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Profiling of 95 MicroRNAs in Pancreatic Cancer Cell Lines and Surgical Specimens by Real Time PCR Analysis

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Abstract

Background—MicroRNAs (miRNAs) are involved in cancer pathogenesis, apoptosis and cell growth, thereby functioning as either tumor suppressors or oncogenes. However, expression alterations and roles of these miRNAs in pancreatic cancer are largely unknown. We hypothesize that pancreatic cancer may have a unique miRNA profile, which may play a critical role in pancreatic cancer development, progression, diagnosis and prognosis.

Methods—Differential expression of 95 miRNAs was analyzed by real time RT-PCR using the QuantiMir System. All 95 miRNAs chosen for the array are based on their potential functions related to cancer biology, cell development and apoptosis. The expression of miRNAs for pancreatic cancer tissue samples or cancer cell lines was normalized to U6 RNA and compared with those in the relatively normal pancreatic tissues or normal human pancreatic ductal epithelial (HPDE) cells. Human pancreatic tissue with chronic pancreatitis was also included for analysis.

Results—In the initial analysis, the expression of most 95 miRNAs was substantially changed in pancreatic cancer tissues (n=5) and cell lines (n=3) compared with relatively normal pancreatic tissues and HPDE cells. However, each pancreatic cancer tissue or cell type had a substantially different profiling pattern with other cases or cell types as well as chronic pancreatitis tissue, indicating the individual diversity of pancreatic cancer. Further analysis was performed on 10 pancreatic cancer cell lines and 17 pairs of pancreatic cancer/normal tissues. Eight miRNAs were significantly upregulated in most pancreatic cancer tissues and cell lines, including miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b and miR-95. The incidence of upregulation of these eight genes between normal controls and tumor cells or tissues was ranging from 70% to 100%. The magnitude of increase of these miRNAs in pancreatic cancer samples was ranging from 3 to 2018 fold of normal controls.

Conclusions—Pancreatic cancer tissues or cell lines have a unique miRNA profiling pattern at the individual basis as compared with relatively normal pancreatic tissues or cells as well as pancreatitis tissue. Upregulation of eight miRNAs occurs in the most of pancreatic cancer tissues and cell types. These miRNAs may share common pathways in pancreatic cancer pathogenesis. This study may provide useful information for further investigations of functional roles of miRNAs in pancreatic cancer development, progression, diagnosis and prognosis.

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Keywords

microRNA; pancreatic cancer; real time PCR

Introduction

Non-coding RNAs are a class of RNAs that do not encode proteins, while possess regulatory functions in gene expression. Non-coding RNAs have drawn a great attention in recent years since. The discovery of small interfering RNAs (siRNAs) and microRNAs (miRNAs) has substantial impact on gene regulation. miRNAs are a novel class of short (typically 18–23 nucleotides) single stranded RNAs, which are identified as a new family of regulatory molecules involved in cancer development [1–4]. miRNAs cause posttranscriptional gene silencing by either inducing target mRNA degradation or by repressing the translation process upon binding to the 3'-untranslational region (UTR) of their target mRNAs [5]. Mature miRNAs are excised from stem-loop precursors, which are transcribed as part of longer primary transcripts. These primary miRNAs appear to be first processed by the RNase Droscha in the nucleus, after which the precursor miRNAs are exported to the cytoplasm where the RNase Dicer further processes them.

Regulation of miRNA expression has been demonstrated to play a key role in development, cell growth and differentiation processes in a variety of eukaryotic organisms [6,7]. Usually, miRNAs are dysregulated in cancers. Some miRNAs are temporally over-expressed in the early stage of cancer progression and they act like oncogenes by promoting proliferation and/or repressing apoptosis. Conversely, some miRNAs with tumor-repressor functions are downregulated in cancers. miRNA expression profiles may be unique in different tumors and from different origins. Both normal and malignant cancer tissues may have specific miRNA expression signatures and show differential expression across tumor types. Several studies have demonstrated altered miRNA expression profile in various hematological and solid tumor entities [1,2]. For example, a unique expression signature of only 13 miRNAs differentiated more aggressive form of chronic lymphocytic leukemia from the benign one and was found to be associated with the cancer progression [8]. Expression alterations of specific miRNAs appear to be correlated with clinically malignancy or metastatic phenotypes, and predict the clinical outcome even better than the mRNA expression data [9–11].

Pancreatic cancer is the fourth leading cause of cancer death in the United States [12]. There was 37,170 new cases diagnosed, and approximately 33,370 deaths due to pancreatic adenocarcinoma in 2007 in the United States [13]. Although surgical resection provides a potential cure, about 70% patients still develop early recurrence within 6–12 months following surgery. Due to lack for reliable early detection markers, pancreatic tumors are usually in the advanced stage upon diagnosis. Moreover, pancreatic tumors have a predilection for early vascular dissemination and metastasis to distant organs. Clearly, the discovery of miRNA alterations in pancreatic cancer not only helps us to better understand the biology of this disease, but more importantly provides new prognostic and diagnostic strategies. Due to the high stability of miRNAs even in poorly preserved specimens, they are expected to be a valuable tool in clinical research and biomarkers discovery.

By Northern blotting analysis, several studies have shown that particular miRNAs were altered in pancreatic cancer tumor tissues [14–20]. However, these data are incomplete for many miRNAs or not consistent among studies due to limitations of methodologies and/or different conditions and sample sizes of cancer tissues and cell lines. In the current study, we used real time quantitative PCR, a more reliable detection method, to detect the expression levels of 95 cancer-related miRNAs in well controlled pancreatic cancer specimens and cell lines as well

as pancreatitis tissues. This study may discover a unique miRNA profiling pattern for pancreatic cancer and identify important molecular targets for further functional investigations and for the developments of new diagnostic tools and therapeutic strategies.

Materials and methods

Cell cultures and tissue collections

Human pancreatic cancer cell lines, Panc-1, MIA PaCa-2, BxPC-3, Hs766T, ASPC-1, Capan-1, Capan-2, Panc3.27, HPAF-II, and PL45, were purchased from the American Type Culture Collection (ATCC, Rockville, MD). The human pancreatic ductal epithelium (HPDE) cells were provided as a generous gift from Dr. Ming-Sound Tsao [21,22]. All cells were cultured as previously described [23–26]. Human pancreatic adenocarcinoma specimens and their adjacent normal pancreatic tissues (17 pairs) and one pancreatic tissue sample with chronic pancreatitis were collected from patients who underwent surgery according to an approved human protocol at the Baylor College of Medicine (Houston, TX).

miRNA extraction and reverse-transcription

Total miRNAs of tissues and cultured cells were extracted and purified using mirVana miRNA Isolation kit (Applied Biosystems/Ambion, Austin, TX) following the manufacturer's instructions. Five μ l of RNA was directly converted to cDNA with the QuantiMir™ RT System (SBI System Biosciences, Mountain View, CA).

