Urologic Cancers
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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers.frontmatter
Urologic cancers are an umbrella term for cancers of the prostate, kidney, bladder, penis, and testis. While cancers of the prostate, testes, and penis are specific to men, kidney cancer and bladder cancer affect both men and women. However, these also have a male-predominance, with men twice as likely to develop kidney and bladder cancer compared to women. The past 20 years have witnessed remarkable advances in the detection and management of urologic cancers. Routine use of advanced imaging modalities for various reasons has increased the detection of some of these cancers at an early stage. Robot-assisted surgery for nephrectomy, prostatectomy, cystectomy, and retroperitoneal lymph node dissection has improved surgical outcomes of localized disease. The introduction of targeted therapies since 2006 has revolutionized the management of metastatic kidney cancer. Despite these advances, the five-year survival of patients with advanced renal cancer is still poor and we need a better understanding of the disease.

The book *Urologic Cancers* provides an up-to-date overview of a wide spectrum of topics that comprise epidemiological, pathological, clinical, and biological aspects of urologic cancers. From the epidemiological spectrum, the most recent information on the epidemiology of testicular cancer and penile cancer are presented. From the clinical spectrum, the characteristics and management of divergent urothelial neoplasms, the role of surgery in the management of testicular and kidney cancer, biopsy approaches for better detection and diagnosis of prostate cancer, the role of family history and germline genetics in prostate cancer disease profiling and screening, and the need for the implementation of quality assurance programs to improve prostate cancer care discussed. The biological aspects of disease mechanisms and potential new therapeutics approaches are reviewed. Disease mechanisms focus on the etiological aspects of bladder cancer, renal cancer and upper tract urothelial carcinoma, the emerging role of microRNAs, metastamiRs, chromatin modifications and epigenetics not only on disease initiation but also on metastatic transformation. Potential therapeutics highlight the advances in radiation oncology and how this will potentially help the identification of novel image-based targets and change future treatment strategies.

The editors of *Urologic Cancers* need to be commended for bringing together an international team of clinicians and basic scientists to cover a range of topics on urologic cancers. The contents of the book will be of interest to healthcare professionals, basic scientists and clinicians interested in urologic cancers.

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers.foreword
Urologic cancers comprise cancers of the kidney, bladder, prostate, testis, and penis. While kidney cancer and bladder cancer affect both men and women, cancers of the prostate, testis and penis are specific to men. Prostate cancer is the fourth common cancer, and the second most cancer diagnosed in men after skin cancer. Urologic cancers are being increasingly diagnosed incidentally, at least in part due to increased use of imaging modalities for various reasons. While novel treatment strategies and management plans introduced in the past two decades have substantially increased patient survival and improved quality of life for localized disease, this has not been the case with metastatic disease highlighting the need for further active research. This book brings together an international team of clinicians and basic scientists to discuss the recent advances in the pathology and management of urologic cancers. The 15 chapters of this book cover a broad array of topics encompassing pathology, management, and potential therapeutic targets of urologic cancers.

The first two chapters provide an account of two unique entities, invasive urothelial carcinoma, and papillary urothelial neoplasms. The histologic subtypes, divergent differentiation, and clinical characteristics, and prognostic features are discussed. Chapters 3–5 are dedicated to bladder cancer, covering various aspects of etiology, the role of microRNA in metastatic phenotype, and how chromatin modifier mutations and impaired epigenetics regulate bladder cancer. Chapters 6–8 cover the etiology of renal cell carcinoma and upper tract urothelial carcinoma, the role of metastamiRs in metastatic kidney cancer, and the new trends in robotic retroperitoneal partial nephrectomy in the management of kidney cancer. Chapters 9–11 cover testicular cancer with a focus on epidemiology and organ sparing surgery, while chapter 11 on a less-known topic, the epidemiology of penile cancer. The remaining four chapters are dedicated to prostate cancer with emphasis on various biopsy approaches for better detection and diagnosis, the role of radiation oncology and how it could help identify novel treatment strategies, the need for implementation of quality assurance programs for better management of prostate cancer, and the role of family history and germline genetics in prostate cancer disease profiling and screening.

We thank the authors for their dedication and professionalism in contributing to this book. We believe this book will encourage readers to delve deeper into this field and take up the critical challenge of working toward effective treatments for urologic cancers.

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers.preface
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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers.contributors
Abstract: Invasive urothelial carcinoma is the most frequent type of bladder cancer and may occur in pure or classical form or with the presence of variant or subtype histology and/or evidence of divergent morphology such as squamous, glandular, or trophoblastic differentiation. Increasingly, it is recognized that certain subtypes impact patient prognosis and outcome hence the need to correctly recognize and document their presence. Certain subtypes and divergent features correlate with the emerging molecular bladder cancer subtypes, which can also influence patient management decisions. The pathologist therefore plays a crucial role in providing clinically relevant information, mostly derived from hematoxylin and eosin slides, which will guide urologists and oncologists in terms of risk stratification and treatment planning.

Keywords: bladder cancer; divergent differentiation of urothelial carcinoma; invasive urothelial carcinoma; molecular classification of urothelial carcinoma; subtypes of urothelial carcinoma


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INTRODUCTION

Bladder cancer is worldwide, the ninth most common adult solid organ malignancy and the fifth most frequent in North America (1, 2). Overwhelmingly, it is a male predominant disease with males more frequently impacted than females in an approximate ratio of 4:1. Diagnosis often occurs in the seventh or eighth decade however can occur earlier including in the pediatric population. The most frequent histologic type of bladder cancer is urothelial carcinoma, not otherwise specified (NOS) which recapitulates the usual urothelial lining of the bladder, urethra and upper urinary tracts. This represents ~80–90% of all bladder cancers worldwide (3). Invasive urothelial carcinoma shows morphologic and molecular heterogeneity along with variability in patient outcome. It can exist in a pure or classical form or may have components of either subtype histology or divergent differentiation (Table 1).

Subtype (variant) histology and divergent differentiation are used interchangeably by some authors, however they are two different processes. The term subtype is now preferred over variant given the use of the word “variant” in molecular terminology and the different implication that this carries. A subtype refers to specific histology features that are urothelial in appearance but have

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<th>Subtype Histology</th>
<th>Proposed Molecular Subtype</th>
<th>Prognosis</th>
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<td>Micropapillary</td>
<td>Luminal</td>
<td>Poor</td>
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<td>Plasmacytoid</td>
<td>Luminal</td>
<td>Poor</td>
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<td>Nested</td>
<td>Luminal or basal</td>
<td>Variable</td>
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<td>Microcystic</td>
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<td>Lymphoepithelioma-like</td>
<td>Basal</td>
<td>Good</td>
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<td>Clear cell</td>
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<td>Lipid rich</td>
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<td>-</td>
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<td>Giant cell</td>
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**Divergent Differentiation**

- Squamous
- Glandular
- Trophoblastic

**Other**

- Neuroendocrine
- Sarcomatoid
- Poorly differentiated

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<th>Proposed Molecular Subtype</th>
<th>Prognosis</th>
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<td>Sarcomatoid</td>
<td>Basal</td>
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<td>Poorly differentiated</td>
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distinct architectural features (e.g., micropapillary or plasmacytoid growth pattern). These subtypes, also referred to as variants by some authors, retain expression of usual markers of urothelial differentiation (4). In contrast, divergent differentiation (also referred to as aberrant differentiation) is used when the histology is no longer urothelial but exhibits a different histogenesis such as squamous, glandular or trophoblastic. These components can also acquire markers of this new histogenesis. With increasing divergence, acquisition of sarcomatoid or neuroendocrine features may be seen. Both subtypes and divergent differentiation may be found within a single tumor.

MICROPAPILLARY UROTHELIAL CARCINOMA

This subtype is frequently admixed with either conventional urothelial carcinoma or another subtype. There is a male predominance of this subtype which often has co-existent carcinoma in situ. The diagnostic features are of small, cohesive nests of carcinoma that are present within an empty space or lacuna which can resemble lymphovascular space invasion. These clusters lack fibrovascular cores and show peripheral orientation of nuclei (Figure 1A). Cytologic atypia may be present and so called “ring forms” are characteristic-cells with cytoplasmic vacuoles with indented nuclei (Figure 1B). Interobserver reproducibility is moderate for diagnosis of this subtype (kappa: 0.54 among expert
urologic pathologists) (5). This subtype expresses typical markers of urothelial differentiation (e.g., CK7, GATA-3, Uroplakin III along with p63 and pankeratin). CA125 may also be expressed and micropapillary subtype consistently shows higher rates of ERBB2 amplifications than conventional urothelial carcinoma (6, 7). Much interest exists in utilizing the Her-2 neu/ERBB2 status as a predictive biomarker however the discordance in Her-2 status by immunostaining and molecular analysis along with lack of urothelial specific reporting guidelines have made this challenging (8, 9). Micropapillary subtype is reported to be associated with poor prognosis (10, 11) and some clinicians will advocate for cystectomy when even a small component is identified (12) although this has been challenged in more recent literature (13).

