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Review

microRNA expression profiles as decision-making biomarkers in the management of bladder cancer

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Summary. Bladder cancer (BC) is generally divided into non-muscle-invasive BC (NMIBC) and muscle-invasive BC (MIBC). The standard treatment protocol for MIBC patients is radical cystectomy preceded by neoadjuvant chemotherapy (NAC). About one-half of the MIBC patients show a priori resistance to chemotherapy, and are therefore exposed to the risks of disease progression and toxicity from ineffective NAC. The discovery of microRNA (miRNA) regulation in tumorigenesis has provided new directions for the development of a new type of BC biomarkers. In this review, we describe the emerging miRNAs as BC biomarkers for different purposes, including diagnosis, prognosis and therapeutic response.

miRNA expression profile changes with alteration of the tissue phenotype. This phenomenon is utilized to predict tumor diagnosis, cancer subclass, disease stage, prognosis and therapeutic response. We classified the miRNAs which are involved in bladder cancer according to malignant potential, chemoresistance, discrimination between normal to cancerous and clinical outcome. Focusing on the major obstacle regarding MIBC patient's NAC response, we summarized the miRNAs that are deregulated and have the potential to identify the patients resistant to NAC, such as miR-34, miR-100, miR-146b and miR-9 and miR-193a-3p. In conclusion, miRNAs expression profile of bladder cancer patient is a promising tool that can serve as biomarker for different aims. Based on this profile we propose upfront radical cystectomy instead of standard NAC to those MIBC patients who are at higher risk for chemoresistance and poor response.

Key words: Bladder cancer, miRNA, Biomarker, Treatment, Neoadjuvant chemotherapy

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Introduction

microRNAs (miRNAs) comprise a class of small, non-coding endogenous RNAs that regulate gene expression by directing their target mRNAs to downregulate gene expression in a variety of ways, including translational repression and mRNA cleavage. Their discovery in 1993 as a novel mechanism of posttranscriptional gene regulation added a new dimension to the understanding of complex gene regulatory networks (Lee et al., 1993). Since then, increasing numbers of miRNAs have been recognized in mammals. Over 3000 miRNAs have been identified and fully sequenced in humans alone. Based on computer models, miRNAs in humans have a direct influence on at least 30% of genes in the whole genome (Zhang, 2008), and 60% of all mRNAs are predicted to be under miRNA control (Bartel, 2009). Those numbers illustrate the apparent importance of miRNA in the regulation of gene expression.

Cancer is an extremely diverse and complex disease that results from alterations at various molecular levels, including miRNAs. miRNAs are directly involved in numerous processes, such as proliferation, apoptosis, cell cycle control, differentiation, migration, and

metabolism, which are all cancer-related pathways. Consequently, the expression profiles of certain miRNAs are closely related to cancer progression. The bulk of studies that have been conducted during the past few decades used DNA microarrays and next-generation sequencing techniques on a genome-wide scale (Li et al., 2015b). They also used microarray expression from a wide spectrum of cancers (Lu et al., 2005; Volinia et al., 2006; Croce, 2009; Munker and Calin, 2011) in order to identify distinct miRNA signatures for cancers. Influxing results have shown that these miRNA are not only differentially expressed in malignant tissues compared to normal tissues, but that they also vary among different types of cancers (Croce, 2009; Heneghan et al., 2010), leading to the recognition that these molecules have the potential to act as oncogenes as well as tumor suppressors.

In this review, we summarize the relevant articles, including original research studies and reviews that describe the relationship between miRNAs and bladder cancer. The key words bladder cancer, urothelium carcinoma, miRNA, biomarker and neoadjuvant chemotherapy were searched in multiple electronic databases through December 2015.

Bladder cancer

Bladder cancer (BC) most commonly refers to carcinoma of the urothelium, the epithelial urinary bladder lining. Less common types of BC include squamous cell carcinoma and adenocarcinoma. The etiology of bladder malignancy includes both genetic and environmental factors.

According to the American Cancer Society, BC is the fourth most common cancer among American men, mainly in older men, who are about three- to four-times more likely to develop the disease than women. Non-muscle invasive BC (NMIBC) represents around 70% of the 70,000 new cases diagnosed in the USA each year (Siegel et al., 2015). Despite medical advances, there has been no reduction in the mortality of bladder cancer patients over the past 20 years (SEER Stat Fact Sheets:

