse, and poor clinical outcomes [10-13]. MiRNAs can be detected by a fully automated, high throughput procedure. MiRNAs produced from disrupted cells are transported in exosomes and released into blood [14, 15]. Being protected by exosomes, circulating miRNAs are stable biomarkers and can potentially be used for cancer detection, prognosis, and therapeutic evaluation [14, 16].

In PDAC, miRNAs have been reported to be responsible for apoptosis escape, proliferation, epithelial mesenchymal transition (EMT), metastasis, invasion, and drug resistance [17, 18]. Besides, it has been shown that they might be used as potential biomarkers for PDAC diagnosis [19]. Although some blood-sample based miRNA expression profiles of PDAC have been reported [20-23], a commonly used miRNAs panel for PDAC detection has not been determined yet.

In this study, we identified miRNA profiles of plasma samples of healthy individuals and PDAC patients by small RNA sequencing and found 165 mature miRNAs that are deregulated in PDAC.

MATERIALS AND METHODS

Patient samples. Five patients were recruited with the diagnosis of PDAC (P group) at the Department of General Surgery in the Second Affiliated Hospital of Zhengzhou University from October 2015 to March 2016. PDAC diagnosis was confirmed by histopathologic examination. Meanwhile, five healthy volunteers (H group) were randomly recruited from the Physical Examination Department or from the Department of Digestive Internal Medicine. The healthy individuals of the H group did not have diagnosis for any type of cancer or prior cancer history. The clinical-epidemiological characteristics of the participants are summarized in Table 1.

All blood samples were collected before surgical operation and gathered into polyethylene tubes that were pre-rinsed with EDTA. The samples were centrifuged at 2500 rpm for 5 min at 4°C. Plasma samples were collected by centrifugation. The plasma samples from each group were immediately collected and stored at –80°C.

RNA extraction and sequencing. Total RNA was extracted using a Norgen Plasma/Serum Circulating RNA Purification Mini Kit (Norgen, Canada) following the manufacturer's procedure. The quantity and purity of total RNA were analyzed by an Agilent Bioanalyzer 2100 and RNA 6000 Nano LabChip Kit (Agilent, USA). The cutoff for RNA sequencing is RIN (RNA Integrity Number) number >7.0. Approximately 0.2-0.7 μg of total RNA was used to prepare a small RNA library according to the manufacturer's protocol of TruSeq Small RNA Sample Prep Kits (Illumina, USA). Briefly, for small RNA library construction, the total RNA was size-fractionated on gel, and the 18-30-nt fraction was excised and purified. The gel-purified small RNAs were ligated to the 5′ RNA adapter and then the 3′ RNA adapter. The ligation products were purified, reverse transcribed, and amplified by RT-PCR. After the PCR products were purified, single-end sequencing (36 bp) was performed on an Illumina Hiseq2500 instrument at LC-BIO (Hangzhou, China) following the vendor's protocol.

Bioinformatics and data processing. In this study, the CAP-miRSeq algorithm was used for a comprehensive analysis of small RNA sequencing reads to identify, annotate, and quantify miRNAs according to the user guide with some modifications [24]. In brief, the pre-processing of sequencing reads was performed by FastQC v. 0.10.1 to clean low-quality sequences and to remove adapter sequences from sequencing reads. Clean reads of more than 17 nt in length were aligned against the human

Table 1. Clinical-epidemiological characteristics of participants

Group	Number	Age, years	Weight, kg	Sex	Clinical stage
H group	ZYFR1	47	67.7	male	–
	ZYFR2	77	63.5	male	–
	ZYFR3	63	59.7	female	–
	ZYFR4	52	57.2	female	–
	ZYFR5	77	56.5	female	–
P group	ZYFR11	77	62.7	male	IV
	ZYFR12	49	67.8	male	III
	ZYFR13	75	62.5	male	IV
	ZYFR14	70	56.5	female	IV
	ZYFR15	29	69.7	male	II

Genome (hg19) and miRBase 20 using Bowtie v. 0.12.7 [25]. Subsequently, identification and quantification of known and predicted potential miRNAs were finished using miRDeep v. 2.0.0.5 [26, 27]. The significance of differential expression of miRNAs was determined using edgeR between healthy volunteers (H group) and PDAC patients (P group) [28]. The small RNA expression profiling data used for this study is publicly accessible through GEO (GSE44853).

Statistical analysis. All results are expressed as mean ± SD. Differences between groups were evaluated by analysis of variance, and post hoc analysis was performed by the Tukey–Kramer test. p value less than 0.05 was considered statistically significant.

