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The role of microRNAs in bladder cancer

Hideki Enokida, Hirofumi Yoshino, Ryosuke Matsushita, Masayuki Nakagawa
Department of Urology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

Bladder cancer (BC) is the fifth most common cancer worldwide and is associated with significant morbidity and mortality. The prognosis of muscle invasive BC is poor, and recurrence is common after radical surgery or chemotherapy. Therefore, new diagnostic methods and treatment modalities are critical. MicroRNAs (miRNAs), a class of small noncoding RNAs, regulate the expression of protein-coding genes by repressing translation or cleaving RNA transcripts in a sequence-specific manner. miRNAs have important roles in the regulation of genes involved in cancer development, progression, and metastasis. The availability of genome-wide miRNA expression profiles by deep sequencing technology has facilitated rapid and precise identification of aberrant miRNA expression in BC. Indeed, several miRNAs that are either upregulated or downregulated have been shown to have associations with significant cancer pathways. Furthermore, many miRNAs, including those that can be detected in urine and blood, have been studied as potential noninvasive tumor markers for diagnostic and prognostic purposes. Here, we searched PubMed for publications describing the role of miRNAs in BC by using the keywords "bladder cancer" and "microRNA" on March 1, 2016. We found 374 papers and selected articles written in English in which the level of scientific detail and reporting were sufficient and in which novel findings were demonstrated. In this review, we summarize these studies from the point of view of miRNA-related molecular networks (specific miRNAs and their targets) and miRNAs as tumor markers in BC. We also discuss future directions of miRNA studies in the context of therapeutic modalities.

Keywords: MicroRNA; Tumor biomarkers; Urinary bladder neoplasms

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INTRODUCTION

Bladder cancer (BC) is one of the leading causes of cancer-related death worldwide, with an estimated 429,800 new cases and 165,100 deaths globally in 2012 [1]. BCs can be categorized into 2 groups: nonmuscle invasive BC (NMIBC) and muscle invasive BC (MIBC). Approximately 70% to 80% of patients are diagnosed with NMIBC, and some of these patients have a high risk of recurrence and a variable risk of progression despite administration of local therapies [2]. The remaining 25% of patients with MIBC are managed by radical surgery or radiotherapy, but often still have poor outcomes, despite administration of these systemic therapies [2]. For patients with advanced BC or failure of first-line chemotherapy, no clearly defined second-line treatments have been shown to prolong overall survival [2]. Therefore, elucidation of novel molecular mechanisms of BC progression and identification of novel tumor markers that can predict recurrence or survival are urgently needed.

Despite its relatively low sensitivity (30%–40%), urinary cytology is a reliable marker for BC diagnosis because of its high specificity (90%–95%). However, because of the

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Corresponding Author: Hideki Enokida
Department of Urology, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
TEL: +81-99-275-5395, FAX: +81-99-275-6637, E-mail: enokida@m.kufm.kagoshima-u.ac.jp

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low sensitivity of urinary cytology, patients are still forced to undergo a painful cystoscopic procedure to confirm the initial diagnosis and to check for recurrence during follow-up examinations after undergoing transurethral resection of bladder tumors. Several new urine-based tests for BC, such as bladder tumor antigen, nuclear matrix protein 22 (NMP22, Sysmex Corp., Kobe, Japan), and FISH (UroVysion, Abbott Molecular Inc, Des Plaines, IL, USA), have been approved for clinical use. However, these new urinary markers have not been widely applied because they have relatively low specificities (60%–80%) compared with urinary cytology, despite their higher sensitivities (50%–70%) [3]. Thus, specificity may come at the cost of sensitivity, and conventional urinary cytology is a good example of this compromise. Accordingly, new effective diagnostic markers are urgently needed for patients with BC.

MicroRNAs (miRNAs) are endogenous small noncoding RNA molecules (19–22 bases in length) that negatively regulate the expression of protein-coding genes in a sequence-specific manner [4]. The nature of miRNAs is unique in that one miRNA can regulate multiple protein-coding RNAs. Bioinformatics predictions have indicated that miRNAs regulate 30% to 60% of the protein-coding genes in the human genome [5,6]. Growing evidence has demonstrated that aberrantly expressed miRNAs can act as oncogenes or tumor suppressors in various types of malignancies [7]. These miRNAs can disrupt tightly controlled RNA networks in cancer cells [8]. Since 2006, we have rigorously investigated the molecular networks of miRNAs and their target genes in clinical BC. Identification of aberrantly expressed miRNAs in BC could provide important clues for the investigation of novel molecular mechanisms of initiation, progression, and metastasis in BC. In this review, we discuss the role of miRNAs in BC on the basis of up-to-date results from our laboratory and other research groups.

CURRENT CONSENSUS OF EPIGENETIC GENE REGULATION BY NONCODING RNA

After the completion of the Human Genome Project in 2003, about 20,000 to 25,000 genes were identified, and the sequences of 3 billion base pairs of human DNA were determined [9]. Surprisingly, only 2% of the human genome encodes functional proteins; 50% to 75% of the human genome is transcribed, and 98% of the transcripts are not translated into protein. Fig. 1 shows the current consensus of epigenetic regulation by noncoding RNAs transcribed from approximately 70% of the genomic region that used to be considered as "junk DNA" [10]. These noncoding RNAs can be roughly classified into two groups according to

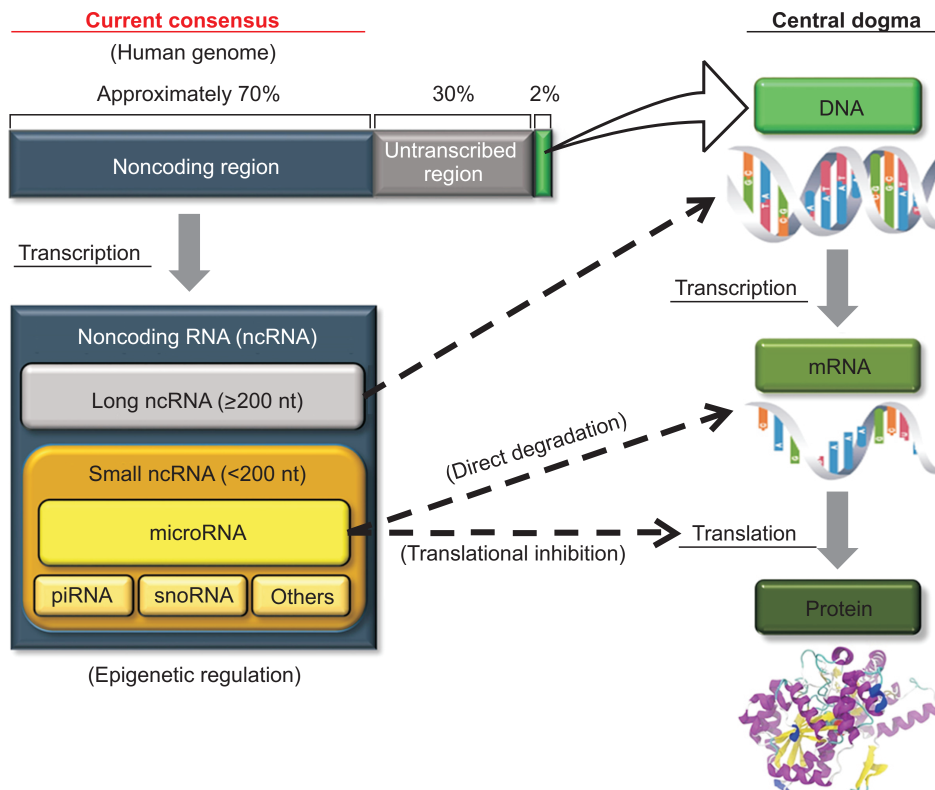

Fig. 1. Current consensus of epigenetic gene regulation by noncoding RNAs. microRNAs is classified as one of small noncoding RNAs, and epigenetically function as negative regulator of protein coding genes by complementary binding to the target messengerRNAs (mRNAs). ncRNA, noncoding RNA.

size. The first group includes short RNAs of less than 200 nucleotides (nt) in length; miRNAs, which are small RNA molecules of around 18 to 22 nt in length, fit into this group, as do other classes of small RNAs, such as piwi-interacting RNAs (around 23–30 nt) [11]. The other group includes long noncoding RNAs (lncRNAs) of around 200 nt or more. lncRNAs have been implicated in a range of developmental processes and diseases and can inhibit or activate gene expression through a range of diverse mechanisms. In contrast to miRNAs, which have been extensively studied and have well-understood functions in gene regulation, lncRNAs are not well studied or characterized.

MICRORNAS BIOGENESIS

miRNAs are evolutionarily conserved and are located either within the introns or exons of protein-coding genes (70%) or in intergenic regions (30%). Most intronic and exonic miRNAs are derived from their host genes, which suggests that they are transcribed concurrently with their host transcripts. Transcripts containing primary miRNAs (pri-miRNAs), which can vary from 200 nt to several kb in length, are capped with a specially modified nucleotide at the 5′-terminus and are polyadenylated with multiple adenosines at the 3′-end. Pri-miRNA is cleaved into precursor-miRNA (pre-miRNA: 60–70 nt in length) by the RNAse III enzyme (also known as Drosha). Pre-miRNA is exported from the nucleus into the cytoplasm by exportin-5. In the cytoplasm, pre-miRNA is cleaved by another RNase III enzyme, known as Dicer, into miRNA duplexes of approximately 19–22 nt in length. One miRNA duplex is then recruited into the RNA-induced silencing complex and functions to recognize complementary sites within the target messenger RNA (mRNA), thereby regulating translation through mRNA cleavage, degradation, or transcriptional repression [12].

