

MicroRNAs as biomarkers associated with bladder cancer

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Abstract

Bladder cancer is the fifth most common cancer with significant morbidity and mortality. Recently, numerous studies demonstrated that microRNAs are emerging as diagnostic biomarkers for bladder cancer. Specific miRNA profiles have been identified for several samples from patients with bladder cancer. MicroRNAs are noncoding RNA molecules of approximately 23 nucleotides that play important roles in multiple steps during the progression of bladder cancer. Here, we review the expression profiles of miRNAs and their biological functions, regulation, and clinical implications in bladder cancer. Either downregulation or upregulation of miRNAs occurs in bladder cancer through epigenetic changes or defects of the biogenesis apparatus. Deregulation of miRNAs is involved in cell cycle arrest, apoptosis, proliferation, metastasis, drug resistance, and other functions in bladder cancer. A number of miRNAs, have been associated with tumor type, stage, or patient survival, and miRNAs might be developed as diagnostic or prognostic markers. A better understanding of the roles of miRNAs in bladder cancer will shed light on the molecular mechanisms of bladder cancer.

Keywords: Bladder cancer, Biomarkers, MicroRNA, Diagnostic.

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Introduction

Bladder cancer (BC) most commonly refers to carcinoma of the epithelial lining of the urinary bladder, the urothelium. Its symptoms are nonspecific and include hematuria, discomfort during urination, and higher frequency and urgency of urination (1). Urothelial bladder cancer constitutes two distinct clinical phenotypes. The common tumors are low grade and non-invasive which may relapse locally but development infrequently; other tumors which are muscle invasive often develop rapidly and have a poor prognosis (2,3). In the United States, an estimated 54,610 new cases of bladder cancer were expected to occur in men in 2013, and 17,960 women presumably acquired this malignancy. The probability of developing this disease seems to increase with age, growing from a 0.02% chance of having bladder cancer by the age of 39 years to a 3.69% (1 in 27) chance of having

this malignancy over the age of 70 years (1, 4). In the most recent national cancer report in Iran, the age-specific incidence rates for bladder cancer were 13.03 in males and 3.32 in females per 100,000 population (Report of National Cancer Registration Iran, 2008) (5). Early diagnosis is crucial and patients with bladder cancer must be followed every 3 to 6 month. The current modalities for diagnosis and follow-up of the bladder cancer are cystoscopy and urine cytology; one an invasive high-cost method and the other a low-sensitive and operator-dependent method (6,7). In our recent project, we are determining the common mutations in FGFR3 and HRAS genes in Iranian patients with bladder cancer. These genes mutations associated with bladder cancer and have been extensively studied. In recent years, there has been a tremendous and growing interest among researchers to investigate the role of miRNAs in

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bladder cancer.

Diagnosis, grading, and staging

Initial diagnostic workup usually involves cystoscopy and urine cytology. Cystoscopy is the gold standard for the initial diagnosis and staging of bladder cancer. Tumors are classified as low-grade or high-grade (8). Grading refers to the degree of aggressiveness of the cancer cells, with low-grade cancer being the less aggressive cells, which grow slower, look quite normal, and act similar to healthy cells, and with high-grade cancer being characterized by fast-growing cells, which look and act in a disorganized manner and are more likely to progress into the muscle layer of the bladder (1). The most widely used classification for grading of nonmuscle invasive urothelial neoplasms has been the 1973 World Health Organization (WHO) classification. This system has designations for papilloma and Grades 1, 2, and 3 carcinomas. In 2004, members of the WHO and International Society of Urologic Pathologists published and recommended a revised consensus classification for papillary neoplasms (9, 10). T-stages 1 through 4 represent the degree to which the cancer has grown in the adjacent layers of tissue, from the connective tissue just beneath the urothelium (T1) to tissue structures located outside the bladder (T4) (Fig. 1) (1). The vast majority (90%) of bladder cancer cases are transi-

tional cell carcinomas (TCC), of which 75–85% present as non-muscle invasive tumors at the time of first diagnosis (Tis/CIS, Ta, and T1) (11-13).

