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MicroRNA profiling of human gastric cancer

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Abstract. MicroRNAs are a group of small non-coding RNAs that modulate gene expression. The de-regulation of microRNA expression has been found in several types of cancer. To study the role of microRNAs in gastric cancer (GC), we analyzed the expression profile of 847 microRNAs in GC from Chinese patients. Total RNA was used for hybridization on the miRCURY LNA Array (v. 11.0), which contains probes specific for 847 human microRNAs. The results from the miRNA microarray analysis were validated by real-time RT-PCR. A total of 24 miRNAs with a more than 2-fold change were differentially expressed between normal gastric tissue and GC. Of these, 22 miRNAs (miR-223, miR-106b, miR-147, miR-34a, miR-130b*, miR-106a, miR-18a, miR-17, miR-98, miR-616*, miR-181a-2*, miR-185, miR-1259, miR-601, miR-196a*, miR-221*, miR-302f, miR-340*, miR-337-3p, miR-520c-3p, miR-575 and miR-138) were significantly up-regulated in GC ($P<0.05$), whereas only miR-638 and miR-378 were significantly down-regulated in GC ($P<0.05$) compared to normal gastric tissue. The expression of miR-185 and miR-638, as measured by miRNA microarray analysis, was in agreement with the expression level of these microRNAs found by real-time RT-PCR in the same samples. Our results show that microRNAs are de-regulated in GC, suggesting the involvement of these genes in the development and progression of gastric cancer.

Introduction

Gastric cancer (GC) is the fourth most prevalent malignancy worldwide and remains the second most common cause of cancer-related death globally. The distribution of GC is not uniform between different populations, as the prevalence in East Asia, including Japan and China (where 42% of cases occur), Eastern Europe and South America is higher than elsewhere (1). The prognosis of GC is poor, with an estimated relative 5-year survival rate of less than 20% (2).

Gastric cancer is a genetic disease that develops from a multi-step process (3). Single or multiple mutations in genes related to growth control, apoptosis, invasion and metastasis form the molecular genetic basis of malignant transformation and tumor progression (4). Therefore, a better understanding of the molecular basis of tumor-host interactions may lead to significant progress in the development of new therapeutic agents.

The discovery of miRNAs has been a landmark milestone in molecular biology. miRNAs can post-transcriptionally regulate the expression of hundreds of their target genes, thereby controlling a wide range of biological functions, such as cellular proliferation (5), differentiation (6) and apoptosis (7). Recent evidence indicates that miRNAs may function as tumor suppressors or oncogenes, and that alterations in miRNA expression may play a critical role in tumorigenesis and cancer progression (8,9). miRNAs have been found to be involved in known oncogenic pathways, including the p53 (10,11), Bcl2 (12) and K-Ras (13) pathways. Finally, miRNAs appear to be markedly significant prognostic factors in patients with various tumors (14-17), and could be useful for treatment (18). However, current and comprehensive data on the miRNA signature of GC in the Chinese population are limited.

In this study, the miRNA expression profile of three pairs of gastric cancer and normal gastric tissue was analyzed. In all three pairs, 20 miRNAs were found to be differentially expressed.

Materials and methods

Patients and tissue specimens. We analyzed frozen specimens of GC tissue and normal tissue from ten patients who underwent surgical resection of GC at the First Affiliated Hospital of Medical College of Xi'an Jiaotong University between November and December 2008. The patients had not received adjuvant chemotherapy. This study was approved by the Institutional Review Board of the Hospital. Written informed consent was obtained from the patients.

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miRNA microarray analysis. Three pairs of specimens were analyzed by miRNA microarray. Total RNA was harvested using TRIzol (Invitrogen) and an RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. After RNA quantification using a Nanodrop spectrophotometer, the samples were labeled using the miRCURY Hy3/Hy5 Power Labeling Kit and hybridized to the miRCURY LNA Array (v. 11.0). The samples were hybridized using a hybridization station and the arrays were scanned with the Axon GenePix 4000B Microarray Scanner. The raw intensity of the image was read using GenePix Pro V6.0. The intensity of the green signal was calculated after background subtraction, and four replicated spots for each probe on the same slide were averaged. The Median Normalization Method was used to obtain 'Normalized Data' [Normalized Data = (foreground-background)/median]. The median was defined as the 50% quantile of microRNA intensity that was >50 in all samples after background correction. The statistical significance of the differentially expressed miRNA was analyzed using the Student's t-test.

