

Review

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Review

# Urinary micro-RNAs as Biomarkers of Urological Cancers: A Systematic Review

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**Abstract: Background:** Micro-RNAs (miRNA) are emerging as biomarkers in the detection and prognosis of cancers due to their inherent stability and resilience. **Methods:** To summarize evidence regarding urinary miRNA (umiRNAs) role in the detection, prognosis and therapeutic management of urological cancers, we performed a systematic review of the most important scientific databases using the following keywords: (urinary mirna)AND(prostate cancer); (urinary mirna)AND(bladder cancer); (urinary mirna)AND(renal cancer); (urinary mirna)AND(testicular cancer); (urinary mirna)AND(urothelial cancer). **Results:** Of all, 1364 articles were initially selected. Only original studies in the English language on human specimens were considered for inclusion in our systematic review. Thus, a convenient sample of 60 original articles was identified. Urinary miRNA (UmiRNAs) are downregulated in prostate cancers and may serve as potential non-invasive molecular biomarkers. Several umiRNAs have been identified as diagnostic biomarkers of urothelial carcinoma and bladder cancer (BCa), allowing to discriminate malignant from non-malignant forms of haematuria. UmiRNAs could serve as therapeutic targets or recurrence markers of non-muscle invasive BCa and could predict the aggressivity and prognosis of muscle invasive BCa. In renal cell carcinoma, miRNAs have been identified as predictors of tumour detection, aggressiveness, and progression to metastasis. **Conclusion:** umiRNAs could play an important role in the diagnosis, prognosis, and therapy of urological cancers.

**Keywords:** Micro-RNAs; biomarkers; urological cancers; prostate cancer; bladder cancer; Upper Tract Urothelial Carcinoma; renal cancer

## 1. Introduction

Urological cancers could affect the kidney, upper urinary tract, bladder, prostate, testis and penis. In developed countries, kidney, bladder, and prostate cancers are the three major types of genitourinary cancers, with 166.440, 228.730, and 486.840 worldwide deaths in 2019, respectively (1). Because of the high morbidity and mortality associated, the improvement in early diagnosis is crucial for better clinical results. Although the identification of emerging targets and novel molecules has resulted in encouraging progress in the management of genitourinary tumors, valuable tools for cancer diagnosis and follow-up continue to be lacking. Therefore, the research for reliable prognostic and predictive biomarkers for the early diagnosis in patients with genitourinary malignancies is actually an evolving landscape. In this context, an ideal biomarker should ensure high-accuracy results and should be as minimally invasive as possible to obtain (2).

In the last few years, microRNAs (miRNAs) emerged as useful markers thanks to their occurrence in all tissues. Indeed, normal and cancerous cells can use exosomes to secrete these molecules into blood or urine as free-circulating miRNAs (3).

MiRNAs are 20-25-nucleotide-long noncoding single-stranded RNA molecules which regulate gene expression, through the breakdown of the mRNA transcript or inhibiting translation of the mRNA to protein (4).

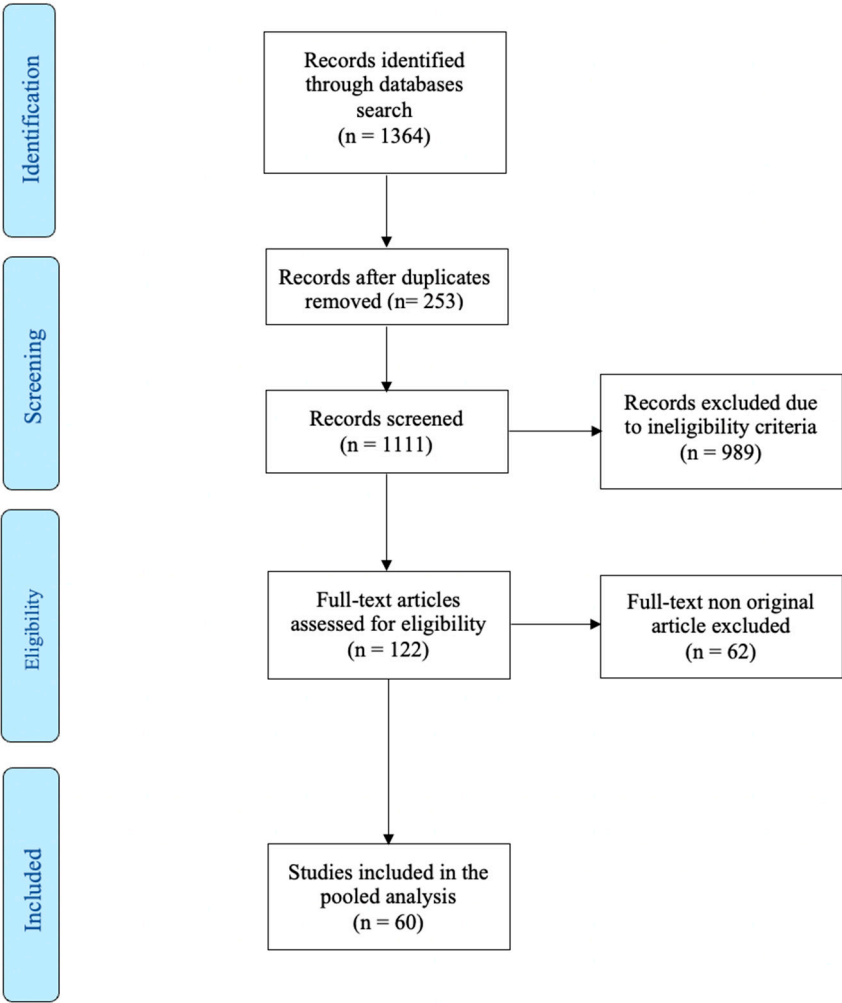
Changes in the expression of miRNAs have been associated with progression of different cancers (5). Indeed, miRNAs can disturb expression of oncogenic or tumor-suppressive target genes implicated in cancer pathogenesis (6). Notably, several miRNAs have been found to be upregulated or downregulated in various tumors, with oncogenes or oncosuppressor role (7).