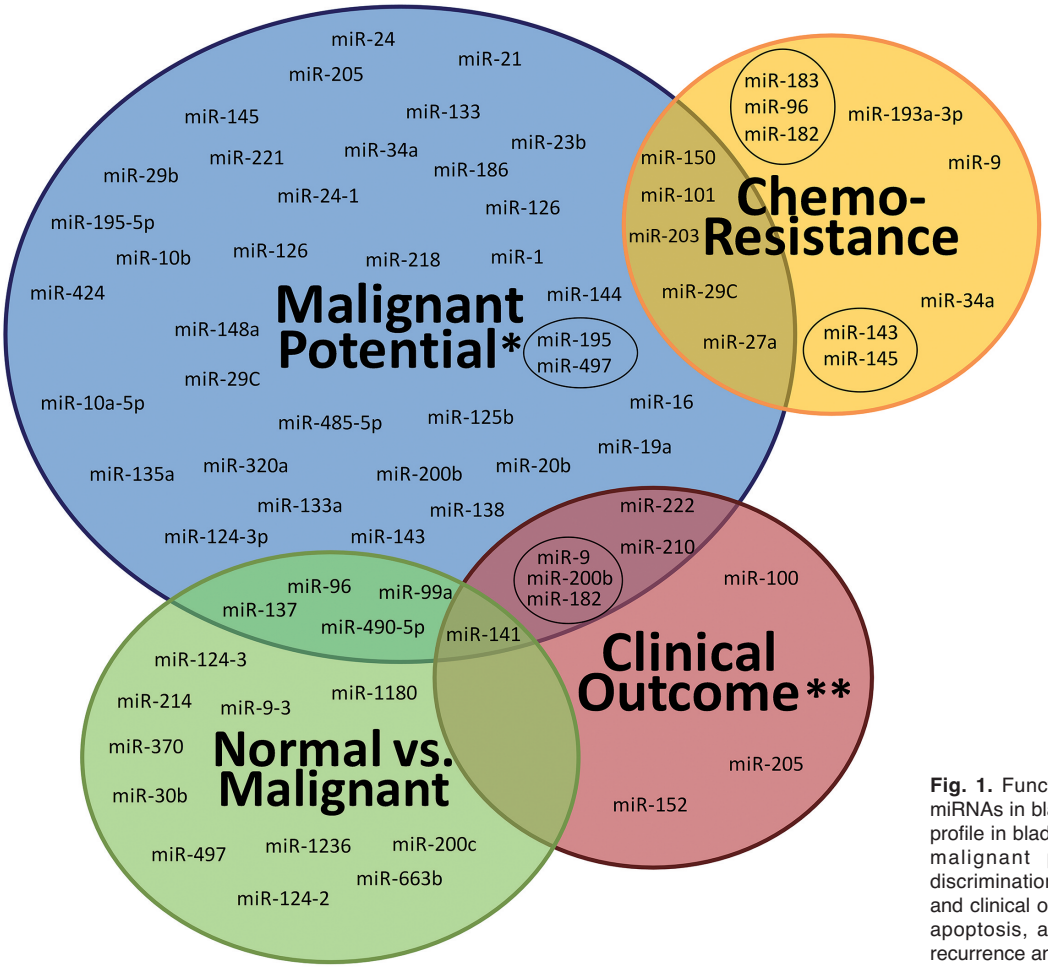

Fig. 1. Functional clustering of deregulated miRNAs in bladder cancer. miRNA expression profile in bladder cancer grouped according to malignant potential, chemoresistance, discrimination between normal to cancerous and clinical outcome. *, invasion, proliferation, apoptosis, angiogenesis and migration. **, recurrence and survival.

Bladder Cancer), a fact that emphasizes the importance of finding innovative research approaches to explore new therapies.

The TNM system is used for the staging of BC, where T (1 to 4) indicates the degree of tumor penetration in adjacent layers of the bladder wall, N (0 to 3) indicates the potential invasion of cancer cells to the lymph nodes, and M (0 or 1) indicates whether the cancer has metastasized. Treatments are generally adjusted according to this TNM staging system (Braicu et al., 2015).

miRNA in BC

The expression pattern of miRNAs varies between tissues and between normal and pathologic developmental stages. Since the miRNA expression profile changes with alteration of the tissue phenotype, it can be used to predict tumor diagnosis, cancer subclass, disease stage, prognosis and therapeutic response (Fig. 1). The expression of miRNAs and their target genes in various aspects of carcinogenesis are listed in Table 1.