Real-time RT-PCR. qRT-PCR was performed in duplicate. Both a minus reverse transcription (RT) control and a no template control were included to assess genomic DNA contamination and to ensure a lack of background amplification, respectively. The RT reaction for miR-551b and miRNA-765 consisted of 2 μl 10X RT Buffer (Epicentre), 2 μl dNTPs (0.25 mM each; HyTest), 1 μl RT Primer (1 μM each; Applied Biosystems), 0.3 μl RNase Inhibitor Protein 40 U/μl (Epicentre), 2 μl MMLV-RT 10 U/μl (Epicentre) and 2 μg total RNA in a final volume of 20 μl. The reactions were incubated at 16˚C for 30 min, 42˚C for 42 min and 85˚C for 5 min. Following the RT reaction, 1 μl of the RT product was transferred into a 25 μl PCR mix containing 2.5 μl 10X PCR Buffer (Epicentre), 1.5 μl 25 mM magnesium chloride (Promega), 2.5 μl dNTPs (2.5 mM each; Ambion), 0.25 μl 10,000X Sybr Green I (Invitrogen), 1 μl forward primer (10 μM), 1 μl reverse primer (10 μM), and 1 unit of Taq (Promega). The sequence of the primers used is listed in Table I. The PCR cycling parameters were: template denaturation at 95˚C for 5 min and then 40 cycles of 95˚C for 10 sec, 60˚C for 20 sec, 72˚C for 20 sec and 78˚C for 20 sec. The PCR was performed on a Rotor-Gene 3000 Real-time PCR Cycler (Corbett Research). The threshold and baseline were manually determined, with the thresholds typically set between 0.05-0.1 paired with a baseline starting at 1-3 and ending at 15-17 Cts.

Real-time RT-PCR data analysis. We chose the relative quantification method to determine the changes in the expression of the target miRNAs (19). The change in amplification was normalized to the expression of the U6 RNA. The fold change in expression was calculated for each sample using $2^{-\Delta\Delta}$Ct, where $\Delta\Delta$CT = (Ct target gene-CtU6) cancer-(Ct target gene-CtU6)normal. A $2^{-\Delta\Delta}$Ct >1.5 or <0.67 was considered differentially expressed miRNA.

Results

Differentially expressed miRNAs in gastric cancer. miRNA expression profiling studies were conducted using the miRCURY LNA microRNA Array (v. 11.0), which contains probes for 847 human microRNAs. A total of 161 miRNAs were overexpressed in GC, while 165 were underexpressed (Fig. 1). Of these, 105 miRNAs with a more than 2-fold change were differentially expressed between the normal gastric tissue and GC, and 24 miRNAs were significantly differentially expressed (P≤0.05). These included miR-223, miR-106b, miR-147, miR-34a, miR-130b*, miR-106a, miR-18a, miR-17, miR-98, miR-616*, miR-181a-2*, miR-185, miR-1259, miR-601, miR-196a*, miR-221*, miR-302f, miR-340*, miR-337-3p, miR-520c-3p, miR-575, miR-138, miR-638 and miR-378. The top five putative targets identified with TargetScan are shown in Tables II and III.

Table I. Sequence of RT-PCR primers.

Primer	Sequence
U6 F	5'GCTTCGGCAGCACATATACTAAAAT3'
U6 R	5'CGCTTCACGAATTTGCGTGTCAT3'
miR-185 GSP	5'GGTGGAGAGAAAGGCAGT3'
miR-185 R	5'TGCGTGTCGTGGAGTC3'
miR-638 GSP	5'AAGGGATCGCGGGCG3'
miR-638 R	5'TGCGTGTCGTGGAGTC3'

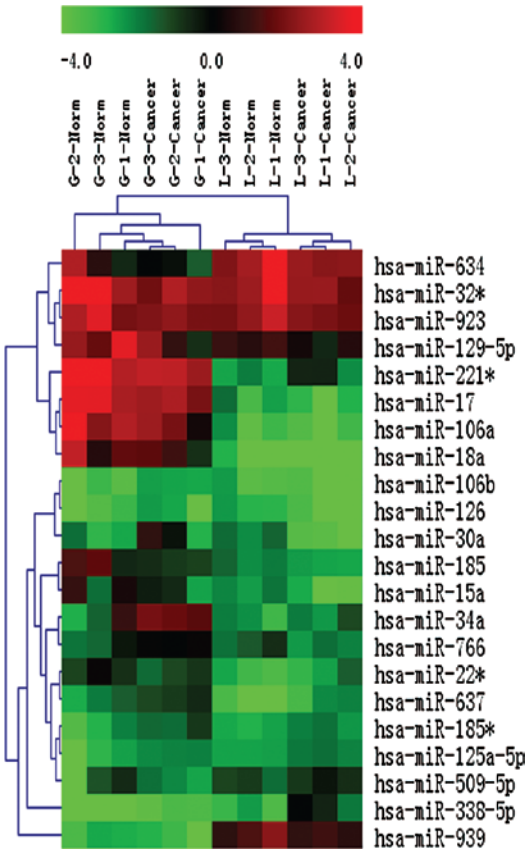

Figure 1. Hierarchical clustering of gastric cancer samples. The analyzed samples are in columns and the micro-RNAs are presented in rows. The miRNA-clustering tree is shown on the left and the sample-clustering tree appears at the top. The color scale shown at the top illustrates the relative expression level of a miRNA; red represents a high expression level, green represents a low expression level.