MICRORNA PROFILING STUDIES IN BLADDER CANCER

miRNA expression profiling by array-based and polymerase chain reaction (PCR)-based methods is not commonly used because these methods only allow for identification of a limited number of known miRNAs. The rapid development of high-throughput, deep sequencing (DS) technology has provided novel information regarding the functions and features of miRNAs. Han et al. [13] first reported miRNA profiling in BC by using an Illumina Genome Analyzer IIX in 2011. Soon thereafter, five studies used DS technology to establish the miRNA profiles of clinical BC samples [14-18]. To date, the largest DS study on BC is a comprehensive investigation by The Cancer Genome Atlas Research Network [18]. Tissues from 131 patients with high-grade MIBC were analyzed by RNA sequencing together with 118 blood samples from the same individuals and 23 adjacent histologically proven normal tissues. The aim of the project was to carry out genetic and epigenetic characterization of the molecular landscape of high-grade MIBC. In our studies, we conducted miRNA profiling by use of both PCR-based methods and DS technology [17,19]. When comparing our 2 studies, the altered expression of miRNAs detected by DS technology was very similar to the results of PCR-based methods. In addition, a considerable number of novel miRNAs have been reported by some studies [13,14,17]. Therefore, this analysis supports that DS technology is superior to previously used methods. Although PCR-based and DS profiles are not comparable from a quantitative point of view, sequencing approaches should provide substantial improvements to transcriptome analyses.

MOLECULAR TARGETS OF MICRORNAS IN BLADDER CANCER

1. Features of miRNA studies in BC

Growing evidence has demonstrated that aberrantly expressed miRNAs can act as oncogenic miRNAs (onco-miRNAs) or tumor-suppressive miRNAs (TS-miRNAs) in BC [12,20]. Because miRNAs can construct miRNA-target gene networks that contribute to tumor initiation, survival, and invasion, many investigators have focused on the genes targeted by aberrantly expressed miRNAs in BC in comparison with normal bladder epithelium. In terms of miRNA analysis, luciferase-reporter assays are useful for confirming the direct binding of miRNAs to the specific 3′-untranslated region of the mRNA representing the target gene. Hence, we have summarized the miRNA studies in BC for which the target genes were validated by luciferase-reporter assays. To date, we have identified 78 studies that satisfied our criteria (Tables 1 and 2). As shown in Table 1, several TS-miRNAs, including *miR-1, miR-16, miR-24, miR-101, miR-125b, miR-129*, and *miR-133a*, have been shown to be located on different chromosomal regions but to have common sequences in their mature miRNAs. This suggests that the expression of these TS-miRNAs may be maintained by another genomic region, even though one region may be functionally disordered because of methylation, deletion, or mutation. This type of backup system is thought to have important roles in protecting normal cells from carcinogenesis.

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Table 1. miRNAs and their target genes validated by lusiferase reporter assay

miRNAs	Stem-loop sequence	Locus	Clustered miRNAs	miRNA type	Target	Reference
miR-1	miR-1-1	20q13	ND	TS	LASP1	38
	miR-1-2	18q11	miR-133a-1		PNP	73
					PTMA	73
					SRSF9	26
					TAGLN2	74
miR-16	miR-16-1	13q14	miR-15a	TS	CCND1	75
	miR-16-2	3q25	miR-15b			
miR-23b	miR-23b	9q22	miR-27b	TS	EGFR	24
			miR-3074		MET	24
			miR-24-1		ZEB1	40
miR-24	miR-24-1	9q22	miR-23b, miR-27b, miR-3074	TS	CARD10	76
	miR-24-2	19p13	miR-23a, miR-27a		FOXM1	32
miR-27a	miR-27a	19p13	miR-23a, miR-24-2	TS	RUNX1	77
					SLC7A11	78
miR-27b	miR-27b	9q22	miR-23b, miR-24-1, miR-3074	TS	DROSHA	79
					EGFR	24
					MET	24
miR-29c	miR-29c	1q32	miR-29b-2	TS	CDK6	80
miR-30a	miR-30a	6q13	ND	TS	NOTCH1	81
miR-34a	miR-34a	1p36	ND	TS	CD44	66
					HNF4G	82
					NOTCH1	83
miR-99a	miR-99a	21q21	let-7c	TS	FGFR3	46
miR-100	miR-100	11q24	ND	TS	MTOR	68
miR-101	miR-101-1	1p31	miR-3671	TS	COX2	84
	miR-101-2	9p24	ND		MET	85
					VEGFC	52
miR-124-3p	miR-124	20q13	ND	TS	ROCK1	86
					CDK4	87
miR-125b	miR-125b-1	11q24	ND	TS	E2F3	31
	miR-125b-2	21q21	ND		MMP13	88
					SPHK1	89
miR-128	miR-128-1	2q21	ND	TS	VEGFC	49
	miR-128-2	3p22	ND			
miR-129	miR-129-1	7q32	ND	TS	GALNT1	25
	miR-129-2	11p11	ND		SOX4	
miR-133a	miR-133a-1	18q11	miR-1-2	TS	EGFR	43
	miR-133a-2	20q13	ND		FSCN1	37
					GSTP1	90
					LASP1	38
					PNP	73
					PTMA	73
					TAGLN2	74
miR-133b	miR-133b	6p12	miR-206	TS	AKT1	47
					BCL2L2	47
					EGFR	43
miR-135a	miR-135a-1	3p21	ND	TS	FOXO1	91
	miR-135a-2	12q23	ND			
miR-138	miR-138-1	3p21	ND	TS	ZEB2	41
	miR-138-2	16q13	ND			

Table 1. Continued

miRNAs	Stem-loop sequence	Locus	Clustered miRNAs	miRNA type	Target	Reference
miR-143	miR-143	5q32	miR-145	TS	SERPIN1	22
					AKT	48
miR-144-5p/3p	miR-144	17q11	miR-451a, miR-451b, miR-4732	TS	CCNE1	28
					CCNE2	
					CDC25A	
					PKMYT1	
miR-145	miR-145	5q32	miR-143	TS	CBFB	92
					CLINT1	92
					FSCN1	37
					ILK	48
					PAK1	93
					PPP3CA	92
					SERPIN1	22
					SOCS7	94
					IGF1R	44
miR-186	miR-186	1p31	ND	TS	HMGN5	95
miR-193a-3p	miR-193a	17q11	ND	TS	LOXL4	96
					PSEN1	52
					HOXC9	97
miR-195	miR-195	17p13	miR-497	TS	BIRC5	17
					CDC42	98
					CDK4	99
					GLUT3	100
					WNT7A	17
miR-200b	miR-200b	1p36	miR-200a, miR-429	TS	MMP16	39
miR-200c	miR-200c	12p13	miR-141	TS	BMI1	30
					E2F3	
miR-203	miR-203	14q32	miR-203b	TS	BCL2L2	65
					BIRC5	
miR-214	miR-214	1q24	miR-199a-2, miR-3120	TS	PDRG1	101
miR-218	miR-218-1	4p15	ND	TS	BMI1	67
	miR-218-2	5q34	ND		LASP1	38
miR-221	miR-221	Xp11	miR-222	TS	STMN1	102
miR-320a	miR-320a	8p21	ND	TS	ITGB3	103
miR-320c	miR-320c	18q11	ND	TS	CDK6	104
miR-449a	miR-449a	5q11.2	miR-449b, miR-449c	TS	CDC25A	105
miR-485-5p	miR-485	14q32	ND	TS	HMGA2	106
miR-490-5p	miR-490	7q33	ND	TS	FOS	107
miR-493	miR-493	14q32	miR-337, miR-665	TS	FZD4	51
					RHOC	
miR-497	miR-497	17p13	miR-195	TS	BIRC5	17
					WNT7A	
miR-574-3p	miR-574	4p14	ND	TS	MESDC1	108
miR-576-3p	miR-576	4q25	ND	TS	CCND1	109
miR-590-3p	miR-590	7q11	ND	TS	TFAM	110
miR-1182	miR-1182	1q42	ND	TS	TERT	111
miR-9	miR-9-1	1q22	ND	Onco	CBX7	112
	miR-9-2	5q14	ND			
	miR-9-3	15q26	ND		CERS2	113

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Table 1. Continued

miRNAs	Stem-loop sequence	Locus	Clustered miRNAs	miRNA type	Target	Reference
miR-10b	miR-10b	2q31	ND	Onco	HOXD10 KLF4	70
miR-19a	miR-19a	13q31	miR-17, miR-18a, miR-20a, miR-19b-1, miR-92a-1	Onco	PTEN	114
miR-96	miR-96	7q32	miR-18, miR-183	Onco	CDKN1A	29
miR-150	miR-150	19q13	ND	Onco	PDCD4	115
miR-155	miR-155	21q21	ND	Onco	DMTF1	69
miR-182-5p	miR-182	7q32	ND	Onco	RECK SMAD4	116
miR-708	miR-708	11q14	ND	Onco	CASP2	27

miRNA, microRNA; TS, tumor suppressive miRNA; Onco, oncogenic miRNA; ND, not determined.