The gold standard for the initial diagnosis of BC is cystoscopy followed by transurethral resection of the bladder tumor (TUR-BT). Posttreatment follow-up includes repeated cystoscopies, which are uncomfortable, costly and, most importantly, invasive methods (Avritscher et al., 2006). There are noninvasive methods for continued follow-up that are comprised of several acceptable bladder tumor antigens, including nuclear matrix protein 22 (NMP22), microsatellite analysis (MSA), hyaluronic acid and hyaluronidase (HA-HAase), survivin and telomerase (Vrooman and Witjes, 2008). Because of their debatable sensitivity and specificity values, however, those markers did not replace cystoscopy for BC follow-up. Therefore, there is a need to develop new accurate biomarkers for BC diagnosis and posttreatment follow-up. The presence of

Table 1. Deregulated miRNAs in Bladder Cancer.

miRNA	Protein	Function	Reference
miR-27a	RUNX-1	chemo-sensitivity	(Deng et al., 2015a,b)
miR-193a-3p	SRSF2; PLAU; HIC2 HOXC9	chemoresistance	(Lv et al., 2015)
miR-21	PTEN/PI3K/AKT/mTOR pathway	aerobic glycolysis	(Yang et al., 2015)
miR-152	NA	NMIBC tumor recurrence	(Jiang et al., 2015)
miR 485 5p	high mobility group; AT hook 2 (HMGA2)	cell metastasis and EMT	(Chen et al., 2015a,b,c)
miR-186	NSBP1	cell proliferation and invasion	(Yao et al., 2015)
miR-20b	MMP-2	cell cycle-modulated proliferation, migration and invasion	(Park et al., 2015)
miR-24	CARMA3	proliferation/ apoptosis; cell cycle; invasion EMT	(Zhang et al., 2015a)
miR-424	DNMT1	aggressive tumor growth, advanced clinical stage; poor prognosis invasion ability	(Wu et al., 2015a)
miR-135a; miR-497	PHLPP2 and FOXO1	cell proliferation	(Mao et al., 2015)
miR-663b	NA	normal/malignant discrimination	(Du et al., 2015)
miR-101	CDK8	poor prognosis	(Li et al., 2015a,b,c)
miR-218	BMI-1	proliferation, migration invasion	(Cheng et al., 2015a,b,c)
miR-200c; miR-141; miR-30b	NA	normal/malignant discrimination	(Mahdavinezhad et al., 2015)
miR-145	HIF-1	cell death	(Blick et al., 2015)
miR-141	NA	Invasion tumor grade	(Mahdavinezhad et al., 2015)
miR-221	STMN1	metastasis	(Liu et al., 2015)
miR-148a	DNMT1	tumor suppressor	(Lombard et al., 2016)
miR-34a	CD44	cell cycle	(Yu et al., 2015)
miR-193a-3p	HOXC9	drug resistance	(Lv et al., 2015)
miR-23b	RAB	invasion, angiogenesis, metastasis	(Ostenfeld et al., 2014)
miR-126	ADAM9	invasion aggressiveness	(Jia et al., 2014)
miR-205; miR-145; miR-125b	ZEB1; ZEB2	histopathological grade	(Lee et al., 2014)
miR-19a	PTEN	aggressiveness	(Feng et al., 2014b)
miR-145	IGF-IR	apoptosis	(Zhu et al., 2014)
miR-24-1	FOXM1	proliferation	(Inoguchi et al., 2014)
miR-133	SFR; HDAC4 cyclin D2	proliferation, migration, invasion	(Yu et al., 2014)
miR-9; miR-29C	SMAD3; SMAD5; SMAD7	cell cycle inflammatory response	(Zhao et al., 2014a,b)
miR-126	PLK2; PI3KR2; Crk	apoptosis/proliferation	(Liu et al., 2014)
miR-29C	BCL-2; MCL-1	apoptosis	(Xu et al., 2014a)
miR-10b	KLF4 HOXD10	migration, invasion	(Xiao et al., 2014)
miR-195; miR-497	NA; Genomic cluster	proliferation, migration, invasion	(Itesako et al., 2014)
miR-27a	AGGF1	cancer aggressive	(Xu et al., 2014b)
miR-200b	MMP 16	migration	(Chen et al., 2014)
miR-99a; miR-125b	NA	tumor grade	(Zhang et al., 2014b)
miR-203	LASP1	tumors progression	(Hailer et al., 2014)
miR-99a	NA	normal/malignant discrimination proliferation, aggressive	(Feng et al., 2014a)
miR-101	NA	tumor diameter, stage, grade, lymph node involvement, metastasis	(Zhang et al., 2014c)
miR-210	NA	progression-free survival relapse-free survival	(Wang et al., 2014b)

miRNAs in body fluids (extracellular miRNA), such as serum, blood and especially in urine was recently discovered, offering an alternative option for noninvasive diagnosis of BC (Hanke et al., 2010; Adam et al., 2013; Jiang et al., 2015). A recent study demonstrates the importance of urine as an miRNA source. In this study miRNA profiles from bladder tumors were identified in urine exosomes and WBCs but not in blood plasma taken from the same patients. The varying relationships between miRNA detected in biological media from the same patient, emphasize the potential of urine-based microRNAs as biomarkers for bladder cancer (Armstrong et al., 2015).

