**Aberrant expression of microRNAs in bladder cancer**

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## Author contributions

H. Yoshino and N. Seki contributed equally to the discussion of content and writing of this article. H. Yoshino, T. Itesako and T. Chiyomaru researched data for the article. H. Enokida contributed to discussion of content. H. Enokida, N. Seki and M. Nakagawa reviewed the manuscript before submission.

## Competing interests

The authors declare no competing interests

**Abstract**

MicroRNAs (miRNAs), a class of small noncoding RNAs, regulate protein-coding gene expression by repressing translation or cleaving RNA transcripts in a sequence-specific manner. A growing body of evidence suggests that miRNAs contribute to bladder cancer progression, development, and metastasis. Genome-wide miRNA expression signatures have been used to rapidly and precisely identify aberrant miRNA expression in bladder cancer. Based on reports describing miRNA signatures, several downregulated and upregulated miRNAs have been discovered. Examination of the differential expression of miRNAs between clinical bladder cancer and normal bladder tissue has led to the elucidation of 11 miRNA expression signatures. miRNAs downregulated in bladder cancer, such as miR-145, miR-143 and miR125b, are known to be tumour suppressors, whereas upregulated miRNAs, such as miR-183, miR-96, miR17-5p and miR-20a are oncogenic. Several studies have demonstrated the potential of miRNAs for providing prognostic information. miR-145 is the most frequently downregulated miRNA in bladder cancer and has been shown to significantly inhibit proliferation, migration, and invasion.

Understanding the role of differentially expressed miRNAs as well as their molecular targets in bladder cancer will provide an effective and promising strategy for miRNA-based therapeutics for the treatment of bladder cancer.

Key points

Most clinical trials of chemotherapeutics for advanced bladder cancer have shown limited benefits, so new prognostic markers and effective treatment strategies are necessary

Examination of the differential expression of miRNAs between clinical bladder cancer and normal bladder tissue has led to the elucidation of 11 bladder-cancer-specific miRNA expression signatures

miRNAs frequently observed in bladder cancer might be the driver molecules for cancer progression

Although some miRNAs might be promising prognostic markers, the results are somewhat inconsistent, and measurement of expression levels of miRNAs was not standardized across studies

miRNAs have been shown to be involved in crucial cell mechanisms, such as apoptosis, the cell cycle, and epithelial–mesenchymal transition

Dysregulation of signalling pathways downstream of miR-145 has been implicated in the progression of bladder cancer

## Introduction

The incidence of bladder cancer varies worldwide, with the highest rates occurring in developed countries.[1] In Europe, bladder cancer is the sixth most commonly diagnosed tumour and the second most common cause of death in patients with genitourinary tract malignancies.[2] Bladder cancer can be classified into two categories: non-muscle-invasive tumours and muscle-invasive tumours. Although 70–80% of patients are diagnosed with non-muscle-invasive tumours, recurrence rates are high (50–70%) in this group. Moreover, 15% of recurrent bladder tumours progress to muscle-invasive disease.[3] The 5-year survival rate for patients with non-muscle-invasive bladder cancer is close to 90%, whereas that of patients with muscle-invasive disease is only approximately 60%.[4] Furthermore, nearly 80% of patients with lymph node metastases die within the first 5 years after diagnosis.[5] Although considerable advances in treatment have been made, including improved surgical techniques and adjuvant chemotherapies, bladder cancer continues to be a common disease with a high mortality rate.[6] Patients with advanced bladder cancer (with or without metastases) are generally treated with combination chemotherapy of gemcitabine and cisplatin, but progression-free survival is limited (approximately 8 months) and there are currently no effective second-line chemotherapy agents. Given that most clinical trials of chemotherapeutics for advanced bladder cancer have shown limited benefits, new prognostic markers and effective treatment strategies are necessary.

Understanding the molecular targets and cancer pathways underlying bladder cancer oncogenesis could tremendously improve diagnosis, therapies, and disease prevention.

To develop novel evidence-based therapies for bladder cancer, understanding at the molecular level is critical. However, most studies on potential biomarkers for human bladder cancer have focused on protein-coding genes, and our understanding of alterations in non-protein-coding sequences in cancer is insufficient. In the post-genome-sequencing era, novel molecular mechanisms should be sought from the results of genome-wide studies. Although protein-coding RNAs are essential for all aspects of cell function, it is also important to examine the functions of noncoding RNAs (ncRNAs) and their associations with human diseases, including cancer.

A growing body of evidence suggests that microRNAs (miRNAs) are aberrantly expressed in many human cancers and have important roles in the initiation, development, and metastasis of these cancers.[7] miRNAs are endogenous small ncRNA molecules (19–22 bases in length) that regulate protein-coding gene expression by repressing translation or cleaving RNA transcripts in a sequence-specific manner.[8] miRNAs are thought to regulate the expression of approximately 30% of all genes.[8] Some highly expressed miRNAs might function as oncogenes by repressing tumour suppressors; conversely, miRNAs expressed at low levels might function as tumour suppressors by negatively regulating oncogenes.[9] Studies of genome-wide miRNA expression

signatures have provided useful data on the aberrant expression of miRNAs in several types of human cancers, with great precision and speed.[10,11]

In this Review, we describe the highlights of recent findings on the aberrant expression of miRNAs in bladder cancer. We will discuss a number of miRNAs that have been identified as potential oncogenes or tumour suppressors, as well as those that have been implicated as prognostic biomarkers. Finally we discuss the molecular targets of miR-145, which is the most frequently identified miRNA that is downregulated in clinical specimens from patients with bladder cancer.

**miRNA biogenesis**

miRNAs are key regulators that contribute to numerous physiological processes, including cell proliferation, differentiation, development, and apoptosis.[12,13] Thus, miRNA biogenesis and its required machinery are tightly controlled in each cell. The disruption of this system can lead to various human diseases, including cancers.

miRNA are evolutionarily conserved and located either within the introns or exons of protein-coding genes (70%) or in intergenic regions (30%).[14] Most intronic and exonic miRNAs are derived from their host gene, suggesting that they are transcribed concurrently with their host transcript. The others are transcribed from intergenic region or gene deserts as separate

transcriptional units.[14] miRNA genes are transcribed by RNA polymerase II or III to form a 33-base-pair hairpin structure (Figure 1).[15-17] RNA polymerase II was the first enzyme to be identified as a transcriber of primary miRNA (pri-miRNA).[15] Transcripts containing pri-miRNA, which can vary from 200 nucleotides to several kilobases in length, are capped with a specially modified nucleotide at the 5′ terminus and are polyadenylated with multiple adenosines at the 3′ end.[16] Pri-miRNA is cleaved into precursor-miRNA (pre-miRNA; 60–70 in length) by the microprocessor complex, which is mainly comprised of the RNAse III enzyme (also known as Drosha) and its cofactor microprocessor complex subunit DGCR8.[18] Pre-miRNA is exported from the nucleus into the cytoplasm by exportin-5 and Ran–GTP.[13,19] In the cytoplasm, pre-miRNA is cleaved by another RNase III enzyme, known as Dicer, into miRNA duplexes approximately 19–22 nucleotides long. One miRNA duplex is then recruited into the RNA-induced silencing complex (RISC), the core component of which is protein argonaute-2 (hAgo2). hAgo2 removes complementary strands by inducing an endonucleolytic cut on the antisense strand.[20] The mature miRNA within RISC protein remains stable and binds its complementary target mRNA. The miRNA then recognizes complementary sites within the target mRNA and regulates translation through mRNA cleavage, degradation, or transcriptional repression.[12,13]