MiRNAs are emerging as diagnostic tools in several tumors. Previous studies assessed their levels in surgical and liquid samples from patients with cancers. However, samples from operative specimens could be altered by coagulation, necrosis, and formalin fixation. In this context urinary miRNAs (umiRNAs) take advantages from bypassing alternating process and from the reduced vulnerability to urinary RNase in urine (8). Furthermore, compared to local tumor sample, urine is a readily accessible source that does not need invasive procedures and represents the genetic profile of the entire tumor (9).

In consequence, considering the high worldwide prevalence of urological cancers and the growing interest in the role of miRNAs, the current systematic review aims at summarizing the role of umiRNAs in the detection of any urological cancers.

## 2. Literature search results

The PRISMA flow chart of the study selection process is shown in Figure 1. Initial search identified 1364 studies. Of these, 253 were excluded for duplication. After applying selection criteria, other 989 records were excluded. A total of 36 studies, including over 3900 patients, were included in the systematic review. Sixteen studies, including 2498 patients, reported data for bladder cancer (Table 1). Ten studies, including 641 patients, reported data on miRNA for prostate cancer (Table 2). Eight studies, including 521 patients, reported data on renal cancer. (Table 3). Two studies with an overall of 240 patients reported results for urothelial cancer (Table 4).



**Figure 1.** Prisma Flow Chart – Study selection with inclusion and exclusion criteria of reviewed studies.

**Table 1.** Characteristics of the included studies of bladder cancer classified according to year of publication (2023-2012).

Authors	Year of Publication	Number of Patients (BC/Ctl)	Study Design	Target (microRNA in PCa)	Primary Findings
Mamdouh et al.	2023	111/25	Retrospective	miR-200 (↑) miR-145 (↑) miR-21(↑)	Positive correlation ( $p=0.02$ ) high and low grade > controls ( $p=0.01$ ) high and low grade > controls ( $p=0.05$ ) high and low grade > controls
Moisoiu et al.	2022	15/16	Retrospective	Panel of three miRNAs: miR-34a-5p (↑) miR-205-5p (↑) miR-210-3p (↑)	AUC 0.92 (miRNA + SERS)
Lin et al.	2021	180/100	Retrospective	let-7c-5p (↑) miR-146a-5p (↑) miR-149-5p (↑) miR-193a-5p (↑) miR-423-5p (↑)	Positive correlation BC > Ctl
Baugmart et al.	2019	37/0	Retrospective	miR-146 (↑)	Positive correlation High grade > low grade
Braicu et al.	2019	23/23	Retrospective	miR-141-3p (↑) miR-205-5p (↑) miR-139-5p (↓) miR-143-5p (↓) miR-200b-3p (↑)	AUC 0.86 (overall) AUC 0.89 (overall) BC < Ctl BC < Ctl BC > Ctl
Pardini et al.	2018	66/48	Retrospective	Panel of three miRNAs: let-7c-5p (↑) miR-30a-5p (↑) miR-486-5p (↓)	AUC 0.70 (overall) AUC 0.73 (low-grade NMIBC) AUC 0.95 (high-grade NMIBC) AUC 0.99 (MIBC)
Huang et al.	2018	80/86	Retrospective	miR-20a (↑)	Positive correlation ( $p<0.001$ )