The miRNAs in Table 1 are listed in order of miRNA number, and the validated target genes are indicated. Interestingly, among the 54 miRNAs examined, 45 were downregulated, whereas 9 were upregulated. In our experience, we often encountered difficulties with the use of anti-miRNAs in *in vitro* studies; however, miRNA restoration systems using miRNA transfection are well established and commercially available. Accordingly, the latter system may be easier to use to examine the potential tumor-suppressive roles of the downregulated miRNAs.

miRNAs have been shown to regulate target gene expression. Several miRNAs and their targets have been identified in BC (Table 2). Among the 61 target genes examined, the expression levels in clinical BC specimens compared with the levels in normal bladder epithelium were validated for 40 genes. The downregulation of several miRNAs, including *miR-1, miR-133a, miR-145, miR-195*, and the *miR-200* family, has been reported, and these miRNAs have been shown to be involved in crucial cellular processes, such as the epithelial-mesenchymal transition (EMT), cell cycle, apoptosis, and cancer signaling pathways. TS-miRNAs and their target genes were classified into functional annotations by using the Kyoto Encyclopedia of Genes and Genomes pathways (Table 2).

2. Clustered miRNAs

Several miRNAs are located in close proximity (within 10 kbp) in the same genomic region; this is called a miRNA cluster. Because of their simultaneous expression, common target genes of miRNAs within a cluster may be important for tumor biology. For example, *miR-145* forms a cluster with *miR-143* on chromosome 5q32, and these clustered miRNAs are regulated by a common promoter [21]. Research indicates that both *miR-145* and *miR-143* are frequently downregulated in a broad range of cancers, including BC. Villadsen et al. [22] suggested that *SERPINE1* is a direct target of both *miR-145* and *miR-143*; this was the first report of an miRNA cluster targeting the same mRNA in BC. Importantly, *miR-145* has been implicated in the p53 regulatory network in human malignancies [23]. p53 transcriptionally induces the expression of *miR-145* by interaction with a potential p53 response element in the *miR-145* promoter; moreover, *MYC* is directly repressed by *miR-145*. Additionally, Chiyomaru et al. [24] found direct binding of the *miR-23b/27b* cluster with *EGFR/MET*, and Itesako et al. [17] found direct binding of the *miR-195/497* cluster with *BIRC5*, which encodes survivin.

3. Cell cycle/apoptosis-related genes

Avoidance of apoptosis and cell cycle acceleration are required for cancer cells to escape cell death. *BCL2L2*, a member of the BCL2 family, can promote cell survival by inhibiting the intrinsic pathway of apoptosis. In contrast, *BIRC5* (*survivin*) is a key member of the inhibitor of apoptosis protein family. Several TS-miRNAs induce apoptosis through targeting *BCL2L2* (*miR-133b, miR-203*) and *BIRC5* (*miR-195, miR-203, miR-497*). Upregulation of *miR-129* may be involved in avoidance of apoptosis through direct targeting of *SOX4* and *GALNT1*, which are involved in transcription and protein expression, respectively [25]. One important pathway is the sequential activation of caspases, which have a central role in the execution of cell apoptosis. *miR-1* is downregulated in both clinical BC tissues and cultured BC cells and has been shown to induce apoptosis by increasing the activity of caspases 3 and 7 through direct targeting of the apoptosis inhibitor *SRSF9* [26]. Additionally, *CASP2* (caspase 2) is directly targeted by an onco-miRNA, *miR-706* [27].

Several miRNAs have been shown to control the cell cycle through their target genes. Matsushita et al. [28] first

Table 2. Functional annotation of the target genes and the relative miRNAs

Annotation	Target	miRNA	miRNA type	Target validation in clinical BCs	Reference
Apoptosis	BCL2L2	miR-133b, miR-203	TS	yes	47,65
	BIRC5	miR-195, miR-203, miR-497	TS	yes	17,65
	BMI1	miR-200c, miR-218	TS	no	30,67
	CASP2	miR-708	Onco	yes	27
	SOCS7	miR-145	TS	no	94
	SOX4	miR-129	TS	yes	25
	SRSF9	miR-1	TS	yes	26
Cell cycle	CCND1	miR-16, miR-576-3p	TS	yes	75,109
	CCNE1	miR-144-5p/3p	TS	yes	28
	CCNE2	miR-144-5p/3p	TS	yes	28
	CDC25A	miR-144-5p/3p	TS	yes	28
	CDC25A	miR-449a	TS	yes	105
	CDK4	miR-124-3p	TS	no	87
	CDK4	miR-195	TS	yes	99
	CDK6	miR-29c, miR-320c	TS	yes	80,104
	CDK6	miR-449a	TS	yes	105
	CDKN1A	miR-96	Onco	yes	29
	E2F3	MiR-125b, miR-200c	TS	yes	30,31
	FOXM1	miR-24	TS	yes	32
	PKMYT1	miR-144-5p/3p	TS	yes	28
	STMN1	miR-221	TS	no	102
EMT	CARD10	miR-24	TS	no	76
	CBX7	miR-9	Onco	yes	112
	FSCN1	miR-133a	TS	yes	37
	FSCN1	miR-145	TS	yes	37
	HMGA2	miR-485-5p	TS	no	106
	HMGN5	miR-186	TS	yes	95
	LASP1	miR-1, miR-133a, miR-218	TS	yes	38
	LOXL4	miR-193a-3p	TS	no	96
	MESDC1	miR-574-3p	TS	no	108
	MMP13	MiR-125b	TS	no	88
	MMP16	miR-200b	TS	no	39
	ZEB1	miR-23b	TS	yes	40
	ZEB2	miR-138	TS	yes	41
Cytokaine-cytokaine receptor intaraction	EGFR	miR-23b, miR-27b, miR-133a, miR-133b	TS	yes	24,85
	IGF1R	miR-145	TS	no	44
	MET	miR-23b, miR-27b, miR-101	TS	yes	24,85
MAPK signaling	FGFR3	miR-99a, miR-100	TS	yes	46
	FOS	miR-490-5p	TS	yes	108
	RECK	miR-182-5p	Onco	yes	116
Notch signaling	NOTCH1	miR-30a, miR-34a	TS	yes	81,83
PI3K-Akt signaling	AKT1	miR-133b	TS	yes	47
	AKT1	miR-143	TS	no	48
	ILK	miR-145	TS	no	48
	MTOR	miR-100	TS	yes	68
	PTEN	miR-19a	Onco	no	114
Ras signaling	CDC42	miR-195	TS	yes	98
	PAK1	miR-145	TS	no	93
	RHOC	miR-493	TS	no	51
	ROCK1	miR-124-3p	TS	yes	86

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Table 2. Continued

Annotation	Target	miRNA	miRNA type	Target validation in clinical BCs	Reference
VEGF signaling	VEGFC	miR-101	TS	yes	49,52
WNT signaling	FZD4	miR-493	TS	no	51
	PSEN1	miR-193a-3p	TS	no	52
	WNT7A	miR-195, miR-497	TS	yes	17
Metabolic	CERS2	miR-9	Onco	yes	113
	COX2	miR-101	TS	no	84
	GALNT1	miR-129	TS	yes	25
ECM-receptor	CD44	miR-34a	TS	yes	66
	ITGB3	miR-320a	TS	yes	103

miRNA, microRNA; TS, tumor suppressive miRNA; Onco, oncogenic miRNA; ND, not determined.

reported that cell-cycle-related genes, such as *CCNE1/2*, *CDC25A*, and *PKMYT1*, are directly regulated by *miR-144-5p*. *CDC25A* is also regulated by *miR-449a*, and *CDK4* and *CDK6* are regulated by *miR-124-3p*, *miR-195*, *miR-29c*, *miR-230c*, and *miR-449a*, inducing G_1-phase arrest. Interestingly, *miR-96*, which is upregulated in BC, directly binds to *CDKN1A* (p21), a well-known tumor-suppressive gene [29]. Moreover, *miR-125b* and *miR-200c* target the oncogene *E2F3*, which is critical for the G_1/S transition and is overexpressed in most high-grade BCs [30,31]. Inoguchi et al. [32] found that *miR-24* regulates *FOXM1*, which is critical for mediating cell cycle progression proteins, such as *Aurora B*, *cyclin B1*, and *CDC25B*, and stimulates the expression of *SKP2/CKS1*, which is involved in the proteolysis of $p27^{kip1}$ and in G_1/S progression.

4. EMT-related genes as targets of miRNAs in BC

We also evaluated whether the expression of the different target genes could be validated in clinical BC samples (Table 2). The EMT is a key progression mechanism that is often activated during cancer invasion and metastasis and is characterized by loss of cell adhesion, repression of E cadherin expression, and increased cell mobility [33]. Members of the *miR-200* family are well-known regulators of the EMT in human malignancies, including BC [34], and some investigators have reported the functions of these miRNAs in BC [35,36].

FSCN1 and LASP1 are components of the filopodia and lamellipodia in various types of cells; both proteins are involved in mediating the dynamics of actin filaments and are activated during the EMT. *miR-133a* and *miR-145* directly regulate *FSCN1* [37], and the *miR-1/133a* cluster and *miR-218* regulate *LASP1* [38]. Restoration of the expression of these miRNAs accelerates cell migration and invasion activity *in vitro* [37,38]. Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing endopeptidases that are capable of degrading various extracellular matrix proteins. MMPs are involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as the FAS ligand), and chemokine/cytokine inactivation. MMPs are also thought to facilitate tumor cell invasion and metastasis via the degradation of the extracellular matrix. Transforming growth factor beta1 reduces the expression level of *miR-200b*, which directly regulates *MMP16*. This then activates other MMPs (e.g., MMP-2 and MMP-9), growth factors, and receptors, thereby facilitating local cellular mechanisms that promote migration [39].