In general, target sites of the miRNA are located within the 3′ untranslated region (UTR)

of mRNA. However, several reports have indicated that functional miRNA binding sites exist in the 5′UTR as well as in the open reading frame.[21,22] The interaction of miRNAs and target mRNAs is tightly regulated. Nucleotides 2–8 (5′) of the miRNA are referred to as the seed sequence and are particularly important for pairing with the target mRNA.[13] Case–control studies have reported associations between miRNAs or miRNA-targeting genes and the risk of bladder cancer.[23,24]

**miRNAs in bladder cancer**

**Aberrant expression of miRNAs**

The association between miRNAs and human cancers was first recognized when miRNA genes were found to be specifically deleted in patients with leukaemia.[25] As a result of this initial discovery, an miRNA-based cancer study was conducted.[25] Subsequently, many studies have demonstrated that overexpressed miRNAs can act as oncogenes by repressing tumour suppressor genes, and underexpressed miRNAs can function as tumour suppressors by negatively regulating oncogenes.[9,26-28] Furthermore, studies have shown that some miRNAs control the activity of major cancer-related signalling molecules,[29] such as p53 family proteins,[30] retinoblastoma protein,[31] and epidermal growth factor receptor (EGFR).[32] Interestingly, expression levels of some miRNAs have been found to be associated with recurrence or metastasis and prognosis of cancers.[33] Thus, identification of aberrant miRNA expression and miRNA-regulated pathways

(oncogenic or tumour suppressive) is necessary for the clinical development of novel cancer therapeutics.

To date, 2,216 human mature miRNAs have been registered at miRBase,[34] which is a biological database that acts as an archive of miRNA sequences and annotations, and screened for differential expression in order to understand their roles in cancer. Genome-wide miRNA expression signatures have been shown to rapidly and precisely reveal aberrant expression of miRNA in cancer cells. Examination of the differential expression of miRNAs between clinical bladder cancer and normal bladder tissue has led to the elucidation of 11 miRNA expression signatures.[35-45] Full details of these expression signatures can be found in Supplementary Table 1 online.

The first report of altered miRNA expression profiles in bladder cancer was reported by Gottardo *et al*.[45] in 2007. They examined 245 human and mouse miRNAs in 27 human bladder specimens (25 urothelial carcinomas and two normal mucosas) and identified 10 miRNAs that were significantly upregulated in bladder cancer compared with normal bladder tissue. Notably, they did not detect any downregulated miRNAs. Downregulated miRNAs in bladder cancer was first reported by Lin and colleagues,[44] who found a total 14 downregulated miRNAs using hybridization-based miRNA array, four of which (miR-143, miR-145, miR-125b, and miR-199b*)* were found to be significantly downregulated on subsequent quantitative reverse transcription

(RT)-PCR analysis of the same samples. The development of array- or PCR-based technology has enabled rapid analysis of aberrant expression of miRNAs in human cancer cells. However, the number of miRNAs which can be analyzed is depended on the platform of the array and PCR tools. The development of high-throughput, deep sequencing technology, has rapidly provided novel information about miRNAs. Deep sequencing analysis seems to be superior to array- or PCR-based methods that determine limited known miRNAs and usually do not contain the full list of known miRNAs sequences. Deep sequencing analysis will become the gold standard method for comprehensive miRNA analysis in cancer genomics. Han *et al*.[36] were the first to report the elucidation of miRNA signatures of bladder cancer by deep sequencing techniques in 2011. At around the same time, Chen *et al*.[35] also reported miRNAs differentially expressed in bladder cancer using the same methods. The altered expression of miRNAs detected by these deep sequencing methods was similar to those previously indicated as downregulated or upregulated by PCR-based methods. However, sequencing-based methods also enable the detection of novel miRNA sequence candidates, although most of these seem to be expressed at very low levels and only in certain samples, or reads number of novel miRNAs in the analysis were much lower than that of the conserved miRNAs.[35] Aberrant expression of miRNAs in clinical specimens can provide important information regarding bladder cancer oncogenesis and sequenced-based analysis of miRNAs is expected to become a standard method in cancer

research.

**miRNAs as oncogenes or tumour suppressors**

miRNAs frequently observed in bladder cancer might be the driver molecules for cancer progression. Thus, miRNAs that have been isolated in three or more expression studies might be assumed to be involved in tumour suppression and oncogenesis (Tables 1 and 2). Expression profile analysis reveals that miR-145, miR-125b, and miR-143 are the most frequently reported downregulated miRNAs,[35,36,39,41,43,44] and miR-182 and miR-183 are the most frequently reported upregulated miRNAs in bladder cancer studies.[35,36,41-43]

miR-145 and miR-143 are clustered at the 5q32 locus and are known to be tumour suppressors in various malignancies, including bladder cancer.[46-48] Downregulation of miR-125b has been observed in bladder cancer specimens and was shown to function as a tumour suppressor by targeting the oncogene *E2F3*.[49] miR-1 and miR-133a have also been reported to be downregulated in clinical specimens in multiple studies (Table 1)[35-37,41,43] and these miRNAs function as tumour suppressors targeting *TAGLN2* in bladder cancer.[37] miR-1 and miR-133a also function as tumour suppressors in prostate cancer, renal cell carcinoma, and maxillary sinus squamous cell carcinoma.[50-52] miR-1-1 and miR-133a-2 are clustered in chromosomal region 20q13.33 and miR-1-2 and miR-133a-1 are clustered in chromosomal region 18q11.2.[53]

Restoration of miR-195 in bladder cells induced G1 arrest by targeting *CDK4*, suggesting that miR-195 acts as a tumour suppressor in bladder cancer.[54] Tumor suppressive functions were reported in miR-100 and miR-133b in bladder cancer.[55,56] Downregulation of miR-26a and miR-29a were recognized in miRNA signatures in bladder cancer, those tumor suppressive functions are unknown enough.

Conversely, of the miRNAs that are upregulated in bladder cancer (Table 2), miR-182, miR-183, and miR-96 form a cluster at the 7q32 locus and have been reported to be oncogenic in some malignancies, such as prostate, lung, and endometrial cancers, and bladder cancer.[57-60] Similarly, miR-17-5p, miR-20a, and miR-18a are found in the miR-17-92 cluster and are described as oncomirs because they target *MYC* in B-cell lymphoma.[61] Other upregulated miRNAs (miR-93, miR-200c and miR-224) has shown in miRNA signature in bladder cancer, however these functions are unknown enough.

Several aberrantly expressed miRNAs in bladder cancer forms miRNA cluster in human genome. Similar thing is recognized for other types of human cancers. Previous report indicated that several miRNAs are frequently located at minimal regions of loss of heterozygosity, amplification, breakpoint and fragile sites, and it is expected that some miRNAs affect human cancers.[62] This report also indicated that miR-24-1, mir-27b, miR-23b, let-7a-1, let-7f-1, and let-7d were shown to be located on a human chromosome involving in bladder cancer.[62]

Aberrant expression of miRNAs and its human chromosome information give a big clue about bladder cancer research.