PLASMACYTOID UROTHELIAL CARCINOMA

This aggressive subtype occurs with conventional urothelial carcinoma in ~50% of cases. It typically presents at an advanced stage with peritoneal spread and frequent positive margins after surgical resection (14, 15). Morphologically, the cells resemble plasma cells with eccentric nuclei and eosinophilic or clear cytoplasm (Figure 1C). Signet ring features with intra-cellular mucin are now recognized as a type of plasmacytoid carcinoma (Figure 1D) when they exist in the absence of extracellular mucin (signet ring cells with extracellular mucin are classified as adenocarcinoma in the bladder). Plasmacytoid carcinoma can grow in linear chains, as single cells or as a solid-sheet like pattern. While the cells are often cytologically bland, increasing atypia can be noted. The presence of a desmoplastic stromal response portends a worse prognosis (16). By immunohistochemistry, the cells express markers of urothelial lineage (e.g., CK7, GATA-3, Uroplakin III along with p63 and pankeratin) along with the plasma cell marker CD138 (17); however, MUM-1 is consistently negative. CDH1 mutations with loss of e-cadherin expression can be seen at a higher frequency than in conventional urothelial carcinoma (70% vs 11%) (18). Outcomes for this subtype are poor with frequent recurrences and lack of chemosensitivity (19, 20) and worse cancer specific mortality than conventional urothelial carcinoma (10).

NESTED UROTHELIAL CARCINOMA

This subtype is also known as a “deceptively bland variant/subtype” of urothelial carcinoma. It may occur as a small nested form (more common) or less frequently as a large nested morphology. The histologic features are of bland nests of urothelial cells (Figure 1E) that recapitulate von Brunn nests. Occasionally there are tubular forms present. There is frequently no atypia or mitotic activity in the superficial portion of these tumors (Figure 1F) with minimal atypia and occasional mitoses observed in the deeper aspects (21). This presents a challenge in superficial resection samples where the diagnosis may be overlooked. Invasion into muscularis propria is most helpful in reaching the
correct diagnosis. The large nested subtype is infrequent and often presents with overlying papillary tumor which has an inverted component (22). Similar to other subtypes, both the small and large nested subtypes express typical urothelial markers. The identification of TERT promoter mutations are helpful in distinguishing this subtype from benign mimics (23). One small study to date found CTNNB1 and JAK3 mutations in nested subtype (24). The outcome for the nested urothelial carcinoma is often poor as it is frequently diagnosed late (25, 26) although when stage matched with conventional urothelial carcinoma, it does no worse (25, 26).

**MICROCYSTIC UROTHELIAL CARCINOMA**

Similar to the aforementioned nested variant, the microcystic subtype also belongs in the “deceptively bland variant/subtype” category and can be admixed with the nested subtype. The morphology of this subtype includes tubular structures along with macro- and microcysts. Typically the urothelial lining is bland and cuboidal but focal higher grade areas can be seen. The lumen may contain calcifications and secretions. Distinguishing microcystic from urothelial carcinoma with a glandular component may be challenging. Further pitfalls with this entity include misinterpretation as cystitis cystica et glandularis or a grade group 1 prostatic adenocarcinoma. This subtype expresses usual markers of urothelial lineage and also MUC5AC (21) and similar to nested subtype, identification of a TERT promoter mutation can be helpful in ruling out a benign lesion. The clinical outcome for these patients is often poor (27).

**LYMPHOEPITHELIOMA-LIKE UROTHELIAL CARCINOMA**

This subtype is so called as it morphologically resembles a lymphoepithelioma of the pharynx. It exhibits a male predominance and can occur as a pure form or admixed with conventional urothelial carcinoma. Unlike lymphepithelioma of the pharynx, no association with Epstein Barr virus has been reported (28, 29). The morphology consists of sheets or nests of large pleomorphic cells arranged in a syncytial manner with indistinct cell borders (Figure 1G). The nuclei are large with prominent nucleoli. An intense inflammatory infiltrate is present comprised of lymphocytes, histiocytes, plasma cells and polymorphs (Figure 1H). Occasionally the inflammation is neutrophil or eosinophil predominant. The differential diagnosis of this entity includes lymphoma and chronic inflammatory processes. The urothelial component expresses pankeratin (helpful to exclude a lymphoproliferative process), CK7, GATA-3 and p63. CK20 is usually negative (21, 29). This subtype exhibits intact mismatch repair expression and has high programmed death-ligand 1 expression (30). In pure form, lymphoepithelioma-like carcinoma is reported to have a good prognosis and response to platinum-based chemotherapy (31) but when co-occurring with conventional urothelial carcinoma, prognosis is determined by the conventional component (29).
CLEAR CELL (GLYCOGEN-RICH) UROTHELIAL CARCINOMA

This infrequent subtype consists of a carcinoma comprised mostly of cells with voluminous clear cytoplasm that resemble clear cell carcinomas of renal origin (Figure 1I). Similar to other subtypes it can exist with a conventional component. The clear appearance is due to cytoplasmic glycogen, which is sensitive to diastase digestion as part of a periodic-acid Schiff with diastase stain. This subtype stains for urothelial markers which helps in differentiation from clear cell adenocarcinomas and metastatic clear cell renal cell carcinomas. There is limited information on the prognostic impact of this subtype due to its rarity however some literature suggests it imparts a worse prognosis (32, 33).

LIPID-RICH UROTHELIAL CARCINOMA

This rare subtype of urothelial carcinoma shows lipid vacuoles which can indent the nucleus and impart a lipoblast-like appearance. The lipid-rich component typically constitutes up to half of the carcinoma. Immunohistochemical analysis demonstrates expression of urothelial markers and electron microscopy confirms the presence of lipid in the vacuoles (34). This subtype is associated with advanced stage and poor prognosis (34).

GIANT CELL UROTHELIAL CARCINOMA

This is another rare subtype that is highly pleomorphic and aggressive. There is a male predominance and it typically occurs with conventional urothelial carcinoma. The morphology consists of pleomorphic giant cells and undifferentiated urothelial carcinoma. There is frequent multinucleation, necrosis and atypical mitoses (35). Urothelial markers are expressed. Patients are frequently late stage at presentation.

UROTHELIAL CARCINOMA WITH SQUAMOUS DIFFERENTIATION

Squamous differentiation is the most frequent line of divergent histology seen in high grade urothelial carcinoma (Figure 2A) and may be present in ~30% of such cases (36). The presence of keratinization and intercellular bridges define squamous histology. The presence of any urothelial carcinoma component (including carcinoma in situ) should be recorded and cases reported as urothelial carcinoma with squamous differentiation as it is thought that pure squamous cell carcinomas show less response to conventional chemotherapy (37, 38). The approximate percentage of the squamous component should be noted in the report. Squamous differentiation may be seen in the context of chronic irritation such as with stones, Schistosoma infection and neurogenic bladder with
in-dwelling catheters (39, 40). Human papilloma virus (HPV) is not thought to be a causative agent of this divergent morphology. Some studies have reported worse outcome when squamous histology is present, which may be related to advanced stage at presentation. Both usual urothelial immunomarkers and squamous markers (desmoglein 3 and CK14) can be expressed (41).

**UROTHELIAL CARCINOMA WITH GLANDULAR DIFFERENTIATION**

A glandular component of urothelial carcinoma is less frequent than a squamous one and frequently recapitulates the appearance of enteric histology, resembling a colonic-type adenocarcinoma (Figure 2B). Another variation of glandular differentiation is the presence of mucinous type carcinoma with mucin pools containing either glands or signet ring cells. The presence of extracellular mucin differentiates signet ring cell glandular differentiation from a plasmacytoid urothelial carcinoma. An in-situ carcinoma with glandular phenotype may be seen in conjunction with invasive glandular differentiation (42). A pseudo-glandular appearance may be seen in conventional urothelial carcinoma whereby cell “dropout” imparts a gland-like appearance. The presence of glandular morphology results in the acquisition of an alternate immuno-phenotype with expression of CK20 and CDX-2, typical of enteric lesions with either co-expression or loss of urothelial markers. The approximate percentage of glandular component should be noted in the report and in the absence of any urothelial carcinoma, the case should be considered as a pure adenocarcinoma. The presence of TERT promoter mutations may be helpful in this context as they are lacking in pure adenocarcinomas but will be present in ~70% of invasive urothelial carcinomas (43).
UROTHELIAL CARCINOMA WITH TROPHOBLASTIC DIFFERENTIATION

Visible syncytiotrophoblast cells are rare in invasive urothelial carcinoma however, HCG staining will often be present in high grade invasive urothelial carcinoma, estimated to be seen in up to 35% of cases. Rarely a choriocarcinoma component may be identified and in up to one third of cases, additional urothelial subtypes may be noted (44). These patients also may show elevated levels of serum HCG which correlate with adverse prognosis (45).