Loss of expression of *miR-23b*, *miR-138*, and *miR-200c* (an *miR-200* family member) is associated with increased expression of *ZEB1*, *ZEB2*, and *ERFFI1*, which facilitate the progression of the EMT [36,40,41]. *ZEB1* and *ZEB2* are known to negatively regulate E cadherin expression and are directly targeted by *miR-23b* and *miR-138*, whereas *ERRFI-1*, a regulator of *EGFR*, is directly targeted by members of the *miR-200* family [36]. *ZEB1* expression has been reported in 22% of clinical BC tissue specimens, but is absent from the bladder mucosa [42]. Expression of the *miR-200* family has been shown to be repressed by hypermethylation of its promoter region, and loss of *miR-200c* expression is significantly associated with subsequent disease progression to MIBC and poor outcomes [35]. These studies implied that many EMT-related genes are activated through downregulation of several TS-miRNAs, and these phenomena may contribute to tumor progression of BC.

5. Others

Downregulated miRNAs have also been reported to target some genes related to cytokine–cytokine receptor interactions, including *EGFR* (*miR-23b, miR-27b, miR-133a, miR-133b*) [24,43], *IGF1R* (*miR-145*) [44], and *MET* (*miR-23b,*

miR-27b, miR-101) [24,43]. Activation of these genes may affect downstream cancer pathways, such as mammalian target of rapamycin signaling, phosphoinositol 3-kinase (PI3K)-Akt signaling, and mitogen-activated protein kinase (MAPK) signaling. *FGFR3*, which belongs to the MAPK signaling pathway, is crucial for BC oncogenesis, and mutations in *FGFR3* have been shown to accelerate proliferation in BC [45]. Downregulation of *miR-99a* and *miR-100* has been shown to cause upregulation of *FGFR3* expression before its mutation, which suggests that the acquisition of mutations may result from increased cell turnover [46]. The expression of *AKT1* and *ILK*, which are involved in the AKT-PI3K pathway, was shown to be repressed by the *miR-143/145* cluster, and proliferation is inhibited in cells transfected with these miRNAs [47,48]. The vascular endothelial growth factor (VEGF) and WNT signaling pathways are also important for BC development, and *VEGFC, FZD4, PSEN1*, and *WNT1* have been reported to be under the control of several TS-miRNAs [17,49-52].

MICRORNAS AS TUMOR MARKERS IN BLADDER CANCER

1. miRNAs as diagnostic markers for BC detection

Many studies have investigated miRNA expression by quantitative real-time PCR of clinical BC specimens [20]. In this review, we focus on studies in which the investigators evaluated diagnostic accuracy by use of receiver-operator curve analyses of the sensitivity and specificity of specific miRNAs in comparison with normal bladder epithelium. We found 18 studies that satisfied our criteria (Table 3). In 2009, Ichimi et al. [19] first evaluated the expression of six miRNAs in tissues as diagnostic markers to distinguish BC (n=104) from non-BC (n=31).

There is a pressing need for highly accurate, noninvasive tests for BC diagnosis. The standard procedures for BC diagnosis and the follow-up for patients depend on urinary cytology and cystoscopy. Urinary cytology is a reliable urine marker for BC diagnosis because of its high specificity (90%–95%). However, this method has low sensitivity (30%–40%), and patients are forced to undergo a painful cystoscopic procedure to confirm the diagnosis. A number of noninvasive urine tests, such as bladder tumor antigen and NMP22, have been developed; however, their diagnostic specificities are inferior to that of urinary cytology tests, and these methods have failed to meet clinical expectations. Therefore, quantitative changes in miRNAs in urine, blood, and tissues are the primary focus in the search for new markers. miRNAs are detectable in urine with pelleted or cell-free urine samples. Hanke et al. [53] evaluated *miR-126* expression in whole urine samples and showed that this miRNA had a sensitivity of 72% and specificity of 82% when used as a diagnostic marker, even though the sample size in their study was small. Yamada et al. [54] also found that *miR-96* detection in the urine including the pellet was a useful diagnostic marker, with a sensitivity of 71.8% and a specificity of 89.2%. Shimizu et al. [55] uniquely reported good sensitivity of 81% and good specificity of 89% by testing a methylation panel of *miR-9-3/miR-124-2/miR-124-3/miR-137*. In terms of miRNA detection in cell-free urine [56-60], several studies have shown that this method provides moderate sensitivity and specificity for distinguishing BC from non-BC but that the results are inferior to those from studies using whole urine samples [53-55,61,62]. In blood tests, Jiang et al. [63] showed dramatic results (sensitivity of 90% and specificity of 90%) using a panel study of *miR-15b-5p/miR-27a-3p/miR-30a-5p/miR-148b-3p/miR-152/miR-3187-3p*.

2. miRNAs for predicting prognosis in patients with BC

Many retrospective studies have suggested that the expression of specific miRNAs in BC specimens may be a good prognostic marker predicting overall survival, disease-free survival, recurrence-free survival, or progression-free survival, as outlined in Table 4. Moreover, several reports analyzing urine and blood samples have shown that *miR-200, miR-214*, and *miR-3187-3p* levels are good prognostic markers in the blood [57,60,63,64]. Surprisingly, *miR-203, miR-214, miR-152*, and *miR-3187-3p* were shown to be independent markers predicting overall survival, progression-free survival, or recurrence-free survival in multivariate studies [63-65]. Unfortunately, no prospective studies have been performed to date. Thus, additional large, prospective studies are needed for the clinical application of miRNA assays for diagnostic and prognostic purposes in BC.

MICRORNAS AS A TREATMENT MODALITY IN BLADDER CANCER

Some TS-miRNAs have been transfected into BC cell lines by using plasmid/virus vectors, followed by subcutaneous injection of the transfected cells into nude mice [27,66,67]. Significant inhibition of tumor growth [27,67] and metastasis [66] was observed. Yu et al. [66] found that the *CD44* gene was a direct target of *miR-34a* and that the pro-apoptotic role of *miR-34a* may be mediated primarily through regulation of CD44, which functions to promote metastasis and angiogenesis. Xu et al. [68] demonstrated that *miR-100-*

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Table 3. miRNAs as diagnostic markers

Sample	Study	Year	Calculator	BC/Cont. (n)	Sensitivity	Specificity	Reference
Tissue	Liu	2015	miR-141	30/30	78.2	51.6	139
			miR-200b		81.3	47	
	Ichimi	2009	miR-30a-3p	104/31	94.3	76.7	19
			miR-125		89.4	77.4	
			miR-133a		93.3	77.4	
			miR-145		90.5	77.4	
			miR-195		91.4	80	
			miR-199a*		72.1	90.3	
Urine	Long	2015	miR-26a/miR-93/miR-191/miR-940	85/45	70	84	56
	Wang	2015	miR-214	192/169	90.5	65.6	57
	Zhang	2014	miR-99a, miR-125b	50	86.7	81.1	58
	Zhou	2014	miR-106b	112/78	76.8	72.4	59
	Tolle	2013	miR-520e	36/19	70	63.2	117
			miR-618		70	68.4	
			miR-1255b-5p		85	68.4	
	Mengual	2013	miR-18a*/miR-25/miR-140-5p/miR-142-3p/ miR-187/miR-204	151/126	84.8	86.5	61
	Shimizu	2013	miR-9-3/miR-124-2/miR-124-3/miR-137	86/20	81	89	55
	Yun	2012	miR-145	207/144	77.8	61.1	60
	Miah	2012	miR-15b/miR-135b/miR-1224-3p	121/53	94.1	51	118
	Dudziec	2011	miR-152/miR-328/miR-122-3p	68/53	81	75	62
			miR-212		40	66	
			miR-328		59	91	
			miR-1224-3p		84	58	
	Yamada	2011	miR-96	100/74	71.8	89.2	54
			miR-183		74	77.3	
	Hanke	2010	miR-126: miR-152 ratio	29/11	72	82	53
			miR-182: miR-152 ratio		55	82	
Blood	Motawi	2016	miR-92a	70/62	97.1	76.7	119
			miR-100		90	66.7	
			miR-143		78.6	93.3	
	Fang	2015	miR-205	89/56	76.4	96.4	120
	Du	2015	miR-497/miR-663b	109/115	69.7	69.6	121
	Jiang	2015	miR-15b-5p/miR-27a-3p/ miR-30a-5p/miR-148b-3p/ miR-152/miR-3187-3p	110/110	90	90	63
	Tolle	2013	miR-26b-5p	38/20	65	94.1	117
			miR-144-5p		70	82.4	
			miR-374-5p		60	94.1	

miRNA, microRNA; BC, bladder cancer; Cont., control.

transfected BC cells exhibited significant growth inhibition in an intravesical orthotopic BC model. In terms of onco-miRNAs, *miR-10b*- and *miR-155*-transfected BC xenografts exhibited significantly increased growth in comparison with controls [69,70]. Inamoto et al. [71] tested the anticancer effects of intravesical injection of *miR-145* by using cationic liposomes (Lipofectamine RNAiMAX, Thermo Fisher Scientific, Waltham, MA, USA) in an intravesical orthotopic BC model. They achieved adequate anticancer effects after intravesical administration of *miR-145*. These miRNAs are promising candidate miRNAs that may be useful for clinical applications in the future.