The standard for the initial diagnosis and prognostic assessment of bladder cancer is cystoscopy and histopathological analysis of biopsy specimens. However, current prognosticators such as tumor grade, size, and multifocality do not accurately reflect clinical outcome and have limited usefulness for a reliable risk-adjusted therapy decision (14,15). Therefore, the identification of new biomarkers to improve the diagnosis and prognosis of different bladder cancer entities is currently a challenge of special interest (6,12,13). Accumulating evidence suggests aberrant miRNA expression patterns in most human malignancies, and some highly expressed miRNAs might function as oncogenes by repressing tumor suppressors; conversely, miRNAs expressed at low levels might function as tumor suppressors by negatively regulating oncogenes (12,13,15). Based on reports describing miRNA signatures, several down-regulated and up-regulated miRNAs have been discovered in bladder cancer (12,13, 15,16). Some of those miRNAs are thought to be potential biomarkers for bladder cancer in diagnosis and prognosis prediction, as well as a treatment target (13,15). Epigenetics has been shown to play a much greater role than previously thought in the

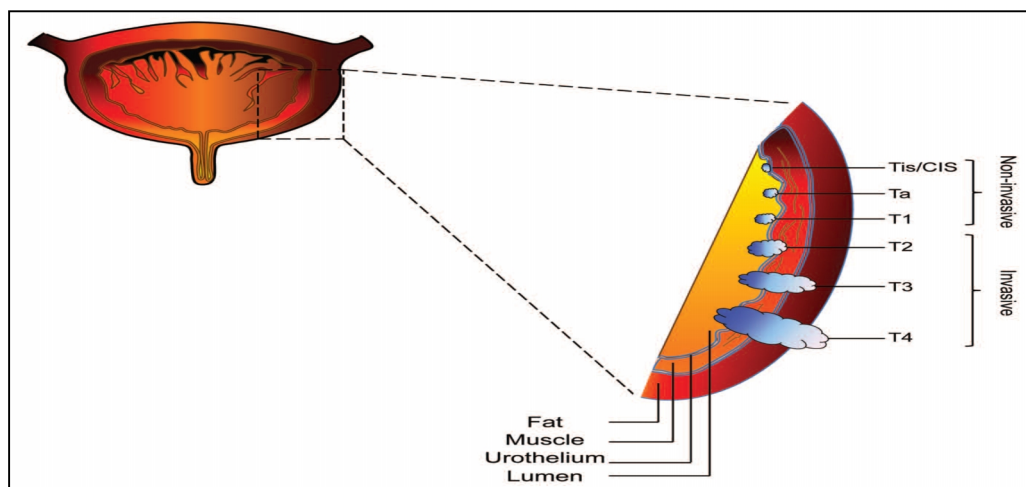


Fig. 1. Diagram of progressive stages of transitional cell carcinoma of the bladder. Non-muscle invasive transitional cell carcinoma is staged at Tis/CIS, Ta, and T1, whereas muscle invasive transitional cell carcinoma is staged at T2, T3 and T4. Tis/CIS, carcinoma in situ.

initiation and propagation of tumors. The use of epigenetics for the diagnosis and treatment of bladder cancer appears to be a promising field. Drugs using epigenetic targets are undergoing a test for their toxicity, safety, and potential synergistic effects with other drugs. Epigenetic profiling also has the potential to identify patients at high risk of bladder cancer through non-invasive urine tests. Such testing could help identify patients at an earlier stage of the disease. The possibility of epigenetic changes being “reversed” makes such epigenetic modification therapy a powerful potential option for cancer treatment in the future (17).

miRNA biogenesis and function

MicroRNAs (miRNAs) are small, non-coding RNA molecules of 21-25 nucleotides in length that regulate the gene expression by base-pairing with the transcripts of their targets i.e. protein-coding genes, leading to down-regulation or repression of the target Genes (18,19). miRNAs are transcribed from the long primary transcript (pri-miRNAs) by RNA polymerase II, in the nucleus and processed into characteristic stem-loop precursor miRNAs (pre-miRNAs) by the enzyme Drosha. Then pre-miRNAs are transported into the cytoplasm, where they are transformed into small, single-stranded miRNAs with the help of Dicer (13,16). Once processed into their mature form, miRNAs enter the miRNA silencing complex (miRISC) to degrade or silence mRNAs. miRNAs regulate gene expression by imperfect or near-perfect base pairing to mostly the 3' untranslated region (UTR) of target mRNAs, thereby inhibiting protein synthesis or causing mRNA degradation(16, 20). The 5' end sequence of miRNA is called “seed” and has a length of 6-8 nucleotides which is energetically favorable for the miRNA target interaction (Fig. 2) (13,20).

