MicroRNA in Prostate, Bladder, and Kidney Cancer: A Systematic Review

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Abstract

Context: MicroRNAs (miRNA) are noncoding RNAs that post-transcriptionally regulate gene expression. Their altered expression and function have been observed in most urologic cancers. MiRNAs represent potential disease biomarkers and novel therapeutic targets.

Objective: To review and evaluate the evidence implicating miRNAs in the pathogenesis of prostate cancer (PCa), bladder cancer (BCa), and renal cancer.

Evidence acquisition: A systematic review was performed using PubMed and Embase to search for reports using strings for microRNA, non-coding RNA, cancer, prostate, bladder, and renal cancer. Identified manuscripts were retrieved and references searched. Selected studies were required to concentrate on the role of miRNA in these urologic cancers.

Evidence synthesis: We reviewed articles that focus on this topic. More than 40 miRNAs have been implicated in urologic cancer and many target common carcinogenic pathways. In particular, apoptosis avoidance, cell proliferation, epithelial-to-mesenchymal transition, angiogenic signalling, and the generation of androgen independence are targeted or facilitated by more than one miRNA. Little work has been done to evaluate the translational applications for this knowledge to date. Novel therapeutic strategies have been developed and are under investigation to selectively modulate miRNAs; such work would potentially enable personalised tumour therapy.

Conclusions: MiRNAs appear to be important modulators of urologic cancer. Their expression is frequently altered in these tumours, and many are functionally implicated in their pathogenesis. They require evaluation to determine the translational role and therapeutic potential for this knowledge.

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1. Introduction

1.1. Epigenetic gene regulation

It is vital for normal cells that protein synthesis occur in a controlled manner. There are numerous tiers to this control, including the structure and expression of a gene. This expression may be regulated at the genetic or epigenetic level. Genetic mechanisms are often irreversible and include DNA mutation or chromosomal translocation, deletion, and amplification. Epigenetic mechanisms are mostly reversible and are defined as inheritable changes that alter expression without changing gene sequence or chromosomal structure. Known epigenetic mechanisms involve biochemical modifications of the histone proteins that support DNA, modification of DNA itself, and expression of noncoding RNAs (ncRNA). Epigenetic changes are dynamic and respond to events such as embryogenesis or environmental factors. Loss of epigenetic control is common in cancer, and here we focus on ncRNA [1].

RNA can be divided into protein-coding and noncoding RNA. Most RNA does not get translated to form proteins, and this ncRNA is classified according to size and location. MicroRNAs (miRNA) are small ncRNAs (on average, 22 bases) transcribed from DNA into RNA hairpins. They were first identified in plants, before their phylogenetic orthologues were discovered in more advanced species. This preservation in primitive species points to their importance in cell function and led to the current interest in their function [2]. MiRNAs are processed, exported outside the nucleus, and cleaved to create mature miRNAs. These miRNAs bind to complementary sequences within protein-coding messenger RNAs to alter their translation.

1.2. MicroRNA function

The 5’ end of an miRNA includes the targeting “seed” region that binds complementary sequences within messenger RNA (mRNA) tails (3’ untranslated region; Fig. 1). The affinity of this bond depends on the sequence and number of complementary seeds [2]. The miRNA–mRNA pair recruits a silencing complex to modulate mRNA expression. Most miRNAs produce a modest reduction (less than two-fold) in their target mRNA concentration (to “fine-tune” protein expression) [2]. A small proportion causes upregulation, or the complete destruction of their target [2–4]. Currently, around 1000 human miRNAs are known, and each may target hundreds to thousands of genes. It should be stated that our knowledge regarding the role of miRNAs in gene regulation is currently incomplete. New evidence regularly appears suggesting further complexity—for example, the targeting of pseudogenes by miRNAs [5]. Coding genes may have defunct siblings, known as pseudogenes, that have lost their ability to code for proteins or are no longer expressed within a cell. Whilst their function is unknown, recent data suggest that they can act as decoys to attract miRNAs and enable the expression of their active sibling genes.