FUTURE PERSPECTIVES

Many studies have examined the roles of miRNAs in human malignancies, including BC. However, the majority of miRNA studies have focused on candidate gene

Table 4. miRNAs as prognostic markers

Sample	Study	Year	Calculator	BC (n)	Valuables	Multivariate	Reference
Tissue	Zhang	2016	miR-30a	50	OS, RFS	N/A	81
	Xu	2015	let-7c, miR-99a, miR-125b-1, miR-193a	202	OS	N/A	124
	Zhang	2015	miR-21	53	OS	N/A	122
	Wang	2015	miR-141	114	DSS	N/A	127
	Avgeris	2015	miR-143, miR-145	133	OS, PFS	N/A	125
	Zhang	2015	miR-203	108	OS, PFS	Independent	65
	Wu	2015	miR-424	124	OS, RFS	N/A	123
	Segersten	2014	miR-10a-5p	86	PFS	N/A	129
	Lin	2014	miR-26a	126	OS, DFS	N/A	126
	Zhang	2014	miR-222	97	OS	N/A	128
	Pignot	2013	miR-9, miR-182, miR-200b	166	OS, RFS	N/A	134
	Majid	2013	miR-23b	20	OS	N/A	40
	Dyrskjot	2009	miR-29c, miR-129, miR-133b	106	PFS	N/A	25
	Rosenberg	2013	miR-29c*	108	PFS	N/A	132
	Wang	2013	miR-31	126	OS, PFS	N/A	131
	Ratert	2013	miR-141, miR-205	40	OS	N/A	130
	Pignot	2012	miR-19a, miR-20a, miR-92a	71	OS	N/A	138
	Zaravinos	2012	miR-21	77	OS	N/A	136
	Wang	2012	miR-100	126	OS, PFS	N/A	133
	Hirata	2012	miR-182-5p	18	OS	N/A	116
	Puerta-Gil	2012	miR-222, miR-143	37	OS, DSS, RFS, PFS	N/A	137
	Majid	2012	miR-1280	21	OS	N/A	135
Urine	Dudziec	2011	miR-152 CpG, miR-1224 CpG	68	PFS	N/A	62
	Yun	2012	miR-200	138	RFS	N/A	60
	Wang	2015	miR-214	192	OS, RFS	N/A	57
	Kim	2013	miR-214	138	RFS	Independent	64
Blood	Jiang	2015	miR-152, miR-3187-3p	110	RFS	Independent	63
	Fang	2015	miR-205	89	DSS	N/A	120

miRNA, microRNA; BC, bladder cancer; OS, overall survival; DSS, disease specific survival; PFS, progression free survival; RFS, recurrence free survival; N/A, not applicable.

approaches with limited whole-genome sequencing, and a detailed characterization of miRNA profiles based on the genomic features of BC has not been reported. Recently, DS technology has been applied for profiling of mRNA and miRNA in human cancers, including BC. However, DS technology has mainly focused on BC tissues without any particular selection according to clinical treatment. Additional DS analyses should be conducted using samples from patients who have undergone different treatments, e.g., chemotherapy regimens, radiation therapies, or immune-checkpoint drugs.

A growing body of evidence has shown that miRNAs have great potential for clinical use as new diagnostic biomarkers. However, detection of single miRNAs in urine samples will not be superior to current urinary cytology tests because of the relatively low diagnostic specificity of such analyses. Some recent studies have attempted to improve the diagnostic specificity of miRNA detection by using a panel of multiple miRNAs [55,56,61,63]. It will be important to select optimal combinations of miRNAs and to perform prospective translational studies in outpatients to validate such miRNA panels.

Moreover, recent studies have shown that some miRNAs control the activity of major cancer-related signaling molecules. Thus, identification of aberrant miRNA expression and oncogenic or tumor-suppressive molecular targets of miRNAs is necessary for the clinical development of novel cancer therapeutics. Because many TS-miRNAs and their target oncogenes are components of complex molecular networks, treatment with multiple miRNAs may provide stronger anticancer effects than treatment with a single miRNA. In an interesting study by Liu et al. [72], new vectors, termed "miRNA-mowers," that contained the entire sequence of the onco-*miR-183-96-182* cluster were constructed. Transfection of the miRNA-mowers strongly inhibited cell growth and migration and induced apoptosis *in vitro*,

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suggesting the usefulness of targeting multiple onco-miRNAs. However, no studies have yet reported successful results for intravenous administration of TS-miRNAs in the induction of anticancer effects *in vivo*. The development of an adequate drug delivery system for TS-miRNAs is needed; otherwise, it will be difficult to determine the value of miRNAs as a therapeutic modality.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
2. Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. Lancet 2009;374:239-49.
3. Schmitz-Drager BJ, Todenhofer T, van Rhijn B, Pesch B, Hudson MA, Chandra A, et al. Considerations on the use of urine markers in the management of patients with low-/intermediate-risk non-muscle invasive bladder cancer. Urol Oncol 2014;32:1061-8.
4. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. Cell 2009;136:642-55.
5. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008;9:102-14.
6. Hobert O. Gene regulation by transcription factors and microRNAs. Science 2008;319:1785-6.
7. Farazi TA, Hoell JI, Morozov P, Tuschl T. MicroRNAs in human cancer. Adv Exp Med Biol 2013;774:1-20.
8. Kong YW, Ferland-McCollough D, Jackson TJ, Bushell M. microRNAs in cancer management. Lancet Oncol 2012;13:e249-58.
9. International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature 2004;431:931-45.
10. Huttenhofer A, Schattner P, Polacek N. Non-coding RNAs: hope or hype? Trends Genet 2005;21:289-97.
11. Bushati N, Cohen SM. microRNA functions. Annu Rev Cell Dev Biol 2007;23:175-205.
12. Yoshino H, Seki N, Itesako T, Chiyomaru T, Nakagawa M, Enokida H. Aberrant expression of microRNAs in bladder cancer. Nat Rev Urol 2013;10:396-404.
13. Han Y, Chen J, Zhao X, Liang C, Wang Y, Sun L, et al. MicroRNA expression signatures of bladder cancer revealed by deep sequencing. PLoS One 2011;6:e18286.
14. Chen YH, Wang SQ, Wu XL, Shen M, Chen ZG, Chen XG, et al. Characterization of microRNAs expression profiling in one group of Chinese urothelial cell carcinoma identified by Solexa sequencing. Urol Oncol 2013;31:219-27.
15. Zhu J, Jiang Z, Gao F, Hu X, Zhou L, Chen J, et al. A systematic analysis on DNA methylation and the expression of both mRNA and microRNA in bladder cancer. PLoS One 2011;6:e28223.
16. Li X, Chen J, Hu X, Huang Y, Li Z, Zhou L, et al. Comparative mRNA and microRNA expression profiling of three genitourinary cancers reveals common hallmarks and cancer-specific molecular events. PLoS One 2011;6:e22570.
17. Itesako T, Seki N, Yoshino H, Chiyomaru T, Yamasaki T, Hidaka H, et al. The microRNA expression signature of bladder cancer by deep sequencing: the functional significance of the miR-195/497 cluster. PLoS One 2014;9:e84311.
18. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 2014;507:315-22.
19. Ichimi T, Enokida H, Okuno Y, Kunimoto R, Chiyomaru T, Kawamoto K, et al. Identification of novel microRNA targets based on microRNA signatures in bladder cancer. Int J Cancer 2009;125:345-52.
20. Matullo G, Naccarati A, Pardini B. MicroRNA expression profiling in bladder cancer: the challenge of next-generation sequencing in tissues and biofluids. Int J Cancer 2016;138:2334-45.
21. Iio A, Nakagawa Y, Hirata I, Naoe T, Akao Y. Identification of non-coding RNAs embracing microRNA-143/145 cluster. Mol Cancer 2010;9:136.
22. Villadsen SB, Bramsen JB, Ostenfeld MS, Wiklund ED, Fristrup N, Gao S, et al. The miR-143/-145 cluster regulates plasminogen activator inhibitor-1 in bladder cancer. Br J Cancer 2012;106:366-74.
23. Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S, Miyazono K. Modulation of microRNA processing by p53. Nature 2009;460:529-33.
24. Chiyomaru T, Seki N, Inoguchi S, Ishihara T, Mataki H, Matsushita R, et al. Dual regulation of receptor tyrosine kinase genes EGFR and c-Met by the tumor-suppressive microRNA-23b/27b cluster in bladder cancer. Int J Oncol 2015;46:487-96.
25. Dyrskjot L, Ostenfeld MS, Bramsen JB, Silahtaroglu AN, Lamy P, Ramanathan R, et al. Genomic profiling of microRNAs in bladder cancer: miR-129 is associated with poor outcome and promotes cell death in vitro. Cancer Res 2009;69:4851-60.
26. Yoshino H, Enokida H, Chiyomaru T, Tatarano S, Hidaka H, Yamasaki T, et al. Tumor suppressive microRNA-1 mediated novel apoptosis pathways through direct inhibition of splicing