The parameters important for biomarker selection are sensitivity [true positives/(true positive + false negative)] and specificity [true negatives/(true negative + false positive)]. Unfortunately, biomarkers with ideal specificity and sensitivity are difficult to find. One potential solution is to use the combined power of different biomarkers, each of which alone may not offer satisfactory specificity or sensitivity. A potential biomarker should be confirmed and validated using hundreds of samples. Among the various studies that focused on this issue, only a few found miRNAs that had satisfactory sensitivity and specificity values, such as miR-200a (Wang et al., 2012a), miR-125b and miR-126 (Snowdon et al., 2012). In addition, it was shown that epigenetic silencing of miRNA genes by methylation could be a useful biomarker for BC detection as well (Yuan et al., 2016). Moreover, miRNA molecules are useful in molecular diagnostics due to their greater stability *in vitro* compared to mRNA molecules (Jung et al., 2010). Yamada and colleagues (Yamada et al., 2011) measured the expression of 27 miRNAs in 104 BC

Table 1. Continuation.

miR-222	NA	tumor grade and stage poorer survival	(Zhang et al., 2014a)
miR-101	COX 2	proliferation chemoresistance	(Bu et al., 2014)
miR-27a	SLC7A11	chemoresistance	(Drayton et al., 2014a)
miR-34a	CD44	chemoresistance	(Li et al., 2014a)
miR-150	PDCD4	invasion chemoresistance	(Lei et al., 2014)
miR-10a-5p	NA	stage and progression	(Segersten et al., 2014)
miR-370; miR-1180; miR-1236	p21	proliferation	(Wang et al., 2014a)
miR-1	NA	cell growth cell motility	(Wang et al., 2014c)
miR-143	mTOR-STAT3	glucose metabolism	(Li et al., 2014b)
miR-145	PAK1	invasion	(Kou et al., 2014)
miR-101	EZH2	apoptosis	(Wang et al., 2014d)
miR-320a	ITGB3	invasion	(Shang et al., 2014)
miR-193a-3p	LOXL4	drug resistance	(Deng et al., 2014)
miR-150	PDCD4	drug resistance	(Lei et al., 2014)
miR-203	bcl-w; Akt2/Src	proliferation	(Bo et al., 2011)
miR-99a; miR-100	FGFR3 and FOXA1	tumor grade	(Drayton et al., 2014b)
miR-137	PAQR3	proliferation invasion	(Xiu et al., 2014)
miR-21	NA	tumor grade	(Monfared et al., 2013)
miR-141; miR-205	NA	survival time	(Ratert et al., 2013)
miR-125b	SIRT7; MALAT1 (long non-coding RNA)	proliferation, motility	(Han et al., 2013)
miR-29b; miR-29c	NA	cell growth, proliferation, cell cycle and apoptosis	(Xu et al., 2013a)
miR-490-5p	c-Fos	cell cycle proliferation	(Li et al., 2013)
miR-124-3p	ROCK1	cell cycle, migration and invasion	(Xu et al., 2013b)
miR-16	Cyclin D1	proliferation	(Jiang et al., 2013)
miR-146b; miR-9	NA	MIBC discrimination	(Pignot et al., 2013)
miR-9; miR-182; miR-200b	NA	MIBC tumor aggressiveness recurrence-free overall survival	(Pignot et al., 2013)
miR-137; miR-124-2; miR-124-3; miR-9-3	methylation	normal/malignant discrimination	(Shimizu et al., 2013)
miR-144	EZH2	proliferation	(Guo et al., 2013)
miR-29c	NA	prognostic, invasiveness	(Rosenberg et al., 2013)
miR-214	NA	normal/malignant discrimination	(Kim et al., 2013)
miR-96	FOXO1	apoptosis	(Guo et al., 2012)
miR-195-5p	GLUT3	glucose uptake cell growth apoptosis	(Fei et al., 2012)
miR-100	NA	Stage, the recurrence, the progression, poorer progression-free survival and overall survival	(Wang et al., 2012a,b)
miR-490-5p; miR-96	NA	normal/malignant discrimination	(Han et al., 2011)
miR-1; miR-133a	TAGLN2	tumor grade	(Yoshino et al., 2011)
miR-145	Syndecan-1	differentiation	(Fujii et al., 2015)
miR-200 family	ZEB-1; ZEB-2; BMI1	MIBC discrimination histopathological grade	(Martinez-Fernandez et al., 2015)
miR-96	CDKN1A	proliferation	(Wu et al., 2015b)
miR-203	Bcl-w; Survivin	chemo-sensitivity	(Zhang et al., 2015b)
miR-138	ZEB-2	metastasis	(Sun et al., 2015a,b)

NA, not analyzed.

tissues and in urine samples and demonstrated that miR-96 and miR-183 concentrations in urine were significantly correlated with tumor stage and grade. Since the expression of those miRNAs was significantly decreased after radical surgery, it was suggested that they can be used as prognostic molecular markers of cancer recurrence (Yamada et al., 2011).