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Bladder cancer associated miRNAs

Many diagnostic and prognostic biomarkers of BC have been identified over the past decade. Most of these are the biomarkers for gene transcription, DNA methylation, or protein expression (21). Each miRNA has the potential to target a

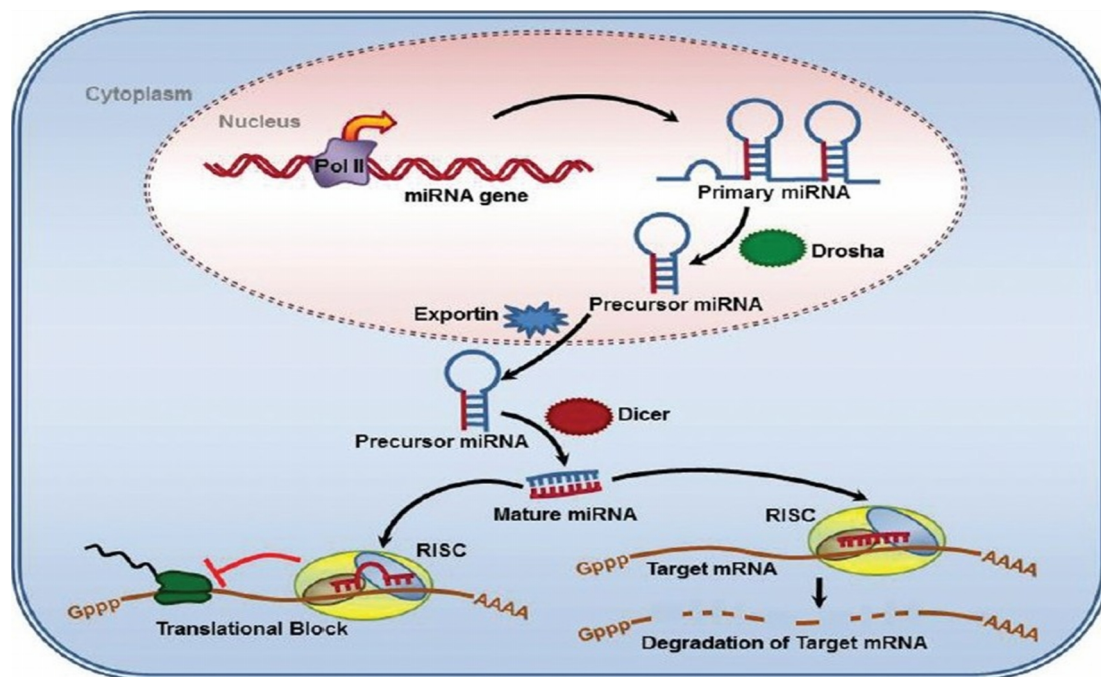


Fig. 2. MicroRNA processing pathway. miRs are processed through multiple steps, starting in the nucleus and continuing in the cytoplasm

large number of genes (on average about 500 for each miRNA family) (22-24). Alteration in miRNA expression may occur earlier in BC and affect carcinogenesis and tumor behavior (20,25,26). Gottardo et al. firstly reported 10 up-regulated miRNAs in BC (25,27). Altered expression of many miRNAs has been identified in Dyrskjot et al. study (25,28). Some miRNAs are related to epithelial mesenchymal transition (25,26,29). Therefore miRNAs expression has a noticeable role in bladder tumor genesis (25). More than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites of the genome. Many types of cancer are associated with aberrantly expressed miRNAs. Both losses and gains of miRNA function contribute to cancer development and continued tumor growth (30,31). miRNAs may act as both oncogenes or tumor suppressors (30,32). Furthermore, different cancer types, stages, and differentiation grades may have unique miRNA expression profiles, which make miRNAs potent biomarkers for cancer diagnosis (30,33,34). Among the studies examined in a review, the most commonly up-regulated miRNA in BC was miR-129 (30,35). In contrast, miR-145 and miR-133a were reported to be down-regulated in cancer tissues, and these two markers allowed the authors to distinguish cancer cells from non-cancer cells with a sensitivity >70% and specificity >75% (30,36). The authors found that the levels of miR-200 family members – miR-141, miR-155, and miR-429 – were lower in urine sediment in BC patients. Levels of miR-200a, miR-200b, miR-200c, miR-141, miR-429, miR-205, miR-192, and miR-146a increased significantly after surgery. These findings suggest that miR-200-family miRNAs (miRs-141, -429, -192, -146a, -141, and others) are promising as non-invasive, diagnostic, and prognostic markers. In another study (28,37), miRNA levels were measured in the plasma of patients with or without BC. The authors identified a total of 79 differentially expressed plasma miRNAs. Some diagnostically relevant miRNAs,