UROTHELIAL CARCINOMA WITH SARCOMATOID DIFFERENTIATION

Sarcomatoid differentiation comprises morphologic features of sarcoma and either histologic or immunohistochemical evidence of an epithelial component (Figure 2C). The sarcomatous areas are frequently undifferentiated, high grade spindle cells or show pleomorphic cells. Heterologous components (osteosarcoma, chondrosarcoma, angiosarcoma etc) may be identified and should be noted in the report. Cytokeratin stains may be required to identify the urothelial/epithelial areas but may also be positive in the sarcomatoid foci, as can p63 and GATA-3 (46). Metastatic disease is frequently present at diagnosis and the 5-year survival is poor (46, 47).

UROTHELIAL CARCINOMA WITH NEUROENDOCRINE DIFFERENTIATION

Small cell neuroendocrine carcinoma (Figure 2D) is much more frequent than large cell neuroendocrine carcinoma and often co-exists with conventional high grade urothelial carcinoma or other divergent morphology. Histologically, it resembles small cell carcinoma of the lung and exhibits staining for neuroendocrine markers (synaptophysin, chromogranin, CD56 etc- Figure E and F). Any amount of small cell morphology needs to be documented as it impacts chemotherapy selection and management. High rates of TP53 and RB1 mutations are noted and in keeping with its origin from urothelial carcinoma, TERT promoter mutations are frequent (48). Patients with small cell differentiation have poor prognosis including overall and disease specific survival (10).

MOLECULAR CLASSIFICATION OF INVASIVE UROTHELIAL CARCINOMA

Multiple classification systems exist for categorizing muscle invasive urothelial carcinoma. Most rely on multi-platform molecular classification techniques such
as transcriptomic analysis with only one system categorizing cases using immunohistochemistry (49). Recently, a consensus classification system was developed utilizing data from six separate systems (50). Irrespective of system, high grade muscle invasive urothelial carcinoma can be broadly categorized as “luminal type”, “basal type” and “other” – this category accounts for neuroendocrine tumors and those with a stromal component. The luminal and basal types have different clinical outcomes with differential responses to various systemic therapies (50, 51). Specific subtypes (variants) cluster within a molecular subgroup, irrespective of technique used to classify cases (50–52) – see Table 1.

CONCLUSION

Urothelial carcinoma subtypes and divergent differentiation impact patient outcome and their presence needs to be recognized and documented by the reporting pathologist. Recognition of these entities guide patient counselling and enable prognostic stratification. It can be envisaged that future bladder cancer pathology reporting should not only include the presence and quantity of subtypes/divergent features but also some adjunctive molecular analysis to further enable optimal and individualized therapy.

Conflict of Interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this chapter.

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Papillary Urothelial Neoplasms: Clinical, Histologic, and Prognostic Features

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Abstract: Primary bladder neoplasms can be divided into two broad categories: flat and papillary lesions. In this chapter, we provide a review of non-invasive papillary urothelial neoplasms of the bladder: urothelial papilloma, inverted urothelial papilloma, papillary urothelial neoplasm of low malignant potential (PUNLMP), non-invasive low grade papillary urothelial carcinoma, and non-invasive high grade papillary urothelial carcinoma. The following is discussed for each entity: clinical features, etiology, microscopic description, ancillary tests, molecular alterations, and prognostic factors.

Keywords: inverted urothelial papilloma; non-invasive papillary urothelial carcinoma; papillary urothelial neoplasm of low malignant potential; papillary urothelial neoplasms; PUNLMP
INTRODUCTION

Non-invasive urothelial neoplasms of the bladder can be divided into two categories: those that are flat and those with papillary configuration. Papillary neoplasms can further be sub-divided into urothelial papilloma, inverted urothelial papilloma, papillary urothelial neoplasm of low malignant potential (PUNLMP), non-invasive low grade papillary urothelial carcinoma, and non-invasive high grade papillary urothelial carcinoma. This chapter discusses each of the aforementioned entities in detail including their clinical features, etiology, microscopic description, ancillary tests, molecular alterations and prognostic factors.

UROTHELIAL PAPILLOMA

Urothelial papilloma is a neoplasm with papillae which contains delicate fibrovascular cores lined by normal urothelium. Urothelial papilloma is a rare benign papillary urothelial neoplasm that accounts for less than 4% of non-invasive urothelial neoplasms (1). It has been described in a wide age range, but patients tend to be younger, and it can be seen in children (2). The exact etiology is largely unknown at this time (3). It is believed that urothelial papilloma shares similar etiologic factors with other urological neoplasms which include smoking (4), occupational exposure to chlorinated hydrocarbons, polycyclic aromatic hydrocarbons, aromatic amines, and arsenic (5, 6). These neoplasms are exophytic lesions with a papillary configuration and normal thickness urothelium (Figure 1A). Dilated lymphatic channels may be found in the fibrovascular cores. There should be no architectural disorder with cells oriented perpendicular to the basement membrane. Umbrella cells are usually present and may display nucleomegaly and multinucleation.

Cytologically, the urothelial cells are bland with no atypia. Mitoses are absent. Immunohistochemistry is not required for the diagnosis. These lesions show positive CK20 expression in the umbrella cells only, similar to the expression of normal urothelium (Table 1). MIB-1 proliferation is usually low (<5%) (1). Urothelial papillomas have FGFR3 mutations (7). Alterations involving TP53 have not been described. The recurrence rate of urothelial papilloma varies from 8 to 14% and the rate of progression to cancer is less than 1% (1, 2, 8). For non-muscle invasive bladder neoplasms, the WHO/ISUP histologic grade correlates with the biological behaviour; higher grade tumours have higher likelihood of recurrence and progression. In the different iterations of the WHO/ISUP grading systems, papilloma is considered a benign neoplasm with a favourable clinical course.

INVERTED UROTHELIAL PAPILLOMA

Inverted urothelial papilloma is a non-invasive urothelial neoplasm with an exophytic or inverted growth pattern and absent or minimal cytological atypia. This is a rare and benign entity. Inverted urothelial papillomas (Figure 1B) are rare and account for less than 1% of all urothelial neoplasms in the bladder (1). Patients typically present in in their fifth to sixth decade and there is a stronger
Papillary Urothelial Neoplasms

**TABLE 1 Expected Immunoprofile of Papillary Urothelial Neoplasms**

<table>
<thead>
<tr>
<th>Entity</th>
<th>Immunoprofile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilloma</td>
<td>CK20+ in umbrella cells only, low Ki-67 proliferation</td>
</tr>
<tr>
<td>Inverted papilloma</td>
<td>CK20-, low Ki-67 proliferation</td>
</tr>
<tr>
<td>Papillary urothelial neoplasm of low malignant potential (PUNLMP)</td>
<td>CK20+ (superficial), FGFR3+/-, low Ki-67 proliferation</td>
</tr>
<tr>
<td>Non-invasive low grade papillary urothelial carcinoma</td>
<td>GATA-3+, p63+, high molecular weight cytokeratin+, CK5/6+ (basal layer) and CK7+. Can have STAG2 loss. Mismatch repair proteins can be lost (Lynch syndrome-associated tumours)</td>
</tr>
<tr>
<td>Non-invasive high grade papillary urothelial carcinoma</td>
<td>GATA3+, CK5/6+, CK7+, CK20+, high molecular weight cytokeratin+, p63+; compared to low grade lesions, can have increased p53 and Ki-67 expression</td>
</tr>
</tbody>
</table>

**Figure 1. Papillary Urothelial Neoplasms.**

A, Urothelial papilloma with normal thickness urothelium, no architectural disorder and bland cytology. B, Inverted urothelial papilloma with an endophytic growth pattern with urothelium organized in trabeculae and anastomosing cords. C, Papillary urothelial neoplasm of low malignant potential (PUNLMP) has thickened urothelium, orderly architecture and uniform cytology. D, An inverted form of PUNLMP. E, Low grade non-invasive papillary urothelial carcinoma with long, slender papillae, higher magnification (F) shows mild loss of polarity, mild cytologic pleomorphism and mitoses in the lower half of the urothelium. G and H, High grade non-invasive papillary urothelial carcinoma with complex papillae with and fused architecture. Architectural disorder and nuclear pleomorphism are visible on low power. I, Concurrent low grade and high-grade lesions can be found.
predilection for males than females (ratio of 5.8 to 1) (9, 10). The most common clinical presentations include hematuria and less commonly, lower urinary tract obstructive symptoms. The most common sites are bladder neck, trigone, lateral and posterior wall (1). On cystoscopy they may appear as raised, polypoid lesions with a smooth surface. The treatment is surgical resection via transurethral approach. Etiology is similar to other urothelial papillary neoplasms which include smoking, occupational exposure to chlorinated hydrocarbons, polycyclic aromatic hydrocarbons, aromatic amines, and arsenic (4–6).