RESULTS

MiRNA expression profiles in blood plasma. To determine whether miRNAs could serve as potential biomarkers for PDAC diagnosis, five PDAC patients and five healthy volunteers were recruited (named as P group and H group, respectively). Blood samples were collected and the miRNAs from the plasma samples were sequenced by an Illumina Hiseq2500 instrument at LC-BIO. We found that a total of 130,162,923 and 163,059,214 reads are produced for the following analysis in P group (samples ZYFR11-15) and H group (samples ZYFR1-5), respectively. Among them, 581 of the known mature miRNAs with ⩾5× coverage are detected in the P group and 558 of those in the H group (Table 2).

After that, the miRNA gene expression profiles were normalized (Fig. 1, a and b). For this purpose, the unsupervised hierarchical clustering of miRNA expression data was performed and presented as a heatmap (Fig. 1c). The p value of comparison of differential expression between H and P groups was calculated and visualized as shown in the volcano plot (Fig. 1d).

Small RNA distribution by genomic annotation. When compared with the human genome annotation (hg19), the small RNAs distributed in 27 types of genomic loci, including the protein coding region, miRNA loci, lncRNA loci, etc. Among them, the top three small RNA mapping regions with the most proportions in the H group were protein-coding ones (73%), miRNA (17%), and lncRNA (5%) loci (Fig. 2a), while the P group consisted of miRNA (44%), lncRNA (38%), and protein-coding (13%) loci (Fig. 2b). Among the 27 genomic loci with small RNAs mapped, proportions of small RNAs from protein-coding (15%) and pseudogene loci (0.2%) in the H group were significantly higher than the ones in the P group (2 and 0.1%) with p value 0.0024 and 0.0419, respectively (Fig. 2, c and d). The data indicated that the miRNAs are significantly differential expressed between PDAC patients and healthy persons.

Differential expression of mature miRNAs and novel miRNA in P versus H groups. To assess potential diagnostic value of the identified small RNAs, differential analysis by edgeR was performed to determine the expression differences between the P and H groups. The analysis revealed that the expression of 165 miRNAs were significantly ($p < 0.05$) different between the P and H groups. From these, mature miRNAs were significantly up- (75) and down-regulated (90) in the P group rather than in the H group ($p < 0.05$; Table S1, see Supplement to this paper on the website of the journal (http://protein.bio.msu.ru/biokhimiya) and Springer site (Link.springer.com)).

In addition, a total of 279 novel miRNAs in the H group and 785 in the P group were found (Tables S2 and S3, see Supplement), of which 35 novel miRNAs were shared by the H and P groups.

DISCUSSION

As mentioned in the introductory part, early diagnosis is extremely important for PDAC patients. Plasma miRNAs have been reported as a type of reliable biomarkers for PDAC early detection due to its sensitivity, specificity, and stability. Although miRNA studies based

Table 2. General information of small RNA sequencing from plasma samples in the H and P groups

Group	Sample	Total reads	Precursor miRNA reads	Mature miRNA reads	Known miRNA with ⩾5× coverage
H group	ZYFR1	26,423,364	2058	1,376,892	637
	ZYFR2	32,198,124	2541	2,273,633	701
	ZYFR3	31,026,114	401	398,921	413
	ZYFR4	38,297,930	1671	328,423	345
	ZYFR5	35,113,682	4372	1,969,065	694
P group	ZYFR11	26,084,797	19,447	1,633,708	527
	ZYFR12	22,687,051	4655	572,342	291
	ZYFR13	30,007,205	13,576	13,892,602	811
	ZYFR14	20,235,930	8966	1,452,705	482
	ZYFR15	31,147,940	7756	15,118,396	795