Real time RT PCR

Differential expression of 95 miRNAs was analyzed by RT-PCR using the QuantiMir System (SBI System Biosciences). All 95 miRNAs chosen for the array are based on their potential roles in cancer, cell development and apoptosis. The array plate also included the U6 transcript as a normalization signal. The miRNA sequences and primer sequences used in RT-PCR were listed in Table 1. cDNAs from different cell lines and tissue samples were mixed with SYBR® Green Mastermix (Bio-Rad Laboratories, Hercules, CA) plus the universal reverse primer. Specific primers (1 μ l) were added each well of the qPCR plate. Expression levels of each mature miRNA were evaluated using comparative threshold cycle (Ct) method as normalized to that of U6 ($2^{-\Delta Ct}$). The fold change of each miRNA was calculated from the expression levels between tumor tissues/cells and normal tissues/cells.

Statistical analysis

The expressions of 8 miRNAs in cancer tissues or cells and normal tissues or cells were compared with paired Student's t-test. Data are presented as means \pm standard deviation (SD). A p value less than 0.05 was considered statistically significant.

Results

The expression of 95 miRNAs in chronic pancreatitis, pancreatic cancer cell lines and surgical specimens

Initially, the expression of 95 miRNAs in 1 pancreatitis tissue, 5 pancreatic cancer tissues and their adjacent benign tissues, 3 human pancreatic cancer cell lines (MIA PaCa-2, Panc-1 and BxPC-3) and HPDE cells was determined by real-time PCR. After normalization to the control U6 expression, the differential expression of miRNAs of pancreatitis tissue compared with normal pancreatic tissues, pancreatic cancer tissues compared with normal pancreatic tissues, and pancreatic cancer cell lines compared with HPDE cells was determined and shown in Fig. 1 and Table 2. Substantial differences of the expression profile of 95 miRNAs were observed between cancer and normal tissues or between cancer cell lines and normal HPED cells at the

individual basis, indicating potential roles of miRNAs in the cancer formation. These differences indicate the individual characteristics and variability of each case compared other cases. The relative expression values for these mature miRNAs spanned 6-logs (from 0.01 to 10000). A number of miRNAs were increased in the most of pancreatic cancer tissues and cell types, but not in normal tissues and cells as well as the pancreatitis sample.

Validation of eight over-expressed miRNAs in more pancreatic cancer cell lines and surgical specimens

From 95 miRNAs, 8 miRNAs (miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b and miR-95) were identified to have high expression levels more than 3.3-fold both in pancreatic cancer tissue samples and cell lines compared with that in normal pancreatic tissues and HPED cells. The expression of these miRNAs was further analyzed in more samples of pancreatic cancer and normal pancreatic tissue pairs (n=17) as well as more pancreatic cancer cell lines (n=10) by real time PCR. The incidence of expression increase and average fold increase of 8 miRNAs were shown in Fig. 2 and Table 3. Compared with normal HPDE cells, the incidence of 10 pancreatic cancer cell lines exhibited elevated levels of miR-196a (100%), miR-190 (100%), miR-186(90%), miR-221(100%), miR-222 (100%), miR-200b (70%), miR-15b (90%) and miR-95 (90%) and the increase levels ranged from 3.3 to 79 fold ($P < 0.01$, n=10, Fig. 2A). For the pancreatic cancer tissues compared with normal pancreatic tissues, the expression increases (incidence and fold increase) of miR-196a (82% and 190), miR-190 (88% and 21), miR-186 (94% and 4.5), miR-221 (88% and 32), miR-222 (88% and 32), miR-200b (76% and 43), miR-15b (82% and 2018) and miR-95 (71% and 468) were also observed ($P < 0.01$, n=17, Fig. 2B). These data indicate that these miRNAs may share common pathways in the pancreatic cancer pathogenesis.

Discussion

In the current study, a unique 95 miRNA expression profile was observed in human pancreatic cancer tissues and cell lines, and eight miRNAs (miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b and miR-95) were significantly increased in the most of pancreatic cancer tissues and cell lines compared with normal pancreatic tissues and cells. Many of these miRNAs have not been reported in pancreatic cancer. This study provides new opportunities for studying novel molecular pathways of pancreatic cancer pathogenesis and for developing new strategies for pancreatic cancer diagnosis and treatment.

The mechanism of action of a specific miRNA is usually involved in its nucleotide complementary pairing to the 3' UTR of its specific targeting mRNAs, primarily functioning as a negative regulator by repressing target mRNA translation. miRNAs may directly regulate tissue or organ development and cell differentiation as well as maintain normal functions of many organ systems [27]. The alterations in miRNA expression may play an important role in many diseases including pancreatic cancer formation. Using the QuantiMir™ RT Kit, we tagged and converted mature miRNAs into detectable and quantifiable cDNAs. We used a highly sensitive real time PCR analysis to profile 95 cancer-related miRNAs. This method is more reliable and accurate for detection of miRNA expression and has much less technical noise, but greater reproducibility than traditional cDNA microarray or northern blot analysis. All 95 miRNAs chosen for the array have functional implications with regard to their potential roles in cancer, cell development and apoptosis. Our expression profiling data indicate a large number of miRNAs that are aberrantly expressed in pancreatic cancer tissues and cell lines compared with normal pancreatic tissues and cells. From these profiling data, we observed a diversity nature of miRNA expression among individual pancreatic cancer tissues or cells, which may support the concept of personalized medicine in care of these patients. However, we also observed the expression pattern of many miRNAs was reserved in the most pancreatic

cancer tissues and cell lines studied in the current study. For example, 8 miRNAs (miR-196a, miR-190, miR-221, miR-222, miR-200b, miR-15b and miR-95) were consistently increased in the majority of pancreatic cancer tissues and cell lines. These data indicate that pancreatic cancer may share some common pathways for cancer pathogenesis by regulation of miRNAs. Many of these miRNAs have not been reported before in pancreatic cancer and their biological functions are largely unknown in pancreatic cancer pathogenesis.