**miRNAs as diagnostic or prognostic markers**

A number of miRNAs show promise as potential diagnostic or prognostic markers for bladder cancer (Table 3).[38,39,42,63-66] Most miRNA profiles have been identified in clinical bladder cancer tissues, but Hanke *et al*.[42] have evaluated miRNA expression in urine samples. They found that miR-126, miR-182, and miR-199a were expressed at higher levels in the urine of patients with bladder cancer than in healthy controls or patients with infections. Of these, miR-126 and miR-182 could be used to detect bladder cancer with high specificity and sensitivity (72% and 82% for miR-126 and 55% and 82% for miR-182, respectively). In another study, miR-96 and miR-183 were detected as highly expressed miRNAs in urine, distinguishing between patients with bladder cancer and healthy controls with sensitivities of 71.0% and 74.0% and specificities of 89.2% and 77.3%, respectively.[63] miR-96, miR-182, and miR-183 have previously been reported to be upregulated in bladder cancer cells (Table 2 and Supplementary Table 1), but miR-126 and miR-199a have not. These findings suggest that the complement of miRNAs found in cancer cells are different from those secreted in the urine, and more studies are necessary to elucidate this distinction.

Several studies have demonstrated the potential of miRNAs for providing prognostic information. Catto *et al*.[38] profiled 72 clinical tissue samples (52 bladder cancer and 20 normal samples) and reported aberrant expression of miRNAs according to clinicopathological findings; low-grade bladder cancer was associated with many downregulated miRNAs, whereas high-grade bladder cancer was characterized by upregulation of related miRNAs. Among these miRNAs, miR-99a and miR-100 were downregulated in low-grade bladder cancer, and their downregulation was associated with better outcomes.[38] On the other hand, miR-21 was upregulated in high-grade bladder cancer, and its upregulation was associated with poor outcomes, though the relationship between tumor grade per se and outcome was not indicated in this study. Furthermore, Dyrskjøt *et al*.[39] performed analysis of miRNA expression signatures in 117 tissue samples (106 bladder cancer and 11 normal samples) and reported that miR-145 was found to be the most downregulated, and miR-21 was the most upregulated in bladder cancers compared with normals.[39] In addition, this study identified several miRNAs as predicting disease progression (miR-29c, miR-129, miR-133b, and miR-518c-5p). Multivariate Cox regression analysis of the study showed that all four miRNAs were significantly associated with disease progression when correcting for disease stage and grade.[39] The patient group with lower expression level of miR-200c among 100 patients diagnosed with stage T1 bladder cancer determined by *in situ* hybridization on tissue microarray was significantly associated with

subsequent disease progression to muscle-invasive bladder cancer and poor outcomes.[65] On the other hand, from the other study based on the miRNA hybridization microarray using 34 patients with bladder cancers, miR-452 was overexpressed in patients with node-positive bladder tumours, and this overexpression was significantly associated with cancer-related death.[66] Finally, the patients with higher expression levels of miR-143 and miR-222 among 113 bladder cancer tissue specimens, as determined by quantitative RT-PCR, were correlated with tumour recurrence and progression.[64]

Although these findings suggest that some miRNAs might be promising prognostic markers, the results are somewhat inconsistent, and measurement of expression levels of miRNAs was not standardized across studies. Further research is necessary to determine the potential clinical applications of miRNAs as prognostic markers for bladder cancer.

**Molecular targets of miRNAs**

miRNAs have been shown to regulate target gene expression.[7] Several miRNAs and their targets have been identified in bladder cancer (Table 4).[37-39,41,43,46-49,54,65,67-80] 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 cell mechanisms, such as apoptosis, the cell cycle, and epithelial–mesenchymal transition

(EMT).

Avoidance of apoptosis and control of the cell cycle are required for cancer cells to escape cell death and many apoptotic signalling pathways have been elucidated in bladder cancer.[37,39,48,49,54,67,69,70,75-77,80] One such pathway is the sequential activation of caspases, which has a central role in the execution of cell apoptosis. miR-1 was markedly downregulated both in clinical tissues and cultured cells of bladder cancer and has been shown to induce apoptosis by increasing the activity of caspases 3 and 7.[67] Upregulation of miR-129 might be involved in apoptosis avoidance as it has been demonstrated to directly target *SOX4* and *GALNT1*, which are involved in transcription and protein expression, respectively.[39] On the other hand, some miRNAs have been demonstrated to control the cell cycle through their target genes.[54,70,77] For example, miR-449a and miR-195 have been reported to inhibit cell growth and induce G1 phase arrest by targeting *CDK6* and *CDC25a* (miR-449a) and *CDK4* (miR-195).[54,70] miR-34a also induces G1 phase arrest, and has been shown to be silenced in several types of cancers including bladder cancer by aberrant CpG methylation of its promoter region.[70] *FGFR3* is crucial for bladder cancer oncogenesis, and its mutation has been shown to accelerate proliferation in bladder cancer.[81] Downregulation of miR-99a and miR-100 has been shown to cause upregulation of *FGFR3* expression prior to its mutation, suggesting that the acquisition of mutations might result from increased cell turnover.[38]

EMT is a key developmental process 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.[82,83] Members of the miR-200 family are well-known regulators of EMT, and some investigators have reported the function of these miRNAs in bladder cancer.[65,73,74] Loss of expression of miR-200 family members (miR-200a/b/c, miR-141 and miR-429) lead to accelerate the expression of *ZEB1*, *ZEB2* and *ERFFI-1*, which facilitates progression of EMT.[65,73,74] *ZEB1* and *ZEB2* are known to negatively regulate E-cadherin expression and mediate EMT, and *ERRFI-1*, a regulator of *EGFR*, is directly targeted by miR-200 family.[73] *ZEB1* expression has been reported in 22% of clinical bladder cancer tissue specimens, but is absent from the bladder mucosa.[74] Moreover, *ZEB1* protein knockdown in UM-UC3 bladder cancer cells suppresses migration and invasion.[74] 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 muscle-invasive bladder cancer and poor outcomes.[65]

**Tumour suppressive miR-145 in bladder cancer**

Among the miRNA expression profiles of bladder cancer that have been published, miR-145 is the miRNA most frequently reported to be downregulated (Table 1). Downregulation of

miR-145 has also been reported in other types of cancer such as prostate, colon, ovary, esophageal cancer, and B-cell malignancies,[84-88] and restoration of miR-145 in cancer cells such as prostate, colon, esophageal cancer, and B-cell malignancies, has been shown to significantly inhibit proliferation, migration, and invasion,[85,87,89] suggesting that miR-145 is a key regulator of tumour suppression in human cancers. Based on these observations, it might be suggested that dysregulation of signalling pathways downstream of miR-145 could be involved in the progression of bladder cancer.