Table II. Statistics, location and putative targets of the of micro-RNAs up-regulated in gastric cancer compared to normal gastric tissue.

Micro-RNA	Value		Fold			P-value	Chromosomal localization	Putative targets
	Normal	SCC	Mean	Min	Max			
hsa-miR-100	0.0287	0.0622	2.17	-7.74	9.40		11q4.1	HS3ST2, SMARCA5, EPDR1, ZZEF1, EIF2C2
hsa-miR-106a	0.07	0.20	2.83	2.12	6.85	0.01	Xq26.2	IL10, KIAA1404, KIAA1196, EDD, RGL1
hsa-miR-106b	0.11	0.24	2.17	1.40	5.76	0.03	7q22.1	p21, PTPN4, GPR6 , SIRT7 , PFKP
hsa-miR-10a	0.04	0.09	2.49	1.13	7.47	0.10	17q21.32	ARSJ, BDNF, SOBP, CRLF3, TFAP2C
hsa-miR-122*	0.08	0.20	2.70	2.17	4.81	0.22	18q21.31	ENOX1, HIST1H2BK, TUBB2B, TP53I13, CNN1
hsa-miR-1248	0.11	0.28	2.66	1.10	40.44	0.08	3q27.3	TPM3, DGAT2, HBEGF, KPNA6, ANKRD13C
hsa-miR-1255b	0.58	1.63	2.83	2.20	4.63	0.08	4p14/1q24.2	DTX4, AGPAT1, NEU3, MORF4L1, IREB2
hsa-miR-1259	0.26	0.78	3.00	2.01	4.78	0.03	20q13.13	CRISPLD1, NR3C2, CD83, PTEN, LRRC2
hsa-miR-125a-5p	0.67	2.02	3.02	2.82	3.74	0.15	19q13.3	STARD13, ZNF792, GCNT1, FUT4, NAIF1
hsa-miR-1263	0.07	0.16	2.64	1.22	12.28	0.09	3q26.1	SLC38A2, CSGALNACT2, ABCE1, DIXDC1, ST8SIA4
hsa-miR-1267	0.08	0.19	2.53	2.08	4.14	0.20	13q33.3	VGLL3, SHROOM2, MAN2A1, SCN1A, TOE1
hsa-miR-1274a	0.09	0.24	2.73	1.22	3.66	0.17	5p13.1	FOXO4, JHDM1D, CUGBP2, TFAP4, MUM1L1
hsa-miR-1291	0.07	0.18	2.42	1.01	4.42	0.11	12q13.11	AQP1, ARID3B, MECP2, MAP3K9, PGM5
hsa-miR-130b*	0.16	0.35	2.19	1.85	2.27	0.03	22q11	IGFBP2, AAMP, CLIC1, AMIGO1, MRO
hsa-miR-138	0.045	0.10	2.21	1.27	3.55	0.04	16q13, 3p21.33	RMND5A, GPR124, CREB3L2, SLC35F1, SYT13
hsa-miR-145	0.04	0.08	2.03	-6.14	4.34	0.42	5q33.1	FAM108C1, FSCN1, SNTB2, SRGAP2, ABCE1
hsa-miR-146b-3p	0.95	2.87	3.01	1.97	3.82	0.08	10q24.32	FMR1, FAM160B1, PTCHD1, CHP, CYFIP2
hsa-miR-147	0.08	0.18	2.30	1.74	2.94	0.002	9q33.2	KIP, SSR1, NF1, ZKSCAN1, TFAP2B
hsa-miR-147b	0.04	0.08	2.23	1.01	5.78	0.16	15q21.1	NDUFA4, HOXC6, BDNF, DIMT1L, MID1IP1
hsa-miR-148a*	0.02	0.05	2.36	-5.14	3.42	0.46	7q15.2	HEATR1, OASL, H2AFY2, IMP3, RG9MTD1
hsa-miR-17	0.07	0.17	2.46	1.61	5.55	0.01	13q31.3	ZNFX1, PKD2, MYT1L, ITGB8, SCN1A
hsa-miR-181a-2*	0.10	0.33	3.29	1.03	25.51	0.05	9q33.3	PCDH11Y, HMGB4, MAGIX, HYI, VPS36
hsa-miR-182	0.02	0.06	3.23	-1.61	9.88	0.27	7q32.2	RGS17, MITF, ACTR2, MFAP3, CTTN
hsa-miR-183	0.09	0.28	3.30	-1.46	9.34	0.17	7q32.2	PIGX, AKAP12, NTRK2, PFN2, SLAIN1
hsa-miR-185	0.48	1.06	2.20	1.33	3.52	0.03	22q11.21	SLC16A2, PALM2, BSN, ABCG4, PCDHAC1
hsa-miR-186*	0.05	0.13	2.59	-1.66	22.00	0.15	1p31.1	PAQR6, BRUNOL5, LRRC41, COX7A2, PPP2R1A
hsa-miR-18a	0.04	0.08	2.08	1.59	3.10	0.01	13q31.3	CCN2a, ANUBL1, PTPN4, GRAMD1A, DDHD1
hsa-miR-193b	0.04	0.11	2.65	1.00	6.69	0.08	16p13.12	ABI2, IL17RD, ERBB4, FHDC1, FLI1
hsa-miR-196a*	0.03	0.13	4.22	2.77	5.93	0.03	12q13.13	UFM1, NGFRAP1, WWTR1, SNCA, NXT1
hsa-miR-198	0.39	0.85	2.19	-1.12	10.73	0.22	3q13.33	OTX1, NRIP1, ADAM12, H3F3A, FUT8
hsa-miR-199a-3p/ hsa-miR-199b-3p	0.03	0.08	2.92	-5.26	6.20	0.24	19p13.2,1q24.3/ 9q34.11	CELSR2, SH3GLB1, BCAR3, KLHL3, UQCRB