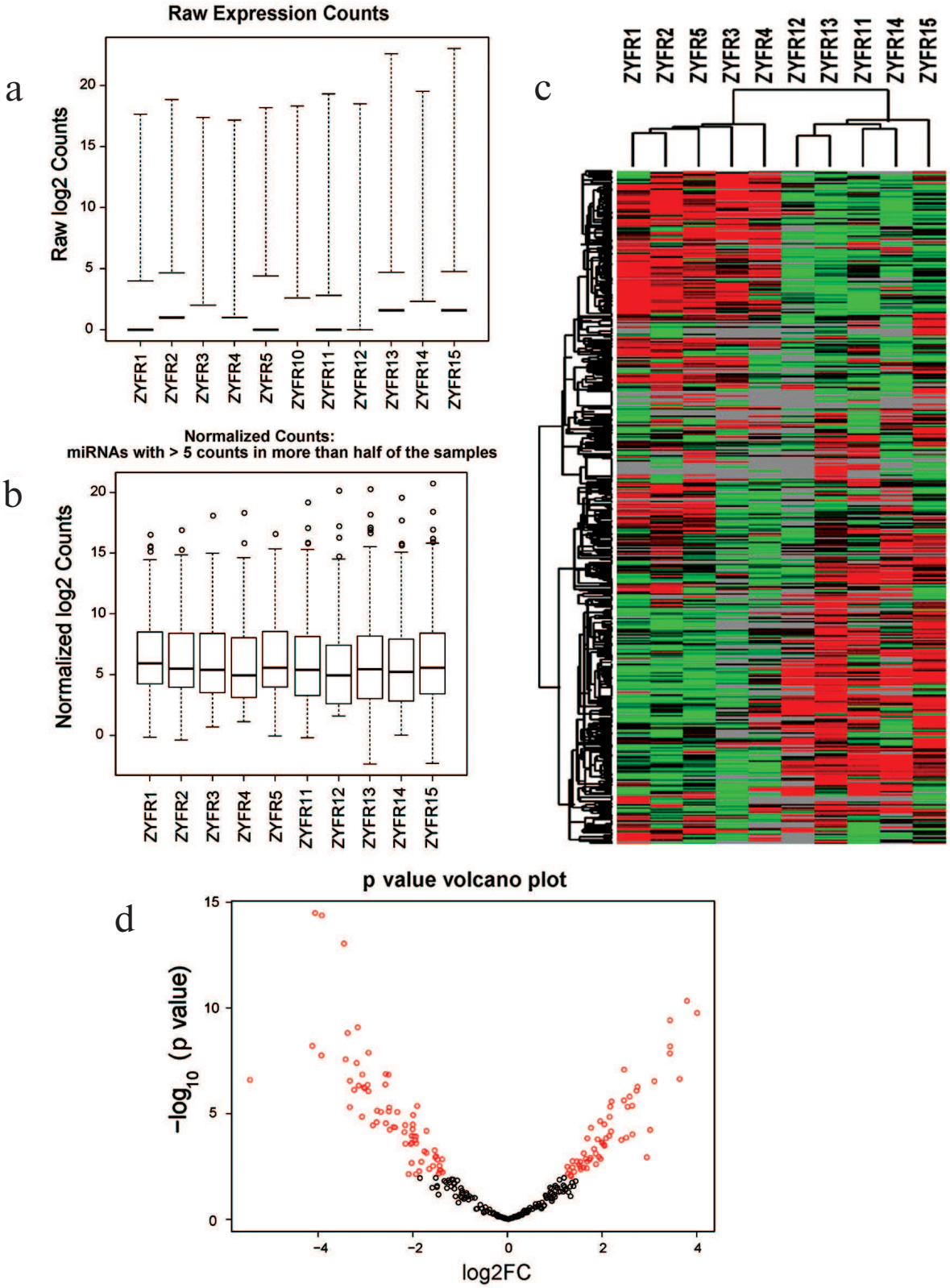

Fig. 1. Statistics and heatmap of sequencing data: a) raw expression counts of each sample; b) normalized counts of each sample; c) heatmap of miRNA expression in H and P groups; d) volcano plot.