To validate the expression signature findings, miR-145 expression was analysed by real-time RT-PCR in bladder cancer tissues ($n$ = 104) and noncancerous bladder tissue specimens ($n$ = 31),[41] and was found to be significantly lower in bladder cancer tissue. A number of target genes controlled by miR-145 have been identified in human cancers.[90] For example, miR-145 has been demonstrated to inhibit cell proliferation and migration through direct regulation of the actin-binding protein, fascin in bladder cancer.[71] Moreover, miR-145 has been shown to induce apoptosis through caspase activation, whereas cell death also proceeds under caspase inhibitor in bladder cancer cell lines, suggesting that the activation of an alternative caspase-independent death signal might be accelerated by miR-145 induction and that miR-145 might regulate two redundant pathways.[48]

Several miRNA clusters have been reported in the human genome that are involved not

only in normal biological processes, but also in the development of cancers.[91,92] For example, the miR-15a and miR-16 clusters are known to act as tumour suppressors,[93] and the miR-17-92 cluster has been shown to function as an oncogene.[94] Human miR-145 forms a cluster with miR-143 on chromosome 5q32–q33, and these clustered miRNAs are regulated by a common promoter.[95] Research indicates that both miR-145 and miR-143 are frequently downregulated in broad range of cancers, including bladder cancer.[39,96] Furthermore, chromosome 5q32–q33 has been reported to be deleted in several types of cancers,[97] although no reports have yet described this phenomenon in bladder cancer. Research suggests that *SERPINE1* is a direct target of both miR-145 and miR-143; this is the first report of a cluster of non-family miRNAs targeting the same mRNA in bladder cancer.[47]

miR-145 has been implicated in the p53 regulatory network in human malignancies.[98] 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.[99] *MYC* is known to be involved in human cancers[100] and is negatively regulated by p53.[101] In addition, downregulation of *MYC* might lead to suppression of oncogenic miR-17-92, which negatively regulates tumour suppressor genes, such as *PTEN*, *E2F-1/2*, and *CDKN1A*.[102] Thus, the tumour suppressor miR-145 is a new member of the p53 regulatory network, contributing to the direct linkage between p53 and *MYC* in human cancer regulatory networks.

**Conclusions**

According to a large number of studies, miRNAs have great potential for clinical use as new diagnostic biomarkers and cancer therapies. Moreover, recent studies have shown that some miRNAs control the activity of major cancer-related signalling molecules, such as p53 family proteins. Thus, identification of aberrant miRNA expression and oncogenic or tumour suppressive molecular targets of miRNAs is necessary for characterization of potential disease biomarkers and the clinical development of novel cancer therapeutics.

Current genome-wide miRNA expression signatures of bladder cancer can drive the discovery of novel cancer pathways regulated by oncogenic and/or tumors suppressive miRNAs. Dysregulation of miR-145 appeared to be the most frequent events in bladder cancer and signaling pathways downstream of miR-145 as a key regulator of tumour suppression in bladder cancer.

In the post-genome-sequencing era, the concept of investigating ncRNAs has permeated the cancer research field. Studies of miRNAs in different cancers are being conducted, and it is hoped that novel molecular networks involving miRNA signalling in bladder cancer will become clear.

**Review criteria**

We searched PubMed for publications on bladder cancer epigenetics using the keywords "bladder cancer," "urothelial carcinoma," "non-coding RNA," "miRNA," and "miRNAs" on June 1, 2012. We selected only articles written in English in which the level of scientific detail and reporting were sufficient to enable our understanding of the study and in which novel findings were demonstrated. Reference lists from selected papers were also analysed for further relevant articles.

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**Table 1** | Downregulated miRNAs found in multiple profiling studies

| Number of studies | Hsa-mature sequence | Stem-loop sequence | Locus | Host genes | | Clustered miRNAs |
|---|---|---|---|---|---|---|
| | | | | Sense | Antisense | |
| Six[35,36,39,41,43,44] | hsa-miR-145-5p | hsa-miR-145 | 5q32 | ND | ND | hsa-miR-143 |
| Five[35,36,39,41,44] | hsa-miR-125b | hsa-miR-125b-1 | 11q24 | RP11 | ND | ND |
| Five[35,36,39,41,44] | hsa-miR-125b | hsa-miR-125b-2 | 21q21 | C21orf34 / LINC00478 | ND | ND |
| Five[35,36,39,43,44] | hsa-miR-143-3p | hsa-miR-143 | 5q32 | ND | ND | hsa-miR-145 |
| Four[35,36,37,43] | hsa-miR-1 | hsa-miR-1-1 | 20q13 | C20orf166/ MIR1-1 | ND | ND |
| Four[35,36,37,43] | hsa-miR-1 | hsa-miR-1-2 | 18q11 | | MIB1 | hsa-miR-133a-1 |
| Three[35,39,40] | hsa-miR-26a | hsa-miR-26a-1 | 3p22 | CTDSPL | ND | ND |
| Three[35,39,40] | hsa-miR-26a | hsa-miR-26a-2 | 12q14 | CTDSP2 | ND | ND |
| Three[35,39,44] | hsa-miR-29a | hsa-miR-29a | 7q32 | MIR29A-201 | AC016831.7 | hsa-miR-29b-1 |
| Three[39,40,43] | hsa-miR-29c | hsa-miR-29c | 1q32 | ND | ND | hsa-miR-29b-2 |
| Three[35,36,41] | hsa-miR-100 | hsa-miR-100 | 11q24 | ND | ND | hsa-let-7a-2 |
| Three[36,37,41] | hsa-miR-133a | hsa-miR-133a-1 | 18q11 | ND | MIB1 | hsa-miR-1-2 |
| Three[36,37,41] | hsa-miR-133a | hsa-miR-133a-2 | 20q13 | C20orf166 | ND | ND |
| Three[37,38,41] | hsa-miR-133b | hsa-miR-133b | 6p12 | ND | RP11 | hsa-miR-206 |
| Three[36,41,44] | hsa-miR-195 | hsa-miR-195 | 17p13 | AC027763.1/MIR497HG | ND | hsa-miR-497 |

Abbreviations: has, Homo sapiens (human); ND, not defined

**Table 2** | Upregulated miRNAs found in multiple profiling studies

| No. of studies | Hsa-mature sequence | Stem-loop sequence | Locus | Host genes | | Clustered miRNAs |
|---|---|---|---|---|---|---|
| | | | | Sense | Antisense | |
| Four[35,36,42,43] | hsa-miR-182 | hsa-miR-182 | 7q32 | ND | ND | hsa-miR-183/hsa-miR-96 |
| Four[35,36,41,43] | hsa-miR-183 | hsa-miR-183 | 7q32 | ND | ND | hsa-miR-182/hsa-miR-96 |
| Three[36,44,45] | hsa-miR-17-5p | hsa-miR-17 | 13q31 | MIR17HG | ND | hsa-miR-18a/hsa-miR-19a/hsa-miR-20a/hsa-miR-19b-1/hsa-miR-92a-1 |
| Three[36,39,44] | hsa-miR-20a | hsa-miR-20a | 13q31 | MIR17HG | ND | hsa-miR-17/hsa-miR-18a/hsa-miR-19a/hsa-miR-19b-1/hsa-miR-92a-1 |
| Three[35,38,44] | hsa-miR-93 | hsa-miR-93 | 7q22 | MCM7 | ND | hsa-miR-106b/hsa-miR-25 |
| Three[35,36,44] | hsa-miR-200c | hsa-miR-200c | 12p13 | U47924.27 | ND | hsa-miR-141 |
| Three[35,41,45] | hsa-miR-224 | hsa-miR-224 | Xq28 | GABRE | ND | hsa-miR-452 |