Histologically, these neoplasms exhibit trabeculae and anastomosing cords of urothelium with an endophytic growth pattern that invaginate into the lamina propria (Figure 1B). Peripheral palisading of the basal cell layer may be seen. There should be a smooth interface with the stroma. The urothelium has normal thickness and lacks cytoarchitectural atypia. Immunohistochemistry is not required for the diagnosis. These neoplasms are usually CK20 negative and show low Ki-67 proliferation index (Table 1). Genetic alterations which have been reported in inverted urothelial papillomas include: \( \text{FGFR3} \) mutations, 9p deletions, 9q deletions, 17p deletions, and \( \text{HRAS} \) mutations (11–13). For non-invasive urothelial neoplasms, the WHO/ISUP histologic grade is a strong prognostic factor. As with conventional urothelial papilloma, inverted papilloma is deemed a benign neoplasm and is not assigned a WHO/ISUP histologic grade. This is a benign neoplasm and reported recurrence rate is less than 1 percent (1).

**PAPILLARY UROTHELIAL NEOPLASM OF LOW MALIGNANT POTENTIAL**

Papillary urothelial neoplasm of low malignant potential (PUNLMP) is a papillary urothelial neoplasm with increased thickness urothelium and minimal cytologic atypia. PUNLMP is a rare tumour, the prevalence is approximately 3 per 100,000 people per year (1). There is a strong male predominance, the male-to-female ratio is 5:1; the mean patient age is 64.6 years (3). The clinical presentation is usually gross or microscopic hematuria. Urine cytology is negative in the majority of cases. On cystoscopy, single or multiple intraluminal bladder papillary masses of variable size may be visualized. The most common locations for PUNLMPs are the lateral and posterior walls of the bladder, although it may be found anywhere along the urinary tract that has urothelium. Treatment option is surgical via transurethral resection. The etiology is similar to other papillary urothelial neoplasms. Specific factors include smoking (4), occupational exposure to certain chemicals such as chlorinated hydrocarbons, polycyclic aromatic hydrocarbons, aromatic amines, and arsenic (5, 6).

Histologically, PUNLMPs are papillary neoplasms with thicker and/or more cellular urothelium (Figure 1C). The architecture has no loss of order or polarity (14–18). The cytology is uniform and monotonous with cells appearing similar to each other. There may be some nuclear crowding and slight enlargement compared to the normal counterparts. Nucleoli should be inconspicuous, and chromatin is evenly distributed. Mitotic activity should be extremely rare and limited to the basal layer (1). An inverted form may also occur (Figure 1D). Immunohistochemistry is not required for the diagnosis of PUNLMP.
Some non-recurrent lesions may show strong positive staining pattern in FGFR3, superficial staining for CK20 and low MIB-1 proliferation index (Table 1) (14). The genetic and cytogenetic changes in PUNLMPs include mutations in FGFR3, TERT promoter mutations and chromosome 9 loss (1, 15, 16). Tumours with nuclear expression of TP53 are correlated with early-onset disease (age less than 45 years old) (17). The WHO/ISUP histologic grade is an important prognostic factor for non-muscle invasive urothelial neoplasms. Very few studies in the literature have looked at prognostic factors specifically for PUNLMPs. A recent study has shown that PUNLMPs have a recurrence rate of 18% and progression rate of 2% (18). Nevertheless, these lesions have a favourable outcome (1). Due to the risk of recurrence, patients typically have long-term follow up (19).

**NON-INVASIVE LOW GRADE PAPILLARY UROTHELIAL CARCINOMA**

Non-invasive low grade papillary urothelial carcinomas have low grade architectural and cytologic abnormality. It is essential that high grade features and invasion through the basement membrane are absent. The incidence of low grade papillary urothelial carcinomas is 5 per 100,000 people per year (3). There is a higher predilection for male (3:1 male-to-female ratio) and the median age is 70 years. Patients with Lynch syndrome may present with earlier stage and low-grade disease (20). Most of the lesions are found in the lateral and posterior walls of the bladder. Painless gross or microscopic hematuria is the most common clinical presentation. Patients who present with gross hematuria may have more advanced disease (21). Patients may initially be diagnosed via cystoscopy, CT urography, ultrasound, or urine cytology. Intraluminal masses, hydronephrosis or filling defects may be detected on imaging (22). Treatment options include transurethral surgical resection and intravesical therapy such as Bacille-Calmette-Guerin or mitomycin C (23). Smoking has been associated with low grade papillary urothelial carcinoma (4). Other etiologic factors include occupational exposure to certain chemicals such as chlorinated hydrocarbons, polycyclic aromatic hydrocarbons, aromatic amines, and arsenic (5, 6).

Histologically, low grade papillary urothelial carcinoma has fibrovascular cores lined by neoplastic urothelium (Figure 1E). Long and slender papillae usually show minimal branching or fusing. At low magnification, the architecture appears mostly orderly. At higher magnification, mild loss of polarity can be seen with some mild cytologic pleomorphism. There may be slight difference in cell size but no significant nucleomegaly or nuclear pleomorphism. Nuclear contour may be slightly irregular. Mitoses may be seen and are usually in the lower half of the urothelium (Figure 1F). There should be no atypical mitotic figures. Inverted growth patterns with both endophytic and exophytic components may also be present. Immunohistochemistry is not routinely utilized in the diagnosis of low grade papillary urothelial carcinomas. GATA-3 is positive in 97.5% of papillary urothelial neoplasms (24). These lesions can show positive staining in p63, high molecular weight cytokeratin, CK5/6 in the basal layer, and CK7 (25–28). STAG2 has been reported to show negative staining in upper tract urothelial malignancies (29). Mismatch repair proteins can be lost in Lynch syndrome-associated tumours (Table 1).
The initial step in the proposed pathogenesis of low-grade urothelial carcinoma involves loss of chromosome 9, which subsequently causes normal urothelium to become hyperplastic. Further genetic alterations such as \textit{FGFR3} mutations, which then activates downstream mitogenic activated protein (MAP) kinase pathway, leading to further development of low grade papillary urothelial carcinoma (15). Mutations in the \textit{TERT} promoter has been shown to be present in 50% of low grade papillary urothelial carcinomas; and these are more likely associated with \textit{FGFR3} mutated tumours (16). \textit{STAG2}, a cohesion complex gene, has been shown to have inactivating mutations in 32 to 36% of low grade and low tumour stage lesions (29). Other genetic alterations include mutations in \textit{CCND1}, loss of 11p chromosome, \textit{PIK3CA} mutations and microRNA alterations (30). Epigenetic silencing via promoter hypermethylation of select tumour suppressor genes have also been reported (31). Adverse prognostic factors for low grade papillary urothelial carcinoma, beyond the WHO/ISUP histologic grade, include multifocal disease, tumour size and the presence of concomitant urothelial carcinoma in situ (1, 23). Multifocal disease is associated with disease progression and higher disease associated mortality. Urothelial carcinoma in situ is associated with higher recurrence rate. High MIB-1 proliferation index is associated with poor prognosis (32). Mutations in \textit{FGFR3} and \textit{PIK3CA} associated tumours show lower rates of recurrence (33); while tumours with \textit{PTEN} deletions show increased rates of recurrence (34).