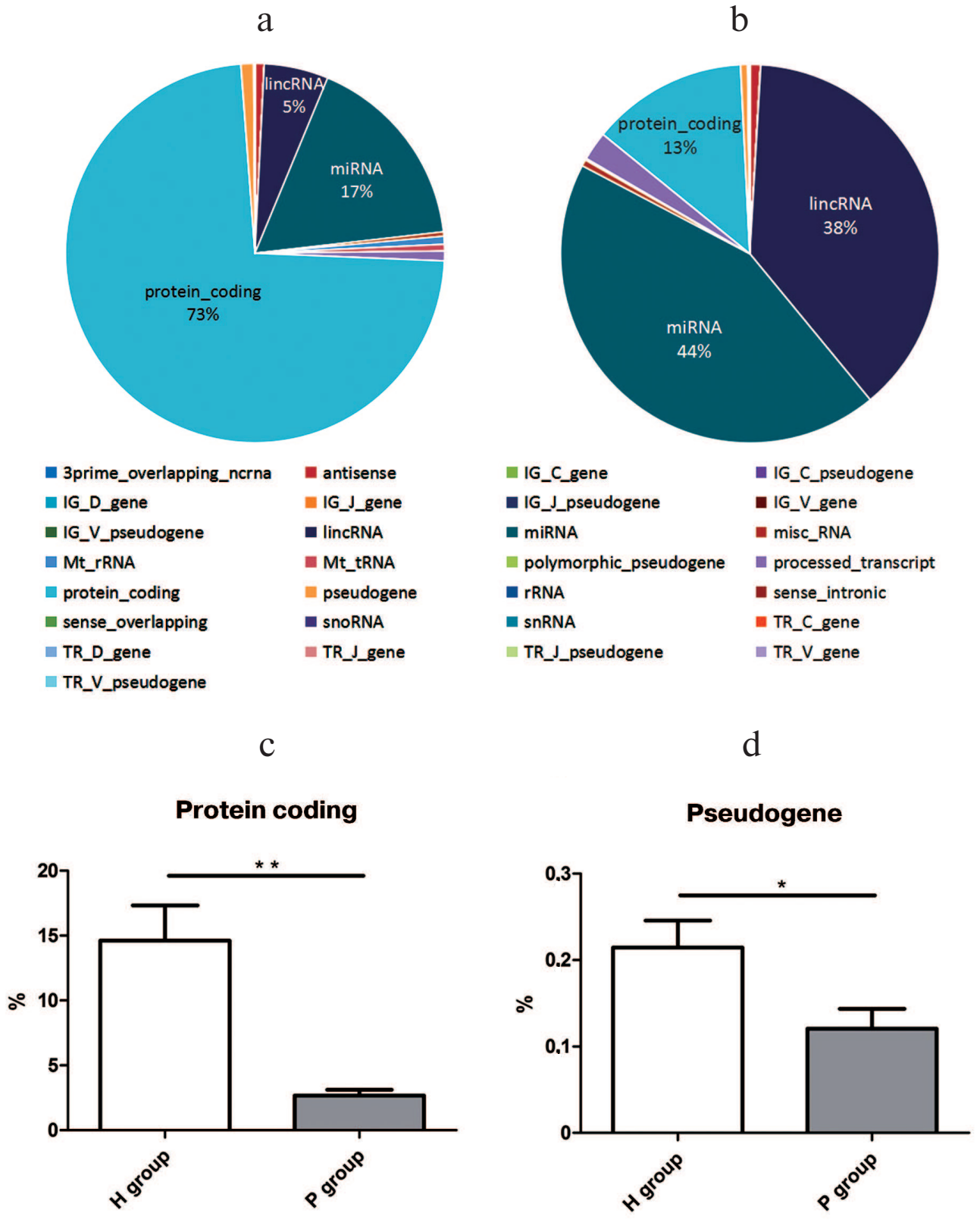

Fig. 2. Small RNA gene loci in the H (a) and P (b) groups; c) proportional difference of protein-coding gene loci between H and P groups, ** $p = 0.0024$; d) proportional difference of pseudogene gene loci between H and P groups, * $p = 0.0422$.

on blood samples have been performed for clinical utilization, there is no reproducible miRNA marker panel shared by all studies [19-23]. In our study, plasma miRNA profiles from healthy individuals (H group) and PDAC patients (P group) in a Chinese Han population were investigated with genome-wide small RNA expression profiling. A total of 165 mature miRNAs are significantly differentially expressed between the P group and H group, among which 75 are up-regulated and 90 are down-regulated in the P group rather than in H group, respectively. The top two significantly up-regulated miRNAs in the P group are hsa-miR-182-5p (logFold-Change = 4.00, p = 1.77E-10) and hsa-miR-4732-5p (logFold-Change = 3.78, p = 4.76E-11), while the two significantly down-regulated miRNAs in P group are hsa-miR-139-5p (logFold-Change = –4.07, p = 3.35E-15) and hsa-miR-23b-3p (logFold-Change = –3.93, p = 4.31E-15).

It has been reported that miR-182-5p (previous ID: miR-182, MiRBase) is overexpressed in PDAC cell lines and tumor tissues and promotes tumor cell proliferation and migration via direct targeting of the β-TrCP/β-catenin pathway [29]. In addition, the overexpression of miR-182-5p could depress FOXO3a (Forkhead box O3) and lead to the BIM/Bax-dependent mitochondrial apoptosis signaling pathway by directly inhibiting FOXO3a expression [30, 31]. Consistent with the above-mentioned studies, plasma miR-182-5p was also found in our study. In addition, Chen et al. found that circulating miR-182 in pancreatic cancer patients was significantly higher than that in healthy controls, which may become useful as a non-invasive tumor marker for diagnosis and prognosis of PDAC [32] after careful further validation in a larger cohort.