**Table 3** | miRNAs associated with bladder cancer diagnosis and prognosis

| miRNA | Expression | Sample | Number of samples (bladder cancer/normal) | Method | Results | Reference |
|---|---|---|---|---|---|---|
| miR-96 | Up | Urine | 100/74 (control 49 + UTI 25) | RT-PCR | Sensitivity 71.0%, Specificity 89.2%, AUC 0.831 | 63 |
| miR-183 | Up | Urine | 100/74 (control 49 + UTI 25) | RT-PCR | Sensitivity 74.0%, Specificity 77.3%, AUC 0.817 | 63 |
| miR-126 | Up | Urine | 29/11 | RT-PCR | Sensitivity 72%, Specificity 82%, AUC 0.768 | 42 |
| miR-182 | Up | Urine | 29/11 | RT-PCR | Sensitivity 55%, Specificity 82%, AUC 0.799 | 42 |
| miR-143 | Up | Tissue | 113/0 | RT-PCR | Associated with recurrence and muscle invasion | 64 |
| miR-222 | Up | Tissue | 113/0 | RT-PCR | Associated with recurrence, muscle invasion, disease-specific survival, and overall survival | 64 |
| miR-200c | Down | FFPE tissue | 100 (diagnosed as T1) /0 | ISH | Weak staining associated with muscle invasion | 65 |
| miR-99a | Down | Tissue | 52/0 | Expression profiling assay | Associated with better progression-free survival | 38 |
| miR-100 | Down | Tissue | 52/0 | Expression profiling assay | Associated with better progression-free survival | 38 |
| miR-21 | Up | Tissue | 52/0 | Expression profiling assay | Associated with worse progression-free survival | 38 |
| miR-29c | Down | Tissue | 106/0 | Expression profiling assay | Associated with worse progression-free survival | 39 |
| miR-129 | Up | Tissue | 106/0 | Expression profiling assay | Associated with worse progression-free survival | 39 |
| miR-133b | Up | Tissue | 106/0 | Expression profiling assay | Associated with worse progression-free survival | 39 |
| miR-518c-5p | Up | Tissue | 106/0 | Expression profiling assay | Associated with worse progression-free survival | 39 |
| miR-452 | Up | Tissue | 34/0 | Expression profiling assay | associated with death from disease | 66 |

Abbreviations: AUC, area under the curve; FFPE, formalin-fixed paraffin-embedded; ISH, *in situ* hybridization; RT-PCR, reverse transcription polymerase chain reaction; UTI, urinary tract infection.

**Table 4 |** Differentially expressed miRNAs in bladder cancer, their targeted mRNAs and associated pathways

| miRNA | Expression | Target mRNA | Target Pathways | Reference |
|---|---|---|---|---|
| miR-1 | Down | SRSF9 | Apoptosis | 67 |
| miRs-1/133a | Down | TAGLN2 | Apoptosis | 37 |
| miRs-1/133a/218 | Down | LASP1 | Cytoskeleton | 68 |
| miR-19a | Down | PTEN | Apoptosis and mTOR | 69 |
| miRs-30a-3p/133a/199a | Down | KRT7 | Differentiation | 41 |
| miR-34a | Down | CDK6 | Cell cycle control | 70 |
| miRs-99a/100 | Down | FGFR3 | Proliferation | 38 |
| miR-101 | Down | EZH2 | Gene expression | 43 |
| miR-125b | Down | E2F3 | Apoptosis and proliferation | 49 |
| miRs-133a/145 | Down | FSCN1 | Cytoskeleton | 71 |
| miR-143 | Down | ERK5 Akt | MAPK | 46 |
| miRs-143/145 | Down | SERPINE1 | Plasminogen activator system | 47 |
| miR-145 | Down | CBFB PPP3CA CLINT1 | Apoptosis/Signal transduction | 48 |
| miR-195 | Down | CDK4 | Cell cycle | 54 |
| miR-195-5p | Down | GLUT3 | Glucose transporter | 72 |
| miR-200 family | Down | ERRFI-1/EMT process | EMT | 73 |
| miRs-200/205 | Down | ZEB1 ZEB2 | EMT | 65,74 |
| miR-203 | Down | bcl-w/Src Akt2 | Apoptosis/PI3K-Akt signaling | 75,76 |
| miR-449 | Down | CDK6 CDC25a | Cell cycle | 77 |
| miR-493 | Down | RhoC FZD4 | Wnt-PCP signaling | 78 |
| miR-1826 | Down | VEGFC CTNNB1 MEK1 | Wnt-beta-catenin, Ras-Raf-MEK-ERK signaling | 79 |
| miR-129 | Up | SOX4 GALNT1 | Signal transduction and protein expression | 39 |
| miR-221 | Up | TRAIL pathway | Apoptosis | 80 |

Abbreviations: EMT, epithelial–mesenchymal transition; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PCP, planar cell polarity; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha.