Associated with larger tumour size and advanced tumour grade in NMIBC (all $p < 0.05$ )					
Ghorbanmehr et al.	2018	45/20	Retrospective	miR-21-5p (↑) miR141-3p (↑) mir205-5p (↑)	Positive correlation 84% SN, 59% SP; AUC 0.76 (overall) 71% SN, 71% SP; AUC 0.74 (overall) 82% SN, 62% SP; AUC 0.73 (overall)
Piao et al.	2018	35/20	Retrospective	miR-6124 to miR-4511 ratio (↑)	Positive correlation (AUC: 0.888, 91.5% SN, 76.2% SP) ( $p < 0.001$ )
Hofbauer et al.	2018	87/115	Retrospective	Panel of six miRNAs: Let-7c (↓) miR-135a (↓) miR-135b (↑) miR-148a (↓) miR-204 (↓) miR-345 (↑)	AUC 0.88 (overall) AUC 0.91 (MIBC)
Andreu et al.	2017	36/9	Retrospective	miR-146 (↑)	Low grade > high grade
Sasaki et al.	2016	28/19	Retrospective	miR-146a-5p (↑)	Positive correlation (AUC=0.773, 95% CI, 0.701-0.892) ( $p=0.014$ ) ( $p=0.0436$ ) (high-grade > low-grade) ( $p=0.1391$ ) (MIBC > NMIBC)
Zhang et al.	2016	162/162	Retrospective	miR-155 (↑)	Positive correlation (AUC=0.804; 95% CI, 0.756-0.845, 80.2% SN, 84.6% SP) (NMIBC)
Kim et al.	2013	138/144	Retrospective	miR-214 (↑)	Positive correlation 20.08±3.21 vs. 18.96±2.68, ( $p=0.002$ ) (NMIBC)
Mengual et al.	2013	181/136	Retrospective	Panel of six miRNAs: miR-18a (↑) miR-25 (↑) miR-140-5p (↓) miR-187 (↑) miR-142-3p (↓)	84.8% SN, 86.5% SP; AUC 0.92 (overall) 87.1% SN, 86.5% SP (MIBC)

				miR-204 (↓)	
					Negative correlation miR-145 (AUC=0.729;77.8% SN, 61.1% SP) (NMIBC < healthy controls) miR-145 (AUC=0.79;84.1% SN, 61.1% SP) (MIBC < healthy controls) miR-145 (p=0.036) (MIBC<NMIBC) miR-200a (p<0.001) (MIBC and NMIBC<healthy controls)
Yun et al.	2012	207/144	Retrospective	miR-145 (↓) miR-200a (↓)	

**Abbreviations:** BC: bladder cancer; Ctl: control participants; NMIBC: non-muscle-invasive bladder cancer; AUC: area under the curve; CI: confidence interval; p: p-value; SN: sensitivity; SP: specificity; SERS: surface-enhanced Raman spectroscopy.

**Table 2.** Characteristics of the included studies of prostate cancer classified according to year of publication (2022-2015).

Authors	Year of Publication	Number of Patients (PCa/Ctl)	Study Design	Target (microRNA in PCa)	Primary Findings
Lee et al.	2022	6/8	Retrospective	miR-21-5p, miR-574-3p, and miR6880-5p (↑)	Positive correlation in CRPC miR-21-5p, miR-574-3p ( $p < 0.05$ ) miR6880-5p ( $p < 0.01$ )
Kang et al.	2022	63/53	Retrospective	miR-H9 to miR-3659 ratio (↑)	Positive correlation (AUC=0.803,95% CI) ( $p = 0.001$ )
Byun et al.	2021	14/5	Retrospective	miR-1913 to miR-3659 ratio (↑)	Positive correlation (AUC=0.7,95% CI, 61.4% SN, 71.8% SP)
Hasanoglu et al.	2021	8/30	Retrospective	miR-320a (↑)	Positive correlation $p=0.0168$
Markert et al.	2021	28/25	Retrospective	miR-19b and miR-26a (↓)	Negative correlation AUC=0.7
Ghorbanmehr et al.	2020	23/42	Retrospective	miR-21-5p (↑) mi-R-141-3p (↑) miR-205p (↑)	Positive correlation $p=0.001$ $p=0.005$ $p=0.020$
Foj et al.	2017	60/10	Retrospective	miR-21, miR-141, and miR-375 (↑) let-7c	Positive correlation miR-21 ( $p=0.001$ ) miR-141( $p=0.033$ ); higher Gleason score ( $p=0.034$ )



					miR-375 ( $p=0.038$ ) let-7c (no correlation)
					Negative correlation
Rodriguez et al.	2017	28/19	Retrospective	miR-196a-5p and miR-501-3p ( $\downarrow$ )	miR-196a-5p (AUC=0.73, 95% CI 0.56 to 0.86) miR-501-3p (AUC=0.69%, 95% CI 0.52 to 0.85)
Salido-Guadarrama et al.	2016	73/70	Retrospective	miR-100 and miR-200b ( $\uparrow$ )	Positive correlation ( $p=0.0355$ ; Spearman coefficient=0.18)
Stephan et al.	2015	38/38	Retrospective	miR-183 and miR-205	No correlation

**Abbreviations:** PCa: prostate cancer; Ctl: control participants; AUC=area under the curve; CI= confidence interval; p= p-value; SN= sensitivity; SP=specificity; CRPC= castration-resistant prostate cancer.

**Table 3.** Characteristics of the included studies of Renal cancer classified according to year of publication (2020-2012).

