RESEARCH COMMUNICATION

Differential miRNA Expression Profiles in Bladder Urothelial Carcinomas

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Abstract

The urothelial carcinoma is the most common pathological type of bladder tumor. Creation of lists of miRNAs differentially expressed between this tumor type and normal tissue might help identify new diagnostic and prognostic markers. We therefore performed the present miRNA microarray analysis with 25 cases of bladder urothelial carcinomas and adjacent normal bladder tissue. The results showed a panel of 51 differentially expressed miRNAs with at least 2-fold differences in expression compared with the normal controls, including 20 up-regulated and 31 down-regulated examples. The expression levels of ten of the top dysregulated miRNAs, mir-1, mir-145, mir-143, mir-100, mir-200b, mir-708, mir-133a, mir-133b, mir-125b and mir-99 were experimentally verified using real-time RT-PCR analysis. These findings suggest that these miRNAs may be involved in bladder urothelial carcinoma pathogenesis and have potential as biomarkers.

Keywords: Bladder urothelial carcinoma - microarray - miRNA

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Introduction

Bladder cancer is one of the world's top ten malignant tumors, among them bladder urothelial carcinoma is the most common pathological type, and accounts for 90% of bladder tumors, with the 50-70% recurrence rate after surgery (Prout et al., 1992). The diagnosis of bladder urothelial carcinoma depends on cystoscopy, but cystoscopy is invasive examination, and there are difficulties for the tumor diagnosis that the naked eye can not see, and also for upper urinary tract tumors can not be diagnosed (Raitanen et al., 2001). The examination of traditional exfoliocytology in urine appears high specificity, and high sensitivity on the high-grade bladder cancer, but for the low-grade tumors it has the lower sensitivity (Inoue et al., 2000). Therefore, it is necessary to explore more effective, convenient, safe diagnostic technology for early detection, early prevention and early treatment and monitoring recurrence of bladder urothelial carcinoma.

MicroRNA (miRNA) is a kind of non-coding small RNA with functions of regulating gene expression in eukaryotes. MicroRNA combining with the RISC can suppress the translation of specific messenger RNA or to cut it. Many researches showed the role of miRNAs in the etiology and pathogenesis of cancer by targeting oncogenes or tumor suppressors. Some miRNAs were demonstrated to be dysregulated in diverse cancer subtypes, including hepatocellular carcinoma, lung cancer, breast cancer, colorectal cancer, and other cancers (Nelson and Weiss, 2008; Osaki et al., 2008). A MicroRNA expression profile defined the invasive bladder tumor phenotype, so the MicroRNA expression profile might be used as diagnostic biomarker of bladder urothelial carcinoma.

In order to find the biomarkers in the early diagnosis, and to further reveal pathogenesis of bladder urothelial carcinoma, the differential expression of miRNA in bladder urothelial carcinoma tissue and adjacent normal bladder tissue was investigated using LC-miRNA microarray and real-time quantitative PCR technology in the present study.

Materials and Methods

Specimen collection

Clinical surgery specimens of bladder urothelial carcinoma tissue were collected in 25 cases, including 15 males and 10 females, aged 40-77 years old, average 60.2 years old. Control group from the adjacent normal bladder tissue.

RNA preparation

Total RNA was isolated from bladder urothelial carcinoma tissue and adjacent normal bladder tissue using TRIZOL Reagent (Invitrogen Cat. No. 15596-026) following the manufacturer's protocol. The integrity and purity of the total RNA was checked on a 1% denaturing agarose gel containing 0.65% (v/v; 0.22 M) formaldehyde. The quality and concentration of total RNA were determined by a DU640 nucleic and protein

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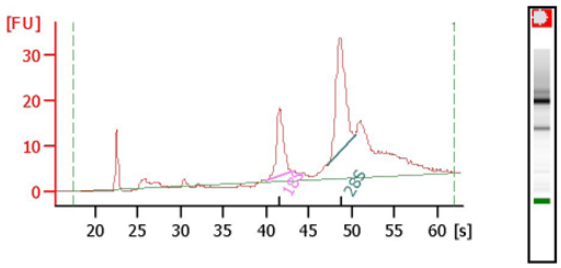

Figure 1. Gel Analysis of Total RNA Extraction from Bladder Urothelial Carcinoma Tissue. The green band on the gel (Right) represents miRNA. The graph is a bio-analysis f the RNA extraction sample, with 18S and 28S used as quality standards.

analyzer (Pharmacia). The quality of extracted RNA was assessed by electropherogram and gel analysis, and an RNA integrity number (RIN) was calculated for every case. Only cases with RINN7 were included (Figure 1).