MiR-4732-5p was reported to be expressed in breast tissue [33]. MiR-4732-5p may directly bind the 5′-UTR region of Wrap53 mRNA in breast cancer and prohibit the binding of p53 mRNA [34], indicating a possible linkage between miR-4732-5p and tumor suppressive protein p53. As recently shown, p53 plays an important role in the late-stage progression of PDAC, indicating that miR-4732-5p may also play an important role in PDAC genesis [35].

The functions of miR-139-5p (previous ID: miR-139, MiRBase) in cancer are still controversial between different studies. Li et al. reported miR-139 is significantly overexpressed in endothelial cells of human pancreatic tumor and may be positively correlated with migration and angiogenesis [36] and could also promote the progression of colorectal cancer and oral tongue squamous cell carcinoma [37, 38]. However, some studies proposed that miR-139 is a tumor suppresser and is significantly down-regulated in non-small cell lung cancer (NSCLC), hepatocellular carcinoma, basal cell carcinoma, and adult acute myeloid leukemia [39-43].

The second most down-regulated miRNAs in the P group is hsa-miR-23b-3p (previous ID miR-23b, MiRBase). However, the function of this miRNA is still to be determined. Previous studies showed that miR-23b-3p was down-regulated in the tissue of NSCLC patients [44], but other researchers reported that miR-23b-3p was an up-regulated factor in circulating exosomes of NSCLC patients [45, 46]. Wang et al. found that miR-23b inhibits autophagy in human pancreatic cancer cell line BxPC3 and Panc-1 by direct targeting of ATG12 (autophagy-related protein 12), and further sensitized pancreatic cancer cells to radiation treatment [47]. An inversely correlated expression pattern of miR-23b and ATG12 was found in human PDAC. In our study, the expression level of miR-23b-3p was significantly decreased in the plasma of PDAC patients, indicating its potential diagnostic role in PDAC detection.

In conclusion, a genome wide small RNA expression profiling was performed for plasma samples from PDAC patients and healthy volunteers, a total of 165 mature miRNAs were differentially expressed between PDAC and healthy groups, and these miRNAs could be potential early and non-invasive diagnostic biomarkers for PDAC when further validated in a large cohort.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

This study was approved (Approval number LL201508001) by the Ethics Committee of the Second

Affiliated Hospital of Zhengzhou University, Zhengzhou, China. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