Bloomston *et al.* reported that the high expression of miR-196a-2 was found to predict poor survival in pancreatic cancer patients [16]. miR-196a involves organ development by negatively regulating Hoxb8 [28]. miR-190 was found to be upregulated in human hepatocellular carcinomas [7]. The miR-200 family has been shown to regulate epithelial to mesenchymal transition (EMT) by targeting ZEB1 and SIP1. However, miR-200b was markedly downregulated in cells that had undergone EMT in response to transforming growth factor (TGF)-beta or to ectopic expression of the protein tyrosine phosphatase Pez [29]. Over-expression of miR-15b sensitized human gastric cancer cells to anticancer drugs by targeting BCL2 [30]. Inhibition of miR-95 decreased cell growth in HeLa cells [31]. miR-221 was reported to be overexpressed in glioblastoma [32] and in thyroid cancer [33]. miR-221 and miR-222 are clustered on the X chromosome, and both of them are predicted to regulate cell cycle by targeting on kit [33] and p27Kip1 [8]. Our data showed that miR-222 was increased in pancreatic cancers at the level similar to miR-221. Based on the miRNA profiling and their functional studies, miRNA/RNAi-based therapeutics could be attractive strategies for pancreatic cancer treatment.

In summary, pancreatic cancer may have a unique miRNA expression pattern at each individual basis. However, common pathways for pancreatic cancer pathogenesis may exist. Our study suggests that the expression of 8 miRNAs (miR-196a, miR-190, miR-221, miR-222, miR-200b, miR-15b and miR-95) was significantly increased in the majority of pancreatic cancer tissues and cell lines. Further investigations are required for determination of their molecular functions and mechanisms as well as characterization of these miRNAs as prognostic and/or diagnostic markers in pancreatic cancer. Since miRNAs may regulate multiple oncogenic pathways, they may serve as potential targets for cancer therapy. For examples, antagomirs and chemically modified antisense nucleotides for miRNAs can be used to silence specific endogenous miRNA *in vivo* [34]. This may provide a novel strategy to treat pancreatic cancer.

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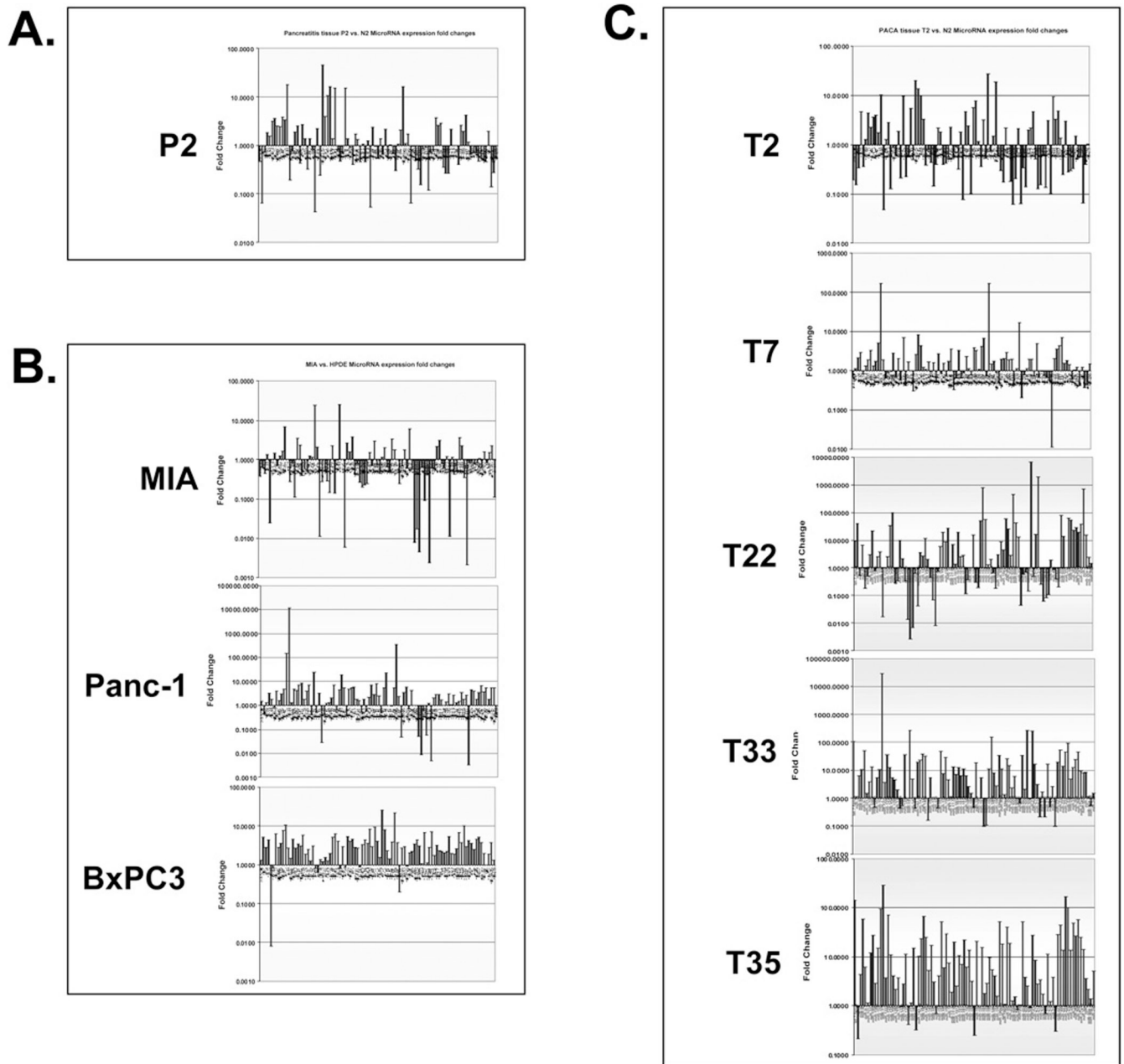
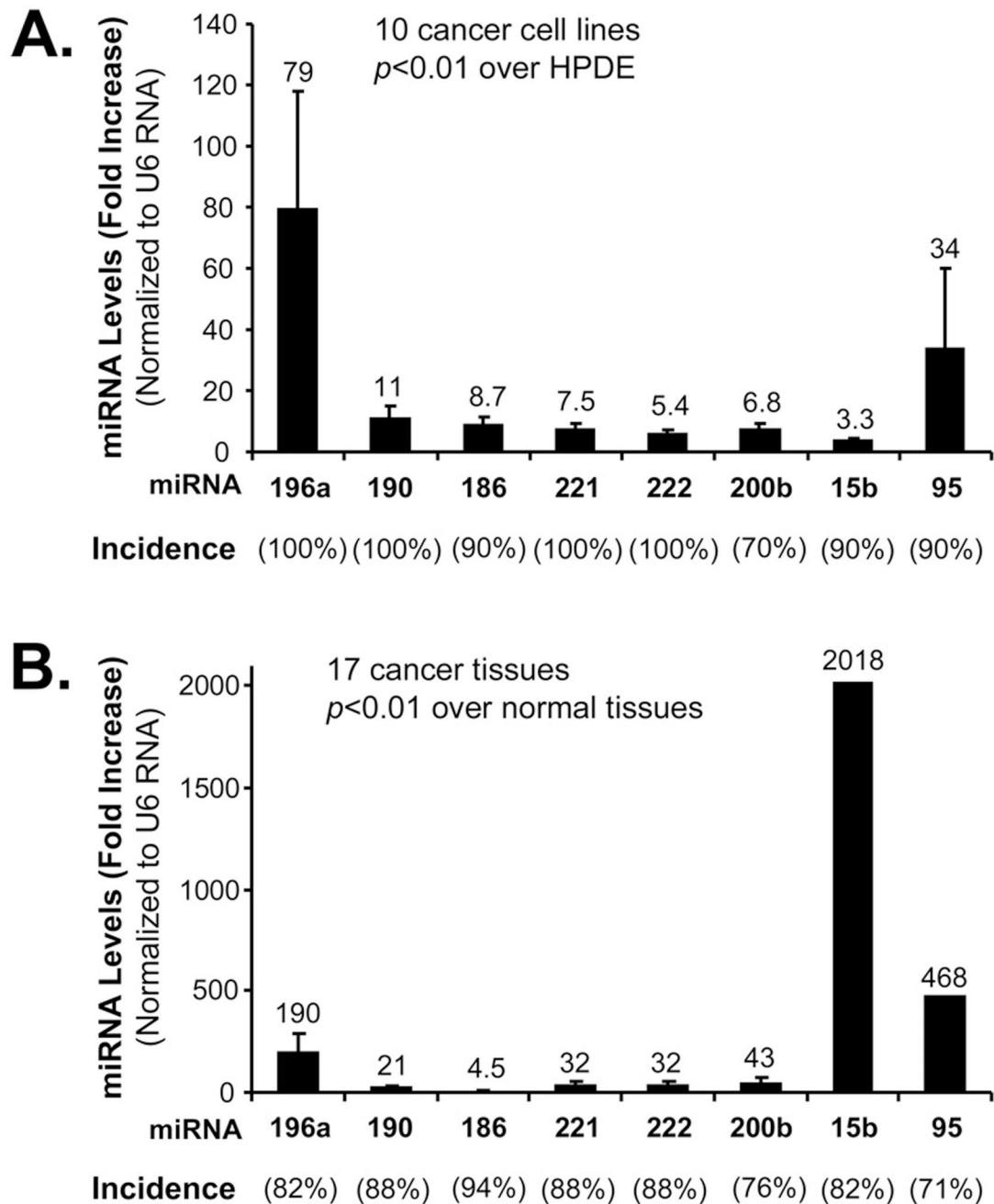


