Review

Diagnostic Strategies for Urologic Cancer Using Expression Analysis of Various Oncogenic Surveillance Molecules—From Non-Coding Small RNAs to Cancer-Specific Proteins

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Abstract: Urinary-tract-related tumors are prone to simultaneous or heterogeneous multiple tumor development within the primary organ. Urologic tumors have a very high risk of recurrence in the long and short term. This may be related to the disruption of homeostasis on the genetic level, such as the induction of genetic mutations due to exposure to various carcinogenic factors and the disruption of cancer suppressor gene functions. It is essential to detect the cancer progression signals caused by genetic abnormalities and find treatment therapies. In this review, we discuss the usefulness of tumor-expressing clinical biomarkers for predicting cancer progression. Furthermore, we discuss various factors associated with disturbed intracellular signals and those targeted by microRNAs, which are representative of non-coding small RNAs.

Keywords: renal cell carcinoma; bladder carcinoma; prostate carcinoma; microRNA; cell-free DNA

1. Introduction

Tumors in urinary-tract-related organs include renal carcinoma, which originates in the renal ureter; urothelial carcinoma, which originates in the urothelium; and prostate cancer in men. Although all these organs are associated with the urinary tract, each tumor has a different histology, different developmental risks, and different treatment strategies. Urine cytological diagnosis can be a trigger for cancer detection. Renal, urothelial, and prostate cancer are often discovered incidentally. Recent improvements in the accuracy of diagnostic imaging have enabled the early detection of renal cancer and have increased the survival rate to more than 70%. However, because distant metastatic recurrence may occur after a long disease-free period, finding a radical cure is of great importance. Urothelial carcinoma may be detected in the presence of hematuria. Tumor cells in urine may be detected by cytology; however, in the case of highly differentiated urothelial carcinoma, it is difficult to distinguish these tumor cells from hyperplasia or papilloma. One major problem is that even if tumor cells are found in the urine they cannot be immediately diagnosed as cancerous. Prostate cancer is one of the most common cancers in the world for men, is the third most common cancer in terms of incidence, and usually progresses slowly and persists within the prostate [1]. However, some tumors are very aggressive and can rapidly metastasize to distant sites such as the bone, lungs, and liver. Serum prostate-specific antigen (PSA) is clinically useful as a marker for prostate cancer, but it does not express tumor characteristics; this means that it is difficult to determine whether follow-up or therapeutic intervention is required.

Many approaches to detecting tumor-related genes based on genetic analyses have been applied. These include the detection of gene mutations based on somatic mutations in tumor cells, increased or reduced protein expression, the detection of fusion genes due to...
miRNAs are low-molecular-weight RNAs consisting of 19–25 bases that bind to the 3′ untranslated region of target mRNAs, inhibit transcription and translation, and degrade mRNAs, thereby contributing to in vivo processes such as development, cell proliferation, and differentiation [17–20]. In various diseases such as cancer and metabolic, neurological, and infectious diseases, miRNAs greatly contribute to cell survival by suppressing or promoting gene expression [17,19,21]. In particular, miRNAs suppress or promote cancer cell proliferation. These miRNAs regulate many biological phenomena, and because they only recognize 7–8 nucleotides of their target mRNAs, the number of genes targeted by a single miRNA can be as many as several hundred. Various life phenomena and diseases, including cancer, in which miRNAs are involved, are extremely diverse (Figure 1). Therefore, when investigating their cellular functions—if the miRNAs are related to cancer from the viewpoint of abnormal expression of miRNAs—it is very useful to elucidate (a) the expression regulation of tumor suppressor genes or oncogenes using miRNAs and (b) the regulation of miRNA dynamics by tumor suppressor genes or oncogenes. This is a practical and useful approach to elucidating the disease mechanism [18,20]. The involvement of miRNAs in cancer stem cell maintenance and carcinogenesis is interesting [22,23]. In particular, the dynamics of miRNAs that regulate the maintenance of pluripotency in cancer stem cells; the expression of cell surface markers such as CD44 and CD133, which are commonly expressed in cancer stem cells; and the expression of stem cell markers such as SOX2, NANOG, and OCT4 may play a significant role in the fate of cancer cells [24,25]. A single miRNA can target tens to hundreds of protein-coding genes in a sequence-dependent manner and regulate them for various functions. Identifying the miRNA expression profiles characteristic of cancers and comprehensively analyzing the regulatory genes in urological cancers will elucidate the molecular mechanisms associated with cancer growth and behavior. It is expected that these will lead to the identification of tumor-specific tumor markers and the establishment of biomarkers that can predict tumor progression and the detect recurrence at an early stage.