Microarray hybridization

Less than 300nt small RNA was isolated from 20μg total RNA by centrifugal filtration column (YM-100, Millipore) and was added the poly (A) tail on the 3 'end by Poly (A) polymerase (Ambion), and then an oligonucleotide tag and the poly (A) tail connection for subsequent fluorescent labeling. Cy3 and Cy5 fluorescent tag were used to label bladder urothelial carcinoma and its control. Hybridization buffer contained 25% formamide in 100 μL 6 × SSPE buffer. Hybridizations were performed on LC-miRNA microarray (μParaflo™ , LC Sciences) for overnight at 34°C in the micro-circulation pump hybrid instrument (LC Sciences). Each region on the LC-miRNA microarray comprises a miRNA probe region, which detects miRNA transcripts listed in Sanger miRBase Release 11.0 (http://www.sanger.ac.uk/Software/Rfam/mirna/).

Data acquisition, processing and analysis

Microarrays were scanned and controlled using a GenePix 4100B microarray scanner (Axon Instruments, Inc.), analyzed by the software Array-Pro (Media Cybernetics). The background was calculated locally for each spot, calculate the average and standard deviation of duplicate points, then data were normalized by LOWESS (Locally-Weighted Regression) filter. The ratio of two test signals (log2) and t-test p-value were calculated, p value <0.01 defined significant differences in expression.

Table 1. Primers Used for Real-time Quantitative PCR

Sequence name	Primer (5'-3')
QmiR-RT	GCGAGCACAGAATTAATACGACTCAC TATAGGTTTTTTTTTTTTTT
QmiR-reverse	GCGAGCACAGAATTAATACGAC
U6-forward	CGCTTCGGCAGCACATATACTA
U6-reverse	CGCTTCACGAATTTGCGTGTCA
hsa-miR-1	TGGAATGTAAAGAAGTATGTAT
hsa-miR-145	GTCCAGTTTTCCCAGGAATCCCT
hsa-miR-143	TGAGATGAAGCACTGTAGCTC
hsa-miR-100	AACCCGTAGATCCGAACTTGTG
hsa-miR-708	AAGGAGCTTACAATCTAGCTGGG
hsa-miR-133a	TTTGGTCCCCTTCAACCAGCTG
hsa-miR-200b	TAATACTGCCTGGTAATGATGA
hsa-miR-133b	TTTGGTCCCCTTCAACCAGCTA
hsa-miR-125b	TCCCTGAGACCCTAACTTGTGA
hsa-miR-99a	AACCCGTAGATCCGATCTTGTG

Real- time quantitative PCR

There are 10 differential expressed miRNA were chosen for quantitative reverse transcription PCR (QRT-PCR) verification, using the same tissues as microarray. Synthesis of single-stranded cDNA from the total RNA was performed using the Reverse Transcriptase (promega). Quantitative PCR was performed on the Mx 3000P fluorescence quantitative PCR instrument (Stratagene Inc.) using the sybr green kit (Qiagen) by primer (Table 1). The amount of miRNA in each sample was normalized to the level of U6.

Results

The creation of lists of miRNAs differentially expressed between tumor and normal tissues gives the chance to identify the miRNAs most probably involved in cancer and to identify new diagnostic and prognostic markers. To identify miRNAs differentially expressed between bladder urothelial carcinoma tissue and adjacent normal bladder tissue, we investigated 25 bladder urothelial carcinoma patients in clinic employed the LC-miRNA microarray and differeitial expressed miRNA were validated by real-time quantitative PCR technique.