Fig. 1. The expression pattern of 95 miRNAs in chronic pancreatitis, pancreatic cancer cell lines and surgical specimens. MiRNAs of tissues and cultured cells were extracted and purified using mirVana miRNA Isolation kit and converted to cDNAs with the QuantiMir™ RT System. Differential expression was analyzed by RT-PCR using QuantiMir 95 microRNAs array System. U6 primer was also included in the array as a normalization control. After normalizing to the control U6 in all samples, the fold change in 95 miRNAs was calculated by comparing the pancreatic cancer tissue or cell lines with normal pancreatic tissues or HPDE cells. **A.** Chronic pancreatitis versus normal pancreatic tissue (n=1). **B.** Pancreatic cancer cell lines versus HPDE cells (n=3). **C.** Surgical specimens of pancreatic cancer tissues versus their adjacent normal pancreatic tissues (n=5).

**Fig. 2.**

The expression of 8 miRNAs in more pancreatic cancer cell lines and surgical specimens. Expression of 8 miRNAs (miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b and miR-95) selected from the 95 miRNAs was determined in 10 pancreatic cancer cell lines and 17 pairs of pancreatic cancer tissues and their adjacent normal pancreatic tissues. **A.** The expression of 8 miRNAs were significantly increased in pancreatic cancer cell lines compared with HPDE cells ($n=10$, $P < 0.01$). **B.** The expression of 8 miRNAs were significantly increased in pancreatic cancer tissues compared with their adjacent normal pancreatic cancer tissues ($n=17$, $P < 0.01$).

Table 1

Mature RNA sequences and real time PCR primers for 95 miRNAs.

miRNA	MirBase #	miRNA Sequence(s)	RT-PCR Primer sequenc(s)
let-7-family	MIMAT0000062, MIMAT0000064, MIMAT0000065, MIMAT0000067	ugagguaguagguuauaguu, ugagguaguagguuauaguu, agagguaguagguugcauagu, ugagguaguaguuauaguu	tgaggtagtaggtgtatagtt, tgaggtagtaggtgtatagtt, agaggtagtaggtgtatagtt, tgaggtagtaggtgtatagtt
miR-7	MIMAT0000252	uggaagacuaguuuuuuug	tggaagactagtgtattttgtg
miR-92	MIMAT0000092	uauugcacuuguccggccug	tattgcactgtcccgcctg
miR-93	MIMAT0000093	aaagugcuguucgucagguag	aaagtctgttcgtcaggtag
miR-9-1	MIMAT0000441	ucuuuuguuauacuagcuguaua	tctttggtatctagctgtatga
miR-101-1	MIMAT0000099	uacaguacugauaacuagaag	tacagtactgtgataactgaag
miR-103	MIMAT0000101	agcagcauugucagggcuaua	agcagcattgtacagggctatga
miR-106a	MIMAT0000103	aaaagugcuuacagucagguagc	aaaagtgttacagtgcaggtagc
miR-106b	MIMAT0000680	uaaagugcugacagucagau	taaagtgtgtacagtgcagat
miR-107	MIMAT0000104	agcagcauugucagggcuaua	agcagcattgtacagggctatca
miR-10b	MIMAT0000254	uaccuguaagaaccgaauuuug	tacctgtgaaccgaattgt
miR-1-1	MIMAT0000416	uggaauuaaagaaguaua	tggaatgtaaagaagtatga
miR-122a	MIMAT0000421	uggagugugacaauuguuuuug	tggagtgtgacaatgggtttgt
miR-125a	MIMAT0000443	ucccugagaccuuuaaccugug	tcctgagacccttaacctgtg
miR-125b	MIMAT0000423	ucccugagaccuuacuuguga	tcctgagacccttaacctgtga
miR-126	MIMAT0000444	cauuuuuuuuuuuuuuuacgcg	cattattactttgtgtacgcg
miR-128b	MIMAT0000676	ucacagugaaccggucucuuc	tcacagtgaaccggctcttttc
miR-132	MIMAT0000426	uaacagucuaacccauggucg	taacagtctacgccatggctg
miR-133a	MIMAT0000427	uuggucccuuuaaccagcugu	ttggtcccctcaaccagctgt
miR-134	MIMAT0000447	ugugacugguuagaccagagg	tgtgactgttgaccagagg
miR-135b	MIMAT0000758	uauggcuuuuauuccuauugug	tatggctttctcctatgtg
miR-136	MIMAT0000448	acuccauuuuuuuuugaugga	actccattgtttgatgatgga
miR-137	MIMAT0000429	uauugcuuaagaauacgcguag	tattgcttaagaatacgcgtag
miR-140	MIMAT0000431	aguguuuuuaccuauugguag	agtgtttttaccctatggtag
miR-141	MIMAT0000432	uaacacugucuguaaagaugg	taacactgtctgtaaagatgg
miR-142-3p	MIMAT0000434	uguaguguuuuuuuuuuuuugga	tgtagtgtttcctactttatgga
miR-143	MIMAT0000435	ugagaugaagcacuguagcuca	tgagatgaagcactgtagctca
miR-145	MIMAT0000437	guccaguuuuuccagaaucuuu	gtccagttttcccagaaatccctt
miR-146a	MIMAT0000449	ugagaacuuaauccauuggguu	tgagaactgaattccatgggtt
miR-149	MIMAT0000450	ucuggcuccgugucuucacucc	tctggtccgtgtcttactcc
miR-150	MIMAT0000451	ucuccaaccuugucacagug	tctccaaccctgtaccagtg
miR-151	MIMAT0000757	acuagacugaagcuccuugagg	actagactgaagctccttgagg
miR-153	MIMAT0000439	uugcauagucacaaaaguga	ttcatagtacacaaaagtga
miR-154	MIMAT0000452	uagguuauccguguugccuucg	taggttatccgtgttccttcg
miR-155	MIMAT0000646	uuauugcuauccgugaugggg	ttaagtctaatcgtgatagggg