Authors	Year of Publication	Number of Patients (RCC/Ctl)	Study design	Target (microRNA in RCC)	Primary Findings
					Positive correlation
					miR-432-5p (AUC: 0.71, 95% CI: 0.59 to 0.83, $p=0.003$ ) miR-532-5p (AUC: 0.70, 95%CI: 0.57–0.82, $p=0.007$ ) miR-10a-5p (AUC: 0.66, 95% CI: 0.53–0.79) miR-144-3p (AUC: 0.68, 95% CI: 0.55–0.81) miR-28-3p (AUC: 0.65, 95% CI: 0.52–0.78) miR-326 (AUC: 0.68, 95% CI: 0.55–0.81) miR-328-3p (AUC: 0.65, 95% CI: 0.52–0.78) miR-603 (AUC: 0.67, 95% CI: 0.55–0.80), and miR-93-3p (AUC: 0.68, 95% CI: 0.54–0.81), all $p<0.05$
Di Meo et al.	2020	6/8	Retrospective	miR-432-5p and miR-532-5p ( $\uparrow\uparrow$ ) miR-10a-5p, miR-144-3p, miR-28-3p, miR-326, miR-328-3p, miR-603, and miR-93-3p ( $\uparrow$ )	
Cochetti et al.	2020	13/14	Retrospective	Panel of: miR-122, miR-1271, miR-15b ( $\uparrow$ )	(100% SN (95% CI 75–100%), and 86% SP (95% CI 57–98%), AUC of 0.96 and $p<0.001$ )
Song et al.	2019	70/30	Retrospective	miR-30c-5p ( $\downarrow$ )	Negative correlation (68.57% SN and 100%SP)
von Brandenstein et al.	2018	26/17	Retrospective	miR-498, miR-183, miR-205, and miR-31( $\uparrow$ )	Positive correlation with Oncocytoma
Mytsyk et al.	2018	67/15	Retrospective	miR-15a ( $\uparrow$ )	Positive correlation between miR-15a levels and tumour size



					(98.1% SP, 100% SN, AUC=0.955, p<0.001)
Li et al.	2017	75/45	Retrospective	miR-210 (↑)	Positive correlation P<0.001 (SN of 57.8% and SP of 80.0%)
Fedorko et al.	2017	69/36	Retrospective	all let-7 miRNAs (let-7a, let-7b, let-7c, let-7d, let-7e and let-7g (↑)	Positive correlation (AUC=0.8307, 71% SN, 81% SP), all p<0.05.
von Brandenstein et al.	2012	25/5	Retrospective	miR-15a (↑)	Positive correlation (p not reported)

**Abbreviations:** RCC: Renal Cell Carcinoma; Ctl: control participants; AUC=area under the curve; CI= confidence interval; p= p-value; SN= sensitivity; SP=specificity.

**Table 4.** Characteristics of the included studies of Upper Tract urothelial carcinoma classified according to year of publication (2017-2011).

Authors	Year of Publication	Number of Patients (UTUC/Ctl)	Study design	Target (microRNA in UTUC)	Primary Findings
Matsuzaki et al.	2017	36/26	Retrospective	miR-155-5p, miR-15a-5p, miR-21-5p, miR-132-3p and miR-31-5p (↑)	Positive correlation in UTUC (all p<0.001) miR-21-5p (AUC=0.900)
Yamada et al.	2011	<104/74	Retrospective	miR-190 (=) miR-96 and miR-183 (↑)	Positive correlation (p=0.006)

**Abbreviations:** UTUC: Upper Tract Urothelial Carcinoma; Ctl: control participants; AUC=area under the curve; CI= confidence interval; p= p-value; SN= sensitivity; SP=specificity.

### 3. Results

#### 3.1. The role of micro-rna in the detection of bladder cancer

Bladder cancer (BC) is one of the most common urogenital cancers worldwide(10) . Among the other types, urothelial carcinoma (UC) is the most common histological type with a prevalence of almost 90% (11). According to European Association of Urology (EAU) guidelines, the diagnosis of BC is actually based on imaging, cytology, cystoscopy and histopathological analysis of sampled tissue from either cold-cup biopsy or trans-urethral resection (TURB) (12,13). Based on histological manifestations and biological traits, BC can be classified in non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) (14).

Being these diagnostic methods uncomfortable for the patients and expensive for the healthcare system, new biomarkers as umiRNAs are crucial in the suspicion of BC (15,16).

Since the 2010s, numerous studies demonstrated how types and urinary concentration of miRNAs could reflect carcinogenicity and invasiveness of BC. For instance, Kim et al. have demonstrated over-expression of miR-214 in the urine samples of the NMIBC patients compared to control specimens ( $20.08 \pm 3.21$  vs.  $18.96 \pm 2.68$ ,  $p=0.002$ ). Furthermore, lower levels of the miR-214 were associated with a significantly longer recurrence-free survival time, making it an independent predictor of NMIBC recurrence ( $p=0.012$ ) (17).

Over-expression of miR-155 in NMIBC patients has been reported by Zhang et al.. The authors showed how the tested umiRNA allow to discriminate between patients with NMIBC from patients with cystitis and healthy controls, with 80.2% of sensitivity and 84.6 % of specificity (18). Nevertheless, the relationship between miR-155 over-expression and bladder cancer development is not fully elucidated. A possible explanation is the action of miR-155 in promoting some tumor cell growth via Wnt/ $\beta$ -catenin signaling activation (19).