MiRNAs differentially expressed in bladder urothelial carcinoma tissue and adjacent normal bladder tissue

When comparing miRNA expression profile of bladder urothelial carcinoma tissue and adjacent normal bladder tissue employing the LC-miRNA microarray, we identified a panel of 51 differentially expressed miRNAs with at least 2-fold differences in expression compared with the normal controls as summarized in Table 2. We performed microarray analysis on three biological replicates. These miRNAs were classified as high and low signal groups. Among the high signal group, 20 miRNAs were up-and 31 down-regulated. Figure 2 shows a representative heat map of statistically significant (p<0.05) results.

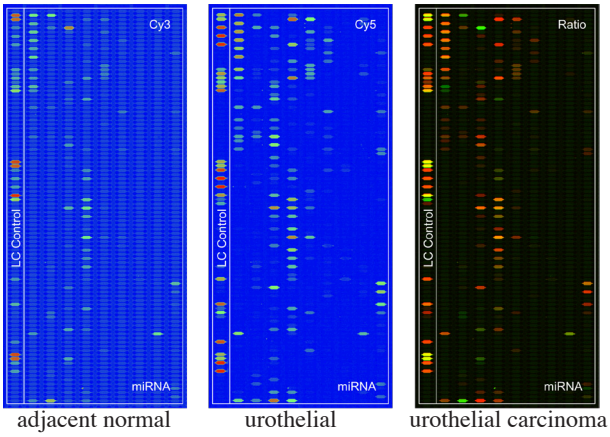

Figure 2. Microarray Heat Map Showing Statistically Significant (p<0.05) Dysregulated miRNAs in Bladder Urothelial Carcinoma

Table 2. miRNAs Differentially Expressed in Bladder Urothelial Carcinoma Tissue and Adjacent Normal Bladder Tissue