**NON-INVASIVE HIGH GRADE PAPILLARY UROTHELIAL CARCINOMA**

Non-invasive high grade papillary urothelial carcinomas are urothelial neoplasms with a papillary configuration and moderate to severe cytoarchitectural disorder. There is no invasion through the basement membrane. There is a stronger predilection for male than female (male-to-female ratio 6 to 8:1) and the mean age of patients is 70 years (1). These lesions are most commonly found in the lateral and posterior walls of the bladder; but it may arise from anywhere on the urinary tract with urothelium. For lesions arising from the renal pelvis, 85% are papillary and 66% are high grade (35). Patients typically present with intermittent, painless hematuria; gross hematuria is associated with higher pathologic stage diseases (21). High grade papillary urothelial carcinomas are associated with high rate of progression to invasion. Patients are diagnosed via cystoscopy, imaging modalities such as CT urography, ultrasound, or urine cytology. On cystoscopy, single or multiple exophytic lesions may be seen. Imaging typically shows filling defects, hydronephrosis or intraluminal masses (22). Treatment options include surgical transurethral resection tumour, intravesical immunotherapy with Bacillus Calmette-Guerin or intravesical chemotherapy with mitomycin C or thiotepa. Similar etiologic factors have been implicated in both high grade and low grade non-invasive papillary urothelial carcinomas: smoking (4), occupational exposure to chlorinated hydrocarbons, polycyclic aromatic hydrocarbons, aromatic amines, and arsenic (5, 6).

Non-invasive high grade papillary urothelial carcinomas show complex papillae with solid to fused architecture (Figures 1G and H). Neoplastic urothelium line fibrovascular cores. On low power, the architectural disorder and nuclear
pleomorphism are visible. The neoplastic cells tend to be crowded and overlapping, with dyscohesion and partial denudation of the epithelium. The nuclei are enlarged with irregular and coarse chromatin. Prominent nucleoli can be present. Mitotic activity may be brisk and atypical forms can be found. Inverted growth pattern with both endophytic and exophytic patterns may also be seen (36).

It is important to note that concurrent low-grade lesions can be found (Figure 1I). Grade heterogeneity is common and can be found in up to one third of non-invasive papillary urothelial carcinomas (37, 38). The grade of the lesion is assigned based on the highest-grade component identified. The general accepted approach is to designate a lesion “high grade” if there is at least 5% high grade histology identified (39). If a lesion comprises less than 5% high grade, it is reported as low-grade tumour with a quantification of the high-grade component present. This is important since such tumours may be more akin to low grade neoplasms in prognosis (38, 40); however, this is still debated (41, 42). Immunohistochemistry is not required for the diagnosis of non-invasive high grade papillary urothelial carcinomas. These lesions are positive for GATA3, CK5/6, CK7, CK20, high molecular weight cytokeratin, and p63 (Table 1). Compared to low grade lesions, high grade papillary urothelial carcinomas can have increased p53 and MIB-1 expression (43). A subset of high-grade lesions will show loss of staining in CK5/6 (44).

Non-invasive high grade urothelial carcinomas have genetic or epigenetic alterations involving the TP53 gene or CDKN2A gene. Somatic mutations in TERT have been reported to be present in 70–80% of non-invasive urothelial carcinomas (1). Mutations in PIK3CA, TSC1, HRAS, APC genes have been reported. Epigenetic silencing via promoter hypermethylation of select tumour suppressor genes are also identified (31). MicroRNA changes and loss of chromosome 9 have been described (30).

The WHO/ISUP histologic grade is an important prognostic factor for non-invasive high grade papillary urothelial carcinomas (45). The presence of nuclear anaplasia is correlated with disease progression and faster recurrence (1). Other adverse prognostic factors include multifocal disease and the presence of concomitant urothelial carcinoma in situ (1). Multifocal disease is associated with disease progression and higher disease associated mortality. Urothelial carcinoma in situ is associated with higher recurrence rate. High MIB-1 proliferation index is associated with poor prognosis (32). Tumours with PTEN deletions show increased rates of recurrence (34). Tumours with TP53 and RB mutations have worse prognosis (46).

**CONCLUSION**

Papillary urothelial neoplasms are commonly encountered genitourinary specimens by the surgical pathologist. Familiarity and an understanding with the clinical features, etiologic factors, histologic appearance, relevant ancillary workup, molecular alterations, and prognosis is important for arriving at the correct diagnosis and guiding appropriate clinical management. A brief overview of what is currently known about papillary urothelial neoplasms is provided in this chapter.
Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

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The Etiology of Bladder Cancer

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers-etiology-bladder-cancer

Abstract: Urothelial cancer of the bladder, known as bladder cancer, is one of the most common cancers in the world. The incidence is rising steadily particularly in developed nations where tobacco smoking is prevalent. With the development of accessible diagnostic modalities, enhanced surgical techniques, and improvement in novel immunotherapy regimes, overall survival rates are improving. Better understanding of the epidemiology and etiology of bladder cancer will lead to improved preventative strategies particularly modifiable risk factors like smoking.

Keywords: etiology of bladder cancer; genetics of bladder cancer; mortality in bladder cancer; schistosomiasis infection and bladder cancer; transitional cancer of the bladder
INTRODUCTION

Bladder cancer is the 10th most diagnosed cancer worldwide, with 573,278 new cases diagnosed in 2020 (1). The major histological type of bladder cancer is transitional cell carcinoma (90%) which arises from urothelial cells in the bladder, while squamous cell carcinoma (due to Schistosomiasis infection and recurrent urinary tract infections) and adenocarcinoma are less common. Typical presentation for bladder cancer is mainly hematuria (either non-visible microscopic or frank macroscopic hematuria), irritative lower urinary tract symptoms, and less commonly, suprapubic pain. Increasingly, more patients have been referred for urological management in the event of non-visible (microscopic) hematuria based on easily accessible use of urine dipsticks. Once confirmed on urine microscopy for the presence of red blood cells, the patient will be subjected to renal tract imaging (either ultrasound of kidneys or computed tomogram urography) and flexible cystoscopy. Obvious frank visible hematuria will certainly trigger the same approach of imaging and flexible cystoscopy. Once a suspicious lesion is seen on imaging or in cystoscopy, resection in the form of transurethral resection of bladder tumor (TURBT) will be performed urgently to attain histopathological confirmation.

With the advancements in radiological imaging coupled with refined diagnostic flexible cystoscopes, bladder cancers are much more likely to be diagnosed at earlier stages. Therefore, most bladder tumors are superficial (without any invasion into deeper muscular layers) on first TURBT and this surgery, if completed successfully with no macroscopic lesion left behind, will usually be sufficient for disease control. Based on the histopathological grade and subtype of bladder cancer, a regimen of follow up involving check cystoscopies and upper tract renal imaging will be implemented to detect any recurrence. The advent of intravesical treatment of mitomycin and Bacillus Calmette-Guerin have proven to be effective in reducing the risk of recurrence and progression of bladder transitional cell carcinoma (TCC).

However, if there was muscle invasive TCC noted on first TURBT, the treatment regimen will then involve either radical radiotherapy to bladder or neoadjuvant chemotherapy followed by radical cystectomy. Muscle invasive TCC are more aggressive and lethal. Metastatic TCC of bladder often carry quite a poor prognosis as the response to cisplatin-based chemotherapy regimen is limited. However, in this new era of immunotherapy, various immune checkpoint inhibitors (programmed cell death protein, PD-1 and cytotoxic T cell antigen, CTLA-4) have provided some modest improvement in survival and bring hope to this cohort of patients with metastatic bladder cancer.

Bladder cancer is the 14th most common cause for cancer-related deaths worldwide with 212,536 deaths in 2020 which equates to 2.1% of all cancer deaths. In men, bladder cancer is the 6th most common cancer and the 9th leading cause of cancer deaths. The incidence of carcinoma of bladder have been rising, especially in Europe and North America, largely due to tobacco smoking. There is an overpowering male predominance in incidence and mortality rates. Elsewhere, in parts of Northern Africa, Schistosomiasis infections still account for bladder squamous cell carcinoma (SCC) cases. Bladder cancer typically presents in the older patients with 80% of diagnoses made in those who are above age 65 in the United States (2). This perhaps reflect the chronicity of carcinogenic exposure to overcome the urothelial tumor suppressor mechanisms leading to carcinogenesis (3).
MORTALITY

Bladder cancer is the 14th most common cause for cancer deaths worldwide with 212,536 deaths in 2020 and this equates to 2.1% of all cancer deaths. However, in men, bladder cancer is the 6th most common cancer and the 9th leading cause of cancer deaths (1). The age standardized rate (ASR) for mortality in men was 3.3 per 100,000 compared to ASR of 0.9 in women. This mortality rate of almost four times more in men than in women perhaps is reflective of the incidence rate disparity where incidence ASR in men is 9.5/100,000 compared to 2.4/100,000 in women (1). The mortality rates in regions with highest incidence (Europe and North America) is about 2.1–3.3 ASR per 100,000; however, the highest mortality rates in the world lies with Northern Africa with a mortality rate of 5.2/100000 (4, 5). The high mortality rates in Europe and North America are reflective of the influence of tobacco smoking whereas the highest incidence in Northern Africa is largely due to Schistosomiasis haematobium infections which causes squamous cell carcinoma of bladder.