Table II. Continued.

Micro-RNA	Value		Fold			P-value	Chromosomal localization	Putative targets
	Normal	SCC	Mean	Min	Max			
hsa-miR-199a-5p	0.04	0.12	2.98	-10.3	5.64	0.25	19p13.2	ZNF763, ZNF776, ZNF439, ZNF468, ZNF563
hsa-miR-221*	0.82	3.24	3.95	1.38	21.73	0.02	Xp11.3	FBXW2, SLN, POU5F1P1, SDCCAG8, RAP1GAP
hsa-miR-223	0.05	0.11	2.38	1.78	3.14	0.04	Xq12	LMO2, RNF32, WDR62 , LELP1 , FBXO8
hsa-miR-302e	0.23	0.93	4.00	1.85	6.39	0.13	11p15.4	TGFBR2, GLIS3, GUCY1A3, CUGBP2, TXNIP
hsa-miR-302f	0.04	0.10	2.50	1.53	4.90	0.05	18q12.1	E2F3, UBE2G1, RCOR3, ELF2, UACA
hsa-miR-337-3p	0.03	0.09	2.78	1.32	3.88	0.05	14q32.31	SORCS1, IL13RA1, FAM76B, FAM104A, ENAH
hsa-miR-338-5p	0.20	0.92	4.66	-1.17	14.14	0.20	17q25.3	DGKB, BAT2D1, LARP4, PPP1R1A, CHL1
hsa-miR-340*	0.11	0.30	2.71	1.58	5.28	0.03	5q35.3	BANP, CETP, EMILIN1, IDS, AMELY
hsa-miR-34a	0.26	0.55	2.10	1.76	3.35	0.03	1p36.22	JAG1, WNT1, c-Met, SIRT1, CCND1
hsa-miR-34c-5p	0.08	0.17	2.23	1.00	6.38	0.10	11q23.1	NAV3, LGR4, MET, NAV1, MMAB
hsa-miR-377	0.03	0.09	2.69	1.46	12.5	0.07	14q32.31	PUM2, ETS1, GLS, XIAP, DCP1A
hsa-miR-423-5p	1.39	2.83	2.03	1.11	4.55	0.15	17q11.2	FOXP4, DMWD, CDC42SE1, BTBD14B, TMEM41A
hsa-miR-506	0.08	0.16	2.03	-1.25	5.16	0.31	Xq27.3	EYA4, PIK3C2A, ASPA, LAMC1, AGXT2L1
hsa-miR-517b	0.03	0.08	2.84	-2.42	9.56	0.19	19q13.41	LEMD3, ISL1, WNT4, LRRTM3, FUSIP1
hsa-miR-519d	0.60	1.54	2.57	1.22	3.62	0.20	19q13.41	EIF5A2, FYCO1, CYBRD1, PLEKHA3, MYT1L
hsa-miR-520c-3p	0.05	0.17	3.44	2.90	5.02	0.05	19q13.41	CROT, ZKSCAN1, TGFBR2, LATS2, RAB22A
hsa-miR-542-3p	1.0984	3.3267	3.03	-2.05	11.79	0.21	Xq26.3	TMEM65, ZNF618, SR140, YPEL5, R3HDM2
hsa-miR-575	0.0892	0.2019	2.26	1.22	4.86	0.05	4q21.22	GCLC, ST7L, RAB6IP1, WDFY3, RIPK4
hsa-miR-589	0.0491	0.1053	2.14	1.42	4.00	0.06	7p22.1	TNRC6A, PSMD9, RFXAP, NPTN, DIP2B
hsa-miR-601	0.23	0.91	3.96	2.30	9.83	0.02	9q33.2	LHFPL2, POU2F2, EEA1, SNN, CUL3
hsa-miR-603	0.03	0.08	2.30	-3.22	16.21	0.39	10p12.1	RIPK5, SOCS6, SP4, BMPR1B, PCNX
hsa-miR-616*	0.02	0.08	4.01	2.77	9.94	0.007	12q13.3	THUMPD2, CCL2, DRG2, PIK3C3, BSND
hsa-miR-618	0.01	0.05	4.48	-1.2	7.51	0.27	12q21.31	ATP11B, KLF9, XIAP, YTHDC1, PSTPIP2
hsa-miR-626	0.03	0.10	3.01	1.29	5.83	0.12	15q15.1	PAPOLB, BACH2, CENPP, ARFGAP3, RBM39
hsa-miR-875-3p	0.08	0.18	2.26	-1.36	7.25	0.14	8q22.2	ZNF654, ONECUT2, PGR, NDRG1, EGLN3
hsa-miR-934	1.11	2.28	2.05	1.30	3.32	0.07	Xq26.3	EAF1, BCLAF1, LRRN1, PTGFR, SLC35F1
hsa-miR-98	0.07	0.19	2.76	1.32	9.50	0.03	Xp11.22	HMGA2, PRTG, ACVR1C, GJC1, KCTD21
hsa-miR-99b	0.06	0.13	2.18	1.24	6.75	0.06	19q13.33	CTDSPL, FZD5, IGF1R, TRIB1, NXF1