![Figure 1. Involvement of miRNAs in physiological processes and diseases.](image)

miRNAs are involved in life processes such as development, proliferation, and differentiation. They are also involved in the processes of diseases such as cancer, metabolic disorders, neurological disorders, and infectious diseases.
2. Importance of miRNAs in Urinary Tract Carcinoma

2.1. Renal Carcinoma

Renal carcinoma is a renal parenchymal tumor with the symptoms of hematuria, lateral abdominal pain, abdominal mass, and fever [26]. It is often detected by imaging studies, such as abdominal ultrasonography, CT, and MRI during medical checkups and other detailed examinations. In advanced stages, tumor-associated syndrome occurs in 20% of patients. Increased erythropoietin activity may also cause hyperplasia. Epidemiologically, renal cell carcinoma affects approximately 6 in 100,000 people—or approximately 1% of all cancers—and is slightly more common in males [27] (Cancer Today; https://gco.iarc.fr/today/fact-sheets-cancers, accessed on 19 June 2022). The incidence rate of renal carcinoma increases around the age of 50 years; as the patient ages, the incident rate increases until they reach 70 years old. The risk factors for renal carcinoma include acquired factors such as smoking, obesity, excessive use of phenacetin, acquired cystic kidney disease, and dialysis kidney disease; various chemical stimuli such as contrast media, asbestos, cadmium, and leather tanning; exposure to petroleum products; and familial syndromes such as von Hippel–Lindau (VHL) disease, which results from genetic abnormalities (Figure 2) [21,26,28–30]. The 5-year relative survival rate for renal carcinoma is 68.6% (70.4% in men and 64.8% in women), while tumors confined to the kidney have a high survival rate of 94.3% [27,31]. Therefore, the prognosis for renal cancer is very favorable if the disease is detected early, even if it is detected incidentally. However, because renal cell carcinoma is a highly vascularized tumor, distant metastasis is also frequent, and there are rare cases in which the carcinoma recurs due to distant metastasis after a long survival period. Once a patient is diagnosed with renal cancer, a highly sensitive and accurate means of diagnosis is needed, even after initial treatment, through screening and recurrence monitoring tests [32–34]. Currently, there is a lack of effective tumor markers in both the blood and urine. In advanced and metastatic renal carcinoma, the presence or absence of anemia, increased calcium levels, LDH, and CRP can predict prognosis to some extent; however, there is no marker for detecting localized tumors or signs of recurrence. Our understanding of miRNA function in renal carcinoma has increased greatly over the past decade, and many reports have been published [35–48]. Most of these studies are beginning to reveal the functions of miRNAs targeting molecules related to proliferation signaling and angiogenesis [37–40,45,47,49]. Nevertheless, we are yet to establish tumor markers specific to renal cancer. This is because the functional analysis of miRNAs associated with vascular endothelial growth factor (VEGF)/VEGFR receptor (VEGFR)-related proliferation signals and VHL gene expression has particularly attracted the attention of the scientific community in the reports to date [37–40,45–47]. Molecular targeted therapies have been commercialized for VEGF/VEGFR signaling, and various kinase inhibitors and immune checkpoint inhibitors have been used as molecular targeted therapies to improve prognoses [36,41–43,50,51]. An increase in proliferation signals is assumed to be a common mechanism in various carcinomas. Although it provides a wide range of therapeutic options, it lacks specificity as a diagnostic or prognostic factor. In order to identify molecules with high specificity for renal cell carcinoma, miRNAs that have important roles in renal cell carcinoma should be identified along with the putative target molecules. After elucidating their functions, these new diagnostic markers specific to renal cell carcinoma can be expected to be put into practical use. Renal cell carcinoma is a type of tubular cancer in which tumor-related molecules may be shed in the urine. Because sampling is easy and most components in urine are unwanted, capturing tumor-produced molecules would be as valuable as detecting cancer cells in very small amounts. miRNAs are very small molecules that are discarded in the urine. Several attempts have been made to identify miRNAs in urine [52–55]. However, urine contains a large pre-existing cell population, including inflammatory cells and exfoliated epithelium, and is affected by the blood component during hemorrhage. Therefore, sensitivity and specificity should be carefully considered after evaluating normal baseline miRNA expression levels.
2.2. Bladder Carcinoma