REFERENCES

1. Raimondi, S., Maisonneuve, P., and Lowenfels, A. B. (2009) Epidemiology of pancreatic cancer: an overview, *Nat. Rev. Gastroenterol. Hepatol.*, **6**, 699-708; doi: 10.1038/nrgastro.2009.177.
2. Hartwig, W., Werner, J., Jager, D., Debus, J., and Buchler, M. W. (2013) Improvement of surgical results for pancreatic cancer, *Lancet Oncol.*, **14**, 476-485; doi: 10.1016/S1470-2045(13)70172-4.
3. Lee, R. C., Feinbaum, R. L., and Ambros, V. (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*, *Cell*, **75**, 843-854; doi: 10.1016/0092-8674(93)90529-Y.
4. Krol, J., Loedige, I., and Filipowicz, W. (2010) The widespread regulation of microRNA biogenesis, function and decay, *Nat. Rev. Genet.*, **11**, 597-610; doi: 10.1038/nrg2843.
5. Van Kouwenhove, M., Kedde, M., and Agami, R. (2011) MicroRNA regulation by RNA-binding proteins and its implications for cancer, *Nat. Rev. Cancer*, **11**, 644-656; doi: 10.1038/nrc3107.
6. Rana, T. M. (2007) Illuminating the silence: understanding the structure and function of small RNAs, *Nat. Rev. Mol. Cell Biol.*, **8**, 23-36; doi: 10.1038/nrm2085.
7. Stefani, G., and Slack, F. J. (2008) Small non-coding RNAs in animal development, *Nat. Rev. Mol. Cell Biol.*, **9**, 219-230; doi: 10.1038/nrm2347.
8. Suzuki, H. I., Arase, M., Matsuyama, H., Choi, Y. L., Ueno, T., Mano, H., Sugimoto, K., and Miyazono, K. (2011) MCPIP1 ribonuclease antagonizes dicer and terminates microRNA biogenesis through precursor microRNA degradation, *Mol. Cell*, **44**, 424-436; doi: 10.1016/j.molcel.2011.09.012.
9. Esquela-Kerscher, A., and Slack, F. J. (2006) Oncomirs – microRNAs with a role in cancer, *Nat. Rev. Cancer*, **6**, 259-269; doi: 10.1038/nrc1840.
10. An, X., Sarmiento, C., Tan, T., and Zhu, H. (2017) Regulation of multidrug resistance by microRNAs in anticancer therapy, *Acta Pharm. Sin. B*, **7**, 38-51; doi: 10.1016/j.apsb.2016.09.002.
11. Jafri, M. A., Al-Qahtani, M. H., and Shay, J. W. (2017) Role of miRNAs in human cancer metastasis: implications for therapeutic intervention, *Semin. Cancer Biol.*, **44**, 117-131; doi: 10.1016/j.semcancer.2017.02.004.
12. Fattore, L., Costantini, S., Malpicci, D., Ruggiero, C. F., Ascierto, P. A., Croce, C. M., Mancini, R., and Ciliberto, G. (2017) MicroRNAs in melanoma development and resistance to target therapy, *Oncotarget*, **8**, 22262-22278; doi: 10.18632/oncotarget.14763.
13. Fabbri, M., Paone, A., Calore, F., Galli, R., Gaudio, E., Santhanam, R., Lovat, F., Fadda, P., Mao, C., Nuovo, G. J., Zanesi, N., Crawford, M., Ozer, G. H., Wernicke, D., Alder, H., Caligiuri, M. A., Nana-Sinkam, P., Perrotti, D., and Croce, C. M. (2012) MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response, *Proc. Natl. Acad. Sci. USA*, **109**, 2110-2116; doi: 10.1073/pnas.1209414109.
14. Cortez, M. A., Bueso-Ramos, C., Ferdin, J., Lopez-Berestein, G., Sood, A. K., and Calin, G. A. (2011) MicroRNAs in body fluids – the mix of hormones and biomarkers, *Nat. Rev. Clin. Oncol.*, **8**, 467-477; doi: 10.1038/nrclinonc.2011.76.
15. Stroun, M., Lyautey, J., Lederrey, C., Olson-Sand, A., and Anker, P. (2001) About the possible origin and mechanism of circulating DNA apoptosis and active DNA release, *Clin. Chim. Acta*, **313**, 139-142; doi: 10.1016/S0009-8981(01)00665-9.
16. Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., Peterson, A., Noteboom, J., O'Briant, K. C., Allen, A., Lin, D. W., Urban, N., Drescher, C. W., Knudsen, B. S., Stirewalt, D. L., Gentleman, R., Vessella, R. L., Nelson, P. S., Martin, D. B., and Tewari, M. (2008) Circulating microRNAs as stable blood-based markers for cancer detection, *Proc. Natl. Acad. Sci. USA*, **105**, 10513-10518; doi: 10.1073/pnas.0804549105.
17. Karius, T., Schnekenburger, M., Dicato, M., and Diederich, M. (2012) MicroRNAs in cancer management and their modulation by dietary agents, *Biochem. Pharmacol.*, **83**, 1591-1601; doi: 10.1016/j.bcp.2012.02.004.
18. Brunetti, O., Russo, A., Scarpa, A., Santini, D., Reni, M., Bittoni, A., Azzariti, A., Aprile, G., Delcuratolo, S., Signorile, M., Gnoni, A., Palermo, L., Lorusso, V., Cascinu, S., and Silvestris, N. (2015) MicroRNA in pancreatic adenocarcinoma: predictive/prognostic biomarkers or therapeutic targets? *Oncotarget*, **6**, 23323-23341; doi: 10.18632/oncotarget.4492.
19. Liu, R., Chen, X., Du, Y., Yao, W., Shen, L., Wang, C., Hu, Z., Zhuang, R., Ning, G., Zhang, C., Yuan, Y., Li, Z., Zen, K., Ba, Y., and Zhang, C. Y. (2012) Serum microRNA expression profile as a biomarker in the diagnosis and prognosis of pancreatic cancer, *Clin. Chem.*, **58**, 610-618; doi: 10.1373/clinchem.2011.172767.
20. Cote, G. A., Gore, A. J., McElyea, S. D., Heathers, L. E., Xu, H., Sherman, S., and Korc, M. (2014) A pilot study to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select miRNA in plasma and bile, *Am. J. Gastroenterol.*, **109**, 1942-1952; doi: 10.1038/ajg.2014.331.
21. Wang, J., Chen, J., Chang, P., LeBlanc, A., Li, D., Abbruzzesse, J. L., Frazier, M. L., Killary, A. M., and Sen, S. (2009) MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease, *Cancer Prev. Res. (Phila)*, **2**, 807-813; doi: 10.1158/1940-6207.CAPR-09-0094.
22. Liu, J., Gao, J., Du, Y., Li, Z., Ren, Y., Gu, J., Wang, X., Gong, Y., Wang, W., and Kong, X. (2012) Combination of plasma microRNAs with serum CA19-9 for early detection of pancreatic cancer, *Int. J. Cancer*, **131**, 683-691; doi: 10.1002/ijc.26422.
23. Schultz, N. A., Dehlendorff, C., Jensen, B. V., Bjerregaard, J. K., Nielsen, K. R., Bojesen, S. E., Calatayud, D., Nielsen, S. E., Yilmaz, M., Hollander, N. H., Andersen, K.