DEMOGRAPHY AND GENDER

The highest incidence rates among both sexes are in Southern Europe (ASR 15.3/100,000), Western Europe (ASR 13/100000) and North America (ASR 10.9/100000), while the lowest rates are from South Central Asia (ASR 1.9/100000) and Middle Africa (ASR 1.6/100000). Greece has the highest incidence rates among men in the world while Hungary has the highest incidence rates among women (4). As mentioned above the incidence and mortality rates in males are approximately 4 times higher compared to females. This marked discrepancy may be largely attributed to patterns of cigarette smoking where men are more likely to smoke longer than women. However, in United States, there has been a rising trend among women smokers with 39% of bladders cancers among women were attributed to smoking in 2014 as compared to 49% among men (6). Another theory for this male predisposition is due to the likelihood of further occupational chemical exposure when working in chemical, dyes, and paints industries.

GENETICS AND HEREDITARY

Genome wide association studies have identified potential genetic loci that has some association with the development of bladder cancers. Some of the genes identified include NAT2 (slow acetylator which detoxifies aromatic amines) (7) and GSTM1 (enzyme which detoxifies environmental carcinogens) (8), with NAT2 and GSTM1 having been shown to have synergistic effect with tobacco smoking (9). Certain hereditary syndromes like Lynch syndrome and Cowden’s syndrome can predispose an individual to develop bladder cancer. In Cowden’s syndrome, there is a defect in the tumor suppressor gene PTEN which predisposes to various tumors including TCC (10). In Lynch syndrome, there is development of non-polyposis colorectal cancer with an increased risk of bladder cancer due to defect in DNA mismatch repair (11).
SMOKING

The most significant modifiable risk factor for bladder cancers is tobacco smoking. Smoking has been shown to increase the risk of developing bladder cancer by up to four times, with mortality from bladder cancer due to smoking is only second to lung cancer due to smoking (12). The well-known carcinogens in tobacco smoke include beta-naphthylamine and polycyclic aromatic polycarbons. These carcinogens promote inflammation, and metabolizes in the bladder leading to DNA-adduct formation and permanent genetic mutations which either suppress tumor suppressor genes or activates oncogenes (3). Another study showed that there exists a difference in types of smoking, with pure tobacco cigarette smokers were at greater risk compared to pure pipe smokers or pure cigar smokers (13). A meta-analysis also revealed that passive second-hand smokers unfortunately develop a 22% increased risk of bladder cancer when compared to unexposed non-smoking individuals (14).

OCCUPATIONAL EXPOSURE

Another important modifiable risk factor for bladder cancer is exposure to hazardous environmental and/or occupational compounds found in dye, paint, rubber, petroleum, and metal industries. These compounds usually contain carcinogens which includes aromatic amines, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons (15). Other professionals who might carry an increased risk due to exposure to such carcinogens include firefighters, hairdressers, and farmers using fungicides. These carcinogens have been estimated to cause about 18% of bladder cancers, usually decades after exposure (16).

SCHISTOSOMIASIS INFECTION

A less common but aggressive bladder cancer is SCC of bladder which is caused by Schistosomiasis haematobium. This trematode is endemic to the Middle East and North Africa and has resulted in bladder SCC to become the second most form of cancer in those regions after hepatic cancer (17). The infection in the bladder caused by Schistosomiasis leads to generation of carcinogens like N-nitroso compounds and also enhances inflammation which induces free oxygen radicals and N-nitrosamines (18). The development of SCC seems to be at a much quicker pace compared to tobacco or hazardous chemicals exposure.

OBESITY

Obesity has been long associated with various forms of cancers including bladder cancers. One meta-analysis showed that obesity is an independent risk factor which increases the risk of bladder cancer by 10%. There is a 4.2% increased risk
of bladder cancer for every increase in 5 kg/m² weight (18). Well-established mechanisms linking obesity to bladder cancers include promotion of chronic inflammation with cascading cytokines, production of insulin-like growth factors, and adipokines, which can affect angiogenesis and apoptosis, leading to carcinogenesis (19, 20).

CONCLUSION

The incidence of urothelial carcinoma of bladder have been rising especially in Europe and North America largely due to tobacco smoking. There is an overpowering male predominance in incidence and mortality rates. In parts of Northern Africa, Schistosomiasis infections still account for bladder SCC cases. The modifiable risk factors in bladder cancers include tobacco smoking, occupational exposure to carcinogenic chemicals and obesity. In Schistosomiasis endemic regions, better water disinfection, avoiding freshwater swimming and mass treatment of praziquantel can help decrease the risk of bladder SCC. Prevention strategies aimed at modifiable risk factors like smoking cessation will continue to reduce the incidence rates. Earlier detection of superficial localized bladder cancers will also lead to improvement in cancer survival rates.

Conflict of Interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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The Role of MicroRNA in the Metastatic Phenotype of Bladder Cancer

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Abstract: Bladder cancer is among the most common cancers globally, with significant mortality associated with more advanced disease. Early detection and diagnostic accuracy are thus fundamental to the clinical pathway for managing bladder cancer. MicroRNA (miRNA) are small, non-coding segments of RNA that regulate gene expression and have been implicated in the process of carcinogenesis. Dysregulation and aberrant expression of miRNAs have been shown to have both oncogenic and tumor suppressive effects. A vast number of miRNAs, across the entire field of cancer biology, have already been identified and characterized, and many of these have been associated with bladder cancer. These miRNAs have furthered our understanding of the genetic profile of bladder cancer, and ultimately, may be utilized in the detection, prognosis, and treatment of this disease. This chapter focuses on the role of miRNA in the pathogenesis of metastatic bladder cancer.
bladder cancer and overviews many of the miRNA thought to be associated with bladder cancer. Additionally, this chapter explores the clinical utilities of miRNAs in bladder cancer to serve as biomarkers and guide individualized treatment.

Keywords: metastamiRs in bladder cancer; metastatic bladder cancer; microRNA in metastatic bladder cancer; miRNAs as prognostic indicators in bladder cancer; therapeutic utility of miRNAs in bladder cancer

INTRODUCTION

There is an ongoing paradigm shift in cancer treatment. The clinical pathways for treating cancer have grown increasingly individualized and the treatments themselves increasingly targeted. Central to these advances is a better understanding of the genetics and the biomolecular mechanisms of different cancers. In practice, characterizing the distinct oncologic profiles of specific tumors has allowed for more personalized therapies. Elucidating the factors that contribute to tumor promotion and suppression is paramount to constructing and understanding these profiles. One of these factors, microRNA (miRNA) is thought to play a major role in gene expression and in the development of cancer.

MiRNAs are small, non-coding segments of RNA approximately 19–22 nucleotides in length. MiRNA regulate messenger RNA (mRNA) in a post-transcriptional, or pre-translational, fashion (1, 2). Approximately 30% of all human genes and 60% of mRNA are regulated by miRNA (3). While miRNAs are evidently responsible for supporting normal human biological functioning, aberrant expression of these non-coding RNA segments may contribute to the pathogenesis of cancer and other diseases (4). Dysregulation of miRNA can trigger both tumor promotion and suppression via a multitude of biomolecular processes and pathways. Alterations to these pathways may significantly impact cancer phenotype, including cell migration and invasion, epithelial-to-mesenchymal transition (EMT) and angiogenesis—all factors that can contribute to metastatic potential (5). MiRNAs that are associated with the promotion or suppression of metastatic potential, when differentially expressed, are known as “metastamiRs” (6). The role of metastamiRs in the detection, prognosis and treatment of cancer continues to be investigated.

In addition to regulating gene expression at the cellular level, miRNAs are often exported from the cell and act as signaling molecules (7). MiRNA is widespread in the human body in both tissue and fluids, and it is relatively stable. The availability and stability of miRNA in easily accessible specimens, such as urine and blood, largely enables the feasibility of miRNA research (8). Basic research into identifying miRNA, their targets, and their downstream oncologic effects, as well as translational research into how these findings can be applied clinically, is ongoing. MiRNA as a non-invasive biomarker presents multiple uses, from detecting cancer to predicting and monitoring treatment response (8–10). Furthermore, the prospective use of miRNAs in personalizing care by identifying individual chemosensitivities to various chemotherapeutic agents, based on the specific genetics of individual tumors, would change the landscape of cancer management (11–13). The clinical utility of miRNA would be particularly welcome in the realm.
of bladder cancer—a cancer that traditionally requires multiple invasive procedures to diagnose as well as to surveille, with early detection and accurate staging vital for long-term survival. This chapter will explore the current knowledge base surrounding miRNA in bladder cancer metastasis and the proposed clinical applications of miRNA in this disease.