In general, the therapeutic modulation of miRNAs is achieved by inhibiting oncogenic miRNAs by miRNA antagonists (termed oncomiR) or by re-introducing tumor suppressor miRNAs. The therapeutic potential of miRNAs in cancer has recently been demonstrated in several published studies which were summarized by Naidu's group (Naidu et al., 2015).

Unlike prognostic biomarkers which are defined as markers that suggest the likely outcome of a disease irrespective of treatment, predictive biomarkers indicate which population of patients is likely to respond to a particular treatment (Drucker and Krapfenbauer, 2013).The major challenges in exploiting miRNA for therapeutics are RNA stability and tumor site-targeted delivery. Multiple approaches were subsequently developed to circumvent these hurdles. For stability, chemical modifications, such as 2'-OH ribose or phosphate backbone (locked nucleic acids, or LNAs), made synthetic miRNAs less susceptible to nuclease degradation (Davis et al., 2006; Obad et al., 2011). For efficient delivery, lentiviral, adenoviral and adeno-associated viral vectors expressing miRNA antagonists or mimics have proven to be effective delivery systems in various cancer models (Liu and Berkhout, 2011). Exosomes and vesicles released by virus-infected cells have recently been shown to enclose and deliver miRNAs into the target cells (Pegtel et al., 2010). Another approach of vectors expressing tandem repeats of miRNA antisense sequences, termed miRNA sponges, were shown to be effective in modulating miRNA expression, and especially effective for targeting a family of miRNAs sharing a common sequence (Ebert and Sharp, 2010). In addition, modified oligonucleotides and various nanomaterial-delivering particles are being used for these purposes, such as polyethylenimine, atelocollagen, as well as gold- and silica-based nanoparticles (Shibata et al., 2013).

Of all the miRNAs that have been proposed as potential candidates for cancer therapy, the most promising is miR-34, a key tumor suppressor miRNA. Ectopic expression of miR-34 was reported to have therapeutic benefits for a variety of cancers (Misso et al., 2014). A liposome-based mimic of miR-34 (MRX34) recently progressed into phase I clinical trials (identifier: NCT01829971).

MIBC vs. NMIBC

Urothelial carcinoma of the bladder is classified according to the differentiation state of the cancer cells, i.e., low-grade *versus* high-grade. Seventy-five percent of diagnosed BC cases are NMIBCs which are confined to the bladder mucosa or lamina propria, whereas the remaining cases are muscle-invasive BCs (MIBCs), which are mostly high-grade tumors. Despite aggressive

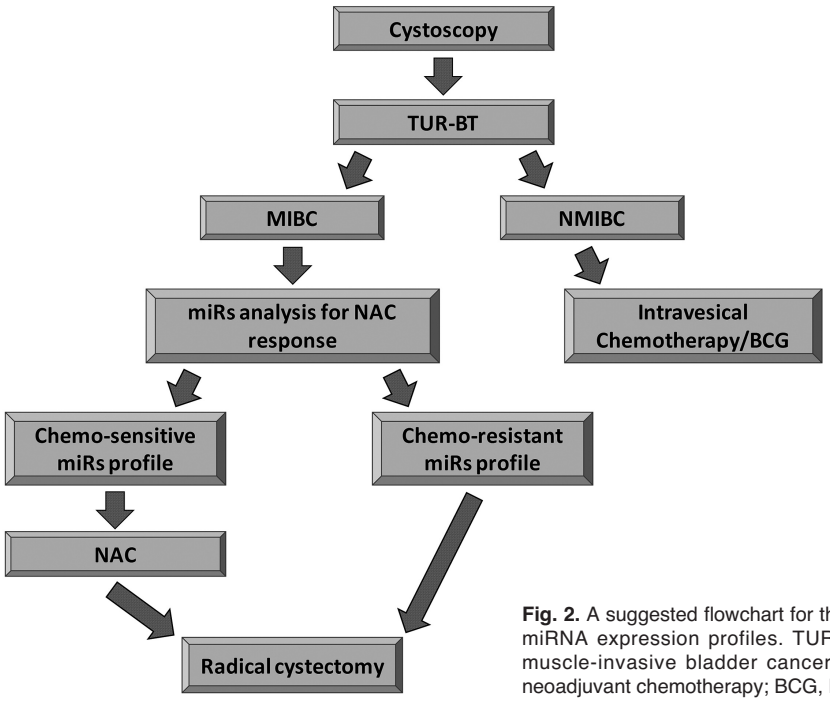

Fig. 2. A suggested flowchart for the management of bladder cancer patients based on their miRNA expression profiles. TUR-BT, transurethral resection of bladder tumor; MIBC, muscle-invasive bladder cancer; NMIBC, nonmuscle-invasive bladder cancer; NAC, neoadjuvant chemotherapy; BCG, Bacillus Calmette Guérin.

local and systemic therapies, MIBC is associated with a high rate of recurrence, poor overall prognosis and mortality (the 5-year mortality rate for patients with MIBC is between 30% to 70%), mainly because of a high rate of systemic failure (Apolo et al., 2015).