**Supplementary Table 1 -** Differentially expressed miRNAs in bladder cancer

| First author (reference) | Year | Sample | Number of tissues (normal-/-cancer) | Downregulated miRNAs | Upregulated miRNAs | Analysis platform |
|---|---|---|---|---|---|---|
| Chen (35) | 2011 | Clinical tissue | 30 (10/20) | let7i, miR-1, miR-100, miR-101, miR-106b, miR-10a, miR-125b, miR-126-3p, miR-126-5p, miR-127-3p, miR-130a, miR-140-3p, miR-141, miR-143-3p, miR-144-5p, miR-145-5p, miR-148a, miR-148b, miR-152, miR-192, miR-199b-3p, miR-200a, miR-22, miR-23b, miR-26a, miR-26b, miR-27a, miR-27b, miR-28-3p, miR-28-5p, miR-29a, miR-30a-3p, miR-30a-5p, miR-30b, miR-30c, miR-30e-5p, miR-379, miR-429, miR-451, miR-452, miR-598 | let-7a, let-7b, let-7c, let-7d, miR-106b-3p, miR-125b-2-3p, miR-1268, miR-1285, miR-1307, miR-146b-5p, miR-155, miR-181a-2-3p, miR-181b, miR-182, miR-183, miR-193a-5p, miR-196a, miR-1974, miR-1979, miR-200b-3p, miR-200b-5p, miR-200c, miR-205, miR-224, miR-25-3p, miR-320a, miR-34c-5p, miR-423-3p, miR-423-5p, miR-744, miR-877, miR-92a, miR-93 | Illumina 1G Genome Analyzer : Deep sequencing |
| Han (36) | 2011 | Clinical tissue | 18 [9/9 (adjacent N9)] | miR-490-5p, miR-99a-3p, miR-490-3p, miR-125b-2-3p, miR-99a-5p, miR-133a, miR-1, miR-125b, miR-145-5p, miR-195, miR-143-5p, miR-145-3p, let-7c, miR-100, miR-143-3p | miR-96, miR-182, miR-183, miR-429, miR-141, miR-200c, miR-200a, miR-200b-3p, miR-18a, miR-7, miR-25-5p, miR-19b, miR-19a, miR-17-5p, miR-20a | Illumina Cluster Station: Deep sequencing |
| Yoshino (37) | 2011 | Clinical tissue | 16 (5/11) | miR-133a, miR-204, miR-1, miR-139-5p, miR-370, miR-133b, miR-574-3p, miR-376c, miR-214, | | TaqMan LDA Human MicroRNA Panel |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | let-7c, miR-140-3p, miR-134, miR-411, miR-218, miR-196b, miR-186, miR-320a | | v2.0 for 665 miRNAs |
| Catto (38) | 2009 | Clinical tissue | 72 [20/52 (adjacent N10)] | miR-133b, miR-204, miR-380-5p | miR-211, miR-549, miR-526b, miR-507, miR-147, miR-517a, miR-556, miR-649, miR-135b, miR-520b, miR-601, miR-646, miR-639, miR-21, miR-644, miR-93, miR-15a, miR-520d, miR-449b | TaqMan MicroRNA Array v1.0 for 354 miRNAs |
| Dyrskjøt (39) | 2009 | Clinical tissue | 117 (11/106) | miR-455-5p, miR-143-3p, miR-145-5p, miR-126-5p, miR-26a, miR-125b, miR-498, miR-489, miR-503, miR-29a, miR-302b, miR-29c | miR-519e-5p, miR-193a-3p, miR-21, miR-20a, miR-198, miR-510, miR-184, miR-492 | Mercury LNA Array for 290 miRNAs |
| Wang (40) | 2009 | Clinical tissue | 14 (7/7) | miR-26a, miR-29c, miR-30c, miR-30e-5p | | miRCURYTM array Microarray Kit for 464 miRNAs |
| Ichimi (41) | 2009 | Clinical tissue | 19 (5/14) | miR-145-5p, miR-30a-3p, miR-100, miR-150, miR-133a, miR-320a, miR-133b, miR-151, miR-195, miR-125b, miR-152, miR-218, miR-199-s, miR-99a-5p, miR-199a-3p, miR-223, miR-139-5p, miR-9, miR-140-5p | miR-96, miR-190, miR-183, miR-130b, miR-124b, miR-215, miR-224, miR-106a | TaqMan MicroRNA Arrays for 156 miRNAs |
| Hanke (42) | 2009 | Urine | 36 (18/18 (9 healthy control/9infectionf)) | | miR-126, miR-182, miR-199a | TaqMan MicroRNA assay - Human Panel-early Assay |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | Kit for 157 miRNAs |
| Friedman (43) | 2009 | Clinical tissue | 18 (9/9 [adjacent N9)] | miR-1, miR-101, miR-143-3p, miR-145-5p, miR-29c | miR-183, miR-182, miR-203, miR-224, miR-196a, miR-10a | LC Sciences for 313 miRNAs |
| Lin (44) | 2009 | Clinical tissue | 12 [6/6 (adjacent N6)] | let-7, miR-23b, miR-29a, miR-30-5p, miR-125a, miR-125b, miR-126-3p, miR-143-3p, miR-145-5p, miR-150, miR-193a, miR-195, miR-199b-5p, miR-221 | miR-17-5p, miR-18a, miR-19, miR-20a, miR-25-3p, miR-31, miR-93, miR-106a, miR-200a, miR-200c, miR-210, miR-324-5p | CapitalBio for 435 miRNAs |
| Gottardo (45) | 2007 | Clinical tissue | 27 (2/25) | | miR-223, miR-26b, miR-221, miR-103-1, miR-185, miR-23b, miR-203, miR-17-5p, miR-23a, miR-205 | Original platform for 245 miRNAs |

Figure legends

Figure 1. The microRNA processing pathway (O'Kelly, F. *et al. Nat. Rev. Urol.* (2012))

**Table 1** | Downregulated miRNAs found in multiple profiling studies

| Number of studies | Hsa-mature sequence | Stem-loop sequence | Locus | Host genes | | Clustered miRNAs |
|---|---|---|---|---|---|---|
| | | | | Sense | Antisense | |
| Six[35,36,39,41,43,44] | hsa-miR-145-5p | hsa-miR-145 | 5q32 | ND | ND | hsa-miR-143 |
| Five[35,36,39,41,44] | hsa-miR-125b | hsa-miR-125b-1 | 11q24 | RP11 | ND | ND |
| Five[35,36,39,41,44] | hsa-miR-125b | hsa-miR-125b-2 | 21q21 | C21orf34 / LINC00478 | ND | ND |
| Five[35,36,39,43,44] | hsa-miR-143-3p | hsa-miR-143 | 5q32 | ND | ND | hsa-miR-145 |
| Four[35,36,37,43] | hsa-miR-1 | hsa-miR-1-1 | 20q13 | C20orf166/ MIR1-1 | ND | ND |
| Four[35,36,37,43] | hsa-miR-1 | hsa-miR-1-2 | 18q11 | | MIB1 | hsa-miR-133a-1 |
| Three[35,39,40] | hsa-miR-26a | hsa-miR-26a-1 | 3p22 | CTDSPL | ND | ND |
| Three[35,39,40] | hsa-miR-26a | hsa-miR-26a-2 | 12q14 | CTDSP2 | ND | ND |
| Three[35,39,44] | hsa-miR-29a | hsa-miR-29a | 7q32 | MIR29A-201 | AC016831.7 | hsa-miR-29b-1 |
| Three[39,40,43] | hsa-miR-29c | hsa-miR-29c | 1q32 | ND | ND | hsa-miR-29b-2 |
| Three[35,36,41] | hsa-miR-100 | hsa-miR-100 | 11q24 | ND | ND | hsa-let-7a-2 |
| Three[36,37,41] | hsa-miR-133a | hsa-miR-133a-1 | 18q11 | ND | MIB1 | hsa-miR-1-2 |
| Three[36,37,41] | hsa-miR-133a | hsa-miR-133a-2 | 20q13 | C20orf166 | ND | ND |
| Three[37,38,41] | hsa-miR-133b | hsa-miR-133b | 6p12 | ND | RP11 | hsa-miR-206 |
| Three[36,41,44] | hsa-miR-195 | hsa-miR-195 | 17p13 | AC027763.1/MIR497HG | ND | hsa-miR-497 |