THE ROLE OF MICRORNAS IN THE METASTATIC PHENOTYPE OF BLADDER CANCER

Bladder cancer is the 10th most common cancer in the world and accounts for 3% of cancer diagnoses globally. In more developed regions of the world, such as Western Europe and the US, bladder cancer is particularly prevalent. In the US, bladder cancer is the 6th most common cancer and represents 4.6% of all cancer diagnoses, clearly outpacing global averages (14). In 2022, approximately 81,000 new cases of bladder cancer will be diagnosed and 17,000 people will die from the disease in the US (15). Ninety percent (90%) of bladder cancer cases are urothelial in origin (UCC or TCC) with additional variants (such as squamous cell carcinoma and adenocarcinoma) being very rare and associated with a worse prognosis. While the overall 5-year survival rate for bladder cancer in the US is 77%, for metastatic bladder cancer the survival rate is about 5% (14). Thus, early detection and diagnosis is vital in addressing bladder cancer before progression to more advanced disease states. Herein we discuss the potential role of miRNAs in revolutionizing how bladder cancer is detected, staged, monitored, and treated.

METASTAMIRS IN BLADDER CANCER

There has been extensive research into the role of miRNAs in bladder cancer, and numerous metastamiRs have been implicated in its progression. Metastasis-promoting miRNAs in bladder cancer downregulate tumor suppressors like PTEN and facilitate increased expression of oncogenes such as the MMP family to facilitate increased cell migration and invasion. Conversely, metastasis-suppressing miRNAs in bladder cancer downregulate genes such as TGFβ1 and E2F3 to inhibit cellular proliferation, migration, and invasion. Tables 1 and 2 provide a summary of specific metastamiRs in bladder cancer and their identified targets and functions (16–94). These tables are far from an all-inclusive list, as the body of research is extraordinarily vast and can be discordant. For example, several studies have reported that miR-200c promotes cell migration and invasion; however, alternate studies posit that downregulation of miR-200c leads to increased cell migration, invasion, and is associated with lung metastases (16–22). While these contradictory findings may be a result of differing methodology or perhaps differences due to tissue origin in the respective studies, the definitive function of miR-200c remains unclear and requires further investigation. Nevertheless, these studies continue to highlight the diverse and multifaceted nature of bladder cancer and how dysregulation of seemingly opposite pathways can still lead to metastasis.
## TABLE 1

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target/Regulator</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-10b</td>
<td>KLF4, HOXD10</td>
<td>Promotes cell migration and invasion</td>
<td>(23)</td>
</tr>
<tr>
<td>miR-21</td>
<td>PTEN</td>
<td>Promotes invasion and migration via upregulation of PI3K/AKT and AKT/STAG3 pathways. Enhances resistance to doxorubicin</td>
<td>(24, 25)</td>
</tr>
<tr>
<td>miR-92b</td>
<td>DAB2IP</td>
<td>Promotes cell migration, invasion and EMT</td>
<td>(26)</td>
</tr>
<tr>
<td>miR-129-5p</td>
<td>SOX4</td>
<td>Promotes cell migration and invasion</td>
<td>(27)</td>
</tr>
<tr>
<td>miR-137</td>
<td>PAQR3</td>
<td>Promotes cell migration and invasion</td>
<td>(28)</td>
</tr>
<tr>
<td>miR-146b</td>
<td>ETS2, MMP2, AUF1</td>
<td>Promotes cell invasion</td>
<td>(29)</td>
</tr>
<tr>
<td>miR-182-5p</td>
<td>RECK, Smad4</td>
<td>Promotes cell migration and invasion</td>
<td>(30)</td>
</tr>
<tr>
<td>miR-200a</td>
<td>PTEN, Dicer/cJun, MMP-2</td>
<td>Promotes cell invasion by downregulating Dicer and its respective downstream targets, leading to MMP-2 upregulation</td>
<td>(31)</td>
</tr>
<tr>
<td>miR-492</td>
<td>GJB4</td>
<td>Promotes cell migration and invasion</td>
<td>(32)</td>
</tr>
<tr>
<td>miR-495</td>
<td>PTEN</td>
<td>Promotes cell invasion</td>
<td>(33)</td>
</tr>
<tr>
<td>miR-516a</td>
<td>MMP9, PHLPP2, SMURF1</td>
<td>Promotes cell migration and invasion via AKT/FOXO3A/SMURF1 pathway and inhibition of MMP-9 degradation</td>
<td>(34)</td>
</tr>
<tr>
<td>miR-556-3p</td>
<td>DAB2IP</td>
<td>Promotes cell proliferation and colony formation via upregulation of Ras-ERK pathways, cell migration and invasion</td>
<td>(35)</td>
</tr>
<tr>
<td>miR-3622a</td>
<td>LASS2</td>
<td>Promotes cell invasion</td>
<td>(36)</td>
</tr>
<tr>
<td>miR-3648</td>
<td>TCF21, KISS1</td>
<td>Promotes cell migration and invasion</td>
<td>(37)</td>
</tr>
<tr>
<td>miR-4295</td>
<td>BTG1</td>
<td>Promotes cell proliferation and migration</td>
<td>(38)</td>
</tr>
</tbody>
</table>

### Diagnostic and therapeutic utility of miRNAs in bladder cancer

Diagnosis of bladder cancer utilizes urine cytology and cystoscopic biopsies of bladder tissue. Further biopsies are occasionally needed for staging and differentiation between muscle invasive (MIBC) and non-muscle invasive bladder cancer (NMIBC) as well as to detect response to intravesical treatment and recurrence. These diagnostic procedures are not without risk, as patients undergoing bladder biopsies require induction with general anesthesia, and the surgeries themselves can cause bleeding, urinary tract infections (UTI), as well as damage to the prostate, bladder, or urethra. Given the physical risks of these procedures, the emotional toll on patients, and the financial burden of diagnosis, an alternative, less invasive diagnostic test utilizing miRNAs would rectify several of these existing issues.
<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target/Regulator</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-15</td>
<td>BMI1</td>
<td>Inhibits cell migration and invasion.</td>
<td>(39)</td>
</tr>
<tr>
<td>miR-22</td>
<td>E2F3, Snail, MAPK</td>
<td>Inhibits cell migration, invasion and EMT.</td>
<td>(40, 41)</td>
</tr>
<tr>
<td>miR-24</td>
<td>CARMA3</td>
<td>Inhibits cell invasion and EMT.</td>
<td>(42)</td>
</tr>
<tr>
<td>miR-26a-5p, -26b-5p</td>
<td>PLOD2</td>
<td>Inhibits cell migration and invasion.</td>
<td>(43)</td>
</tr>
<tr>
<td>miR-34a-5p</td>
<td>TCF1, LEF1, DNMT3B, MMP-2</td>
<td>Inhibits cell migration and invasion. Enhances epirubicin sensitivity.</td>
<td>(44–46)</td>
</tr>
<tr>
<td>miR-101</td>
<td>FZD4, c-FOS, VEGF-C</td>
<td>Inhibits cell migration and invasion. Increases cisplatin sensitivity.</td>
<td>(47–50)</td>
</tr>
<tr>
<td>miR-124-3p</td>
<td>ITGA3</td>
<td>Inhibits cell migration, invasion, and EMT via downregulation of FAK/PI3K/AKT pathway.</td>
<td>(51)</td>
</tr>
<tr>
<td>miR-125b-5p</td>
<td>SIRT7, MALAT1, MMP13, HK2</td>
<td>Inhibits proliferation, cell migration, and invasion via the PI3K/AKT pathway. Promotes apoptosis.</td>
<td>(52–54)</td>
</tr>
<tr>
<td>miR-132</td>
<td>TGFβ1</td>
<td>Inhibits cell migration, invasion and EMT via TGFβ1/SMAD2 pathway.</td>
<td>(55)</td>
</tr>
<tr>
<td>miR-138</td>
<td>ZEB2</td>
<td>Inhibits cell migration and invasion.</td>
<td>(56)</td>
</tr>
<tr>
<td>miR-140-3p</td>
<td>FOXQ1</td>
<td>Inhibits cell invasion.</td>
<td>(57)</td>
</tr>
<tr>
<td>miR-145</td>
<td>N-cadherin, MMP9</td>
<td>Inhibits cell migration and invasion.</td>
<td>(58)</td>
</tr>
<tr>
<td>miR-146a-3p</td>
<td>PTTG1</td>
<td>Inhibits cell migration and invasion.</td>
<td>(59)</td>
</tr>
<tr>
<td>miR-154</td>
<td>ATG7</td>
<td>Inhibits cell migration and invasion.</td>
<td>(60)</td>
</tr>
<tr>
<td>miR-186</td>
<td>VEGF-C</td>
<td>Inhibits cell migration, invasion, angiogenesis.</td>
<td>(61)</td>
</tr>
<tr>
<td>miR-194-5p</td>
<td>E2F3</td>
<td>Inhibits cell migration and invasion.</td>
<td>(62)</td>
</tr>
<tr>
<td>miR-199a-5p</td>
<td>CCR7, MMP9</td>
<td>Inhibits cell migration, invasion, EMT.</td>
<td>(63)</td>
</tr>
<tr>
<td>miR-200b</td>
<td>TGF-β1</td>
<td>Inhibits cell migration and invasion. Enhances cisplatin sensitivity.</td>
<td>(64, 65)</td>
</tr>
<tr>
<td>miR-203a</td>
<td>SIX4</td>
<td>Inhibits cell migration, invasion and EMT.</td>
<td>(66)</td>
</tr>
<tr>
<td>miR-204</td>
<td>ROBO4</td>
<td>Inhibits cell migration and invasion.</td>
<td>(67)</td>
</tr>
<tr>
<td>miR-210-3p</td>
<td>FGFR1</td>
<td>Inhibits cell invasion.</td>
<td>(68)</td>
</tr>
<tr>
<td>miR-223</td>
<td>WDR62</td>
<td>Inhibits cell migration and invasion.</td>
<td>(69)</td>
</tr>
<tr>
<td>miR-300</td>
<td>SP1/MMP9 pathway</td>
<td>Inhibits cell migration.</td>
<td>(70)</td>
</tr>
<tr>
<td>miR-325-3p</td>
<td>MT3</td>
<td>Inhibits cell migration, invasion, and EMT.</td>
<td>(71)</td>
</tr>
</tbody>
</table>
## TABLE 2 Summary of metastasis-suppressing miRNAs involved in BC with impacted pathways, targets, and resulting oncologic outcome (Continued)