No.	Probe_ID	adjacent normal bladder sample signal	bladder urothelial carcinoma sample signal	log2 (bladder urothelial carcinoma / adjacent normal bladder)
1	hsa-miR-1	3,604.26	4.93	-9.36
2	hsa-miR-145	53,946.14	596.19	-6.54
3	hsa-miR-143	30,713.93	535.24	-5.81
4	hsa-miR-100	1,883.02	42.47	-5.47
5	hsa-miR-708	4.16	124.64	5.09
6	hsa-miR-133a	479.04	15.42	-5.05
7	hsa-miR-200b	433.21	14,445.37	5.01
8	hsa-miR-133b	413.85	13.53	-4.95
9	hsa-miR-125b	14,177.02	481.57	-4.88
10	hsa-miR-99a	757.71	27.93	-4.81
11	hsa-miR-214	3,476.09	169.48	-4.34
12	hsa-miR-200c	854.31	16,316.92	4.22
13	hsa-miR-199a-3p	3,794.09	220.34	-4.06
14	hsa-miR-199a-5p	431.76	27.40	-3.93
15	hsa-miR-182	149.80	2,338.14	3.90
16	hsa-miR-205	548.30	6,696.52	3.58
17	hsa-miR-429	56.34	531.88	3.55
18	hsa-miR-584	41.20	284.40	3.40
19	hsa-miR-10a	91.80	955.89	3.39
20	hsa-miR-532-5p	19.05	121.90	3.37
21	hsa-miR-145*	191.49	17.84	-3.30
22	hsa-miR-195	4,141.61	442.95	-3.14
23	hsa-miR-497	138.17	15.17	-3.11
24	hsa-miR-455-3p	27.67	169.96	3.11
25	hsa-miR-92b	345.30	2,897.06	3.09
26	hsa-miR-652	11.63	96.08	3.05
27	hsa-miR-183	108.83	910.72	3.03
28	hsa-miR-638	11,242.05	1,455.05	-2.96
29	hsa-miR-505*	27.55	134.01	2.83
30	hsa-miR-146a	139.85	972.84	2.81
31	hsa-miR-20b	57.10	365.90	2.75
32	hsa-miR-422a	14.19	86.68	2.73
33	hsa-miR-374b	95.99	626.50	2.67
34	hsa-miR-25	445.08	2,653.87	2.65
35	hsa-miR-30e*	41.01	204.13	2.61
36	hsa-miR-425	114.00	662.46	2.59
37	hsa-miR-155	133.73	734.10	2.56
38	hsa-miR-768-5p	139.63	806.12	2.55
39	hsa-miR-150	243.02	1,305.44	2.50
40	hsa-miR-210	19.39	99.10	2.45
41	hsa-miR-200b*	86.94	447.47	2.38
42	hsa-miR-15b	993.78	5,044.81	2.37
43	hsa-miR-92a	1,156.69	5,929.08	2.37
44	hsa-miR-222	3,263.41	588.22	-2.36
45	hsa-miR-130a	118.48	25.27	-2.23
46	hsa-miR-200a	233.19	1,070.48	2.22
47	hsa-miR-221	2,034.33	418.27	-2.18
48	hsa-miR-139-5p	85.36	19.55	-2.16
49	hsa-miR-141	63.39	277.70	2.11
50	hsa-miR-148b	31.35	127.21	2.02
51	hsa-miR-663	837.86	201.94	-2.01
52	hsa-miR-15a	115.23	445.59	1.98
53	hsa-miR-421	28.24	109.21	1.97
54	hsa-miR-146b-5p	67.31	227.41	1.84
55	hsa-miR-152	153.18	41.72	-1.84
56	hsa-miR-21	3,149.21	11,183.35	1.83
57	hsa-miR-342-3p	91.07	318.24	1.72
58	hsa-miR-93	169.55	562.73	1.71
59	hsa-miR-98	195.78	634.68	1.70
60	hsa-miR-486-5p	58.32	189.16	1.70
61	hsa-miR-181b	52.82	191.85	1.69
62	hsa-miR-193a-5p	169.79	53.50	-1.67
63	hsa-miR-151-3p	162.05	489.70	1.67
64	hsa-miR-22	364.21	114.65	-1.67
65	hsa-miR-30c	1,348.57	4,228.91	1.66
66	hsa-miR-191	1,439.11	4,437.86	1.66
67	hsa-miR-361-5p	656.52	1,974.17	1.63
68	hsa-miR-29a	3,001.22	965.11	-1.62
69	hsa-miR-106a	253.90	758.16	1.61
70	hsa-miR-331-3p	165.22	54.45	-1.60
71	hsa-miR-149*	1,072.24	335.25	-1.60
72	hsa-miR-1280	249.27	729.91	1.57
73	hsa-miR-20a	320.98	937.61	1.56
74	hsa-miR-10b	273.44	728.63	1.56
75	hsa-miR-17	310.66	826.22	1.49
76	hsa-miR-24	4,763.84	1,730.10	-1.46
77	hsa-miR-106b	182.76	499.12	1.43
78	hsa-miR-768-3p	239.29	608.96	1.40
79	hsa-miR-30e	118.68	303.23	1.39
80	hsa-miR-99b	862.82	317.89	-1.38
81	hsa-miR-151-5p	870.13	2,222.20	1.37
82	hsa-miR-423-5p	539.07	1,287.77	1.27
83	hsa-miR-30b	1,476.99	3,551.86	1.26
84	hsa-miR-128	131.30	308.99	1.26
85	hsa-miR-34a	191.84	453.40	1.22
86	hsa-miR-27b	3,757.88	1,643.90	-1.17
87	hsa-miR-26b	2,627.39	5,559.10	1.11
88	hsa-miR-31	171.27	362.07	1.08
89	hsa-miR-483-5p	365.15	174.17	-1.08
90	hsa-let-7g	3,147.08	6,232.04	1.05
91	hsa-miR-185	111.92	226.09	1.04
92	hsa-miR-181a	168.45	338.48	1.00
93	hsa-miR-1826	6,115.63	10,529.93	0.82
94	hsa-miR-23b	12,715.32	7,017.57	-0.80
95	hsa-miR-923	7,218.06	4,336.55	-0.73
96	hsa-miR-29c	987.77	1,511.75	0.68
97	hsa-miR-320a	1,753.31	1,116.31	-0.66
98	hsa-miR-1275	518.04	797.47	0.66
99	hsa-miR-378	229.31	351.53	0.62
100	hsa-miR-1246	3,389.79	5,220.49	0.60
101	hsa-miR-16	3,155.27	4,601.37	0.58
102	hsa-miR-720	364.66	538.18	0.57
103	hsa-miR-27a	2,363.82	1,518.44	-0.54
104	hsa-miR-320c	1,673.40	1,142.01	-0.54
105	hsa-miR-30a	578.70	822.51	0.52
106	hsa-let-7e	3,897.02	5,407.56	0.47
107	hsa-miR-320d	1,130.11	820.75	-0.46
108	hsa-miR-320b	1,384.77	1,025.15	-0.43
109	hsa-let-7i	3,279.81	2,423.36	-0.40
110	hsa-let-7f	12,074.60	9,468.13	-0.35
111	hsa-let-7b	6,146.29	8,144.66	0.32
112	hsa-miR-26a	13,583.91	10,704.62	-0.32
113	hsa-miR-23a	9,208.11	6,984.74	-0.32
114	hsa-let-7a	14,314.01	11,660.58	-0.30
115	hsa-let-7d	7,606.45	9,244.20	0.28