Abbreviations: has, Homo sapiens (human); ND, not defined

**Table 2** | Upregulated miRNAs found in multiple profiling studies

| No. of studies | Hsa-mature sequence | Stem-loop sequence | Locus | Host genes | | Clustered miRNAs |
|---|---|---|---|---|---|---|
| | | | | Sense | Antisense | |
| Four[35,36,42,43] | hsa-miR-182 | hsa-miR-182 | 7q32 | ND | ND | hsa-miR-183/hsa-miR-96 |
| Four[35,36,41,43] | hsa-miR-183 | hsa-miR-183 | 7q32 | ND | ND | hsa-miR-182/hsa-miR-96 |
| Three[36,44,45] | hsa-miR-17-5p | hsa-miR-17 | 13q31 | MIR17HG | ND | hsa-miR-18a/hsa-miR-19a/hsa-miR-20a/hsa-miR-19b-1/hsa-miR-92a-1 |
| Three[36,39,44] | hsa-miR-20a | hsa-miR-20a | 13q31 | MIR17HG | ND | hsa-miR-17/hsa-miR-18a/hsa-miR-19a/hsa-miR-19b-1/hsa-miR-92a-1 |
| Three[35,38,44] | hsa-miR-93 | hsa-miR-93 | 7q22 | MCM7 | ND | hsa-miR-106b/hsa-miR-25 |
| Three[35,36,44] | hsa-miR-200c | hsa-miR-200c | 12p13 | U47924.27 | ND | hsa-miR-141 |
| Three[35,41,45] | hsa-miR-224 | hsa-miR-224 | Xq28 | GABRE | ND | hsa-miR-452 |

**Table 3** | miRNAs associated with bladder cancer diagnosis and prognosis

| miRNA | Expression | Sample | Number of samples (bladder cancer/normal) | Method | Results | Reference |
|---|---|---|---|---|---|---|
| miR-96 | Up | Urine | 100/74 (control 49 + UTI 25) | RT-PCR | Sensitivity 71.0%, Specificity 89.2%, AUC 0.831 | 63 |
| miR-183 | Up | Urine | 100/74 (control 49 + UTI 25) | RT-PCR | Sensitivity 74.0%, Specificity 77.3%, AUC 0.817 | 63 |
| miR-126 | Up | Urine | 29/11 | RT-PCR | Sensitivity 72%, Specificity 82%, AUC 0.768 | 42 |
| miR-182 | Up | Urine | 29/11 | RT-PCR | Sensitivity 55%, Specificity 82%, AUC 0.799 | 42 |
| miR-143 | Up | Tissue | 113/0 | RT-PCR | Associated with recurrence and muscle invasion | 64 |
| miR-222 | Up | Tissue | 113/0 | RT-PCR | Associated with recurrence, muscle invasion, disease-specific survival, and overall survival | 64 |
| miR-200c | Down | FFPE tissue | 100 (diagnosed as T1) /0 | ISH | Weak staining associated with muscle invasion | 65 |
| miR-99a | Down | Tissue | 52/0 | Expression profiling assay | Associated with better progression-free survival | 38 |
| miR-100 | Down | Tissue | 52/0 | Expression profiling assay | Associated with better progression-free survival | 38 |
| miR-21 | Up | Tissue | 52/0 | Expression profiling assay | Associated with worse progression-free survival | 38 |
| miR-29c | Down | Tissue | 106/0 | Expression profiling assay | Associated with worse progression-free survival | 39 |
| miR-129 | Up | Tissue | 106/0 | Expression profiling assay | Associated with worse progression-free survival | 39 |
| miR-133b | Up | Tissue | 106/0 | Expression profiling assay | Associated with worse progression-free survival | 39 |
| miR-518c-5p | Up | Tissue | 106/0 | Expression profiling assay | Associated with worse progression-free survival | 39 |
| miR-452 | Up | Tissue | 34/0 | Expression profiling assay | associated with death from disease | 66 |

Abbreviations: AUC, area under the curve; FFPE, formalin-fixed paraffin-embedded; ISH, *in situ* hybridization; RT-PCR, reverse transcription polymerase chain reaction; UTI, urinary tract infection.

**Table 4 |** Differentially expressed miRNAs in bladder cancer, their targeted mRNAs and associated pathways

| miRNA | Expression | Target mRNA | Target Pathways | Reference |
|---|---|---|---|---|
| miR-1 | Down | SRSF9 | Apoptosis | 67 |
| miRs-1/133a | Down | TAGLN2 | Apoptosis | 37 |
| miRs-1/133a/218 | Down | LASP1 | Cytoskeleton | 68 |
| miR-19a | Down | PTEN | Apoptosis and mTOR | 69 |
| miRs-30a-3p/133a/199a | Down | KRT7 | Differentiation | 41 |
| miR-34a | Down | CDK6 | Cell cycle control | 70 |
| miRs-99a/100 | Down | FGFR3 | Proliferation | 38 |
| miR-101 | Down | EZH2 | Gene expression | 43 |
| miR-125b | Down | E2F3 | Apoptosis and proliferation | 49 |
| miRs-133a/145 | Down | FSCN1 | Cytoskeleton | 71 |
| miR-143 | Down | ERK5 Akt | MAPK | 46 |
| miRs-143/145 | Down | SERPINE1 | Plasminogen activator system | 47 |
| miR-145 | Down | CBFB PPP3CA CLINT1 | Apoptosis/Signal transduction | 48 |
| miR-195 | Down | CDK4 | Cell cycle | 54 |
| miR-195-5p | Down | GLUT3 | Glucose transporter | 72 |
| miR-200 family | Down | ERRFI-1/EMT process | EMT | 73 |
| miRs-200/205 | Down | ZEB1 ZEB2 | EMT | 65,74 |
| miR-203 | Down | bcl-w/Src Akt2 | Apoptosis/PI3K-Akt signaling | 75,76 |
| miR-449 | Down | CDK6 CDC25a | Cell cycle | 77 |
| miR-493 | Down | RhoC FZD4 | Wnt-PCP signaling | 78 |
| miR-1826 | Down | VEGFC CTNNB1 MEK1 | Wnt-beta-catenin, Ras-Raf-MEK-ERK signaling | 79 |
| miR-129 | Up | SOX4 GALNT1 | Signal transduction and protein expression | 39 |
| miR-221 | Up | TRAIL pathway | Apoptosis | 80 |

Abbreviations: EMT, epithelial–mesenchymal transition; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PCP, planar cell polarity; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha.

**Supplementary Table 1 -** Differentially expressed miRNAs in bladder cancer

| First author (reference) | Year | Sample | Number of tissues (normal-/-cancer) | Downregulated miRNAs | Upregulated miRNAs | Analysis platform |
|---|---|---|---|---|---|---|
| Chen (35) | 2011 | Clinical tissue | 30 (10/20) | let7i, miR-1, miR-100, miR-101, miR-106b, miR-10a, miR-125b, miR-126-3p, miR-126-5p, miR-127-3p, miR-130a, miR-140-3p, miR-141, miR-143-3p, miR-144-5p, miR-145-5p, miR-148a, miR-148b, miR-152, miR-192, miR-199b-3p, miR-200a, miR-22, miR-23b, miR-26a, miR-26b, miR-27a, miR-27b, miR-28-3p, miR-28-5p, miR-29a, miR-30a-3p, miR-30a-5p, miR-30b, miR-30c, miR-30e-5p, miR-379, miR-429, miR-451, miR-452, miR-598 | let-7a, let-7b, let-7c, let-7d, miR-106b-3p, miR-125b-2-3p, miR-1268, miR-1285, miR-1307, miR-146b-5p, miR-155, miR-181a-2-3p, miR-181b, miR-182, miR-183, miR-193a-5p, miR-196a, miR-1974, miR-1979, miR-200b-3p, miR-200b-5p, miR-200c, miR-205, miR-224, miR-25-3p, miR-320a, miR-34c-5p, miR-423-3p, miR-423-5p, miR-744, miR-877, miR-92a, miR-93 | Illumina 1G Genome Analyzer : Deep sequencing |
| Han (36) | 2011 | Clinical tissue | 18 [9/9 (adjacent N9)] | miR-490-5p, miR-99a-3p, miR-490-3p, miR-125b-2-3p, miR-99a-5p, miR-133a, miR-1, miR-125b, miR-145-5p, miR-195, miR-143-5p, miR-145-3p, let-7c, miR-100, miR-143-3p | miR-96, miR-182, miR-183, miR-429, miR-141, miR-200c, miR-200a, miR-200b-3p, miR-18a, miR-7, miR-25-5p, miR-19b, miR-19a, miR-17-5p, miR-20a | Illumina Cluster Station: Deep sequencing |
| Yoshino (37) | 2011 | Clinical tissue | 16 (5/11) | miR-133a, miR-204, miR-1, miR-139-5p, miR-370, miR-133b, miR-574-3p, miR-376c, miR-214, | | TaqMan LDA Human MicroRNA Panel |