hsa, homo sapiens; Max, maximum; Min, minimum. The top putative targets identified with TargetScan were included.

Table III. Statistics, location and putative targets of the micro-RNAs down-regulated in gastric cancer compared to normal gastric tissue.

Micro-RNA	Value		Fold			P-value	Chromosomal localization	Putative targets
	Normal	SCC	Mean	Min	Max			
hsa-let-7b*	0.43	0.04	10.86	1.22	22.34	0.16	22q13.31	RABGGTB, NPY5R, PCDH8, EPC1, MARK1
hsa-miR-103	0.70	0.29	2.37	1.34	3.62	0.26	20p13/5q35.1	DICER1, TMEM16C, NF1, FOXP1, EIF5
hsa-miR-107	0.43	0.14	3.10	1.17	4.45	0.25	10q23.31	HRB, AMMECR1, IGSF3, KIAA1804, CLCN5
hsa-miR-125b-1*	0.61	0.30	2.03	-1.14	4.66	0.10	11q24.1	IFITM5, SLC7A14, COL7A1, DDX49, RBP7
hsa-miR-1261	0.40	0.12	3.21	1.48	6.23	0.23	11q14.3	MIPOL1, SLC2A12, THAP6, MAML2, IGF1
hsa-miR-1275	0.44	0.17	2.60	1.75	3.44	0.12	6p21.31	IGF1, VAMP2, ABCF3, NFIX, PKNOX2
hsa-miR-1280	0.12	0.06	2.00	-1.59	4.94	0.49	3q21.3	TARDBP, ETS1, CREBL1, SCD, NCOR2
hsa-miR-1281	0.16	0.05	3.53	1.28	5.84	0.11	22q13.2	DAG1, HDAC4, LSM12, NRL, RTN2
hsa-miR-1290	1.52	0.71	2.14	1.20	3.05	0.13	1p36.13	EHHADH, RTKN2, ONECUT2, CBFA2T3, JARID1A
hsa-miR-129-5p	9.40	3.54	2.65	-1.28	5.70	0.21	7q32.1/11p11.2	TNRC6B, HRNBP3, TCF4, CACNG2, LDB3
hsa-miR-133b	0.77	0.06	13.18	-1.49	66.67	0.12	6p12.2	SYT2, LHFP, CCBL2, BRUNOL4, TTPAL
hsa-miR-141	2.55	1.14	2.23	1.81	2.65	0.24	12p13.31	ABL2, ZEB2, ATP8A1, RANBP6, KLF12
hsa-miR-149*	7.27	3.59	2.03	-1.41	6.11	0.24	2q37.3	G6PC3, ATN1, LMTK3, NRBP1, MCF2L
hsa-miR-150	2.72	1.27	2.14	-1.06	4.55	0.29	19q13.33	MYB, ADIPOR2, PDCD4, CBL, GABRA4
hsa-miR-155	0.19	0.07	2.61	1.37	4.00	0.23	21q21.3	IKIP, GABRA1, BACH1, JARID2, ZNF652