factor serine/arginine-rich 9 (SRSF9/SRp30c) in bladder cancer. Biochem Biophys Res Commun 2012;417:588-93.
27. Song T, Zhang X, Zhang L, Dong J, Cai W, Gao J, et al. miR-708 promotes the development of bladder carcinoma via direct repression of Caspase-2. J Cancer Res Clin Oncol 2013;139:1189-98.
28. Matsushita R, Seki N, Chiyomaru T, Inoguchi S, Ishihara T, Goto Y, et al. Tumour-suppressive microRNA-144-5p directly targets CCNE1/2 as potential prognostic markers in bladder cancer. Br J Cancer 2015;113:282-9.
29. Wu Z, Liu K, Wang Y, Xu Z, Meng J, Gu S. Upregulation of microRNA-96 and its oncogenic functions by targeting CDKN1A in bladder cancer. Cancer Cell Int 2015;15:107.
30. Liu L, Qiu M, Tan G, Liang Z, Qin Y, Chen L, et al. miR-200c inhibits invasion, migration and proliferation of bladder cancer cells through down-regulation of BMI-1 and E2F3. J Transl Med 2014;12:305.
31. Huang L, Luo J, Cai Q, Pan Q, Zeng H, Guo Z, et al. MicroRNA-125b suppresses the development of bladder cancer by targeting E2F3. Int J Cancer 2011;128:1758-69.
32. Inoguchi S, Seki N, Chiyomaru T, Ishihara T, Matsushita R, Mataki H, et al. Tumour-suppressive microRNA-24-1 inhibits cancer cell proliferation through targeting FOXM1 in bladder cancer. FEBS Lett 2014;588:3170-9.
33. Yun SJ, Kim WJ. Role of the epithelial-mesenchymal transition in bladder cancer: from prognosis to therapeutic target. Korean J Urol 2013;54:645-50.
34. Abba ML, Patil N, Leupold JH, Allgayer H. MicroRNA regulation of epithelial to mesenchymal transition. J Clin Med 2016; 5:8.
35. Wiklund ED, Bramsen JB, Hulf T, Dyrskjot L, Ramanathan R, Hansen TB, et al. Coordinated epigenetic repression of the miR-200 family and miR-205 in invasive bladder cancer. Int J Cancer 2011;128:1327-34.
36. Adam L, Zhong M, Choi W, Qi W, Nicoloso M, Arora A, et al. miR-200 expression regulates epithelial-to-mesenchymal transition in bladder cancer cells and reverses resistance to epidermal growth factor receptor therapy. Clin Cancer Res 2009;15:5060-72.
37. Chiyomaru T, Enokida H, Tatarano S, Kawahara K, Uchida Y, Nishiyama K, et al. miR-145 and miR-133a function as tumour suppressors and directly regulate FSCN1 expression in bladder cancer. Br J Cancer 2010;102:883-91.
38. Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Uchida Y, Kawahara K, et al. Functional role of LASP1 in cell viability and its regulation by microRNAs in bladder cancer. Urol Oncol 2012;30:434-43.
39. Chen MF, Zeng F, Qi L, Zu XB, Wang J, Liu LF, et al. Transforming growth factor β1 induces epithelial mesenchymal transition and increased expression of matrix metalloproteinase 16 via miR 200b downregulation in bladder cancer cells. Mol Med Rep 2014;10:1549-54.
40. Majid S, Dar AA, Saini S, Deng G, Chang I, Greene K, et al. MicroRNA-23b functions as a tumor suppressor by regulating Zeb1 in bladder cancer. PLoS One 2013 2;8:e67686.
41. Sun DK, Wang JM, Zhang P, Wang YQ. MicroRNA-138 regulates metastatic potential of bladder cancer through ZEB2. Cell Physiol Biochem 2015;37:2366-74.
42. Kenney PA, Wszolek MF, Rieger-Christ KM, Neto BS, Gould JJ, Harty NJ, et al. Novel ZEB1 expression in bladder tumorigenesis. BJU Int 2011;107:656-63.
43. Zhou Y, Wu D, Tao J, Qu P, Zhou Z, Hou J. MicroRNA-133 inhibits cell proliferation, migration and invasion by targeting epidermal growth factor receptor and its downstream effector proteins in bladder cancer. Scand J Urol 2013;47:423-32.
44. Zhu Z, Xu T, Wang L, Wang X, Zhong S, Xu C, et al. MicroRNA-145 directly targets the insulin-like growth factor receptor I in human bladder cancer cells. FEBS Lett 2014;588:3180-5.
45. Gomez-Roman JJ, Saenz P, Molina M, Cuevas Gonzalez J, Escuredo K, Santa Cruz S, et al. Fibroblast growth factor receptor 3 is overexpressed in urinary tract carcinomas and modulates the neoplastic cell growth. Clin Cancer Res 2005;11(2 Pt 1):459-65.
46. Catto JW, Miah S, Owen HC, Bryant H, Myers K, Dudziec E, et al. Distinct microRNA alterations characterize high- and low-grade bladder cancer. Cancer Res 2009;69:8472-81.
47. Chen XN, Wang KF, Xu ZQ, Li SJ, Liu Q, Fu DH, et al. MiR-133b regulates bladder cancer cell proliferation and apoptosis by targeting Bcl-w and Akt1. Cancer Cell Int 2014;14:70.
48. Noguchi S, Yasui Y, Iwasaki J, Kumazaki M, Yamada N, Naito S, et al. Replacement treatment with microRNA-143 and -145 induces synergistic inhibition of the growth of human bladder cancer cells by regulating PI3K/Akt and MAPK signaling pathways. Cancer Lett 2013;328:353-61.
49. Zhou XU, Qi L, Tong S, Cui YU, Chen J, Huang T, et al. miR-128 downregulation promotes growth and metastasis of bladder cancer cells and involves VEGF-C upregulation. Oncol Lett 2015;10:3183-90.
50. Ueno K, Hirata H, Majid S, Yamamura S, Shahryari V, Tabatabai ZL, et al. Tumor suppressor microRNA-493 decreases cell motility and migration ability in human bladder cancer cells by downregulating RhoC and FZD4. Mol Cancer Ther 2012; 11:244-53.
51. Deng H, Lv L, Li Y, Zhang C, Meng F, Pu Y, et al. The miR-193a-3p regulated PSEN1 gene suppresses the multi-chemoresistance of bladder cancer. Biochim Biophys Acta 2015;1852:520-8.
52. Lei Y, Li B, Tong S, Qi L, Hu X, Cui Y, et al. miR-101 suppress-