K., and Johansen, J. S. (2014) MicroRNA biomarkers in whole blood for detection of pancreatic cancer, *JAMA*, **311**, 392-404; doi: 10.1001/jama.2013.284664.
24. Sun, Z., Evans, J., Bhagwate, A., Middha, S., Bockol, M., Yan, H., and Kocher, J. P. (2014) CAP-miRSeq: a comprehensive analysis pipeline for microRNA sequencing data, *BMC Genomics*, **15**, 1-10; doi: 10.1186/1471-2164-15-423.
25. Langmead, B., Trapnell, C., Pop, M., and Salzberg, S. L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome, *Genome Biol.*, **10**, R25; doi: 10.1186/gb-2009-10-3-r25.
26. An, J., Lai, J., Lehman, M. L., and Nelson, C. C. (2013) miRDeep*: an integrated application tool for miRNA identification from RNA sequencing data, *Nucleic Acids Res.*, **41**, 727-737; doi: 10.1093/nar/gks1187.
27. Friedlander, M. R., Mackowiak, S. D., Li, N., Chen, W., and Rajewsky, N. (2012) miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades, *Nucleic Acids Res.*, **40**, 37-52; doi: 10.1093/nar/gkr688.
28. Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010) edgeR: a bioconductor package for differential expression analysis of digital gene expression data, *Bioinformatics*, **26**, 139-140; doi: 10.1093/bioinformatics/btp616.
29. Chen, X., Su, Z., Wang, S., and Xu, H. (2016) Clinical and prognostic significance of Arl4c expression in colorectal cancer, *Cancer Biomark*, **16**, 253-257; doi: 10.3233/cbm-150562.
30. Li, Y., Li, A., Wu, J., He, Y., Yu, H., Chai, R., and Li, H. (2016) MiR-182-5p protects inner ear hair cells from cisplatin-induced apoptosis by inhibiting FOXO3a, *Cell Death Dis.*, **7**, e2362; doi: 10.1038/cddis.2016.246.
31. Czabotar, P. E., Colman, P. M., and Huang, D. C. (2009) Bax activation by Bim? *Cell Death Differ.*, **16**, 1187-1191; doi: 10.1038/cdd.2009.83.
32. Chen, Q., Yang, L., Xiao, Y., Zhu, J., and Li, Z. (2014) Circulating microRNA-182 in plasma and its potential diagnostic and prognostic value for pancreatic cancer, *Med. Oncol.*, **31**, 225; doi: 10.1007/s12032-014-0225-z.
33. Persson, H., Kvist, A., Rego, N., Staaf, J., Vallon-Christersson, J., Luts, L., Loman, N., Jonsson, G., Naya, H., Hoglund, M., Borg, A., and Rovira, C. (2011) Identification of new microRNAs in paired normal and tumor breast tissue suggests a dual role for the *ERBB2/Her2* gene, *Cancer Res.*, **71**, 78-86; doi: 10.1158/0008-5472.CAN-10-1869.
34. Pouladi, N., Kouhsari, S. M., Feizi, M. H., Gavgani, R. R., and Azarfam, P. (2013) Overlapping region of p53/wrap53 transcripts: mutational analysis and sequence similarity with microRNA-4732-5p, *Asian Pac. J. Cancer Prev.*, **14**, 3503-3507; doi: 10.7314/APJCP.2013.14.6.3503
35. Maitra, A., Adsay, N. V., Argani, P., Iacobuzio-Donahue, C., De Marzo, A., Cameron, J. L., Yeo, C. J., and Hruban, R. H. (2003) Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray, *Mod. Pathol.*, **16**, 902-912; doi: 10.1097/01.MP.0000086072.56290.FB.
36. Li, L., Li, B., Chen, D., Liu, L., Huang, C., Lu, Z., Lun, L., and Wan, X. (2015) miR-139 and miR-200c regulate pancreatic cancer endothelial cell migration and angiogenesis, *Oncol. Rep.*, **34**, 51-58; doi: 10.3892/or.2015.3945.