NMIBCs and MIBCs have different genetic backgrounds: NMIBC is often associated with mutations in H-RAS and PI3K, while TP53, RB and PTEN mutations are detected in most of the high-grade MIBCs (Castillo-Martin et al., 2010). Fibroblast growth factor receptor 3 (FGFR3) has an important role in both grades of BCs, either by mutation in NMIBCs or by increased expression in MIBCs (Tomlinson et al., 2007). FGFR3 is one of four members of the FGFR family of receptor tyrosine kinases that serve as cell surface receptors for the FGF ligands. The prognostic role of FGFR3 as a marker for recurrence, progression, and survival has been extensively investigated, and it has been suggested that there is a negative correlation between FGFR3 mutation and disease progression (Kompier et al., 2009; van Oers et al., 2009). It has also been shown that miR-100 is downregulated in NMIBCs, which probably causes increased expression of FGFR3 (Catto et al., 2009).

An extensive study which attempted to identify miRNAs involved in bladder carcinogenesis found a discrimination expression miRNA pattern between normal and BC samples, including between NMIBC and MIBC. Except for two miRNAs, miR-146b and miR-9, which were specifically upregulated in MIBC, the majority of miRNAs were deregulated in the same way in both types of bladder tumors. Furthermore, the ratio between miR-21 and miR-205 may differentiate between these two cancer grades (Neely et al., 2010). In terms of pathological stage, a 3-miRNA signature (miR-9, miR-182 and miR-200b) was significantly related to MIBC tumor aggressiveness and associated with both recurrence-free and overall survival (Pignot et al., 2013). A recent study suggested that a four-miRNA signature (let-7c, mir-125b-1, mir-193a, and mir-99a), which is associated with the progression and aggressiveness of MIBC, could be a promising prognostic marker of MIBC (Xu et al., 2015).

Hypoxia-related miRNAs in BC

Hypoxia is a major feature that occurs in most solid tumors, BC among them. Intratumoral hypoxia is primarily mediated by the key transcription factor, hypoxia-inducible factor 1 (HIF-1) to activate the "survival machinery" in cancer cells, including angiogenesis, invasion, proliferation and metabolic adaptation (Mabjeesh and Amir, 2007). HIF-1 is a heterodimeric transcription factor composed of a HIF-1α subunit and a HIF-1β subunit. HIF-1α is constitutively produced and degraded under normal O_2 conditions (normoxia), while HIF-1β expression is unaffected by O_2 levels (Hirota and Semenza, 2005).

There are some hypoxia-regulated miRNAs (HRMs) that have been recognized as being cancer-related. For example, HIF-1is was shown to regulate miR-210 in renal cancer (McCormick et al., 2013). Renal cancer tissues have high miR-210 expression compared with normal renal cortex tissue. That expression was associated with better clinico-pathological prognostic factors (McCormick et al., 2013). HIF and miR-210 were also identified as being a part of tumorigenesis in colon and head and neck cancers (Gee et al., 2010; Neal et al., 2010; Sun et al., 2015b). In addition, miR-338 inhibited migration and proliferation by targeting HIF-1α in nasopharyngeal carcinoma (Shan et al., 2015). Hypoxic regions have been demonstrated in both the luminal surface on NMIBC and around the peripheral necrotic areas and hypoxic cores in larger MIBCs (Turner et al., 2002; Theodoropoulos et al., 2004).

As mentioned above, there is a negative correlation between miR-100 and FGFR3 expression in NMIBCs (Catto et al., 2009). Blick and colleagues (Blick et al., 2013) investigated the effects of hypoxia on both miR-100 and FGFR3 expressions (Blick et al., 2013) and found that FGFR3 expression was induced by hypoxia in a transcriptional and HIF-1α-dependent manner in NMIBC cell lines. In addition, miR-100 levels decreased in the same hypoxic condition, which indicates that the induction of FGFR3 expression was also dependent on miR-100. The HRM signature for BC cell lines includes miR-210, miR-193b, miR-145, miR-125-3p, miR-708 and miR-517a. Interestingly, the results were similar for both the MIBC and NMIBC cell lines (Blick et al., 2015), but not for miR-145 which was uniquely overexpressed in the NMIBC cell line. Focusing on miR-145 revealed that this miRNA is a direct HIF-1α target gene and that it is correlated with apoptosis. Finally, increased miR-145 level in hypoxia was shown to contribute to cell death as an adaptive mechanism in tumorigenesis (Blick et al., 2015).