In 2018, Piao et al. explored a novel method to discriminate bladder cancer from benign hematuria by measuring the urinary miR-6124 to miR-4511 ratio. The capacity of this proposed diagnostic tool enabled the discrimination of BC from patients with hematuria under nonmalignant conditions with a sensitivity higher than 90% (AUC: 0.888, 91.5% SN, 76.2% SP) ( $p<0.001$ ) (20) .

Differences in miR-20a expression in the urine samples from 80 NMIBC patients and 86 healthy individuals were investigated by Huang et al. They found that urinary concentration of miR-20a were significantly higher in NMIBC patients than in healthy controls ( $p<0.001$ ). Moreover, they showed that larger tumor size and advanced tumor grade were associated with high expression of this umiRNA (all  $p<0.05$ )(21).

Sasaki et al. demonstrated that the expression level of miR-146a-5p in patients with BC was higher than healthy individuals (AUC=0.773,95% CI, 0.701-0.892)( $p=0.014$ ). Higher umiR-146a-5p concentrations were displayed in patients with high-grade BC and with MIBC with respect to those with low-grade tumors ( $p=0.0436$ ) or NMIBC ( $p=0.1391$ ). Moreover, the authors showed that levels of miR-146a-5p decreased to the normal range after TURB (22).

In the multitude of studies on BC, umiR-146 showed the most overlap. In this context, Andreu et al. reported over-expression in low-grade rather than in high-grade disease, whilst Baumgart et al. found overabundance in high-grade more than in lower-graded disease (23,24). Nevertheless, both the research groups showed that this umiR-146 in BC is indeed an inflammasome and the discordance of results might be explained by the inflammatory status in BC and not directly to the aggressiveness of the disease, thus further studies are needed to clarify its role.

Therefore, in addition to the over-expression of umiRNAs in BC, several studies have shown down-regulation of tumor suppressor miRNAs in urinary samples. In 2012, Yun et al. reported down-regulation of miR-145 in NMIBC and MIBC patients compared to healthy controls (77.8% sensitivity and 61.1% specificity for NMIBC, AUC 0.729; 84.1 and 61.1% for MIBC, respectively, AUC 0.790,  $p<0.001$ ). Moreover, miR-145 urinary levels were lower in MIBC patients than in NMIBC patients ( $p=0.036$ ). In addition, in the same study the authors reported that also the levels of miR-200a were significantly decreased in NMIBC and MIBC than healthy controls ( $p<0.001$ ) (25).

In contrast to the previous finding, in 2023 Mamdouh et al. reported that the urinary concentration of miR-200, miR-145 and miR-21 were higher in case of low and high grade BC compared to the controls, depicting a possible oncogene role of those miRNAs ( $p=0.02$ ,  $0.01$  and  $0.05$  respectively)(26).

To improve accuracy of umiRNAs for the detection of BC, numerous studies analysed combination tests utilizing multiple umiRNAs. For instance, Mengual et al. identified a subset of six umiRNAs (miR-187, miR-18a\*, miR-25, miR-142-3p, miR-140-5p, and miR-204) founding a specificity of 86.5% and a sensibility of 84.8% (AUC 0.92) in the diagnosis of BC (27).

Three urine microRNAs, miR-21-5p, miR-141-3p, and miR-205-5p, have been found by Ghorbanmehr et al. as prospective non-invasive diagnostic biomarkers candidates for the identification of both bladder and prostate cancer (28).

Hofbauer et al. achieved comparable results with 88.3% sensitivity using six different umiRNAs (let-7c, miR-135a, miR-135b, miR-148a, miR-204, and miR-345), which can predict the presence of BC from urine samples, independently from grading and staging (AUC 0.88) (29).

Pardini et al. also confirmed, by firstly using the Next-Generation Sequencing (NGS), that the combination of specific miRNAs profiles may provide more robust results than individual miRNAs. Indeed, the authors showed a statistically significant improvement in the AUC discrimination between BC and controls (from 50% to 70%), using a set of three umiRNAs (miR-30a-5p, let-7c-5p and miR-486-5p) (30). Accordingly, Braicu et al. proposed interactions between the genes associated to BC carcinogenesis (TP53, FGFR3, KDR, PIK3CA, and ATM) and altered miRNAs expressions (miR-139-5p, miR-143-5p, miR-23a-3p, miR-141-3p, miR-205-5p). In particular, three up-regulated miRNAs (miR-141b, miR-200 s or miR-205) and two downregulated (miR-139-5p and miR-143-5p) target these multiple genes involved in the carcinogenesis of bladder cancer (31).

Likewise, Lin et al. in 2021 concluded that let-7b-5p, miR-149-5p, miR-146a-5p, miR-193a-5p, and miR-423-5p were significantly increased in BC compared with healthy specimens. Moreover, these umiRNAs had a significant impact on cancer-related signaling pathways implied in cell growth, proliferation and survival, such as: PI3K/AKT, MAPK, focal adhesion and Erb(32,33).