37. Miyoshi, J., Toden, S., Yoshida, K., Toiyama, Y., Alberts, S. R., Kusunoki, M., Sinicrope, F. A., and Goel, A. (2017) MiR-139-5p as a novel serum biomarker for recurrence and metastasis in colorectal cancer, *Sci. Rep.*, **7**, 43393; doi: 10.1038/srep43393.
38. Chen, Z., Yu, T., Cabay, R. J., Jin, Y., Mahjabeen, I., Luan, X., Huang, L., Dai, Y., and Zhou, X. (2017) miR-486-3p, miR-139-5p, and miR-21 as biomarkers for the detection of oral tongue squamous cell carcinoma, *Biomark. Cancer*, **9**, 1-8; doi: 10.4137/BIC.S40981.
39. Sun, C., Sang, M., Li, S., Sun, X., Yang, C., Xi, Y., Wang, L., Zhang, F., Bi, Y., Fu, Y., and Li, D. (2015) Hsa-miR-139-5p inhibits proliferation and causes apoptosis associated with down-regulation of *c-Met*, *Oncotarget*, **6**, 39756-39792; doi: 10.18632/oncotarget.5476.
40. Mo, Y., Lu, Y., Wang, P., Huang, S., He, L., Li, D., Li, F., Huang, J., Lin, X., Li, X., Che, S., and Chen, Q. (2017) Long non-coding RNA XIST promotes cell growth by regulating miR-139-5p/PDK1/AKT axis in hepatocellular carcinoma, *Tumour Biol.*, **39**, 1010428317690999; doi: 10.1177/1010428317690999.
41. Sand, M., Skrygan, M., Sand, D., Georgas, D., Hahn, S. A., Gambichler, T., Altmeyer, P., and Bechara, F. G. (2012) Expression of microRNAs in basal cell carcinoma, *Br. J. Dermatol.*, **167**, 847-855; doi: 10.1111/j.1365-2133.2012.11022.x.
42. Xu, K., Shen, K., Liang, X., Li, Y., Nagao, N., Li, J., Liu, J., and Yin, P. (2016) MiR-139-5p reverses CD44+/CD133+-associated multidrug resistance by downregulating NOTCH1 in colorectal carcinoma cells, *Oncotarget*, **7**, 75118-75129; doi: 10.18632/oncotarget.12611.
43. Chen, H., Xu, H., Meng, Y. G., Zhang, Y., Chen, J. Y., and Wei, X. N. (2016) miR-139-5p regulates proliferation, apoptosis, and cell cycle of uterine leiomyoma cells by targeting TPD52, *Onco. Targets Ther.*, **9**, 6151-6160; doi: 10.2147/OTT.S108890.
44. Janikova, M., Zizkova, V., Skarda, J., Kharaishvili, G., Radova, L., and Kolar, Z. (2016) Prognostic significance of miR-23b in combination with P-gp, MRP and LRP/MVP expression in non-small cell lung cancer, *Neoplasma*, **63**, 576-587; doi: 10.4149/neo_2016_411.
45. Cinegaglia, N. C., Andrade, S. C., Tokar, T., Pinheiro, M., Severino, F. E., Oliveira, R. A., Hasimoto, E. N., Cataneo, D. C., Cataneo, A. J., Defaveri, J., Souza, C. P., Marques, M. M., Carvalho, R. F., Coutinho, L. L., Gross, J. L., Rogatto, S. R., Lam, W. L., Jurisica, I., and Reis, P. P. (2016) Integrative transcriptome analysis identifies deregulated microRNA-transcription factor networks in lung adenocarcinoma, *Oncotarget*, **7**, 28920-28934; doi: 10.18632/oncotarget.8713.
46. Begum, S., Hayashi, M., Ogawa, T., Jabboure, F. J., Brait, M., Izumchenko, E., Tabak, S., Ahrendt, S. A., Westra, W. H., Koch, W., Sidransky, D., and Hoque, M. O. (2015) An integrated genome-wide approach to discover deregulated microRNAs in non-small cell lung cancer: clinical significance of miR-23b-3p deregulation, *Sci. Rep.*, **5**, 13236; doi: 10.1038/srep13236.
47. Wang, P., Zhang, J., Zhang, L., Zhu, Z., Fan, J., Chen, L., Zhuang, L., Luo, J., Chen, H., Liu, L., Chen, Z., and Meng, Z. (2013) MicroRNA 23b regulates autophagy associated with radioresistance of pancreatic cancer cells, *Gastroenterology*, **145**, 1133-1143; doi: 10.1053/j.gastro.2013.07.048.