1.3. MicroRNA in cancer

The importance of miRNA in cancer was suggested when miRNA genes were found to be specifically deleted in leukaemia [1,3]. Subsequent reports have shown that miRNAs are altered in many cancers, and they can initiate carcinogenesis or drive progression [1]. MiRNA expression is dynamic, so their expression or target may be altered within the same cell depending on circumstance. This variability makes them potent modulators of cellular behaviour, as a single protein may cascade its message using few miRNAs onto many genes. For example, miR-34a/b/c are directly regulated by p53 within a positive feedback loop to affect numerous proapoptotic proteins [6]. However, this feature is also a vulnerability that cancers can exploit. Thus, loss of p53 prevents the production of miR-34a/b/c and so enables a cell to avoid apoptosis at numerous levels and affect other targets of these miRNAs.

Alterations of miRNA expression have been described in most cancers and can arise from either genetic or epigenetic means. Many miRNAs are located within fragile chromosomal sites, and these are often deleted or rearranged in cancer [7]. Rarely, miRNA mutations have been found [8], which may reflect their low specificity for gene targeting and thus the low selection pressure associated with their mutation. A more important event may be mutation within the mRNA target site for specific miRNAs. With respect to epigenetic regulation, between 20% and 40% of miRNA genes are located close to CpG islands [9], suggesting that...
they may be susceptible to epigenetic silencing; reports have demonstrated this event in urologic cancers [10–12]. Of note, the reciprocal regulation of the DNA and histone methylation machinery by miRNAs is also seen [13].

MiRNA genes can be located within coding mRNAs (40% are intronic or exonic) or on their own (in the intergenic regions) [9]. They are either solitary or grouped into clusters. Around one-third of miRNAs are clustered, and in these clusters, a single event may affect several miRNAs to alter thousands of protein targets [10]. For example, the oncogene MYC transcriptionally activates the miR-17-92 cluster on chromosome 13 to initiate carcinogenesis [14].

Humans have several large clusters, including those at chromosome 14q32 and chromosome 19q13 (each with >50 miRNAs). Many miRNAs have two or more duplicate genes that encode their mature RNA. This redundancy ensures that loss at one region has little impact on a cell but doubles the chance of upregulation through chromosomal gain or amplification. Notable examples include miR-1302, which has eight miRNA genes. In general, the expression of an intronic/exonic miRNA and its host coding gene are linked [15]. For example, in prostate cancer (PCa), two of the most upregulated miRNAs are located within highly expressed protein-coding genes (MCM7 [miR-106b-25 cluster] and C9orf5 [miR-32]) [16].

The translational applications for knowledge about miRNAs include their use as disease biomarkers, in prognostic prediction, and as novel treatments. This last application is potentially the most exciting, as it enables disease-specific individualised therapeutic targeting. This personalised medicine represents the future of oncology. Examples of miRNA therapies include the administration of synthesised anti-miRNAs to normalise disease-related upregulated miRNA expression [17,18] and the manipulation of lethal gene expression using endogenous miRNAs [19,20].

2. Evidence acquisition

2.1. Literature search

We undertook a detailed literature search of the Embase and Pubmed repositories, with strings for microRNA, non-coding RNA, miRNAs, mRNA, cancer, prostate, bladder, urothelial, kidney, and renal on 18 October 2010.

2.2. Literature selection

We selected articles written in English in which scientific detail and reporting were sufficient to enable our understanding and that had novel findings. In cases of multiple or serial reports, we selected either the first or the most detailed report for inclusion. We preferentially chose articles in which mechanistic data supported observational findings. Each report was extracted in full and its references searched for relevant missing manuscripts.