Validation of differentially expressed miRNAs by the real time quantitative fluorescence PCR

We used the real-time RT-PCR analysis for precursor miRNAs to validate the results from microarray analysis (Table 2). The expression levels of ten of the top dysregulated miRNAs, mir-1, mir-145, mir-143, mir-100, mir-200b, mir-708, mir-133a, mir-133b, mir-125b, and mir-99 were experimentally verified on bladder urothelial

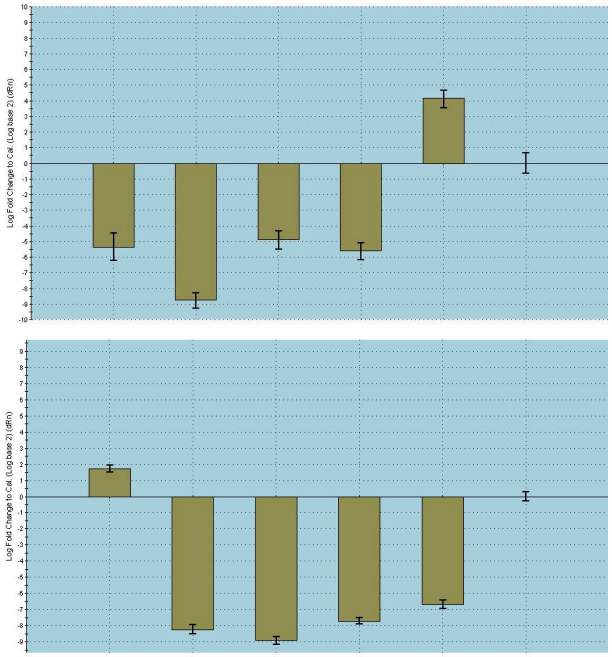

Figure 3. Quantitative RT-PCR Validation of the Expression of mir-1, mir-145, mir-143, mir-100, mir-200b, mir-708, mir-133a, mir-133b, mir-125b, and mir-99 in Bladder Urothelial Carcinoma

carcinoma tissue and adjacent normal bladder tissue with miRNA-specific primer using qRT-PCR, and then, real-time RT-PCR analysis for these miRNAs and U6 as an endogenous control were performed. Results (Figure 3) showed that the mir-200b and mir-708 expression were up-regulated in bladder urothelial carcinoma, the expression of mir-1, mir-145, mir-143, mir-100, mir-133a, mir-133b, mir-125b, and mir-99 were down-regulated, as was seen in the results by using microarray analysis. Therefore, the analyses confirmed the microarray data for these ten miRNAs. And, microarray data were believable.