Bladder carcinoma is clinically detected by identifying urinary irritation symptoms such as hematuria, frequency, and urinary urgency. Risk factors include smoking; excessive use of fanacetin; prolonged use of cyclophosphamide; parasites; prolonged placement of urinary catheters; chronic irritation caused by bladder stones; exposure to hydrocarbons, tryptophan metabolites, and industrial chemicals, especially aromatic amines (such as naphthylamine, which is used in the dye industry and aniline dyes); and exposure to chemicals used in the rubber, electrical wire, paint, and textile industries (Figure 2) [56–59]. Most bladder carcinomas are urothelial carcinomas (transitional epithelial carcinomas), but squamous cell carcinomas and adenocarcinomas are also frequently observed and can be easily differentiated into various types [60,61]. Bladder carcinomas tend to occur simultaneously or heterochronously. Heterochronous cancers are poorly differentiated carcinomas or small-cell carcinomas that may develop into more aggressive types with an early transition to invasive carcinoma [60–64]. Carcinoma in situ is noninvasive; however, it is more malignant and often causes multiple lesions. In bladder carcinoma, cells often pass into the urine with the detached urothelium, but in the case of low atypical papillary carcinoma at the level of specific genes at an early stage, attempts have been made to detect miRNA in urine [14,72–77]. Moreover, miRNAs are extracted and analyzed in cells and outside cells in the form of cell-free and endoplasmic reticulum miRNAs [73,75,77–80]. Among the thousands of miRNAs in urine, those specific to bladder cancer have been identified
through comparative studies between non-cancer and cancer patients [66,79,81]. This will lead to the discovery of cancer-specific miRNAs. We believe that the use of urine, which contains relatively few cellular components, will improve the specificity and sensitivity of urinary-tract-system tumor detection.

2.3. Prostate Carcinoma

Prostate cancer is the most common slow-growing type of urinary tract tumor. PSA is a widely used serum tumor marker that is useful for diagnosing and predicting prostate cancer recurrence. Prostatic acid phosphatase, another prostate tumor marker, is not as sensitive or specific as PSA [82]. Nevertheless, PSA is a marker of prostate tissue rather than cancer, and invasive testing cannot be avoided since it is elevated even in noncancerous lesions such as prostate hyperplasia and prostatitis [83]. In prostate cancer, the presence of cancer stem cells, which are normally dormant and have low proliferative activity, can initiate cluster formation and the proliferation of cancer cells upon stimulation, thereby contributing to tumorigenesis [84]. In other words, the differentiation and activation of dormant cancer stem cells are expected to trigger cancer. Various functional analyses of miRNAs, which play important roles in prostate carcinoma, have been performed, and numerous reports have been published [24,48,85–103]. Due to the importance of cancer stem cells in the prostate, miRNAs associated with the expression of stem cell markers and regulating the expression of DICER, which is involved in miRNA biosynthesis, have also been shown to regulate prostate cancer growth and EMT [24,89]. Several urinary molecular markers of prostate carcinoma have been reported [96,104–108]. In the case of prostate carcinoma, cytological diagnoses are of minimal validity; this is because, in contrast to bladder carcinomas, tumor cells appear in the urine in the early stage and do not appear when tumors are present. Therefore, detecting secreted molecular markers can identify prostate cancer at an early stage. Previous studies have reported that metabolites excreted in urine may be effective markers for prostate cancer, as some metabolites are involved in the growth and invasion of prostate cancer [109]. Therefore, detecting the various products produced in urine by cancer can also be used for cancer diagnosis. New nucleic acid detection techniques can also distinguish between cancerous and noncancerous lesions by detecting copy number variations in the very small amount of nucleic acids excreted in urine by quantitative PCR or next-generation sequencing (NGS) analysis [105]. Genetic abnormalities identified in cancer tissues, such as chromosomal instability, can be noninvasive and convenient diagnostic tools for cancer by extracting and detecting the small amounts of nucleic acid in urine [105,110,111]. Because miRNAs are small molecules and are part of the small number of nucleic acids excreted in the urine, they have been extensively studied as diagnostic tools for prostate cancer, and functional analyses have reported their roles and target molecules [96,97,112–119]. In the case of prostate carcinoma, although it is very difficult to detect tumor cells in urine, the detection of extracellular nucleic acids and transcripts or metabolic products could be established as a powerful diagnostic marker.