<table>
<thead>
<tr>
<th>MiRNA Expression</th>
<th>MRNA target Pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCa:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-20a Up</td>
<td>E2F1-3</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>miR-21 Up</td>
<td>PTEN, AKT, androgen pathway</td>
<td>Apoptosis, mTOR pathway, androgen independence</td>
</tr>
<tr>
<td>miR-24 Up</td>
<td>FAF1</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>miR-32 Up</td>
<td>BCL2L11(Bim)</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>miR-106b Up</td>
<td>P21, E2F1</td>
<td>Cell cycle control/apoptosis and proliferation</td>
</tr>
<tr>
<td>miR-125b Up</td>
<td>PS3, BCC3(Puma), BAK1</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>miR-146a Up</td>
<td>CAND1</td>
<td>Cell cycle control</td>
</tr>
<tr>
<td>miR-221 Up</td>
<td>p27(kip1)</td>
<td>Cell cycle control and androgen independence</td>
</tr>
<tr>
<td>miR-222 Up</td>
<td>p27(kip1)</td>
<td>Cell cycle control and androgen independence</td>
</tr>
<tr>
<td>miR-521 Up</td>
<td></td>
<td>DNA repair</td>
</tr>
<tr>
<td>miR-1 Down</td>
<td>Exportin-6, tyrosine kinase 9</td>
<td>Gene expression</td>
</tr>
<tr>
<td>miR-7 Down</td>
<td>ERBB-2 (EGFR, HER2)</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>miR-15a-16 cluster Down</td>
<td>CCND1 and WNT3a</td>
<td>Cell cycle regulation, apoptosis and proliferation</td>
</tr>
<tr>
<td>miR-34a Down</td>
<td>Hr(Bcl2)/SIRT1-&gt;p53/p21/BBC3</td>
<td>Apoptosis and drug resistance</td>
</tr>
<tr>
<td>miR-101 Down</td>
<td>E2F1</td>
<td>Apoptosis and proliferation</td>
</tr>
<tr>
<td>miR-107 Down</td>
<td>Granulin</td>
<td>Gene expression</td>
</tr>
<tr>
<td>miR-143 Down</td>
<td>MYO6, ERK5</td>
<td>Proliferation</td>
</tr>
<tr>
<td>miR-145 Down</td>
<td>MYO6, BNI3L-&gt;AIFM1, CCNA2, TNFSF10</td>
<td>Cell migration, proliferation</td>
</tr>
<tr>
<td>miR-146a Down</td>
<td>ROCK1</td>
<td>Cell migration, apoptosis, cell cycle control</td>
</tr>
<tr>
<td>miR-148a Down</td>
<td>MSK1</td>
<td>Cell growth and invasion, EMT</td>
</tr>
<tr>
<td>miR-205 Down</td>
<td>IL-24 and IL-32, Cepson</td>
<td>Signal transduction, cell cycle control</td>
</tr>
<tr>
<td>miR-331-3P Down</td>
<td>ERBB-2, CDC5, KIF23</td>
<td>Gene expression</td>
</tr>
<tr>
<td>miR-449a Down</td>
<td>HDAC-1</td>
<td>DNA replication</td>
</tr>
<tr>
<td>miR-1296 Down</td>
<td>MCM family</td>
<td>Cell cycle control and proliferation</td>
</tr>
<tr>
<td>Let-7a Down</td>
<td>E2F2 and CCND2</td>
<td>Cell cycle control and proliferation</td>
</tr>
</tbody>
</table>

MiRNA = microRNA; mRNA = messenger RNA; PCa = prostate cancer; PTEN = phosphatase and tensin homologue; mTOR = mammalian target of rapamycin; IL = interleukin; EMT = epithelial-to-mesenchymal transition.

3. Evidence synthesis

Our literature search retrieved 237 manuscripts. We selected 89 that were of sufficient reporting rigor or novelty. We mostly used articles that revealed mechanistic data about the biology of these cancers.