In 2022, Moisoiu et al. firstly demonstrated an (AUC) of  $0.92 \pm 0.06$  in discriminating patients with BC from controls by the combination of surface-enhanced Raman spectroscopy (SERS) with three differentially expressed miRNAs (miR-34a-5p, miR-205-3p, miR-210-3p). This unique method seems to guarantee a better BC's diagnostic and molecular stratification, even if studies in larger cohorts should be performed to confirm these results (34).

In conclusion, in relation to T stage, De Long et al. identified seven miRNAs over-expressed in the bladder cancer group ( $p<0.05$ ). Of the RNA analyzed, miR-940 was differentially expressed between patients with MIBC compared with patients with NMIBC. In particular, miR-940 level was the highest in advanced disease (pT1 G3 and  $\geq$  pT2) and the lowest in absence of tumor (healthy volunteers with or without history of urothelial carcinoma) (35). Contrarily, Baumgart et al. demonstrated a downregulation of miR-138-5p between pT2 and pT3–4 tumors, indicating that low expression correlates with an aggressive phenotype(24). It might be explained by the fact that low expression of this miRNA, as shown in another two studies, results in higher expression of the EMT-associated protein ZEB2(36,37).

### 3.2. The role of micro-RNA in the detection of prostate cancer

Prostate cancer (PCa) is the most commonly diagnosed malignancy among men and the fifth cause of male cancer-related death worldwide (11,38).

The suspicion of prostate cancer arises from an abnormal digit-rectal examination or/and an elevated PSA value (39). The gold standard for the diagnosis is then obtained by transperineal or transrectal prostate biopsy(40). Multi-parametric magnetic resonance imaging (mpMRI) should be recommended before prostate biopsy(41).

Although serum prostate-specific antigen (PSA) is the most widely used biomarker for prostate cancer (PCa) screening, it has several limitations. The lack of specificity and the limited ability of this

serum marker to distinguish between malignant and benign causes of its elevation, might in fact result in overdiagnosis and a significant risk of false-positive results (42).

To overcome these limitations, numerous studies have been conducted to identify new biomarkers and several miRNAs have been shown to be involved in the development and progression of PCa(43–45).

The first studies investigated the miRNAs profile directly in prostate carcinoma tissue. Indeed, in 2009, Schaefer et al. firstly showed the upregulation of miR-183 and the downregulation of miR-205 in PCa tissues (46).

After that, in 2015 Stephan et al. aimed to translate these results into a urine-based testing procedure. They enrolled 38 patients with PCa and 38 without PCa to test the clinical utility of miR-183 and miR-205 in urines samples founding that urinary concentration of those miRNAs were comparable in patients with and without PCa (47).

Salido-Guadarrama et al. showed that elevated urinary levels of miR-100 and miR-200b were associated with advanced PCa (48). Furthermore, miR-100 remained upregulated throughout the carcinogenic process and its downregulation has been observed for hormone-refractory PCa (49,50).

In 2017, Rodriguez et al. showed that miR-196a-5p and miR-501-3p, downregulated in urinary exosomes, are promising biomarkers for PCa (51). In the same year, Foj et al. observed that, when compared to samples from healthy men, the urinary pellet of PCa patients had higher concentrations of miR-21, miR-141, and miR-375 ( $p$  0.001, 0.033, and 0.038, respectively). On the other hand, based on the study by Nadiminty et al. (52), they found no significant differences on the expression of let-7c. Moreover, they found also an higher expression of miR-141 in patients with higher Gleason score ( $p=0.034$ ) (53).

Supporting these notions, Ghorbanmehr et al. collected urines samples from 110 men with BC ( $n = 45$ ), PCa ( $n = 23$ ) cancer, benign prostatic hyperplasia (BPH) ( $n = 22$ ) and healthy men ( $n = 20$ ). They assessed the expression of miR-21-5p, miR-141-3p, and miR-205p to identify and discriminate PCa patients from those with BPH ( $p$  0.001, 0.005, and 0.020, respectively). Moreover, the authors reported how the upregulation of those miRNAs in urine sample was associated with higher cancer detection specificity in PCa compared to PSA test (28).

Markert et al. analyzed urine samples of 53 patients (25 with BPH and 28 with PCa) and showing that miR-19b and miR-26a were significantly downregulated in PCa patients compared to BPH patients (54). These microRNAs seem to play a role in regulating PTEN (Phosphatase and tensin homolog enzyme), whose mutation is a common event in Pca (55).

In 2021, Hasanoglu et al. identified miR-320a as a valuable biomarker in the diagnosis of PCa, reporting higher concentrations in PCa patients compared to healthy controls ( $p= 0.0168$ ) (56). The upregulation of this microRNA confirmed what Porkka et al. reported in a previous study (50).

Over the years, ratio analysis has been used to improve results in microRNA research. It consists of measuring and comparing the expression ratios of up-regulated to down-regulated miRNAs in PCa and control patients. Using this approach, Byun et al. observed that the urinary miR-1913 to miR-3659 ratio was increased in PCa (AUC=0.7,95% CI, 61.4% SN, 71.8% SP), declaring a particular utility in patients within the PSA gray zone ( defined as total serum PSA between 3 to 10 ng/mL) (57).