3. Problems and Prospects of Genetic Analysis via Liquid Biopsies

As the detection of molecular diagnostic markers and oncogene panel tests for nucleic acids derived from cancer cells have played an important role in cancer diagnosis, the quality of DNA and RNA must also be improved [120,121]. The ability to extract nucleic acids from raw or frozen pathology specimens as well as DNA and RNA from FFPE stored for several years has advanced high-quality nucleic acid extraction techniques [120]. Despite the increasing usefulness of liquid biopsies, which perform genetic analyses using the nucleic acids derived from bodily fluid samples, such as urine, ascites, and pleural fluid, the principles and methods of analysis are less clear as a specialized genetic analysis technique, in the manner of a black box. However, the ability of pathologists to perform genetic analyses from the same perspective as immunohistochemical staining is an extremely important revolutionary diagnostic tool. In addition, it is now possible to detect
free nucleic acids in liquid samples, whereas in the past genetic diagnosis was only possible by extracting nucleic acids from tissues or cells. Similarly, the ability to use genetic analysis to evaluate the expression levels of watch molecules such as miRNAs, which are considered important small RNAs and miRNA target molecules, is another important advantage of genetic analysis methods. Furthermore, it is highly useful to detect somatic gene mutations, which are expected to be used as molecular targeted therapy in tissues and as free nucleic acids in urine. Ideally, genetic analysis, including quantitative PCR and NGS, should be commonly used by pathologists, as it provides an opportunity to determine how histopathology can be related to genetic abnormalities and to increase the basis for diagnosis. To this end, it is necessary to devise various methods to extract high-quality nucleic acids from both tissues and cells, as well as from liquid specimens subjected to liquid biopsy. To accomplish this, methods for DNA and RNA extraction from FFPE for tissues and liquid-based cytology for cells have been investigated [120–123]. Although many nucleic-acid-based biomarkers have been discovered in research settings and presented from a functional analysis or clinical point of view, very few biomarkers in the field of urology are useful for companion diagnostics. In bladder cancer, the genetic damage caused by external factors, such as smoking and chemical substances, may be more problematic than various hereditary factors [58], and genomic analyses focusing on gene repair mechanisms will be useful to target sighting genes. For this purpose, it is important to extract sufficient amounts of nucleic acids and to maintain their quality. Although urine is a convenient specimen that can be obtained noninvasively, the problems of maintaining its quality and quantity must be solved. Single-nucleotide polymorphism-based oncogenes in bladder cancer include MYC, TP53, PSCA, TERT, FGFR3, TACC3, NAT2, CBX6, APOBEC3A, CCNE1, and UGT1A [124–127]. These genes are involved in carcinogen detoxification, cell cycle regulation, and apoptosis, and their collective analysis has the potential to comprehensively detect risk factors with a genetic background; however, their therapeutic application is still under development [124,125]. An alternative strategy would be to establish combinations of miRNAs involved in the function of these genes and then to build an algorithm for diagnosis.

Prostate cancer progresses slowly and has a long course, which is highly stressful due to treatments such as brachytherapy, hormonal therapy, and chemotherapy, which may cause various genetic alterations. Therefore, it is important to understand the genetic changes and select treatments for castration-resistant and other treatment-resistant prostate cancers. Germline mutations and genetic polymorphisms are also present in prostate cancer, and BRCA gene abnormalities have been shown to be involved in the risk of prostate cancer development and prognosis [3,6,10]. The role of DNA repair genes such as BRCA 2 and ATM are shown in Figure 3. In prostate cancer, approximately half of BRCA1/2 gene mutations are somatic mutations; therefore, finding miRNAs associated with molecules that may be useful as companion diagnostics can also increase the significance of urinary miRNA detection [11,13].

VHL is found in more than 50% of cases of indolent renal cell carcinoma [47]. In renal cell carcinoma, the activation of VEGF and its receptors, VEGF-R, HIFs, and Akt, is involved in the mechanisms of tumor growth, progression, and angiogenesis, and finding clusters of miRNAs involved in their signaling is expected to help establish a diagnostic algorithm [36,41,45,46].
Targeting cell-free DNA (cfDNA) is another powerful approach to establishing tumor-specific molecules as diagnostic markers in urine. Although DNA derived from circulating tumor cells (CTCs) is very scarce, the amount of cfDNA is significantly increased in the plasma of patients with inflammation and tumors to begin with [65]. In patients with tumors, the main body of the DNA is derived from CTCs; that is, circulating tumor DNA (ctDNA). Since cfDNA suggests tumor heterogeneity and the origin of the lesion from a site distant from that of the biopsy, it may contain mutations not found by the genetic testing of tissue using biopsy materials; in addition, because cfDNA is more abundant than CTCs, the genetic analysis of cfDNA is also highly useful. However, the half-life of ctDNA is not known exactly, but fragmentation by the action of nucleases and other degrading enzymes in body fluids is inevitable and is generally around 150 bp [126,127]. In a large-scale DNA or RNA panel analysis for cancer, it is important to ensure the quantity and quality of nucleic acids; however, by limiting the panel size as a companion diagnosis, it is possible to analyze even a small amount of DNA or RNA. Therefore, even fragmented nucleic acids can be strong candidates for molecular pathology biomarkers. Cancer diagnosis and treatment strategies using molecular pathology biomarkers require the rapid detection of genetic changes that may affect cancer progression. In metastatic or recurrent tumors, tumor changes are generally detected. It is customary to collect tumors by tissue biopsy to identify the primary site and to detect various biomarkers by IHC or tissue in situ hybridization to detect cancer-type-specific biomarkers. Multiple miRNAs combine the changes in the expression of several kinds of miRNAs, which improves the specificity. Furthermore, a number of miRNAs have already been identified as candidate biomarkers for urologic tumors (Figure 4, Table 1). In addition to the histopathological diagnosis method, a new technique—non-invasive urine collection for the analysis of a large number of target genes by NGS and genetic analysis by liquid biopsy technology—is expected to be a more useful and higher-quality histopathological diagnosis with molecular pathological support.
Figure 4. The function of cancer-related proteins and miRNAs in tumor cells and the detection of target genes by genetic analysis. Tumor-associated proteins and miRNAs control cell proliferation, invasion, and prognosis. Genetic analyses of DNA/RNA in tumor cells and cell-free DNA in body fluids are effective for diagnoses and prognoses.