3.1. MicroRNA and prostate cancer

The first systematic profiling report detailing miRNA expression in PCa was published in 2007 [21]. Using a self-synthesised microarray, the authors reported both up- and downregulation of many miRNAs when malignant and benign prostate cells were compared. Of interest were cells clustered according to their malignant state and their androgen dependence. To date, >100 reports have examined miRNA expression in PCa (Table 1). Two key themes have emerged that require discussion. The first is generic to human cancer and is the coordinated targeting of common cellular defences. Although many miRNAs are differentially expressed in PCa, these miRNAs often converge on key pathways necessary for carcinogenesis. For example, the ability to avoid apoptosis is an important carcinogenic event (Fig. 2) facilitated by numerous miRNAs in PCa.

3.1.1. MicroRNA and apoptosis avoidance in prostate cancer

Apoptosis avoidance can be facilitated by many miRNAs in PCa. For example, upregulation of the miR-17-92 cluster (on chromosome 13) leads to the overexpression of miR-20a, which targets the E2F1-3 transcription factors [22]. Depending on cell cycle phase, reduced E2F1-3 may promote cell proliferation or reduce p53 and caspase-mediated apoptosis—an autoregulatory loop, as E2F1-3 directs miR-20a transcription. E2F1 is a common target also downregulated by increased miR-25 [16] and miR-205 [23]. Further apoptosis avoidance is generated by miR-21. This important miRNA is upregulated in many cancers and has antiapoptotic mechanisms mediated through the p53 network [24]. In PCa, miR-21 has been shown to target both programmed cell death 4 (PDCD4) [25] and the phosphatase and tensin homologue (PTEN) [26] mRNAs to attenuate apoptosis. To date, at least 10 miRNAs have been implicated in apoptosis avoidance, including those with multiple targets and in feedback loops. For example, the miR-34 family is expressed partly under p53 control. Loss of p53 leads to reduced miR-34a expression and decreased targeting of the silent information regulator 1 (SIRT1) [6]. In turn, upregulated SIRT1 causes further downregulation of p53 and may reduce apoptosis whilst facilitating paclitaxel resistance [27]. As a consequence of this loop, miR-34a/b/c are downregulated and induce their own phenotypic affects [28].

Other common cellular targeted pathways include the cell cycle, intracellular signalling, DNA repair, and adhesion/migration. For example, upregulation of miRs-221/222 (located on chromosome X) occurs in PC3 PCa cells [29]. These miRNAs directly target p27(kip1) to induce cell proliferation by inhibiting this cell cycle check. Proliferation also results from loss of miR-15a/16-1, which are clustered together on chromosome 13q14 and downregulated in 80%
of prostate tumours [30]. MiR-15a/16-1 loss produces upregulation of cyclin D1 to facilitate G1/S transition. These miRNAs also target WNT3a, and their loss facilitates procarcinogenic Wnt pathway activation.

By targeting cellular pathways, miRNAs can affect a tumour’s response to treatment. Radiation induces DNA damage detected by the cell’s repair machinery to cause either repair or cell death. In PCa, upregulation of miR-521 reduces the response to damage by targeting Cockayne syndrome protein A [31]. A similar effect is seen with the p53-regulated miR-34c. There is crossover between miRNAs and the key carcinogenic pathways. For example, miR-21 upregulation may reduce apoptosis, induce proliferation, and facilitate cell migration [32].

3.1.2. MicroRNA and androgen signalling

The second observation deserving of discussion is the relationship between miRNA and androgen signalling. This relationship forms a complex feedback loop, with androgen-responsive miRNAs and miRNAs modulating the androgen pathway. For example, miR-125b is regulated by androgens [33] via an androgen-responsive element (ARE) within the promoter of the miR-125b-2 gene. Upregulation of miR-125b facilitates androgen-independent growth in LNCaP cells and attenuates apoptosis by targeting BAK1, BBC3, and p53 [34]. MiR-21 also has an ARE within its promoter and can mediate androgen insensitivity through various pathways [35]. Downregulation of miR-146a was found when androgen-independent and sensitive cells were compared [36]. In fact, miR-146a targets ROCK1, a kinase partly responsible for the development of castration-resistant PCa. Most recently, Waltering et al profiled androgen-responsive miRNAs in cells and xenografts [37]. Of 55 candidates, miR-141 was found to be most strongly regulated by androgens and also overexpressed in PCa. Androgen receptor amplification increased miR-141 expression 8- to 10-fold in a direct transcriptional manner. As discussed later, miR-141 is upregulated in human PCa [38]. Importantly, androgen-mediated miRNA expression is affected by the integrity of the androgen pathway. Consequently, expression is more responsive in androgen-dependent than insensitive cells [16].