http://dx.doi.org/10.4111/icu.2016.57.S1.S60

es vascular endothelial growth factor C that inhibits migration and invasion and enhances cisplatin chemosensitivity of bladder cancer cells. PLoS One 2015;10:e0117809.
53. Hanke M, Hoefig K, Merz H, Feller AC, Kausch I, Jocham D, et al. A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. Urol Oncol 2010;28:655-61.
54. Yamada Y, Enokida H, Kojima S, Kawakami K, Chiyomaru T, Tatarano S, et al. MiR-96 and miR-183 detection in urine serve as potential tumor markers of urothelial carcinoma: correlation with stage and grade, and comparison with urinary cytology. Cancer Sci 2011;102:522-9.
55. Shimizu T, Suzuki H, Nojima M, Kitamura H, Yamamoto E, Maruyama R, et al. Methylation of a panel of microRNA genes is a novel biomarker for detection of bladder cancer. Eur Urol 2013;63:1091-100.
56. Long JD, Sullivan TB, Humphrey J, Logvinenko T, Summerhayes KA, Kozinn S, et al. A non-invasive miRNA based assay to detect bladder cancer in cell-free urine. Am J Transl Res 2015;7:2500-9.
57. Wang J, Zhang X, Wang L, Dong Z, Du L, Yang Y, et al. Downregulation of urinary cell-free microRNA-214 as a diagnostic and prognostic biomarker in bladder cancer. J Surg Oncol 2015;111:992-9.
58. Zhang DZ, Lau KM, Chan ES, Wang G, Szeto CC, Wong K, et al. Cell-free urinary microRNA-99a and microRNA-125b are diagnostic markers for the non-invasive screening of bladder cancer. PLoS One 2014;9:e100793.
59. Zhou X, Zhang X, Yang Y, Li Z, Du L, Dong Z, et al. Urinary cell-free microRNA-106b as a novel biomarker for detection of bladder cancer. Med Oncol 2014;31:197.
60. Yun SJ, Jeong P, Kim WT, Kim TH, Lee YS, Song PH, et al. Cell-free microRNAs in urine as diagnostic and prognostic biomarkers of bladder cancer. Int J Oncol 2012;41:1871-8.
61. Mengual L, Lozano JJ, Ingelmo-Torres M, Gazquez C, Ribal MJ, Alcaraz A. Using microRNA profiling in urine samples to develop a non-invasive test for bladder cancer. Int J Cancer 2013;133:2631-41.
62. Dudziec E, Miah S, Choudhry HM, Owen HC, Blizard S, Glover M, et al. Hypermethylation of CpG islands and shores around specific microRNAs and mirtrons is associated with the phenotype and presence of bladder cancer. Clin Cancer Res 2011;17:1287-96.
63. Jiang X, Du L, Wang L, Li J, Liu Y, Zheng G, et al. Serum microRNA expression signatures identified from genome-wide microRNA profiling serve as novel noninvasive biomarkers for diagnosis and recurrence of bladder cancer. Int J Cancer 2015;136:854-62.
64. Kim SM, Kang HW, Kim WT, Kim YJ, Yun SJ, Lee SC, et al. Cell-free microRNA-214 from urine as a biomarker for non-muscle-invasive bladder cancer. Korean J Urol 2013;54:791-6.
65. Zhang X, Zhang Y, Liu X, Fang A, Li P, Li Z, et al. MicroRNA-203 is a prognostic indicator in bladder cancer and enhances chemosensitivity to cisplatin via apoptosis by targeting Bcl-w and survivin. PLoS One 2015;10:e0143441.
66. Yu G, Yao W, Xiao W, Li H, Xu H, Lang B. MicroRNA-34a functions as an anti-metastatic microRNA and suppresses angiogenesis in bladder cancer by directly targeting CD44. J Exp Clin Cancer Res 2014;33:779.
67. Cheng Y, Yang X, Deng X, Zhang X, Li P, Tao J, et al. MicroRNA-218 inhibits bladder cancer cell proliferation, migration, and invasion by targeting BMI-1. Tumour Biol 2015;36:8015-23.
68. Xu C, Zeng Q, Xu W, Jiao L, Chen Y, Zhang Z, et al. miRNA-100 inhibits human bladder urothelial carcinogenesis by directly targeting mTOR. Mol Cancer Ther 2013;12:207-19.
69. Peng Y, Dong W, Lin TX, Zhong GZ, Liao B, Wang B, et al. MicroRNA-155 promotes bladder cancer growth by repressing the tumor suppressor DMTF1. Oncotarget 2015;6:16043-58.
70. Xiao H, Li H, Yu G, Xiao W, Hu J, Tang K, et al. MicroRNA-10b promotes migration and invasion through KLF4 and HOXD10 in human bladder cancer. Oncol Rep 2014;31:1832-8.
71. Inamoto T, Taniguchi K, Takahara K, Iwatsuki A, Takai T, Komura K, et al. Intravesical administration of exogenous microRNA-145 as a therapy for mouse orthotopic human bladder cancer xenograft. Oncotarget 2015;6:21628-35.
72. Liu Y, Han Y, Zhang H, Nie L, Jiang Z, Fa P, et al. Synthetic miRNA-mowers targeting miR-183-96-182 cluster or miR-210 inhibit growth and migration and induce apoptosis in bladder cancer cells. PLoS One 2012;7:e52280.
73. Yamasaki T, Yoshino H, Enokida H, Hidaka H, Chiyomaru T, Nohata N, et al. Novel molecular targets regulated by tumor suppressors microRNA-1 and microRNA-133a in bladder cancer. Int J Oncol 2012;40:1821-30.
74. Yoshino H, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Nishiyama K, et al. The tumour-suppressive function of miR-1 and miR-133a targeting TAGLN2 in bladder cancer. Br J Cancer 2011;104:808-18.
75. Jiang QQ, Liu B, Yuan T. MicroRNA-16 inhibits bladder cancer proliferation by targeting Cyclin D1. Asian Pac J Cancer Prev 2013;14:4127-30.
76. Zhang S, Zhang C, Liu W, Zheng W, Zhang Y, Wang S, et al. MicroRNA-24 upregulation inhibits proliferation, metastasis and induces apoptosis in bladder cancer cells by targeting CARMA3. Int J Oncol 2015;47:1351-60.
77. Deng Y, Bai H, Hu H. rs11671784 G/A variation in miR-27a decreases chemo-sensitivity of bladder cancer by decreasing

miR-27a and increasing the target RUNX-1 expression. Biochem Biophys Res Commun 2015;458:321-7.
78. Drayton RM, Dudziec E, Peter S, Bertz S, Hartmann A, Bryant HE, et al. Reduced expression of miRNA-27a modulates cisplatin resistance in bladder cancer by targeting the cystine/glutamate exchanger SLC7A11. Clin Cancer Res 2014;20:1990-2000.
79. Yuan L, Chu H, Wang M, Gu X, Shi D, Ma L, et al. Genetic variation in DROSHA 3'UTR regulated by hsa-miR-27b is associated with bladder cancer risk. PLoS One 2013;8:e81524.
80. Zhao X, Li J, Huang S, Wan X, Luo H, Wu D. MiRNA-29c regulates cell growth and invasion by targeting CDK6 in bladder cancer. Am J Transl Res 2015;7:1382-9.
81. Zhang C, Ma X, Du J, Yao Z, Shi T, Ai Q, et al. MicroRNA-30a as a prognostic factor in Urothelial Carcinoma of Bladder inhibits cellular malignancy by antagonizing Notch1. BJU Int 2016 Jan 18 [Epub]. http://dx.doi.org/10.1111/bju.13407.
82. Sun H, Tian J, Xian W, Xie T, Yang X. miR-34a inhibits proliferation and invasion of bladder cancer cells by targeting orphan nuclear receptor HNF4G. Dis Markers 2015;2015:879254.
83. Zhang C, Yao Z, Zhu M, Ma X, Shi T, Li H, et al. Inhibitory effects of microRNA-34a on cell migration and invasion of invasive urothelial bladder carcinoma by targeting Notch1. J Huazhong Univ Sci Technolog Med Sci 2012;32:375-82.
84. Bu Q, Fang Y, Cao Y, Chen Q, Liu Y. Enforced expression of miR-101 enhances cisplatin sensitivity in human bladder cancer cells by modulating the cyclooxygenase-2 pathway. Mol Med Rep 2014;10:2203-9.
85. Hu Z, Lin Y, Chen H, Mao Y, Wu J, Zhu Y, et al. MicroRNA-101 suppresses motility of bladder cancer cells by targeting c-Met. Biochem Biophys Res Commun 2013;435:82-7.
86. Xu X, Li S, Lin Y, Chen H, Hu Z, Mao Y, et al. MicroRNA-124-3p inhibits cell migration and invasion in bladder cancer cells by targeting ROCK1. J Transl Med 2013;11:276.
87. Zhang T, Wang J, Zhai X, Li H, Li C, Chang J. MiR-124 retards bladder cancer growth by directly targeting CDK4. Acta Biochim Biophys Sin (Shanghai) 2014;46:1072-9.
88. Wu D, Ding J, Wang L, Pan H, Zhou Z, Zhou J, et al. microRNA-125b inhibits cell migration and invasion by targeting matrix metallopeptidase 13 in bladder cancer. Oncol Lett 2013;5:829-34.
89. Zhao X, He W, Li J, Huang S, Wan X, Luo H, et al. MiRNA-125b inhibits proliferation and migration by targeting SphK1 in bladder cancer. Am J Transl Res 2015;7:2346-54.
90. Uchida Y, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Kawahara K, et al. MiR-133a induces apoptosis through direct regulation of GSTP1 in bladder cancer cell lines. Urol Oncol 2013;31:115-23.
91. Mao XP, Zhang LS, Huang B, Zhou SY, Liao J, Chen LW, et al. Mir-135a enhances cellular proliferation through post-transcriptionally regulating PHLPP2 and FOXO1 in human bladder cancer. J Transl Med 2015;13:86.
92. Ostenfeld MS, Bramsen JB, Lamy P, Villadsen SB, Fristrup N, Sorensen KD, et al. miR-145 induces caspase-dependent and -independent cell death in urothelial cancer cell lines with targeting of an expression signature present in Ta bladder tumors. Oncogene 2010;29:1073-84.
93. Kou B, Gao Y, Du C, Shi Q, Xu S, Wang CQ, et al. miR-145 inhibits invasion of bladder cancer cells by targeting PAK1. Urol Oncol 2014;32:846-54.
94. Noguchi S, Yamada N, Kumazaki M, Yasui Y, Iwasaki J, Naito S, et al. socs7, a target gene of microRNA-145, regulates interferon-β induction through STAT3 nuclear translocation in bladder cancer cells. Cell Death Dis 2013;4:e482.
95. Yao K, He L, Gan Y, Zeng Q, Dai Y, Tan J. MiR-186 suppresses the growth and metastasis of bladder cancer by targeting NSBP1. Diagn Pathol 2015;10:146.
96. Deng H, Lv L, Li Y, Zhang C, Meng F, Pu Y, et al. miR-193a-3p regulates the multi-drug resistance of bladder cancer by targeting the LOXL4 gene and the oxidative stress pathway. Mol Cancer 2014;13:234.
97. Lv L, Li Y, Deng H, Zhang C, Pu Y, Qian L, et al. MiR-193a-3p promotes the multi-chemoresistance of bladder cancer by targeting the HOXC9 gene. Cancer Lett 2015;357:105-13.
98. Zhao C, Qi L, Chen M, Liu L, Yan W, Tong S, et al. microRNA-195 inhibits cell proliferation in bladder cancer via inhibition of cell division control protein 42 homolog/signal transducer and activator of transcription-3 signaling. Exp Ther Med 2015;10:1103-8.
99. Lin Y, Wu J, Chen H, Mao Y, Liu Y, Mao Q, et al. Cyclin-dependent kinase 4 is a novel target in micoRNA-195-mediated cell cycle arrest in bladder cancer cells. FEBS Lett 2012;586:442-7.
100. Fei X, Qi M, Wu B, Song Y, Wang Y, Li T. MicroRNA-195-5p suppresses glucose uptake and proliferation of human bladder cancer T24 cells by regulating GLUT3 expression. FEBS Lett 2012;586:392-7.
101. Wang J, Zhang X, Wang L, Yang Y, Dong Z, Wang H, et al. MicroRNA-214 suppresses oncogenesis and exerts impact on prognosis by targeting PDRG1 in bladder cancer. PLoS One 2015;10:e0118086.
102. Liu J, Cao J, Zhao X. miR-221 facilitates the TGFbeta1-induced epithelial-mesenchymal transition in human bladder cancer cells by targeting STMN1. BMC Urol 2015;15:36.
103. Shang C, Zhang H, Guo Y, Hong Y, Liu Y, Xue Y. MiR-320a down-regulation mediates bladder carcinoma invasion by targeting ITGB3. Mol Biol Rep 2014;41:2521-7.
104. Wang X, Wu J, Lin Y, Zhu Y, Xu X, Xu X, et al. MicroRNA-320c inhibits tumorous behaviors of bladder cancer by targeting