such as miR-200b, were up-regulated in the BC patients, whereas others, such as miR-92 and miR-33, were inversely correlated with the clinical stage of cancer. These findings support the notion that cell-free circulating miRNAs in the blood can be released in the bladder, as well as in many other tissues (30,38). By comparing the miRNA panel in nonmalignant and malignant bladder tissue, the researchers identified seven significantly up-regulated and eight down-regulated miRNAs. miR-100, miR-125b, miR-130a, miR-139-5p, miR-145*, miR-199a-3p, miR-214 and miR-222 were found to be down-regulated in invasive bladder tumors compared to superficial tumors, as well as in other tumor entities. The expressions of miR-20a, miR-106b, miR-141, miR-130b, miR-200a, miR-200a* and miR-205 were found to be up-regulated in bladder cancer (14). miR-10b, 19a, 126, 145, 221, 296-5p and 378 were significantly down-regulated in bladder cancer compared to adjacent normal urothelium. miR-145 was the most down-regulated microRNA of this group. miR-19b, 21, 205 and 210 showed no significant difference between the 2 tissue types. High miR-21 expression correlated with worse overall patient survival ($p = 0.0099$). Multivariate analysis revealed that miR-21, 210 and 378 may serve as independent prognostic factors for overall patient survival ($p = 0.005, 0.033$ and 0.012 , respectively). miR-21 and 378 may serve as independent prognostic factors for recurrence ($p = 0.030$ and 0.031 , respectively) (Fig. 3) (Table 1) (39). To date, the majority of miR studies in BC use profiling to describe dynamic changes in miR expression across stage and grade. Generalized down-regulation of miRs, including those that target the fibroblast growth factor 3 (FGFR3) pathway such as miR-145, miR-101, miR-100 and miR-99a, has been observed in low-grade, non-muscle invasive bladder cancer (NMIBC). In contrast, generalized increased expression of miRs is observed in high grade, muscle invasive bladder cancer (MIBC) compared with adjacent normal bladder