MiRNAs are also important in modulating the androgen pathway. When examining expression in matched androgen-sensitive and androgen-resistant cells, Sun et al observed upregulation of miRs-221/222 [39]. Manipulating these miRNAs in vivo altered the response of cells to dihydrotestosterone, as measured by prostate-specific antigen (PSA) expression, and facilitated the development of androgen independence. MiRNAs also regulate androgen signalling through shared transcriptional factors with other signalling pathways. ERBB-2 (Her2-neu) is a tyrosine kinase receptor overexpressed in a subgroup of PCa types [40]. One mechanism of ERBB-2 upregulation appears to be the loss of miR-331-3p expression [41]. In vitro expression of miR-331-3p reduced ERBB-2 signalling by targeting this receptor, and also prevented androgen signalling. This effect was independent of androgen receptor expression and could be enhanced by bicalutamide, suggesting shared downstream effectors. Indeed miR-331-3p may be central to prostate carcinogenesis. Wang et al analysed mRNA and miRNA expression in low- and high-risk PCa [42]. Using a systems biology approach, they defined networks of related genes with altered expression. Central to these networks were 20 hub RNAs that included miRs-145/331-3p.

3.1.3. MicroRNA and prostate cancer outcomes

We have detailed that altered miRNA expression is common and appears important in PCa. With this in mind, groups have evaluated miRNA expression in the clinical context. MiRNAs appear to be promising biomarkers, as their structure and size protects them from RNase attack and delays their degradation (S. Miah and J. Catto, unpublished findings). In addition, they are secreted within protective exosomal packages in relative abundance into bodily fluids [43]. For example, MiR-141 is upregulated in primary PCa and was found to be detectable in circulating serum from affected patients [38]. Brase et al screened the serum for 667 miRNAs in a small sample of localised and metastatic cancer patients, and that miR-141 (and miR-375) appeared to be robust diagnostic and prognostic biomarkers [44]. Lodes et al analysed the sera from patients with five different cancers and found that miR-141 was only associated with advanced PCa. They further identified a different panel of 15 miRNAs upregulated in PCa patients [45].

We have discussed the importance of miR-21 in cancer and its androgen regulation. Zhang et al measured serum miR-21 and found that it closely correlated with serum PSA and docetaxel resistance [46]. Moltzahn et al recently defined a new diagnostic miRNA panel that included different miRNAs to those previously identified [47]. This work is important methodologically and reveals the heterogeneity among studies and the need for larger validating reports. Of interest, some of these miRNAs could identify other malignancies from serum. Saini et al implicated miR-203 in the development and propagation of bone metastases in the disease [48].

As discussed, numerous miRNAs have been implicated in prostate carcinogenesis. This reflects different profiling strategies, differences in analytical thresholds and study design, and disease heterogeneity. MiRNA expression is dynamic and will differ according to the phases of prostate carcinogenesis (eg, initiation vs progression vs metastasis), dependence upon androgens for growth, treatment exposure (radiation therapy or chemotherapy), and the molecular pathway for that tumour. One would expect that cancers characterised by the TMPRSS2–ERG fusion to have different miRNA expression profiles to those without this translocation.

3.2. MicroRNA and bladder cancer

The first report of altered miRNA expression in bladder cancer (BCa) appeared in 2007 and detected upregulation of 10 miRNAs [49]. To date, several large-scale profiling experiments have been described (Table 2). Dyrskjøt et al analysed 117 samples and found altered expression of many miRNAs, including downregulation of miR-145 and
upregulation of miR-21 [50]. Prognostic relationships were seen with miRs-133b/129/518c, but it was miR-129 that appeared of most interest. Functional analysis implicated upregulation of this miRNA in apoptosis avoidance and revealed direct targeting of the SOX4 transcription factor and GALNT1 (involved in protein expression). Catto et al profiled 78 urothelial samples and observed phenotypic differential miRNA expression [51].