hsa-miR-155*	0.32	0.10	3.09	-8.25	4.52	0.36	21q21.3	CA14, TPM1, ZNF669, ZNF124, ZNF560
hsa-miR-15b	0.33	0.13	2.53	-5.27	4.15	0.30	3q26.1	SLC11A2, TMEM16C, SPRED1, PLAG1, TNRC6B
hsa-miR-185*	3.07	0.75	4.07	1.62	7.89	0.30	22q11.2	ZNFN1A4, AQP5, ESRRA, RAC3, RGS14
hsa-miR-200b*	1.69	0.74	2.30	1.28	3.46	0.35	1p36.33	ANKRD56, OR6X1, DAP3, PNN, SLC22A4
hsa-miR-219-2-3p	0.23	0.051	4.51	-1.01	6.44	0.32	9q34.11	SERP1, C1QTNF7, P2RY13, SEPHS1, SUV39H2
hsa-miR-24-2*	0.55	0.10	5.76	1.32	7.87	0.36	19p13.13	FOXA3, DNM3, SLC39A6, TMEM125, OSBPL9
hsa-miR-27a*	0.85	0.20	4.25	-1.66	5.91	0.38	19p13.13	CDK5, IFRD2, CDS2, KIAA1586, TRH
hsa-miR-320c	1.49	0.63	2.36	1.59	2.82	0.32	18q11.2	ONECUT2, KITLG, ABHD13, RIT1, SLC5A3
hsa-miR-34b	4.15	1.78	2.33	1.10	5.09	0.33	11q23.1	SLITRK3, KLHL28, FURIN, AZI2, BCAT1
hsa-miR-374a	0.73	0.08	8.83	-2.48	18.85	0.13	Xq13.2	ACVR2B, SPOPL, YOD1, STK38L, PAQR3
hsa-miR-374b	0.43	0.06	6.75	-2.57	56.59	0.16	Xq13.2	KIAA1333, TXLNB, TACC1, RRP15, TMEM123
hsa-miR-378	0.16	0.04	3.99	2.20	6.94	0.05	5q33.1	TOB2, KIAA1522, SDAD1, METTL4, CDC40
hsa-miR-423-3p	0.13	0.05	2.64	1.23	4.80	0.21	17q11.2	PABPC1, PANX2, BCORL1, NPHP4, NR1H2
hsa-miR-489	0.12	0.05	2.54	1.37	3.44	0.14	7q21.3	ETNK1, ALS2CR13, HRH4, LONRF2, SFRS7
hsa-miR-490-5p	0.23	0.08	2.88	-1.14	3.79	0.29	7q33	FOS, AFF2, RPS6KA3, ESR2, ARHGAP26
hsa-miR-509-5p	6.31	1.51	4.17	-1.14	7.35	0.12	Xq27.3	FIGN, FOXP1, TET1, ANKRD50, AFF3
hsa-miR-557	0.08	0.04	2.05	-1.33	3.48	0.35	1q24.2	BACH2, PRKCE, CAMK4, RBMS3, ADAM17
hsa-miR-574-5p	3.58	1.54	2.33	-1.16	3.69	0.17	4p14	CALCOCO1, RFX4, CD96, FOXN3, DHX40
hsa-miR-585	0.185	0.061	3.03	1.64	3.25	0.14	5q35.1	SMG1, FLRT3, FJX1, UBXD1, MLSTD1

Table III. Continued.