miRNA	MirBase #	miRNA Sequence(s)	RT-PCR Primer sequenc(s)
miR-15a	MIMAT0000068	uagcagcacauaauguuugug	tagcagcacataatggtttgtg
miR-15b	MIMAT0000417	uagcagcacaucauguuuaca	tagcagcacatcatggtttaca
miR-16	MIMAT0000069	uagcagcacguaaaauuuggcg	tagcagcacgtaaatattggcg
miR-17-3p	MIMAT0000071	acugcagugaaggcacuugu	actgcagtgaaggcactgt
miR-17-5p	MIMAT0000070	caaagugcuuacagucagguagu	caaagtctctacagtgcaggtagt
miR-181a	MIMAT0000256	aacauucaacgcugucggugagu	aacattcaacgtctcgggtgagt
miR-181b	MIMAT0000257	aacauucauugcugucgguggg	aacattcattgctcgggtggg
miR-181c	MIMAT0000258	aacauucaaccugucggugagu	aacattcaacgtctcgggtgagt
miR-181d	MIMAT0002821	aacauucauugucgguggguu	aacattcattgtctcgggtggtt
miR-183	MIMAT0000261	uauaggcacugguagaauucacug	tatggcactgtgagaattcactg
miR-185	MIMAT0000455	uggagagaaggcaguuc	tggagagaaggcagttc
miR-186	MIMAT0000456	caaagaauucuccuuugggcuu	caaagaattctcttttggcgtt
miR-188	MIMAT0000457	caucccuugcauguggagggu	catcccttgcattggtggagggt
miR-18a	MIMAT0000072	uaaggugcaucuaugucagaua	taaggtgcatctagtgcagata
miR-190	MIMAT0000458	ugauauuuugauauuuaggu	tgatattgttatattagggt
miR-191	MIMAT0000440	caacggaaucacaaagcagcu	caacggaaucacaaagcagct
miR-192	MIMAT0000222	cugaccuaugaauugacagcc	ctgacctatgaattgacagcc
miR-194	MIMAT0000460	uguuacagcaacucaugugga	tgtaacagcaactccatgtgga
miR-195	MIMAT0000461	uagcagcacagaaaauuuggc	tagcagcacagaaaatattggc
miR-196a	MIMAT0000226	uagguaguuucauguuuggg	taggtagtctcatgttgggtg
miR-197	MIMAT0000227	uucaccaccuuccaccaccagc	ttcaccaccttccaccaccagc
miR-198	MIMAT0000228	gguccagaggggagauagg	ggfccagaggggagatagg
miR-199a+b	MIMAT0000231, MIMAT0000263	cccaguuucagacuaccuuguc, cccaguuuagacuauucguuc	cccagtttcagactacctgttc, cccagttttagactatctgttc
miR-30b	MIMAT0000420	uguuacauccuacacucagcu	tgtaaacatctcactcagct
miR-19a+b	MIMAT0000073, MIMAT0000074	ugugcaaaucuaugcaaacuga, ugugcaaaucuaugcaaacuga	tgtgcaaatctatgcaaaactga, tftgcaaatccatgcaaaactga
miR-95	MIMAT0000094	uucaacggguuuuuuugagca	ttcaacgggtattatttagca
miR-20a	MIMAT0000075	uaaagugcuuauagucagguag	taaagtgtctatagtgcaggtag
miR-200a	MIMAT0000682	uaacacugucguaacgaugu	taacactgtctgtaacgatgt
miR-200b	MIMAT0000318	uaauacugccgguaaugaugac	taatactgcctgtaaatgatgac
miR-200c	MIMAT0000617	uaauacugccgguaaugaugg	taatactgccggtaaatgatgg
miR-202	MIMAT0002811	agagguauaggcgaugggaaa	agaggfatagggcatgggaaa
miR-203	MIMAT0000264	gugaaauguuuaggaccacuag	gtgaaatgttagaccactag
miR-204	MIMAT0000265	uuccuuuugucauccuaugccu	ttcctttgtcatcctatgcct
miR-205	MIMAT0000266	uccuauuccaccggagucug	tccttaccaccggagtctg
miR-206	MIMAT0000462	uggaauuaaggaugugugg	tggaatgaaggaaagtgtgtgg
miR-21	MIMAT0000076	uagcuuacagacugauuuga	tagcttatcagactgatgtga
miR-210	MIMAT0000267	cugucgugugacagcggcuga	ctgtgcgtgtgacagcggctga
miR-214	MIMAT0000271	acagcaggcacagacagcag	acagcaggcacagacagcag
miR-215	MIMAT0000272	augaccuaugaauugacagac	atgacctatgaattgacagac