Discussion

Micro-RNAs (miRNAs) regulate one-third of all protein-coding genes and are fundamental in the pathophysiology of a wide range of diseases. Studies showed miRNAs have been related to the regulation of different biological processes, such as insulin regulation (Poy et al., 2004; Baroukh et al., 2007), lipid metabolism (Esau et al., 2006), synaptic activity (Ashraf and Kunes, 2006), cell differentiation (Sugatani and Hruska, 2007) and inflammatory response (O'Connell et al., 2007; Thai et al., 2007). Therefore, the creation of lists of miRNAs differentially expressed between tumor and normal tissues gives the chance to identify the miRNAs most probably involved in cancer and to identify new diagnostic and prognostic markers. The majority of the published papers that reported gene expression profiling analysis were performed using microarray technology. The present study employed it also. We reported here that the expression profile of miRNAs was significantly different between bladder urothelial carcinoma tissue and adjacent normal bladder tissue using microarray technology. The microarray data were validated by real-time RT-PCR analysis for 10 miRNAs, mir-1, mir-145, mir-143, mir-100, mir-200b, mir-708, mir-133a, mir-133b, mir-125b, and mir-99. These miRNAs may be involved in bladder urothelial carcinoma pathogenesis and have the potential to be biomarkers.

miR-1 and miR-133a clustered on the same chromosomal loci, have been described as muscle, in regulation of muscle cell growth and differentiation, or cardiac specific that the altered expression of miR-1 and miR-133 in hearts also contributed to cardiovascular diseases, such as heart ischemia, cardiac hypertrophy, and arrhythmias (Cai et al., 2010). However, miR-1 and miR-133 were correlation with the different cancer. For example, miR-1 and miR-133 seem to be important regulators of heart adaptation after ischaemic stress (Bostjancic et al., 2010), miR-1 and miR-133a were disregulation in colon cancer (Sarver et al., 2009) and miR-133a tends to be higher in patients with coronary artery disease (Fichtlscherer et al., 2010). Levels of miR-1 and miR-133a are drastically reduced in representative cell lines from each major rhabdomyosarcoma subtype (embryonal and alveolar), mRNA targets of miR-1 and miR-133a are up-regulated in rhabdomyosarcomas (Rao et al., 2010). Moreover, miR-1 and miR-143 were down-regulated in lung tumors relative to normal lungs (Melkamu et al., 2010), and miR-1 were significantly associated with overall survival of non-small-cell lung cancer (NSCLC) (Hu et al., 2010). Muscle-specific miR-1 was highly upregulated in differentiated cardiomyocyte progenitor cells (CMPCs), transient transfection of miR-1 in CMPC reduced proliferation rate by 25% and 15%, respectively, and enhanced differentiation into cardiomyocytes in human CMPCs and embryonic stem cells. So by modulating miR-1 expression levels, human CMPC function can be altered and differentiation directed, thereby enhancing cardiomyogenic differentiation (Sluijter et al., 2010). Studies also showed miR-145, miR-133a and miR-133b as candidate tumor suppressors have conserved sequences in the 3'UTR of FSCN1 (actin-binding protein, Fascin homolog 1), inhibited of FSCN1 expression and target FSCN1 in esophageal squamous cell carcinoma (Kano et al., 2010). In bladder cancer (BC), miR-145 and miR-133a were down-regulated and directly control oncogenic FSCN1 (Chiyomaru et al., 2010). Moreover in bronchial smooth muscle (BSM), miR-133a negatively regulated RhoA expression, and IL-13 might, at least in part, contribute to the reduction of miR-133a (Chiba et al., 2009). Our present study showed miR-1, miR-133a mir-133b, miR-143 and miR-145 were down-regulated in bladder urothelial carcinoma. Taken together, these miRNAs may contribute to tumorigenesis, might be serve as biomarkers for tumor.

A computational survey of the human and mouse genomes revealed that miR-125b are members of a multigene family located in paralogous clusters. The miR-125b cluster on chromosome 21 includes miR-99a and miR-let-7c. Study showed miR-125b promotes B lymphocyte diversification in GC by inhibiting premature utilization of essential transcription factors for plasma cell differentiation (Gururajan et al., 2010). Moreover, miR-125b was implicated to have close relationship with cell proliferation and differentiation and down-regulation of