In addition, in Kang and colleagues study the expression ratio of urinary miR-H9 to miR-3659 was quantified, and they affirmed that the ratio was significantly higher in the PCa group than healthy men group (AUC=0.803,95% CI) ( $p< 0.001$ ) and that it could represents a non-invasive biomarker for PCa (58).

In conclusion, umiRNAs could serve as a supplemental biomarker to PSA for the diagnosis but also in the prediction of cancer progression according to the latest studies. Indeed, in 2022 Lee et al. reported that miR-21-5p, miR-574-3p, and miR6880-5p were significantly higher in patients with CRPC (castration-resistant prostate cancer) and they could be used as potential biomarkers for the prognosis of CRPC (59). In particular, overexpression of miR-21-5p downregulates programmed cell death protein 4, which is a regulator of PCa cell growth and castration resistance, whilst the overexpression of miR-574-3p reflect the down-regulation of the Notch signaling pathway, DNA damage and apoptosis (60,61).

### 3.3. The role of micro-RNA in the detection of renal cancer

Renal cell carcinoma (RCC) is the sixth most frequently diagnosed cancer in men and the 10th in

women representing the third most frequent urological malignancy worldwide and the 13th most common cause of cancer death worldwide (62–64).

Symptoms related to RCC are usually rare and occur in the late stages(65–67). In this context, several micro-RNA have been tested and identified as early diagnostic markers or as useful tools in the follow-up of treated patients(68). Overall, Cui and Cui, observed a significant positive correlation between human tissue miRNAs and the ones from urine specimen in patients with renal cancer ( $\rho=0.51$ ,  $p<0.001$ ) (69).

In 2012, von Brandenstein et al. enrolled 25 patients with ccRCC and 5 healthy volunteers. They found that miR-15a levels from paraffin-embedded tissue and from urine samples are inversely related in malignant versus benign renal tumours. Thus, the authors suggested miR-15a as a potential new preoperatively urinary marker of patients with renal cancer (70).

Fedorko et al. analysed the role of the miRNA let-7 family which are widely accepted as a tumour suppressor miRNAs. Indeed, downregulation of the members of let-7 family has been observed in various types of tumour tissue including RCC, while the upregulation has been observed in BCa (71,72). For the specific purpose of their study, the authors analysed urine samples of 69 patients with non-metastatic ccRCC and 36 healthy controls. They identified 6 let-7 miRNA (let-7 let-7a, let-7b, let-7c, let-7d, let-7e, and let-7g) highly expressed in the urine of ccRCC patients with respect to healthy controls (all  $p<0.015$ ), an in particular let-7a outperforms the others and may be considered as a promising non-invasive biomarker for the detection of clear-cell RCC (73).

Li et al. collected urinary samples from 75 patients diagnosed with ccRCC, 45 healthy volunteers and, to determine a decrease of umiRNAs concentration after surgery, they repeated the collection of urinary samples in 15 patients 7 days after tumour resection. The authors identified that free miR-210 levels were significantly higher in patients with ccRCC than in control subjects ( $p<0.001$ ) regardless of tumour staging. Moreover, miR-210 levels were significantly reduced one week after surgery, thus directly reflecting the presence of ccRCC (74).

In 2018, Mytsyk et al. aimed at testing the utility of urinary miR-15a as a diagnostic molecular biomarker of ccRCC. They collected urinary samples from 67 patients with various solid renal tumours and 15 healthy controls. MirR-15a allowed to discriminate between malignant and benign renal masses ( $p<0.01$ ) and its levels were significantly reduced after one week from tumour surgery. Thus, the authors affirmed that mir-15a could be used as a reliable marker for the diagnosis of ccRCC (75).

Song et al. detected the expression of dysregulated miRNAs in urine exosomes of ccRCC patients and healthy individuals, in order to identify a specific dysregulated miRNA. They identified several umiRNAs in patients with ccRCC, PCa, BCa and healthy individuals. Among them the expression levels of miR-30c-5p in the urinary exosomes of ccRCC patients were significantly lower than that of normal individuals. The sensitivity and specificity of urinary exosome miR-30c-5p in the diagnosis of ccRCC were found to be 68.57% and 100%, respectively (76).

In 2020, Cochetti et al. identified twenty-seven significantly overexpressed, and 30 significantly underexpressed, umiRNAs in ccRCC. Among them, they tested the two most overexpressed umiRNAs (miR-122 and miR-15b) plus four more randomly chosen overexpressed miRNAs (miR-1271, miR-629, miR-625, and miR-93), and the most underexpressed miRNA (miR-1260a) plus another randomly chosen underexpressed miRNA (miR-369). The authors compared urinary expression levels in patients vs. healthy controls and concluded that the combined use of urinary miR-122, miR-1271, miR-15b, together with imaging controls, allow to diagnose ccRCC with high sensitivity and specificity (77).