Although low-grade tumours had downregulation of many miRNAs, in high-grade BCa, upregulation was most common. Clinicopathologic observations suggest that low- and high-grade BCa have different molecular pathways [52,53]. When these pathways were compared, few altered miRNAs were shared between low- and high-grade cancers (only miR-153b), in contrast to high-grade noninvasive and invasive high-grade BCa types (which shared many miRNA alterations). Two of the most downregulated were miR-99a/100, and these were shown to target FGFR3, whose expression is lost in high-grade BCa. The authors found that these miRNAs targeted ERRF-1 [56], a regulator of epidermal growth factor receptor (EGFR)-independent growth and mediator of epithelial-to-mesenchymal transition (EMT). Thus, loss of miR-200 expression facilitates EMT and resistance to EGFR-based therapies. Wiklund et al demonstrated CpG hypermethylation of the promoter for miR-200 [11] and an inverse association with a promesenchymal transcription factor, TWIST1. Kenney et al supported these observations by demonstrating the expression of ZEB1 in 22% of BCa cases and an inverse association with miR-200 [57]. ZEB1 is a transcriptional repressor of E-cadherin and so mediates EMT. These data suggest that TWIST1 may silence members of the miRNA-200 family, with a consequential upregulation of ZEB1 and repression of E-cadherin. This is a feedback loop, with ZEB1 also repressing miRs-141/200 expression [58].

With respect to translational applications, few authors have evaluated miRNA expression in the urine of patients with BCa [43]. Hanke et al revealed the stability of urinary miRNAs and identified miRs-126/152, with promising diagnostic performance characteristics [59]. These data suggest that more studies are required to evaluate this topic.

As with PCa, miRNA expression in BCa varies with tumour type, molecular pathways, and treatment exposure. As with genetic events, the profile of altered miRNAs appears distinct between low- and high-grade cancers and supports the close homology between noninvasive (including carcinoma in situ [CIS]) and muscle-invasive high-grade cancers. When compared with PCa, many miRNAs have shared altered expression (such as miRs-21/34a,b,c/133a,b/135b/143/145/221/222/200), suggesting common carcinogenic roles and the opportunity for novel treatment.

Table 2 – Altered microRNA (miRNA) expression in bladder and renal cancer. A summary of miRNAs with altered expression, including their targeted messenger RNAs and pathways