Cyclin-dependent kinase 6. J Exp Clin Cancer Res 2014;33:69.
105. Chen H, Lin YW, Mao YQ, Wu J, Liu YF, Zheng XY, et al. MicroRNA-449a acts as a tumor suppressor in human bladder cancer through the regulation of pocket proteins. Cancer Lett 2012;320:40-7.
106. Chen Z, Li Q, Wang S, Zhang J. miR 485 5p inhibits bladder cancer metastasis by targeting HMGA2. Int J Mol Med 2015;36:1136-42.
107. Li S, Xu X, Xu X, Hu Z, Wu J, Zhu Y, et al. MicroRNA-490-5p inhibits proliferation of bladder cancer by targeting c-Fos. Biochem Biophys Res Commun 2013;441:976-81.
108. Tatarano S, Chiyomaru T, Kawakami K, Enokida H, Yoshino H, Hidaka H, et al. Novel oncogenic function of mesoderm development candidate 1 and its regulation by MiR-574-3p in bladder cancer cell lines. Int J Oncol 2012;40:951-9.
109. Liang Z, Li S, Xu X, Xu X, Wang X, Wu J, et al. MicroRNA-576-3p inhibits proliferation in bladder cancer cells by targeting cyclin D1. Mol Cells 2015;38:130-7.
110. Mo M, Peng F, Wang L, Peng L, Lan G, Yu S. Roles of mitochondrial transcription factor A and microRNA-590-3p in the development of bladder cancer. Oncol Lett 2013;6:617-23.
111. Zhou J, Dai W, Song J. miR-1182 inhibits growth and mediates the chemosensitivity of bladder cancer by targeting hTERT. Biochem Biophys Res Commun 2016;470:445-52.
112. Xie D, Shang C, Zhang H, Guo Y, Tong X. Up-regulation of miR-9 target CBX7 to regulate invasion ability of bladder transitional cell carcinoma. Med Sci Monit 2015;21:225-30.
113. Wang H, Zhang W, Zuo Y, Ding M, Ke C, Yan R, et al. miR-9 promotes cell proliferation and inhibits apoptosis by targeting LASS2 in bladder cancer. Tumour Biol 2015;36:9631-40.
114. Cao Y, Yu SL, Wang Y, Guo GY, Ding Q, An RH. MicroRNA-dependent regulation of PTEN after arsenic trioxide treatment in bladder cancer cell line T24. Tumour Biol 2011;32:179-88.
115. Lei Y, Hu X, Li B, Peng M, Tong S, Zu X, et al. miR-150 modulates cisplatin chemosensitivity and invasiveness of muscle-invasive bladder cancer cells via targeting PDCD4 in vitro. Med Sci Monit 2014;20:1850-7.
116. Hirata H, Ueno K, Shahryari V, Tanaka Y, Tabatabai ZL, Hinoda Y, et al. Oncogenic miRNA-182-5p targets Smad4 and RECK in human bladder cancer. PLoS One 2012;7:e51056.
117. Tolle A, Jung M, Rabenhorst S, Kilic E, Jung K, Weikert S. Identification of microRNAs in blood and urine as tumour markers for the detection of urinary bladder cancer. Oncol Rep 2013;30:1949-56.
118. Miah S, Dudziec E, Drayton RM, Zlotta AR, Morgan SL, Rosario DJ, et al. An evaluation of urinary microRNA reveals a high sensitivity for bladder cancer. Br J Cancer 2012;107:123-8.
119. Motawi TK, Rizk SM, Ibrahim TM, Ibrahim IA. Circulating microRNAs, miR-92a, miR-100 and miR-143, as non-invasive biomarkers for bladder cancer diagnosis. Cell Biochem Funct 2016;34:142-8.
120. Fang Z, Dai W, Wang X, Chen W, Shen C, Ye G, et al. Circulating miR-205: a promising biomarker for the detection and prognosis evaluation of bladder cancer. Tumour Biol 2015 Dec 29 [Epub]. http://dx.doi.org/10.1007/s13277-015-4698-y.
121. Du M, Shi D, Yuan L, Li P, Chu H, Qin C, et al. Circulating miR-497 and miR-663b in plasma are potential novel biomarkers for bladder cancer. Sci Rep 2015;5:10437.
122. Zhang HH, Qi F, Cao YH, Zu XB, Chen MF. Expression and clinical significance of microRNA-21, maspin and vascular endothelial growth factor-C in bladder cancer. Oncol Lett 2015;10:2610-6.
123. Wu CT, Lin WY, Chang YH, Lin PY, Chen WC, Chen MF. DNMT1-dependent suppression of microRNA424 regulates tumor progression in human bladder cancer. Oncotarget 2015;6:24119-31.
124. Xu Z, Yu YQ, Ge YZ, Zhu JG, Zhu M, Zhao YC, et al. MicroRNA expression profiles in muscle-invasive bladder cancer: identification of a four-microRNA signature associated with patient survival. Tumour Biol 2015;36:8159-66.
125. Avgeris M, Mavridis K, Tokas T, Stravodimos K, Fragoulis EG, Scorilas A. Uncovering the clinical utility of miR-143, miR-145 and miR-224 for predicting the survival of bladder cancer patients following treatment. Carcinogenesis 2015;36:528-37.
126. Lin R, Shen W, Zhi Y, Zhou Z. Prognostic value of miR-26a and HMGA1 in urothelial bladder cancer. Biomed Pharmacother 2014;68:929-34.
127. Wang XL, Xie HY, Zhu CD, Zhu XF, Cao GX, Chen XH, et al. Increased miR-141 expression is associated with diagnosis and favorable prognosis of patients with bladder cancer. Tumour Biol 2015;36:877-83.
128. Zhang DQ, Zhou CK, Jiang XW, Chen J, Shi BK. Increased expression of miR-222 is associated with poor prognosis in bladder cancer. World J Surg Oncol 2014;12:241.
129. Segersten U, Spector Y, Goren Y, Tabak S, Malmström PU. The role of microRNA profiling in prognosticating progression in Ta and T1 urinary bladder cancer. Urol Oncol 2014;32:613-8.
130. Ratert N, Meyer HA, Jung M, Lioudmer P, Mollenkopf HJ, Wagner I, et al. miRNA profiling identifies candidate mirnas for bladder cancer diagnosis and clinical outcome. J Mol Diagn 2013;15:695-705.
131. Wang S, Li Q, Wang K, Dai Y, Yang J, Xue S, et al. Decreased expression of microRNA-31 associates with aggressive tumor progression and poor prognosis in patients with bladder cancer. Clin Transl Oncol 2013;15:849-54.
132. Rosenberg E, Baniel J, Spector Y, Faerman A, Meiri E, Aharonov R, et al. Predicting progression of bladder urothelial car-

cinoma using microRNA expression. BJU Int 2013;112:1027-34.
133. Wang S, Xue S, Dai Y, Yang J, Chen Z, Fang X, et al. Reduced expression of microRNA-100 confers unfavorable prognosis in patients with bladder cancer. Diagn Pathol 2012;7:159.
134. Pignot G, Cizeron-Clairac G, Vacher S, Susini A, Tozlu S, Vieillefond A, et al. microRNA expression profile in a large series of bladder tumors: identification of a 3-miRNA signature associated with aggressiveness of muscle-invasive bladder cancer. Int J Cancer 2013;132:2479-91.
135. Majid S, Dar AA, Saini S, Shahryari V, Arora S, Zaman MS, et al. MicroRNA-1280 inhibits invasion and metastasis by targeting ROCK1 in bladder cancer. PLoS One 2012;7:e46743.
136. Zaravinos A, Radojicic J, Lambrou GI, Volanis D, Delakas D, Stathopoulos EN, et al. Expression of miRNAs involved in angiogenesis, tumor cell proliferation, tumor suppressor inhibition, epithelial-mesenchymal transition and activation of metastasis in bladder cancer. J Urol 2012;188:615-23.
137. Puerta-Gil P, Garcia-Baquero R, Jia AY, Ocana S, Alvarez-Mugica M, Alvarez-Ossorio JL, et al. miR-143, miR-222, and miR-452 are useful as tumor stratification and noninvasive diagnostic biomarkers for bladder cancer. Am J Pathol 2012;180:1808-15.
138. Pignot G, Vieillefond A, Vacher S, Zerbib M, Debre B, Lidereau R, et al. Hedgehog pathway activation in human transitional cell carcinoma of the bladder. Br J Cancer 2012;106:1177-86.
139. Liu W, Qi L, Lv H, Zu X, Chen M, Wang J, et al. MiRNA-141 and miRNA-200b are closely related to invasive ability and considered as decision-making biomarkers for the extent of PLND during cystectomy. BMC Cancer 2015;15:92.

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