In conclusion, one of the opened challenge in renal cancer identification, is the differentiation with benign masses, such as Oncocytoma (78). To this regard, in 2018 von Brandenstein et al. aimed at finding urinary miRNA allowing to discriminate benign and malign masses. Thus, they collected urinary samples from 26 patients with renal masses and 17 urine samples of healthy volunteers or



patients with other pathologies. They found that miR-498 (associated with formation of the oncocytoma-specific splice-form of vimentin, Vim3), miR-183 (associated with increased CO<sub>2</sub> levels), miR-205, and miR-31 were specific urinary miRNA guiding the diagnosis for benign Oncocytoma (79). Accordingly, Di Meo et al. tested the sensibility of mi-RNA in discriminating benign oncocytoma from early-stage ccRCC, identifying miR-432-5p and miR-532-5p as presenting the higher discriminatory power, followed by miR-10a-5p, miR-144-3p, miR-28-3p, miR-326, miR-603, and miR-93-3p. In particular, miR-93-3p was identified as the only miRNA associate with progressive ccRCC when downregulated ( $p=0.042$ ) and with longer overall survival when upregulated ( $p=0.016$ ) (80).

### 3.4. The role of micro-RNA in the detection of upper tract urothelial carcinoma

Urothelial carcinomas (UCs) are the sixth most common tumours in developed countries (12). They can be localised in the lower (bladder and urethra) and/or the upper (pyelo-caliceal cavities and ureter) urinary tract. While BCa account for 90–95% of UCs, upper tract UCs (UTUCs) are uncommon and account for only 5–10% of UCs (81,82).

In this section, we aimed at focalising on the role of miRNA in UTUCs detection.

Back in 2011, Yamada et al. evaluated miRNAs expression in clinical samples, using specimens from 104 UC patients underwent cystectomy, between 2003 and 2007, and urine samples from another series of UCs patients (BCa, renal pelvic and ureter (UC)) who had undergone cystectomy, TUR-BT or nephroureterectomy, between 2008 and 2010. Moreover, they collected urine samples from 49 health volunteers and 25 urine samples from patients with urinary tract infections (UTIs). They tested miR-96, miR-183 and miR-190 which appeared upregulated in a previous study based on urine from UC patients (83). Urinary concentration of miR-190 presented no clinically significant difference between patients and controls, while miR-96 and miR-183 were significantly higher in UCs patients than controls or UTI samples ( $p<0.006$ ) (84).

Matsuzaki et al. analysed, in 2017, urinary sample of 36 patients diagnosed with UC, and 24 controls (defined as without history of UC), and selected 5 miRNAs that showed a more than 2.5-fold higher expression and  $p$ -value  $<0.1$  in urinary extracellular vesicles of UC patients, compared to those of healthy volunteers. The authors identified miR-155-5p, miR-15a-5p, miR-21-5p, miR-132-3p and miR-31-5p as all significantly more expressed in urinary extracellular vesicles of UC patients compared to those of the control (all  $p<0.0001$ ). Through a logistic multivariate analyses, the authors found that miR-21-5p was the most important predictor of UC (AUC=0.900) and could be a candidate to early diagnosis of UC even in patients with negative urine cytology (85).

## 4. Materials and methods

A systematic review of the literature was performed in March 2023 using the PubMed®, Scopus®, Web of Science®, Clinical trial.gov, Cochrane Library® databases [MEDLINE, EMBASE, and Web of Science databases]. Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) recommendations were followed to design the search strategies, selection criteria, and evidence report. The International Prospective Register of Systematic Review (PROSPERO) protocol number is CRD42023402737. Patient-related and intervention search terms were combined to build the following search string: [(urinary miRNA) AND (prostate cancer); (urinary miRNA) AND (bladder cancer); (urinary miRNA) AND (renal cancer); (urinary miRNA) AND (testicular cancer); (urinary miRNA) AND (urothelial cancer)].

Search results were filtered by language (English only), species (human), publication type (article). Study eligibility was defined using the PICOS (patient, intervention, comparator, outcome, study type) approach. Inclusion criteria were:

(P) studies focused on adults ( $>18$  yr of age) with a diagnosis of kidney, bladder, or prostate cancers.

(I) identification of miRNAs as diagnostic biomarkers.

(C) in which controls as healthy subjects were used as a comparator.

(O) evaluating one or more of the following outcomes: in the diagnosis, prognosis, and therapy of urological cancers.

(S) retrospective or prospective comparative studies, with a minimum cohort size of 10 patients.

Exclusion criteria were: (1) SP on animal or cadaveric models; (2) studies reporting fewer than five cases; and (3) non-original studies including editorial comments, meeting abstracts, case reports, or letters to the editor or any form of grey literature because of the general lack of details or peer review.

## 5. Conclusions

The development of brand-new diagnostic tools allowing the early detection of cancers is still evolving in the everyday clinical research. In this context, urinary miRNA are emerging as important and reliable tools which could help physicians in the diagnosis, prognosis, and in the therapeutic management of urological cancers.

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