miRNA	MirBase #	miRNA Sequence(s)	RT-PCR Primer sequenc(s)
miR-372	MIMAT0000724	aaagugcugcgacauuugagcgu	aaagtgctgcgacatttgagcgt
miR-373	MIMAT0000726	gaagugcuucgauuuuggggugu	gaagtgtctcgatttgggggtgt
miR-218	MIMAT0000275	uugugcuugaucuaaccaugu	ttgtgcttgatctaacctgt
miR-219	MIMAT0000276	ugauuguccaaacgcaauucu	tgattgtccaacgcaattct
miR-22	MIMAT0000077	aagcugccaguugaagaacugu	aagctgccagttgagaactgt
miR-488	MIMAT0002804	cccagauaauggcacucuaa	cccagataatggcactctcaa
miR-221	MIMAT0000278	agcuacaauugucugcggguuc	agctacattgtctgtgggttc
miR-222	MIMAT0000279	agcuacaucuggcuacugggucuc	agctacatctggctactgggtctc
miR-223	MIMAT0000280	ugucaguuuguaaaacccc	tgctagttgtcaaatcccc
miR-224	MIMAT0000281	caagucacuagguuccguua	caagtcactagtgttccgtta
miR-23a	MIMAT0000078	aucacauugccagggaauucc	atcacattgccagggtttcc
miR-24	MIMAT0000080	uggcucaguucagcaggaacag	tggtcagttcagcaggaacag
miR-25	MIMAT0000081	cauugcacuugucuggucuga	cattgcactgtctcggctctga
miR-26a	MIMAT0000082	uucaaguaauccaggauaggc	ttcaagtaatccaggataggc
miR-26b	MIMAT0000083	uucaaguaauccaggauagguu	ttcaagtaatccaggataggtt
miR-27a+b	MIMAT0000084, MIMAT0000419	uucacaguggcuaaguuccgc, uucacaguggcuaaguucgc	ttcacagtggttaagtccgc, ttcacagtggttaagtctgc
miR-30c	MIMAT0000244	uguaaacuuccacucucagc	tgtaaacatcctacactctcagc
miR-29a+b+c	MIMAT0000086, MIMAT0000100, MIMAT0000681	uagcaccuucgaaucgggu, uagcaccuuugaaucaguguu, uagcaccuuugaaucgggu	tagcaccatctgaaatcggtt, tagcaccattgaaatcagttt, tagcaccattgaaatcggt
miR-30a-3p	MIMAT0000088	cuuucagucggauguuugcagc	ctttcagtcggatgttgcagc
miR-30a-5p	MIMAT0000087	uguaaacuuccgacuggaag	tgtaaacatcctcactggaag
miR-296	MIMAT0000690	aggcccccccucauuccugu	aggccccccctcaatcctgt
U6 snRNA	NCBI: X07425.1	caccacguuuauacgccggug	caccacgtttatagccgggtg

Table 2
The expression of 95 miRNAs in chronic pancreatitis, pancreatic cancer cell lines* and surgical specimens***

miRNA	P2	MIA	Panc-1	BxPC-3	T2	T7	T22	T33	T35
let-7-family	0.476319	0.3737123	1.494849	1.328686	0.186856	0.707107	9.3178687	146.0178	106.89125
miR-7	0.065154	0.6328783	0.432269	5.098243	0.151774	1.156688	39.396621	0.210224	1.1095695
miR-92	0.721965	0.4537596	1.292353	2.789487	0.339151	2.158456	0.5212329	4.287094	6.2333166
miR-93	1.827663	1.3755418	3.24901	4.40762	4.69134	2.948538	6.6345564	58.89201	10.556063
miR-9-1	1.569168	0.0245183	1.717131	0.008201	0.358489	0.82932	0.1780063	6.233317	51.268472
miR-101-1	3.226567	0.9930925	0.823591	0.835088	1.265757	1.337928	0.4863275	1.148698	1.4948492
miR-103	3.5801	1.4742692	3.89062	6.276673	4.316913	1.853176	2.9690471	11.87619	3.810552
miR-106a	2.514027	0.7120251	1.569168	2.828427	2.297397	3.317278	21.406841	27.47409	12.996038
miR-106b	2.42839	1.2483305	3.031433	3.506423	3.630077	1.337928	0.8010699	2.86791	0.463294
miR-107	3.810552	1.6245048	4.823231	7.674113	4.027822	1.79005	2.5315132	14.82541	5.464161
miR-10b	3.363586	6.7739625	153.2773	10.33882	1.777685	5.063026	3.732132	95.00951	10.126053
miR-1-1	17.87659	∞	11268.44	2.657372	10.26741	165.4212	0.016176	286.0255	28724.616
miR-122a	0.19751	0.2793218	1.347234	1.494849	0.047696	1.918528	∞	3.680751	3.4822023
miR-125a	0.784584	0.8408964	4.756828	4.594793	1.265757	0.632878	2.4794154	70.0348	36.758347
miR-125b	1.905276	0.1088188	4.198867	2.770219	2.80889	0.907519	33.128478	11.00433	12.295001
miR-126	2.514027	3.4822023	6.868523	3.458149	0.126745	0.946058	100.42676	4.112455	5.5021673
miR-128b	0.432269	2.3949574	8.339726	2.989698	0.876606	2.751084	0.283221	2.17347	4.5630549
miR-132	2.639016	0.3977682	1.729074	5.61778	0.61132	0.707107	0.3391511	3.732132	1.9724654
miR-133a	1.385109	0.5864175	3.758091	1.840375	1.827663	2.07053	9.9176616	0.959264	0.4506252
miR-134	0.316439	0.5358867	7.110741	2.462289	0.211686	0.578344	2.1584565	2.80889	0.5471469
miR-135b	1.375542	1.2570134	0.384219	1.265757	9.781122	7.012846	0.3321715	11.23556	37.271475
miR-136	0.80107	1.1647336	23.58831	3.052518	0.225313	0.41466	0.0133224	0.406126	∞
miR-137	0.042689	23.917588	0.503478	0.795536	0.570382	1.658639	0.0025772	1.140764	261.3791
miR-140	2.234574	2.0562277	3.160165	0.628507	5.540438	0.993092	0.0066612	14.92853	4.9588308
miR-141	0.246558	0.0113592	0.028756	1.375542	0.493116	0.309927	0.6285067	0.323088	0.4413515
miR-142-3p	45.25483	0.2812646	0.406126	1.205808	19.83532	2.657372	0.0418102	10.12605	19.835323
miR-143	3.917681	0.5212329	1.214195	1.526259	13.73705	8.168097	3.4105396	23.75238	22.943284