Micro-RNA	Value		Fold			P-value	Chromosomal localization	Putative targets
	Normal	SCC	Mean	Min	Max			
hsa-miR-602	0.80	0.33	2.45	-1.2	4.93	0.27	9q34.3	NOG, ODZ4, HTT, HABP4, FOXG1
hsa-miR-610	0.21	0.08	2.68	1.13	4.46	0.39	11p14.1	NIPA2, CREB5, NECAB1, MEF2A, LNPEP
hsa-miR-628-3p	16.02	1.87	8.57	4.63	11.84	0.08	15q21.3	PAIP1, CCDC4, MAMDC2, ATRX, FAM60A
hsa-miR-637	1.17	0.52	2.23	-2.49	7.49	0.27	19p13.3	RBM9, MNT, DAGLA, SGTA, GLP1R
hsa-miR-638	7.60	2.47	3.08	1.54	7.24	0.03	19p13.2	STARD10, NPAS4, PGK1, MKLN1, FAM80B
hsa-miR-656	0.32	0.09	3.74	-1.39	6.44	0.14	14q32.31	CPEB4, ARHGAP20, PURA, ARID2, CNTN4
hsa-miR-708	0.21	0.09	2.19	-1.34	3.89	0.37	11q14.1	GON4L, FOXJ3, JMJD6, GPM6A, NNAT
hsa-miR-720	2.00	0.59	3.40	1.97	3.85	0.12	3q26.1	DNMT3A, DCUN1D4, SAMD4B, KCTD15, HNRNPA2B1
hsa-miR-744	1.34	0.40	3.35	-1.52	6.78	0.23	17p12	KCNAB3, SH3BGRL3, KLC2, LRP3, GRIN2D
hsa-miR-874	0.22	0.12	1.87	2.22	3.23	0.39	5q31.2	HSPB7, RGS4, HEG1, SORCS1, FMR1
hsa-miR-933	3.57	1.41	2.52	1.02	4.83	0.22	2q31.1	BDNF, COL12A1, RAP2B, KCMF1, KPNA1
hsa-miR-943	13.16	3.65	3.61	2.19	5.69	0.19	4p16.3	ICK, BMP3, TMEM165, TMED5, GAS2

hsa, homo sapiens; Max, maximum; Min, minimum. The top putative targets identified with TargetScan were included.

Figure 2. Validation of miRNA microarray results by qRT-PCR in the gastric cancer sample. (A) The expression level of miR-638 in three pairs of samples analyzed by miRNA microarray and by qRT-PCR. (B) The expression level of miR-185 in three pairs of samples analyzed by miRNA microarray and by qRT-PCR.

Based on the hierarchical clustering observed in the miRNA expression patterns, the samples were divided into two groups: GC and normal tissue. Among the different GC samples, the miRNA expression profile was consistent. Cancer-associated genes were primarily up-regulated and miR-18a (20), miR-302f, miR-337-3p, miR-196a* and miR-616* were clustered into one group, while miR-17 (21), miR-106a (22), miR-223 (23), miR-520c-3p and miR-98 (24) were clustered into the other group.

Validation of miRNA microarray results by qRT-PCR. In order to confirm the results obtained from the miRNA microarray, the expression of miR-638 and miR-185 was analyzed by qRT-PCR in the samples analyzed on the microarray. Consistent with the results from the miRNA microarrays, miR-185 was up-regulated and miR-638 was down-regulated in each of the three gastric cancer samples (Fig. 2).

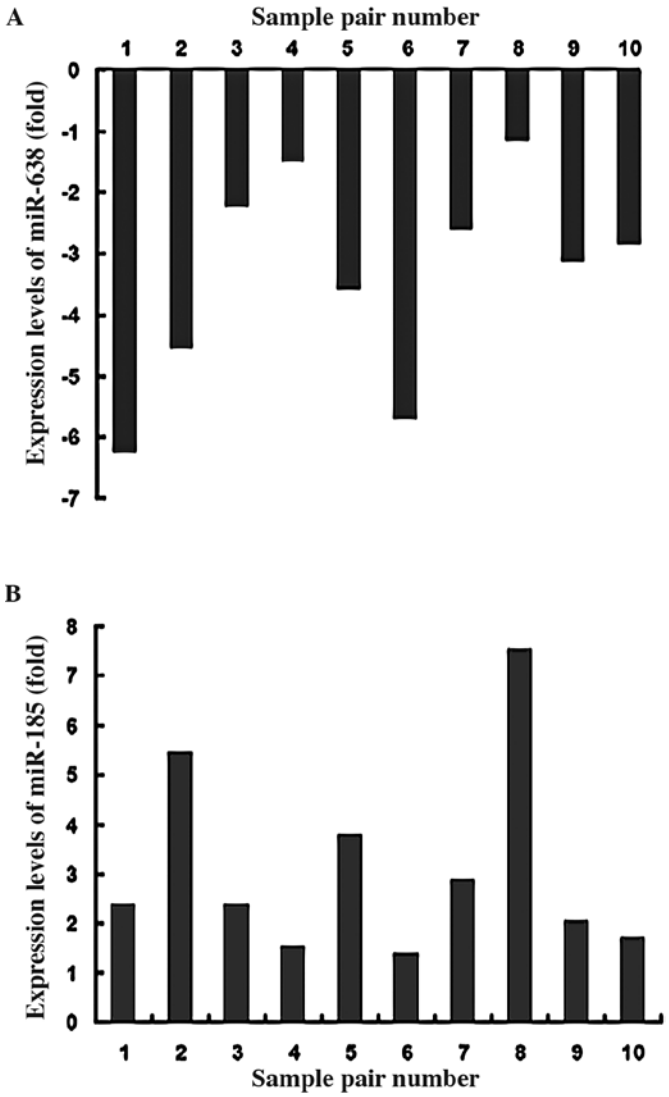

Figure 3. The expression level of miR-638 and miR-185 in ten pairs of gastric cancer and normal tissue samples. (A) The expression level of miR-638 in ten pairs of GC and normal tissue samples. (B) The expression levels of miR-185 in ten pairs of GC and normal tissue samples.