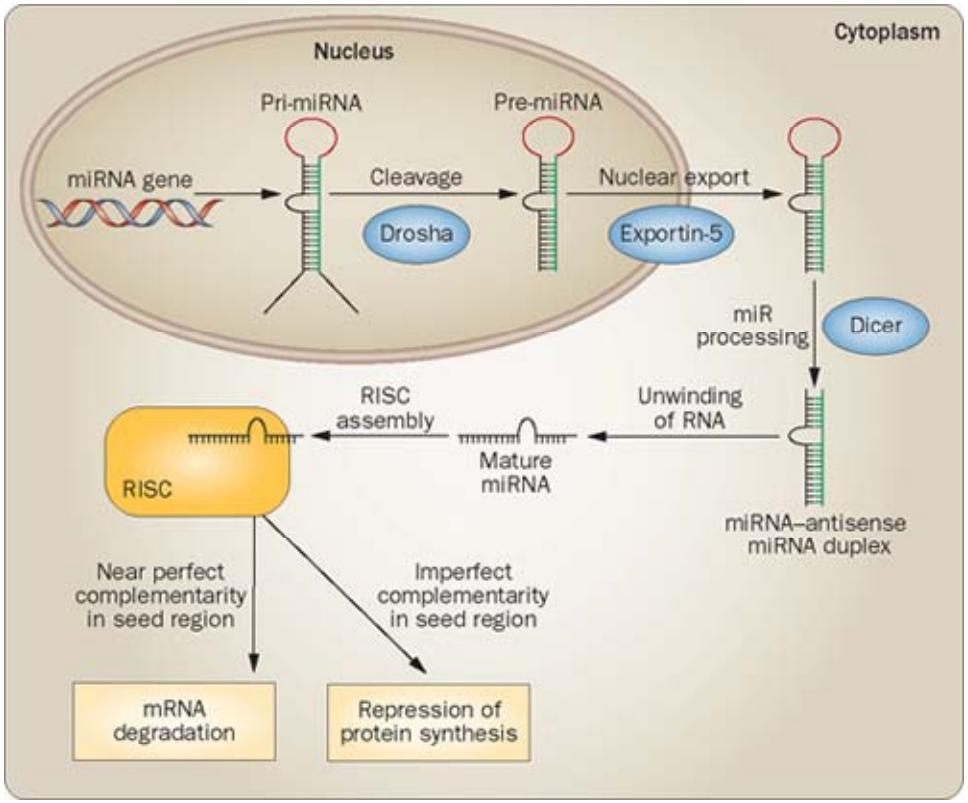
| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | let-7c, miR-140-3p, miR-134, miR-411, miR-218, miR-196b, miR-186, miR-320a | | v2.0 for 665 miRNAs |
| Catto (38) | 2009 | Clinical tissue | 72 [20/52 (adjacent N10)] | miR-133b, miR-204, miR-380-5p | miR-211, miR-549, miR-526b, miR-507, miR-147, miR-517a, miR-556, miR-649, miR-135b, miR-520b, miR-601, miR-646, miR-639, miR-21, miR-644, miR-93, miR-15a, miR-520d, miR-449b | TaqMan MicroRNA Array v1.0 for 354 miRNAs |
| Dyrskjøt (39) | 2009 | Clinical tissue | 117 (11/106) | miR-455-5p, miR-143-3p, miR-145-5p, miR-126-5p, miR-26a, miR-125b, miR-498, miR-489, miR-503, miR-29a, miR-302b, miR-29c | miR-519e-5p, miR-193a-3p, miR-21, miR-20a, miR-198, miR-510, miR-184, miR-492 | Mercury LNA Array for 290 miRNAs |
| Wang (40) | 2009 | Clinical tissue | 14 (7/7) | miR-26a, miR-29c, miR-30c, miR-30e-5p | | miRCURYTM array Microarray Kit for 464 miRNAs |
| Ichimi (41) | 2009 | Clinical tissue | 19 (5/14) | miR-145-5p, miR-30a-3p, miR-100, miR-150, miR-133a, miR-320a, miR-133b, miR-151, miR-195, miR-125b, miR-152, miR-218, miR-199-s, miR-99a-5p, miR-199a-3p, miR-223, miR-139-5p, miR-9, miR-140-5p | miR-96, miR-190, miR-183, miR-130b, miR-124b, miR-215, miR-224, miR-106a | TaqMan MicroRNA Arrays for 156 miRNAs |
| Hanke (42) | 2009 | Urine | 36 (18/18 (9 healthy control/9infectionf)) | | miR-126, miR-182, miR-199a | TaqMan MicroRNA assay - Human Panel-early Assay |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | Kit for 157 miRNAs |
| Friedman (43) | 2009 | Clinical tissue | 18 (9/9 [adjacent N9)] | miR-1, miR-101, miR-143-3p, miR-145-5p, miR-29c | miR-183, miR-182, miR-203, miR-224, miR-196a, miR-10a | LC Sciences for 313 miRNAs |
| Lin (44) | 2009 | Clinical tissue | 12 [6/6 (adjacent N6)] | let-7, miR-23b, miR-29a, miR-30-5p, miR-125a, miR-125b, miR-126-3p, miR-143-3p, miR-145-5p, miR-150, miR-193a, miR-195, miR-199b-5p, miR-221 | miR-17-5p, miR-18a, miR-19, miR-20a, miR-25-3p, miR-31, miR-93, miR-106a, miR-200a, miR-200c, miR-210, miR-324-5p | CapitalBio for 435 miRNAs |
| Gottardo (45) | 2007 | Clinical tissue | 27 (2/25) | | miR-223, miR-26b, miR-221, miR-103-1, miR-185, miR-23b, miR-203, miR-17-5p, miR-23a, miR-205 | Original platform for 245 miRNAs |

Figure legends

Figure 1. The microRNA processing pathway (O'Kelly, F. *et al. Nat. Rev. Urol.* (2012))

**Figure 1** The miRNA processing pathway




O'Kelly, F. *et al.* (2012) MicroRNAs as putative mediators of treatment response in prostate cancer *Nat. Rev. Urol.* doi:10.1038/nrurol.2012.104

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