miRNA	P2	MIA	Panc-1	BxPC-3	T2	T7	T22	T33	T35
miR-145	10.48315	0.289172	1.319508	1.231144	10.12605	4.316913	2.6390158	67.64915	38.054628
miR-146a	16.56424	0.1486509	2.013911	1.958841	3.271608	1.214195	11.551434	24.93327	31.77896
miR-149	1.356604	2.250117	6.773962	5.028053	0.376312	1.057018	2.0139111	5.169411	0.1582196
miR-150	15.03236	0.1396609	0.63728	6.408559	0.784584	1.717131	0.4444213	17.14838	5.5789747
miR-151	0.806642	0.9726549	4.469149	3.97237	0.441351	0.920188	0.0674518	3.07375	∞
miR-153	0.473029	25.457167	18.12614	0.790041	0.144586	1.580083	0.0083732	0.687771	∞
miR-154	0.673617	1.0352649	5.278032	2.969047	0.395021	0.554785	0.7219646	4.112455	0.4383029
miR-155	15.03236	0.0060872	0.395021	0.835088	2.158456	2.732081	5.6177795	52.34573	48.840295
miR-15a	1.375542	2.6026837	4.789915	5.426417	1.802501	0.870551	18.507011	5.979397	7.674113
miR-15b	0.90125	1.5583292	5.388934	4	0.395021	1.547565	8.6338259	29.04061	28.442966
miR-16	0.403321	3.6807506	6.020987	4.563055	0.411796	0.721965	27.09585	7.568461	4.4382779
miR-17-3p	1.729074	0.4537596	1.729074	2.828427	0.566442	1.729074	1.0352649	1.866066	1.1095695
miR-17-5p	1.301342	0.8066418	1.591073	2.751084	2.281527	3.482202	7.061624	20.11221	13.269113
miR-181a	0.463294	0.2660925	0.532185	0.876606	0.535887	0.320856	1.3286858	2.549121	7.1107414
miR-181b	1.071773	0.2016604	1.717131	3.271608	0.888843	0.632878	18.635737	10.62949	12.728584
miR-181c	0.450625	0.2284579	0.607097	4.346939	0.316439	3.317278	2.5847057	6.868523	6.4531341
miR-181d	1.257013	0.25	2.12874	3.5801	1.802501	0.632878	2.8088898	21.70567	11.2355559
miR-183	0.05366	1.5052467	6.821079	8.168097	0.075363	0.913831	0.1111053	6.19026	6.1475007
miR-185	2.34567	0.7169776	2.828427	2.969047	4.626753	2.313376	0.3634931	13.73705	2.6390158
miR-186	0.97942	2.907945	7.727491	9.38268	2.361985	1.109569	∞	3.09513	1.4640857
miR-188	0.539614	0.7474246	2.445281	3.97237	0.10083	0.779165	14.928528	0.246558	0.4600938
miR-18a	1.385109	0.4569157	0.496546	1.515717	5.61778	3.863745	0.2973018	20.25211	18.126142
miR-190	0.659754	1.1647336	5.35171	25.63424	7.835362	3.294364	0.1907824	∞	∞
miR-191	2.12874	1.9724654	22.94328	7.78124	1.164734	1.086735	49.52208	15.13692	5.5021673
miR-192	0.554785	0.659754	0.986233	2.297397	0.363493	4.257481	781.44471	1.79005	0.0973956
miR-194	1.021012	0.4796321	0.946058	1.42405	3.182146	6.773962	55.330383	2.828427	0.1111053
miR-195	0.664343	3.2716082	5.464161	3.837056	0.528509	0.716978	1.2657566	9.57983	10.777869
miR-196a	0.301452	1.8150383	349.7063	21.85664	27.09585	168.897	2.0139111	5.314743	152.21851
miR-197	1.071773	1.0210121	2.361985	3.758091	0.732043	1.474269	0.6643429	4.112455	8.1116758
miR-198	2.042024	0.2414841	0.049037	0.198884	1.474269	1.753211	0.1755556	1.591073	2.7894873

miRNA	P2	MIA	Panc-1	ExPC-3	T2	T7	T22	T33	T35
miR-199a+b	16.56424	0.6925547	3.458149	2.732081	18.50701	0.888843	3.0314331	51.62507	33.824577
miR-30b	1.101905	1.8150383	5.696201	2.969047	0.578344	0.482968	8.8152409	18.50701	10.852835
miR-19a+b	1.70527	0.6029039	0.737135	0.913831	0.297302	1.94531	4.3771748	1.071773	1.3472336
miR-95	0.065154	6.1050368	4.027822	2.013911	0.175556	2.042024	58.89201	40.22443	24.933267
miR-20a	0.959264	0.5904963	1.028114	2.188587	2.158456	2.989698	26.354913	18.76536	14.723002
miR-200a	0.486327	0.0079767	0.986233	3.41054	0.993092	1.866066	2.8878584	1.239708	2.2815274
miR-200b	0.325335	0.017337	0.052193	4.316913	0.180491	1.958841	464.64981	1.515717	5.8158901
miR-200c	0.153893	0.0044253	0.008669	3.07375	0.060371	0.441351	41.642939	0.835088	0.9265881
miR-202	0.835088	0.6285067	0.598739	1.042466	0.204476	1.148698	12.906268	1.028114	0.6551967
miR-203	0.895025	0.0915054	0.05672	6.868523	2.12874	16.67945	0.0429857	52.34573	33.590934
miR-204	0.11908	0.417544	1.189207	1.101905	0.063813	0.208772	0.5823668	3.89062	2.0849315
miR-205	0.716978	0.0024381	0.004809	2.828427	0.334482	0.80107	0.7071068	2.496661	268.72747
miR-206	0.80107	0.5946036	2.084932	7.210004	0.139661	1.109569	0.1425955	0.926588	0.952638
miR-21	3.732132	0.6198538	2.789487	1.753211	2.013911	1.958841	6888.6234	27.66519	256
miR-210	2.620787	2.1584565	2.80889	3.160165	2.17347	1.972465	0.5	8.633826	16
miR-214	2.86791	3.1166583	1.658639	2.265768	4.563055	0.732043	16	2.770219	3.09513
miR-215	0.353553	0.7900413	∞	2.188587	0.514057	4.890561	2005.8528	3.41054	0.208772
miR-372	0.264255	0.6029039	1.385109	2.042024	0.125869	0.668964	0.2448551	1.70527	1.6586391
miR-373	0.264255	1.1809927	3.031433	2.188587	0.153893	0.939523	0.060371	0.687771	0.2102241
miR-218	2.114036	0.0112028	1.079228	5.314743	0.594604	0.747425	0.0824692	1.131371	16
miR-219	0	1.1647336	2.751084	1.972465	0.134904	0.678302	0.1073207	1.231144	0.5034778
miR-22	0.993092	0.4263174	1.347234	1.879045	3.138336	0.582367	1.8531761	3.863745	2.6026837
miR-488	0.403321	0.5509526	1.515717	2.639016	0.102238	0.011518	0.8585654	0.303549	0.0940779
miR-221	2.602684	3.6553258	3.340352	6.680703	9.12611	2.07053	0.3977682	28.05138	19.159659
miR-222	1.972465	2.2657678	1.765406	3.758091	3.271608	3.630077	0.2016604	43.71329	54.1917
miR-223	4.14106	0.3535534	2.566852	10.05611	4.723971	4.469149	79.341293	13.54792	13.737047
miR-224	1.148698	0.0021373	0.003262	2.86791	1.36604	6.868523	14.025692	168.897	45.886568
miR-23a	0.655197	0.8408964	4.626753	4.169863	0.241484	1.569168	∞	101.1253	95.670352
miR-24	0.702222	0.8293195	3.944931	3.605002	3.031433	1.840375	62.682899	13.8326	4.6913398
miR-25	0.707107	0.7791646	1.815038	2.445281	0.279322	1.453973	52.709825	48.50293	12.295001