The expression of miR-185 and miR-638 in ten pairs of samples. The miRNAs with a more than 2-fold change were considered to be differentially expressed between the gastric tissue and gastric cancer. We evaluated the expression of miR-185 and miR-638 in ten pairs of samples by real-time PCR. miR-638 was down-regulated in eight of ten GC samples and miR-185 was up-regulated in seven of ten GC samples (Fig. 3).

Discussion

There have been a number of studies that have directly profiled miRNA expression in cancer, including head and neck squamous cell carcinoma (25) and lung (26), hepatocellular (27), breast (28) and colon (29) cancer. Furthermore, groups of miRNAs have been identified that either characterize neoplastic tissue or act as prognostic markers for patients (30,31). However, current and comprehensive data on a microRNA signature of GC in the Chinese population have not been reported.

We used a miRNA expression array to determine the miRNA profiles of GC and normal gastric tissue. Our results show that the miRNA expression profile can distinguish GC from normal gastric tissue. Furthermore, GC samples can be grouped into one cluster (Fig. 1). The expression level of miR-638b and miR-185 was verified by qRT-PCR and was consistent with the results obtained using miRNA microarray in the same samples (Fig. 2). Most of the miRNAs that were differentially expressed in GC showed an expression pattern similar to other cancers in previously published studies.

The microRNAs encoded by the oncogenic miR-17-92 cluster and its paralog, the miR-106b-25 cluster, are among those that have been found to be differentially expressed in human cancers. The oncogenic miR-17-92 cluster is composed of miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1. Recently, a large-scale analysis of the miRNA profiles of solid tumors detected an up-regulation of the human miR-17-92 cluster in many cancers, including lung (32) and colorectal cancer (33). The miR-17-92 paralog is composed of the highly conserved miR-106b, miR-93 and miR-25 genes, which accumulate in different types of cancer, including gastric, prostate and pancreatic neuroendocrine tumors, neuroblastoma and multiple myeloma. Both the miR-106b-25 and the miR-17-92 clusters have been shown to regulate the MYC/E2F1/TGFh network. MYC and E2F1 induced the expression of the MCM7 and C13ORF25 miRNA host genes. The subsequent overexpression of the miR-106 family (miR-106b, miR-93, miR-17 and miR-20a) down-regulated the cell cycle inhibitor p21, thereby impairing TGFh-dependent cell cycle arrest. In contrast, the overexpression of miR-25/miR-92 interfered with TGFh-induced apoptosis and inhibited BIM expression (34). In this study, we observed the up-regulation of miR-18a, miR-17, miR-106a and miR-106b (Table II), which is consistent with a study by Guo *et al* (35). miR-18a expression has been shown to be significantly higher in various cancer tissues compared to normal tissue (36,37). Furthermore, a miR-18a inhibitor moderately attenuated anaplastic thyroid cell growth (38), and recent studies have identified estrogen receptor-α (ERα) as a target of miR-18a. The overexpression of miR-18a decreased the ERα level but stimulated the proliferation of hepatoma cells (20). Some additional miRNAs identified in our study, including miR-147 (39), miR-185, miR-340* (40) and miR-575 (41), have been shown to be highly expressed in other types of cancer as well. Furthermore, miR-616*, miR-181a-2*, miR-1259, miR-601, miR-196a*, miR-221*, miR-302f, miR-337-3p and miR-520c-3p have also been found to be highly expressed in cancer cells.

Some putative tumor-suppressor miRNAs were up-regulated in GC (Table II), including miR-138 (42), miR-223 (43) and miR-34a (44); however, only miR-638 and miR-378 were significantly down-regulated in GC (Table III). The up-regulation of miR-638 has been observed in lung fibroblasts upon hydrogen peroxide-induced premature senescence. In our study, miR-638 was down-regulated in eight of ten GC samples (Fig. 3A). The down-regulation of miR-378 was also found in a study by Guo *et al* (35).

Our results show that the expression of microRNAs is de-regulated in GC, suggesting the involvement of these genes in the development and progression of GC.

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