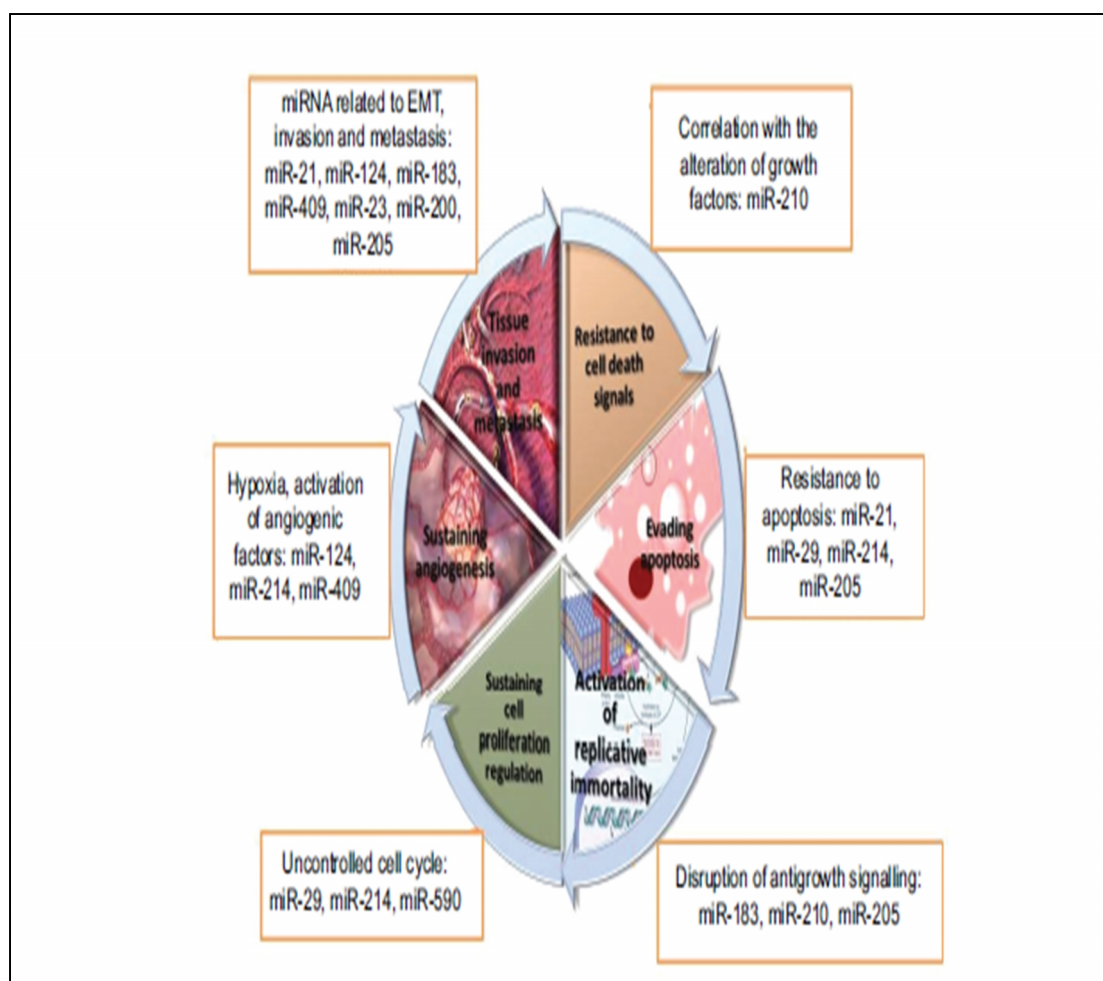


Fig. 3. Hallmarks of bladder cancer beyond miRNAs perspective

urothelium, including miRs predicted to target p53, such as miR-21 and miR-373. Furthermore, p53 suppresses transcriptional factors which promote mesenchymal differentiation, ZEB-1 and ZEB-2, through regulation of the miR200 family (40).

Database of miRNAs related to BC progression

miRNAs can potentially be used as biomarkers for BC diagnosis, prognosis, and to inform treatment strategies. For this purpose, we performed a search in the MedLine database for papers using the search terms microRNA, miRNA, bladder, urothelial, and cancer (30). miRNA-target interactions were extracted from TarBase 5.0 (41,42) and miRTarBase (41, 43). Different databases use varying symbols to represent miRNAs and genes. miRNAs and their host genes were extracted

from miRBase (41,44) and NCBI. miRBase provides a collection of confirmed human miRNAs. Official symbols and IDs from NCBI were used to verify host genes and their miRNAs. Differentially expressed genes of BC were extracted from the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database (45) and the Cancer Genetics Web which can be accessed online at <http://www.cancerindex.org> (see some miRNAs related databases in Tables 2 & 3).

Conclusion

The field of miR research has advanced significantly within the last decade, and more recent investigations are focusing on the role of miRs in BC. Aberrations in miR expression identified in NMIBC and MIBC support and provide insight into molecular alterations known to distinguish the two