MIBC neoadjuvant chemotherapy response

The current standard treatment for MIBC (T2-T4/N0/M0) is radical surgery preceded by neoadjuvant chemotherapy (NAC). Therefore, an important issue in this setting is the response of MIBC patients to NAC. Administration of NAC is thought to eradicate undetected micrometastases (Sternberg et al., 2013). An acceptable NAC regimen includes cisplatin, methotrexate, vinblastine, and doxorubicin. Two large, well-designed, randomized trials using cisplatin-based combinations (Grossman et al., 2003; International Collaboration of et al., 2011) and two meta-analyses of NAC in MIBC (Advanced Bladder Cancer Meta-analysis, 2005a, b) support this treatment concept based on improved survival and down-staging compared to surgery alone. One Phase III clinical trial on neoadjuvant cisplatin-based chemotherapy demonstrated a survival benefit (median 77 months *versus* median 46 months) and down-staging (T2-T4 to ≤T1 disease at cystectomy) compared to surgery alone (Grossman et al., 2003).

According to the National Cancer Database reports, NAC in MIBC increased from 10.2% in 2006 to 20.9% in 2010 (Zaid et al., 2014).

The major obstacle associated with this NAC regimen is that not all patients respond to NAC, with an estimated response rate of approximately 50% of cases (Hurst and Knowles, 2014). For the NAC-resistant group of patients, the significant concern is disease progression due to delay in surgery and the potential toxicity of NAC. This emphasizes the need to define molecular response predictors for patient selection. Predicting response to NAC will potentially allow avoidance of disease progression among patients unlikely to respond. Indeed, several studies tried to define the molecular signatures of NAC-responsive and NAC-resistant cases as a subtype of MIBC (Takata et al., 2005; Kato et al., 2011; Sjodahl et al., 2012; Baras et al., 2015).

Choi's group's recent breakthrough publication has characterized 73 MIBC tissues by using whole-genome gene expression profile to implicate it in different aspects of treatment response (Choi et al., 2014). Cluster analysis revealed 3 major subtypes which the authors termed basal, luminal and "p53-like". One of the features of the "p53-like" subtype was NAC resistance. To confirm that observation, Choi group conducted another investigation that focused on this specific question with an expanded NAC cohort, 23 archival tumors from a phase III chemotherapy trail and some BC cell lines. The "p53-like" molecular signature was indeed associated with resistance to NAC (Choi et al., 2014).

The role of miRNAs in chemoresistance of BC

The role of miRNAs in regulating BC progression has been extensively studied, but little is known about their involvement in the mechanisms underlying treatment response in BC. The few published studies on the potential of miRNAs as therapeutic targets to overcome this obstacle are summarized in Table 2.

The most studied miRNA in the context of chemoresistance is miR-193a-3p. It was found to be a direct target of the LOXL4 (Deng et al., 2014), PSEN1 (Deng et al., 2015a), SRSF2, PLAU and HIC2 genes (Lv et al., 2014), which affect oxidative stress, DNA damage, Notch, NF-κB, and Myc/Max signaling pathways. In addition, cisplatin and paclitaxel were reported to alter the expression of miR-143/145 and miR-183/96/182 clusters (Papadopoulos and Scorilas, 2015). Another study demonstrated the importance of the natural miRNAs processes in response to chemotherapy (Deng et al., 2015b). A single nucleotide polymorphism in pre-miR-27a, rs11671784, significantly decreased mature miR-27a expression, resulting in increased RUNX-1 expression and was followed by weakened chemosensitivity in BC (Deng et al., 2015b).

The pressing need to elucidate the cause underlying the high rate of chemotherapy failures among MIBC patients led many researchers to focus on miR-34a, whose expression is low in MIBC compared to NMIBC. Li and colleagues (Li et al., 2014a) demonstrated that miR-34a was frequently decreased in human MIBC tissues and cell lines through promoter hypermethylation. In addition, they also showed that cisplatin can upregulate miR-34a by promoter demethylation in MIBC cell lines, thereby sensitizing the cells to chemotherapy (Li et al., 2014a). Their research attributed the chemoresistant phenomenon to the CD44 target gene. miR-34a had earlier been described as a

Table 2. Deregulated miRNAs in Bladder Cancer Chemoresistance.