### Table 1. miRNA for molecular biomarker.

| miRNA       | Target Molecules/Function                               | Target Cancer         | Reference |
|-------------|--------------------------------------------------------|-----------------------|-----------|
| miR-145     | Syndecan-1/suppressed cell proliferation               | Bladder carcinoma     | [25]      |
| miR-1826    | β-catenin, MEK1                                        | renal cell carcinoma  | [37]      |
| miR-185     | VEGFR/Inhibits cell proliferation and induces cell apoptosis | renal cell carcinoma  | [39]      |
| miR-204     | Suppress tumor growth                                   | renal cell carcinoma  | [40]      |
| miR-224-5p  | Cyclin D1/Regulates PD-L1 expression                    | renal cell carcinoma  | [42]      |
| miR-497-5p  | Regulates PD-L1 expression                             | renal cell carcinoma  | [43]      |
| miR-107     | Tumor suppressor                                        | renal cell carcinoma  | [44]      |
| miR-92      | Regulates VHL gene expression                           | renal cell carcinoma  | [46]      |
| miR-15a     |                                                        | renal cell carcinoma  | [52]      |
| miR-20a-5p  |                                                        | renal cell carcinoma  | [53]      |
| miR-30a-5p  |                                                        | renal cell carcinoma  | [54]      |
| miR-96-5p   |                                                        | Bladder carcinoma     | [73]      |
| miR-192     |                                                        | Bladder carcinoma     | [74]      |
| miR-214     |                                                        | Bladder carcinoma     | [76]      |
| miR-99a, 125b |                                                      | Bladder carcinoma     | [77]      |
| miR-106b    |                                                        | Bladder carcinoma     | [80]      |
| miR-23a     | suppressed cell proliferation                           | Prostate carcinoma    | [86]      |
| miR-194     | Post-transcriptional regulation                        | Prostate carcinoma    | [87]      |
| miR-125-5p  | NAIIF1/Regulates cell proliferation and migration      | Prostate carcinoma    | [88]      |
| miR-185     | Androgen receptor                                      | Prostate carcinoma    | [92]      |
| miR-122     | Rho associated protein kinase 2/Inhibits cell proliferation | Prostate carcinoma    | [93]      |
| miR-149-5p  | RGS17/Suppress cancer malignancy                       | Prostate carcinoma    | [94]      |
| miR-183, 205 |                                                      | Prostate carcinoma    | [96]      |
| miR-26a     | Modulates the metastasis and tumor growth               | Prostate carcinoma    | [99]      |
| miR-145     | suppressed cell proliferation                           | Prostate carcinoma    | [100]     |
| miR-205, 338-3p |                                                  | Prostate carcinoma    | [102]     |
| miR-188     | MARCKS / suppressed cell proliferation                  | Prostate carcinoma    | [103]     |
| miR-203     |                                                        | Bladder carcinoma     | [116]     |

### 5. Conclusions

Although there are currently no candidate genes available for molecular targets that are useful as companion diagnoses for urologic tumors, many researchers have shown
that urinary miRNAs and cell-free DNA/RNA have the potential to serve as biomarkers and may be useful in predicting prognoses. It is expected that various molecular targeted therapies and tumor-specific biomarkers based on the functions of miRNAs and their target molecules will be used in practice in the future.

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**Informed Consent Statement:** Patient consent was waived by disclosing the opt-out statement on our institution’s website and on the bulletin board of the institution’s affiliated hospital.

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