miRNA	P2	MIA	Panc-1	BxPC-3	T2	T7	T22	T33	T35
miR-26a	0.479632	0.8293195	3.434262	4.531536	0.29937	0.61132	22.161751	26.72281	23.26356
miR-26b	0.586417	1.0717735	6.498019	5.205367	0.325335	1.042466	29.040613	56.49299	43.411338
miR-27a+b	0.496546	0.6417129	3.605002	3.732132	1.494849	1.214195	19.027314	24.42015	9.3178687
miR-30c	0.450625	1.591073	5.426417	1.931873	0.539614	0.423373	37.530718	14.02569	8.168097
miR-29a+b+c	1.972465	0.8408964	1.729074	0.993092	0.566442	1.22264	704.27741	3.5801	8.3977335
miR-30a-3p	0.140632	1.4948492	5.540438	1.879045	0.065154	0.376312	15.242208	2.188587	1.1647336
miR-30a-5p	0.273573	2.2657678	5.314743	3.706352	0.389582	0.435275	2.3133764	1.375542	0.528509
miR-296	0.539614	0.1103379	0.432269	1.310393	1.013959	1.505247	1.4539725	5.028053	1.4640857

Chronic pancreatitis tissue (P2). Pancreatic cancer cell lines (MIA-CaPa2, Panc-1 and BxPC-3). Surgical specimens of pancreatic cancer tissues (T2, T7, T22, T33 and T35).

[∞] the miRNA was increased in cancer tissues or cells while normal controls had no expression. The expression of all miRNAs was normalized to the U6 level in all tissue samples and cell types.

* Compared with the relatively normal human pancreatic ductal epithelium (HPDE).

** compared with the relatively normal pancreatic tissues.

Table 3
Eight commonly increased miRNAs in more pancreatic cancer cell lines* and surgical specimens**

Sample	miR-196a	miR-190	miR-186	miR-221	miR-222	miR-200b	miR-15b	miR-95
1 P2	0.301452	0.659754	0.97942	2.602684	1.972465	0.325335	0.90125	0.065154
2 Panc-1	349.7063	5.35171	7.727491	3.340352	1.765406	0.052193	5.388934	4.027822
3 MIA	1.815038	1.164734	2.907945	3.655326	2.265768	0.017337	1.558329	6.105037
4 BxPC-3	21.85664	25.63424	9.38268	6.680703	3.758091	4.316913	4	2.013911
5 ASPC-1	234.753	∞	21.18542	19.63016	13.54792	21.55574	11.75335	273.4247
6 Capan1	13.68952	∞	4.228072	2.505329	1.802501	8.724062	0.737135	11.08088
7 Capan2	7.43844	∞	1.918528	7.361501	7.210004	11.3924	1.840375	9.849155
8 Panc3.27	9.849155	∞	1.328686	2.799172	1.898684	1.765406	1.068065	0.747425
9 Hs766T	36.12686	∞	27.28432	14.17228	10.81529	0.031577	2.838247	16.39291
10 PL45	2.666597	∞	0.70466	2.694467	2.099433	1.252664	1.630145	4.084049
11 HPAFII	117.784	∞	10.30305	11.71269	8.969329	19.15966	1.879045	8.724062
12 T7	168.897	3.294364	1.109569	2.07053	3.630077	1.958841	1.547565	2.042024
13 T13	648.0674	21.63057	6.680703	17.75311	21.70567	9.000468	15.03236	7.412704
14 T18	0.353553	0.503478	0.018517	25.45717	0.63728	10.30305	261.3791	61.60604
15 T19	1.094294	4.773343	1.500039	1.69937	2.313376	3.680751	9.986644	9.094536
16 T22	2.013911	0.190782	∞	0.397768	0.20166	464.6498	8.633826	58.89201
17 T29	1.125058	4.890561	2.020903	3.271608	2.531513	1.01748	1.261377	1.049717
19 T31	101.8287	8.574188	1.287882	1.986185	2.938337	0.784584	4.789915	1.248331
20 T32	7.727491	6.083915	1.168777	1.419123	1.404445	1.132884	1.314943	0.787308
21 T33	5.314743	∞	3.09513	28.05138	43.71329	1.515717	29.04061	40.22443
22 T34	0.809442	6.940309	7.412704	21.03908	6.84476	6.750526	3.193194	31.45026
23 T35	152.2185	∞	1.464086	19.15966	54.1917	5.81589	28.44297	24.93327
24 T36	11.31371	174.8532	8.196455	32.1111	22.08508	1.086735	62.03455	19.7667
25 T37	140.0696	32.67239	18.37917	76.63864	325.1587	61.1805	68.35619	21.78103
26 T38	1557.482	∞	11.91742	292.0355	40.64483	150.6441	33806.19	7669.942
27 T39	2.938337	1.433955	1.172835	0.760489	1.132884	1.85961	0.750019	0.78187
28 T40	401.7071	21.48116	3.797368	7.210004	8.969329	9.679953	0.604997	0.982821

Sample	miR-196a	miR-190	miR-186	miR-221	miR-222	miR-200b	miR-15b	miR-95
29 T2	27.0958	7.8356	2.362	9.1261	3.2716	0.1805	0.395	0.1756

Chronic pancreatitis tissue (row 1), Pancreatic cancer cell lines (rows 2–11), Surgical specimens of pancreatic cancer tissues (rows 12–29).

[∞] the miRNA was increased in cancer tissues or cells while normal controls had no expression. The expression of all miRNAs was normalized to the U6 level in all tissue samples and cell types.

* Compared with the relatively normal human pancreatic ductal epithelium (HPDE).

** compared with the relatively normal pancreatic tissues.