<table>
<thead>
<tr>
<th>MiRNA</th>
<th>Expression</th>
<th>MRNA target</th>
<th>Pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCa:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-129</td>
<td>Up</td>
<td>SOX4, GALNT1</td>
<td>Signal transduction, protein expression</td>
<td>[50]</td>
</tr>
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<td>miR-221</td>
<td>Up</td>
<td>TRAIL pathway</td>
<td>Apoptosis</td>
<td>[89]</td>
</tr>
<tr>
<td>miRs-1/133a/218</td>
<td>Down</td>
<td>LASP1</td>
<td>Cytoskeleton</td>
<td>[90]</td>
</tr>
<tr>
<td>miR-19a</td>
<td>Down</td>
<td>PTEN</td>
<td>Apoptosis and mTOR pathway</td>
<td>[91]</td>
</tr>
<tr>
<td>miRs-30a-3p/133a/199a</td>
<td>Down</td>
<td>KRT7</td>
<td>Differentiation</td>
<td>[92]</td>
</tr>
<tr>
<td>miR-34a</td>
<td>Down</td>
<td>CDK6</td>
<td>Cell cycle control</td>
<td>[93]</td>
</tr>
<tr>
<td>miRs-99a/100</td>
<td>Down</td>
<td>FGFR3</td>
<td>Proliferation</td>
<td>[11,59]</td>
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<tr>
<td>miR-101</td>
<td>Down</td>
<td>EZH2</td>
<td>Gene expression</td>
<td>[94]</td>
</tr>
<tr>
<td>miR-125b</td>
<td>Down</td>
<td>EZF3</td>
<td>Apoptosis and proliferation</td>
<td>[95]</td>
</tr>
<tr>
<td>miRs-145/133a</td>
<td>Down</td>
<td>FSCN1</td>
<td>Cytoskeleton</td>
<td>[90]</td>
</tr>
<tr>
<td>miR-145</td>
<td>Down</td>
<td>CBFB, PPP3CA, CLINT1</td>
<td>Signal transduction</td>
<td>[96]</td>
</tr>
<tr>
<td>miRs-200/205</td>
<td>Down</td>
<td>ZEB1 and ZEB2</td>
<td>EMT</td>
<td>[11,57]</td>
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<td>miR-200 family</td>
<td>Down</td>
<td>ERRF-1/EMT process</td>
<td>EMT</td>
<td>[56]</td>
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<td>Renal cancer:</td>
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<tr>
<td>miRs-141/200</td>
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<td>ZEB2</td>
<td>EMT</td>
<td>[63]</td>
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<td>miR-23b</td>
<td>Up</td>
<td>Proline oxidase</td>
<td>Apoptosis and hypoxic signalling</td>
<td>[69]</td>
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<td>[67]</td>
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<tr>
<td>miR-438-3p</td>
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<td>BBC3</td>
<td>Apoptosis</td>
<td>[72]</td>
</tr>
</tbody>
</table>

MiRNA = microRNA; mRNA = messenger RNA; BCa = bladder cancer; PTEN = phosphatase and tensin homologue; mTOR = mammalian target of rapamycin; EMT = epithelial-to-mesenchymal transition.
3.3. MicroRNA in kidney cancer

Although BCa and PCa share many miRNAs, the first functional report of miRNA expression in renal cell carcinoma (RCC) found contrasting patterns of miR-34a expression. This miRNA is reduced in most cancers (under p53 regulation), but Dutta et al found miR-34a overexpression in RCC [60], and knockdown of miR-34a slowed proliferation. These data are supported by further reports on RCC [61,62] and contradict the role of miR-34 in other malignancies, suggesting a cell-specific effect. The first systematic profiling experiment compared clear cell RCC with chromophobe tumours [63] (Table 2). The authors found numerous miRNAs with altered expression, which may be a consequence of the modest sample size, and that the two tumour subtypes differed significantly. MiRs-141/200c were the most downregulated types in RCC and were found to target ZEB2 (Fig. 3), a transcriptional repressor from the EMT pathway.

Petillo et al also found that each histologic subtype of renal tumour had a distinct miRNA profile [64], which is to be expected, as the biology of each histologic tumour type differs and can be used to differentiate tumours pathologically. For example, five miRNAs (including miR-200b) can separate histologically similar tumours, such as oncocytesomas and chromophobe RCCs. Juan et al defined a panel of 10 miRNAs (miR-200c, miR-185, miR-34a, miR-142-3p, miR-21, miR-155, miR-224, miR-210, and miR-592) that could distinguish RCC from normal kidney [61]. These results are interesting and include miR-21 (upregulated in many cancers) and miR-34a (more specifically upregulated in RCC). MiR-210 is regulated, in part, by hypoxic stimuli, and so this discovery integrates miRNAs into angiogenic pathways in RCC. Weng et al have profiled both frozen and paraffin-embedded RCC samples using microarrays and next-generation sequencing. These complementary technologies agree in their findings and confirm the importance of the downregulation of miRs-141/200 [65]. Perhaps the most important message from this report, however, was technological. The authors showed that formalin-fixed paraffin-embedded tissues could be used to profile small RNAs. This finding is counterintuitive to the historical view and unlocks large tumour repositories with documented long-time follow-up.