miRNA	Target genes	Chemotherapeutic drug	Reference
miR-193a-3p	SRSF2 PLAU HIC2	Pirarubicin Paclitaxel Adriamycin Epirubicin Hydrochloride Cisplatin	Lv et al., 2014
miR-193a-3p	LOXL4		Deng et al., 2014
miR-193a-3p	PSEN1		Deng et al., 2015a
miR-193a-3p	IGF5		Li et al., 2015a,b
miR-143/145 miR-183/96/182	NA	Cisplatin Paclitaxel	Papadopoulos and Scorilas, 2015
miR-34a	CDK6 SIRT1	Cisplatin	Vinall et al., 2012
miR-34a	CD44	Cisplatin	Li et al., 2014
miR-101	COX-2	Cisplatin	Bu et al., 2014
miR-27a	RUNX-1	Paclitaxel Rifampin Cisplatin Adriamycin	Deng et al., 2015b
miR-27a	SLC7A11	Cisplatin	Drayton et al., 2014a,b
miR-150	PDCD4	Cisplatin	Lei et al., 2014

NA, not analyzed.

direct target of two other genes, CDK6 and SIRT1, in the p53 signaling pathway (Vinall et al., 2012).

miR-150 expression was recently found to be significantly increased in MIBC cell lines (Lei et al., 2014). Treatment with a miR-150 inhibitor strongly sensitized MIBC cells to cisplatin. In addition, PDCD4 was identified as a direct target of miR-150, while transfection with pLEX-PDCD4 plasmid efficiently sensitized MIBC cells to cisplatin. That work showed promise as a therapeutic strategy for MIBC (Lei et al., 2014).

Cisplatin is the most commonly used NAC that induces apoptosis through DNA cross-linking. Overexpression of miR-383 has been shown following cisplatin treatment. Gadd45g, a stress-response protein, which has been implicated in several biological processes, including DNA repair, the cell cycle and cell differentiation, is a direct target of miR-383 (Zhao et al., 2014a). Reduced expression of miRNA-27a reportedly modulated cisplatin resistance in BC by targeting the cystine/glutamate exchanger, SLC7A11 (Drayton et al., 2014b).

One of the prominent features of BC is the epithelial mesenchymal transition (EMT) process, in which cells convert from the epithelial to the mesenchymal state. At the molecular level, EMT is characterized by the loss of E-cadherin as the result of increased expression of several transcriptional repressors of E-cadherin expression. EMT is associated with BC progression, metastasis, poor clinical outcome and drug sensitivity (McConkey et al., 2009). The relationship between EMT and the development of drug resistance has been demonstrated in lung, colorectal and breast cancer as well. ZEB1 and ZEB2 are E-cadherin repressors which were found to be a direct target of 4 miRNAs, miR-145, miR-205, miR-125b and miR-200c (Zaravinos, 2015).

NAC administration before surgery is an acceptable procedure in breast cancer as well (Cleator et al., 2002). Two miRNAs, miR-34a and miR-122, were reportedly upregulated in breast cancer patients' plasma after NAC (Freres et al., 2015). Those authors considered that NAC-induced miRNA expression might facilitate tumor response to chemotherapy. At the therapeutic level, an ovarian cancer model study demonstrated that *in vivo* delivery of miR-31 resulted in increased sensitivity for paclitaxel (Mitamura et al., 2013).

Conclusion

The heterogeneity and even contradictory publications on miRNA involvement in BC led to the performance of 2 recent meta-analyses (Cheng et al., 2015b; Ouyang et al., 2015). Ouyang's group summarized 41 studies of miRNA in urologic cancers and came to two important conclusions. Firstly, a subgroup of multiple miRNAs has significantly better diagnostic specificity than a single miRNA subgroup. Secondly, there is a significant difference in biomarker sensitivity among groups of individuals with a variety of ethnic origins. Cheng and colleagues based their meta-analysis on 23 studies (719 BC patients and 494 controls), which focused on urine miRNAs as biomarkers. They summarized the pooled sensitivity, specificity, positive and negative likelihood ratios, diagnostic odds ratio, and area under the curve parameters in order to determine diagnostic accuracy. Their final calculation revealed that urine miRNA assays were more sensitive than the traditional urine cytology testing in BC diagnosis. In addition, urine sediment showed a different miRNA profile compared to urine supernatant, which apparently derives from diverse cell types, and urine sediment miRNAs reflected intracellular expression, whereas urine supernatant miRNAs reflected cell surface and systemic circulation expression (Wang et al., 2012a). Altogether, these results indicate the applicability of miRNA analysis in the setting of BC.

In this review, we focused on a well-known phenomenon regarding the NAC response of MIBC patients. Approximately 50% of the patients respond to the NAC regimen, which means that the NAC-resistant patients are at risk of disease progression and potential toxicity from ineffective NAC. Based on the published data, we recommend performing miRNA expression profiles on all MIBC patients in order to select those who would be most likely to respond to NAC, and referring chemoresistant patients to immediate radical cystectomy (Fig. 2). We believe that miRNA expression analyses will become part of our clinical practice in the field of BC in the near future.

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