miR-125b was observed in various types of cancers. For example, In 160 paired samples of non-tumour mucosa and cancer, miR-125b, miR-199a, and miR-100 were the most important microRNAs involved in the progression-related signature (Ueda et al., 2010). Otherwise, miR-125b plays a central role in conferring Taxol resistance through the suppression of Bak1 expression, might be important for the development of targeted therapeutics for overcoming Taxol resistance in a number of different tumor histologies (Zhou et al., 2010). Research in animal showed that the expression of miR-125b was significantly decreased in bladder cancer tissues and four bladder cancer cell lines. Moreover, miR-125b could suppress bladder cancer cells to form colonies in vitro and to develop tumors in nude mice. miR-125b may regulate G1/S transition through the E2F3-Cyclin A2 signaling pathway (Huang et al., 2010). Result of the present study is the same as this. Taken together, miR-125b may act as a tumor suppressor in bladder urothelium and down-regulation of miR-125b may contribute to the tumorigenesis of bladder cancer.

miR-143 and miR-145, which are located approximately 1.3 kb from each other at chromosome 5q33, are highly expressed in several tissues, but down-regulated in most cancers. miR-143 is a miRNA involved in tumorigenesis in multiple types of cancer, smooth muscle cell fate and adipocyte differentiation. miR-145 was under-expressed in all cancer cell lines, including breast cancer, prostate cancer, glioblastoma, and lung cancer (Roa et al., 2010). miR-143 and miR-145, which are coexpressed from a single promoter, have been shown to play a crucial role in regulating smooth muscle cells (SMCs) phenotypes and control neointima formation and atherosclerosis (Bonauer et al., 2010). Such as, miR-143 and miR-145 were found to be downregulated in the squamous cell lung carcinoma tissues compared with normal tissues (Gao et al., 2010). Down-regulation of miR-143 and miR -145 regulates the switch from contractile to synthetic phenotype, allowing SMCs to migrate and proliferate. Moreover, platelet-derived growth factor (PDGF) mediates podosome formation in SMCs through the regulation of miR-143/145 expression via a pathway involving Src and p53, and key podosome regulators as targets of miR-143 (PDGF receptor alpha and protein kinase C epsilon) and miR-145 (fascin) (Quintavalle et al., 2010). Moreover, study showed p53/p73/p63 appears to regulate the processing of miR-143 and miR-145 (Boominathan, 2010). miR-143 and miR-145 are involved in the regulation of mRNA for myosin VI (MYO6) expression and possibly in the development of prostate cancer (Szczyrba et al., 2010). Otherwise, miR-143 and -145 are important onco-related genes for the initiation step of colorectal tumor development and that the chemically modified synthetic miR-143 may be a hopeful candidate as an RNA medicine for the treatment of colorectal tumors (Akao et al., 2010). Combining with our results, modulation of miR-143 and miR-145 may be an important therapeutic approach for the management of cancer including bladder urothelial carcinoma.

The miR-200 family involve in the many kinds of disease. For example, miR-200 was upregulated early after ischemic preconditioning and the miR-200 family was neuroprotective mainly by downregulating prolyl hydroxylase 2 levels (Lee et al., 2010). The development of nonalcoholic steatohepatitis (NASH) was accompanied by prominent changes in the expression of miR-200b (Pogribny et al., 2010). Inhibition of miR-200b increased sensitivity to gemcitabine in cholangiocarcinoma cell lines (Hummel et al., 2010). Intrarenal expression of miR-200a and miR-200b were increased in hypertensive nephrosclerosis, and the degree of upregulation correlated with disease severity (Wanga et al., 2010). Urinary expression of miR-200a and miR-200b were down-regulated in patients with IgA nephropathy (IgAN), and the degree of reduction correlated with disease severity and rate of progression (Wangb et al., 2010). Besides above all, the expression of miRNAs of the miR-200 family has a tight association with epithelial phenotype, and sensitivity to epidermal growth factor receptor (EGFR) inhibitors-induced growth inhibition in bladder carcinoma cell lines. Members of the miR-200 family appear to control the epithelial-to-mesenchymal transition (EMT) process and sensitivity to EGFR therapy in bladder cancer cells and the expression of miR-200 is sufficient to restore EGFR dependency at least in some of the mesenchymal bladder cancer cells. The targets of miR-200 include ERRFI-1, which is a novel regulator of EGFR-independent growth (Adam et al., 2009). Expression of miR-200a and miR-200b was decreased in EBV-associated gastric carcinoma, as compared with that in EBV-negative carcinoma (Shinozaki et al., 2010). miR-200b and miR-200c up-regulated E-cadherin through direct targeting of transcriptional repressors of E-cadherin, ZEB1, and ZEB2, inhibits EMT, a crucial process in the tumor progression (Tryndyak et al., 2010). Moreover, study showed combination of miR-21, miR-486, miR-375, and miR-200b produced the best prediction in distinguishing lung adenocarcinoma patients from normal subjects with 80.6% sensitivity and 91.7% specificity (Yu et al., 2010). miR-200c, miR-141, and miR-30b showed a sensitivity of 100% and a specificity of 96.2% in the diagnostic test, such a panel of miRNAs has the potential to identify invasive bladder tumors misclassified in pathologic assessment of bladder biopsy specimens (Wszolek et al., 2009). This study was supplementary for these results.