Table 1. miRNAs associated with Bladder cancer

MicroRNA	Chromosome location	Level	Sample	Target gene	Biological role	Clinical significance
Mir-21	Chr 17:57918627-57918698	Increase	Fresh tissue and bladder cell lines	P53, AKT, PTEN	Apoptosis, mesenchymal transition	Diagnostic, prognostic
Mir-18396-182 cluster	Chr 7: 129414745-129414854 Chr 7: 129414532-129414609 Chr 7: 129410223-129410332	Increase	Fresh tissue, urine and bladder cell lines	PI3K/AKT/mTOR pathway	Cell growth, migration, apoptosis	Diagnostic, prognostic
Mir-29c	Chr 1: 207975197-207975284	Decrease	Fresh tissue and FFPE	methyltransferase	Maintain normal methylation profile	Stratification for high and low risk groups, good prognosis
Mir-210	Chr 11: 568089-568198	Increase	Tissue and bladder cell lines	E2F3, FGFR1, HOXA1	Cell growth, migration, apoptosis	Diagnostic, prognostic
Mir-124	Chr 20: 61809852-61809938	Decrease	Tissue and bladder cell lines	ROCK1	Migration and invasion	Biomarker for Diagnostics or prediction the response to therapy
Mir-409	Chr 14: 101531637-101531715	Decrease	Tissue and bladder cell lines	c-Met and c-Fos	Migration and invasion	prognostic
Mirs-23b family	chr 9: 9784749097847586	Decrease	Fresh tissue and FFPE	ZEB1	Epithelial-mesenchymal transition	Therapeutic target
Mirs200 family	Chr 12: 7072862-7072929	Decrease	Tissue, serum and urine	ZEB1, ZEB2 and ERFF-1	Epithelial-mesenchymal transition	Diagnostic, prognostic
Mir-205	Chr 1: 209605478-209605587	Increase	Tissue and urine	PTEN, AKT, VEGF	Apoptosis cell cycle invasion, Epithelial-mesenchymal transition	Prognostic, therapeutic target
Mir-214	Chr 1: 172107938-172108047	Decrease	Tissue, serum and urine	B-catenin	Inhibition of cell proliferation, migration and invasion	Diagnostic, prognostic
Mir-5903p	Chr 7: 73605528-73605624	Decrease	Fresh tissue	TFAM, (PI3K), AKT, MMP2 and MMP9	Tumorogenesis	Therapeutic target

Abbreviation: Chr: Chromosome. FFPE: Formalin-fixed paraffin embedded

parallel pathways of bladder carcinogenesis. miRs that target the FGFR3 pathway, specifically miR-99a, miR-100, miR-101, and miR-145, are among the most altered in

non-muscle invasive disease with reduced expression. In contrast, miRs predicted to target p53, including miR-21 and miR-373, are the most up-regulated in MIBC, and

Table 2. MicroRNA target prediction tools

Algorithm	Web link
TargetScan	http://genes.mit.edu/targetscan
miRanda	http://www.microna.org
PicTar	http://pictar.bio.nyu.edu
RNAhybrid	http://bibiserve.techfak.uni-bielefeld.de/mahybrid
Diana-microT	http://www.diana.pcbi.upenn.edu/cgi-bin/micro_t.cgi
Target Boost	http://demo1.interagon.com/demo
Rna 22	http://cbcsrv.watson.ibm.com/rna_22_target.html
MicroTar	http://tiger.dbs.nus.edu.sg/microtar/
NBmiRTar	http://wotan.wistar.upenn.edu/NBmiRTar
miRecords	http://mirecords.umn.edu/miRecords/

Table 3. Databases for microRNA and targets

Database	Web link
MiRBase	http://microna.sanger.ac.uk/
TarBase	http://diana.cslab.ece.ntua.gr/tarbase/
Argonaute	http://www.ma.uni-heidelberg.de/apps/zmf/argonaute/
miRecords	http://mirecords.umn.edu/miRecords/

p53 suppresses transcriptional factors which promote mesenchymal differentiation, ZEB-1, and ZEB-2, through regulation of the miR-200 family. The discovery of additional changes in miR expression in BC may ultimately expand what is known about molecular drivers of BC pathogenesis outside of the FGFR3 and p53 pathways. A number of questions remain regarding factors that regulate miR expression, including changes in miR processing machinery and potentially dynamic alterations in response to stressors such as chemotherapy. Thus far, a complex network of interactions is emerging that implicates novel mechanisms of gene modulation in BC. It is hoped that better understanding of these mechanisms will lead to unique opportunities for the development of diagnostic biomarkers and targeted therapy across the spectrum of BC. The heterogeneity of tumor specimens and research methods limits the reproducibility of changes in miRNAs expression profiles between studies and underscores the importance of in vivo validation in a field that utilizes in silico miR target prediction models. In this review we summarized the current knowledge and concept concerning the involvement of miRNAs in bladder cancer.

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