The molecular biology of RCC is characterised by hypoxia and angiogenesis. Indeed, it was the identification of the causative genes for von Hippel-Lindau (VHL) syndrome that unlocked much of the hypoxic signalling pathway. Profiling reports have identified hypoxia-regulated miRNAs that are upregulated in RCC (eg, miR-210). With this in mind, RCC miRNA expression has been used to explore the angiogenesis. Wurdinger et al found that hypoxic growth factors increased miR-296 expression in vascular endothelial cells [66]. This miRNA directly targets the hepatocyte growth factor–regulated tyrosine kinase substrate to reduce the degradation of two angiogenic growth factor receptors (VEGFR2 and PDGFR-β) and promote angiogenesis. Expression of miR-296 has not been described in RCC. Sinha et al examined miRNA expression in RCC cells expressing high levels of VHL protein [67]. They identified high levels of miR-29b and found that this miRNA targeted TIS11B, a negative regulator of vascular endothelial growth factor, to promote angiogenesis.

A similar approach has been taken by Neal et al. [68]. Using another RCC cell line, they identified 10 miRNAs under VHL regulation (including miR-210). The prognosis of RCC varied according to miR-210 expression. Liu et al identified upregulated miR-23b in RCC and showed that it targeted proline oxidase, a mediator of apoptosis and hypoxia-inducible factor (HIF) signalling HIF1 [69].

Several authors have examined miRNA expression in Wilms' nephroblastoma. The first report identified E2F3...
regulation of the miR-17-92 cluster. This axis increased as tumours progressed and facilitated the oncogenic properties of miR-17-92 [70]. The same cluster is upregulated in RCC and may potentially mediate VHL [71]. Veronese et al examined Wilms’ tumour and found that miR-438-3p was overexpressed in all cases; this overexpression was highly correlated with the host IGF2 gene [72]. MiR-438-3p was found to target the proapoptotic protein BBC3 (Fig. 2).

3.4. MicroRNA-based therapies

Novel therapies are perhaps the most exciting aspect in the developing field of miRNAs in cancer. RNA profiling and selective targeting would enable a personalised approach to each tumour. As discussed, reversing the malignant expression of a single miRNA could have beneficial effects on hundreds of genes and affect numerous key pathways. However, a complete discussion of RNA targeting is beyond the scope of this review (although see Bader et al. [73]). In general, technological advances are enabling the synthesis of pre- or anti-RNA molecules within carrier vehicles that can be safely delivered into patients. These molecules can be administered topically or systematically to induce generalised cell targeting. If the targeted event is cancer specific, then the affects should be harmless to normal cells and antineoplastic.

Several groups have evaluated miRNA therapies in urologic cancer. In mice, the administration of miR-16 embedded within atelocollagen (a water-soluble collagen molecule) reversed its low malignant expression and attenuated the growth of PCa metastases [74]. Lee et al engineered the herpes simplex virus-1 (HSV-1) to incorporate miRs-143/145 seed regions into an essential gene [19]. These miRNAs are abundant in normal tissues but lacking in PCa [75]. Thus, PCa-specific expression of HSV-1 should occur from the lack of these suppressive miRNAs. In a mouse model, this virus reduced tumour volume by 80%. Yosomaki et al engineered an adenovirus to express miR-122 target sequences with another essential viral gene (E1A). This miRNA is expressed abundantly within the liver, and thus the virus could be delivered systemically without hepatic toxicity (where suppression of E1A prevents viral replication) [20].

3.5. Future perspectives

Technological advances have enabled the dissection of the role of miRNAs in human disease. This technology will become less expensive and more prevalent, and so it is likely that further depth of knowledge will be added. With respect to urologic cancers, miRNAs hold the promise to be potent biomarkers and therapeutic targets. Clinical trials using RNA therapies are now opening, and it is likely that within the next few years, these trials will affect urology [76].

4. Conclusions

MiRNAs are important modulators of gene expression. They are frequently altered in urologic cancers and as such offer the potential to be used as biomarkers or novel therapeutic targets. Better evidence is required to test their use in both of these fields.

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