Functional analysis of miR-99a and miR-100 in adrenocortical tumors (ACT) cell lines showed that they coordinately regulate expression of the insulin-like growth factor-mammalian target of rapamycin (mTOR)-raptor signaling pathway through binding sites in their 3'-untranslatedregions (Doghman et al., 2010). Study showed also mir-100, a putative tumor suppressor, was down-regulated miRNA in human clear cell ovarian cancer cell lines, and its up-regulated target, FRAP1/mTOR (Nagaraja et al., 2010). Otherwise, miR-100 was required for proper differentiation of mouse embryonic stem cells (ESCs), and function in part by targeting Smarca5 mRNAs (Tarantino et al., 2010). miR-100 was differentially expressed in medulloblastoma specimens versus normal non-tumorous cerebellum tissues (Liu et al., 2009). Besides, the down-regulation of miR-125b and miR-100 in oral squamous cell carcinoma (OSCC) appears to play an important role in the development and/or progression

of disease and may contribute to the loss of sensitivity to ionizing radiation (Henson et al., 2009). miR-100 was significantly overexpressed by all localized high GS, pT3 prostate carcinoma (PC) in comparison with metastatic carcinoma (Leite et al., 2009). These results showed miR-100 involved in different tumor. Our results showed mir-100 was down-regulated in bladder urothelial carcinoma and suggested mir-100 might play important roles in the pathophysiology of bladder urothelial carcinoma.

Studies showed that miR-99 was significantly associated with pericellular fibrosis in non-alcoholic steatohepatitis (NASH) patients (Estep et al., 2010), remarkably down-regulated in lung cancer (Izzotti et al., 2009). A panel of miR-205, miR-210 and miR-708 in combination produced the best prediction in distinguishing lung squamous cell carcinoma patients from normal subjects with 73% sensitivity and 96% specificity. The sputum markers showed the potential to improve the early detection of lung squamous cell carcinomas (Xing et al., 2010). miR-708 involved in the acute lymphoblastic leukemia (ALL) (Schotte et al., 2009) and stage I non-small cell lung cancer (NSCLC) (Patnaik et al., 2010). Our results showed mir-708 expression were up-regulated in bladder urothelial carcinoma, while mir-99 were down-regulated. This suggested these miRNAs may contribute to tumorigenesis.

In summary, we believe miRNA expression patterns may give important clue for better prognostication of tumor. We identify a panel of 51 differentially expressed miRNAs in bladder urothelial carcinoma, that is 20 up-regulated miRNAs and 31 down-regulated, including mir-200b and mir-708 expression were up-regulated in bladder urothelial carcinoma, while the expression of mir-1, mir-145, mir-143, mir-100, mir-133a, mir-133b, mir-125b, and mir-99 were down-regulated. The present study indicated that these miRNAs may play important roles in the pathophysiology of bladder urothelial carcinoma, and suggested these miRNAs may contribute to tumorigenesis, serve as prognostication, diagnostic and prognostic biomarkers, also as therapeutic targets for bladder urothelial carcinoma. Further studies are needed to clarify the role of these miRNAs involved bladder urothelial carcinoma.

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