<table>
<thead>
<tr>
<th>miR</th>
<th>Target(s)</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-338-3p</td>
<td>ETS1</td>
<td>Inhibits cell proliferation, metastasis and EMT.</td>
<td>(72)</td>
</tr>
<tr>
<td>miR-370</td>
<td>SOX12</td>
<td>Inhibits cell migration and invasion.</td>
<td>(73)</td>
</tr>
<tr>
<td>miR-372/373</td>
<td>CUL4B</td>
<td>Inhibits cell migration via downregulation of PI3K/AKT pathway.</td>
<td>(74)</td>
</tr>
<tr>
<td>miR-375-3p</td>
<td>FZD8</td>
<td>Inhibits cell migration.</td>
<td>(75)</td>
</tr>
<tr>
<td>miR-379-5p</td>
<td>MDM2</td>
<td>Inhibits cell migration and invasion.</td>
<td>(76)</td>
</tr>
<tr>
<td>miR-381-3p</td>
<td>BM1, Rho/ROCK, CCNA2, MET</td>
<td>Inhibits cell invasion, migration and EMT.</td>
<td>(77, 78)</td>
</tr>
<tr>
<td>miR-429</td>
<td>MMP2, E-cadherin</td>
<td>Inhibits cell migration, invasion, and EMT via E-cadherin upregulation.</td>
<td>(79, 80)</td>
</tr>
<tr>
<td>miR-485-5p</td>
<td>HMGA2</td>
<td>Inhibits cell invasion and metastatic potential. Inhibits cancer cell adhesion and EMT.</td>
<td>(81, 82)</td>
</tr>
<tr>
<td>miR-486-5p</td>
<td>ROCK, CD44, MMP9</td>
<td>Inhibits cell migration. Enhances cisplatin sensitivity.</td>
<td>(83)</td>
</tr>
<tr>
<td>miR-497</td>
<td>Vimentin, α-SMA, E-cadherin, E2F3</td>
<td>Inhibits cell migration, invasion, and EMT by downregulating vimentin and α-SMA and upregulating E-cadherin.</td>
<td>(84, 85)</td>
</tr>
<tr>
<td>miR-502-5p</td>
<td>CCND1, NOP14, DNMT3B</td>
<td>Inhibits cell migration.</td>
<td>(86)</td>
</tr>
<tr>
<td>miR-539</td>
<td>IGF-1R</td>
<td>Inhibits cell proliferation and invasion.</td>
<td>(87)</td>
</tr>
<tr>
<td>miR-612</td>
<td>ME1</td>
<td>Inhibits cell migration, invasion, and EMT.</td>
<td>(88)</td>
</tr>
<tr>
<td>miR-613</td>
<td>SphK1</td>
<td>Inhibits cell migration, invasion, and EMT.</td>
<td>(89)</td>
</tr>
<tr>
<td>miR-621</td>
<td>TRIM29, Wnt/β-catenin</td>
<td>Inhibits cell proliferation and metastatic potential by downregulation of the Wnt/β-catenin pathway.</td>
<td>(90)</td>
</tr>
<tr>
<td>miR-1182</td>
<td>hTERT</td>
<td>Inhibits cell proliferation and invasion. Enhances cisplatin sensitivity.</td>
<td>(91)</td>
</tr>
<tr>
<td>miR-1280</td>
<td>ROCK1</td>
<td>Inhibits cell proliferation, migration and invasion.</td>
<td>(92)</td>
</tr>
<tr>
<td>miR-3619-5p</td>
<td>β-catenin, CDK2</td>
<td>Inhibits cell migration, invasion, and reduces metastatic potential by upregulating p21.</td>
<td>(93)</td>
</tr>
<tr>
<td>miR-4324</td>
<td>RACGAP1</td>
<td>Inhibits cell colony formation, migration, invasion and EMT. Enhances doxorubicin sensitivity.</td>
<td>(94)</td>
</tr>
</tbody>
</table>
miRNAs as diagnostic biomarkers

A urine-based diagnostic test utilizing miRNAs as biomarkers for bladder cancer would be an ideal clinical tool, given the ease and non-invasive nature of specimen collection. Several pilot studies have investigated the feasibility of detecting miRNAs in urine to diagnose bladder cancer (95–100), and a meta-analysis found that urine-based miRNA assays were more sensitive than urine cytology in diagnosis of bladder cancer (101). Implementing a urine-based miRNA test in the pathway of bladder cancer management could prove invaluable if it lessens the burden of repetitive invasive testing as required in NMIBC. Furthermore, tissue-based miRNA profiles may have a role in bladder cancer diagnostics as well. Patients often require repeat resections to ensure the presence of muscle in the specimen—the differentiator between NMIBC and MIBC. To this end, a tissue-based miRNA test could preclude the need for repeat resection if the presence of specific miRNAs in the initial specimen can predict muscle invasion (20) or risk of recurrence.

Utilizing miRNAs to screen and assess treatment response in bladder cancer

Treatment algorithms differ between NMIBC and MIBC. While bladder tumor resections, intravesical chemotherapy, and immunotherapy are mainstay treatments for NMIBC, surgical removal of the bladder via radical cystectomy and urinary diversion, often after neoadjuvant cisplatin-based chemotherapy, is the standard of care for MIBC (102–103). However, roughly 60% of all patients subjected to neoadjuvant chemotherapy fail to have an adequate response to systemic treatment and still have invasive disease upon cystectomy (104). Thus, a large percentage of patients with MIBC receiving neoadjuvant chemotherapy are subjected to the morbid side-effects of these chemotherapeutic agents, while not benefiting from any considerable response. Furthermore, completion of systemic therapy in this population serves to delay definitive management via surgery.

Utilizing a biomarker to identify patients who are likely to respond to chemotherapy prior to its initiation could transform the utility of neoadjuvant treatment by both minimizing unnecessary chemotherapy exposure and expediting surgery for those who are unlikely to respond. Current research suggests miRNAs may be particularly suited for this purpose. For example, miR-101, -1182, -200b and -486-5p are all implicated in tumor suppression and have been associated with cisplatin sensitivity (47, 48–50, 65, 83, 91). As such, these miRNAs could potentially be utilized to screen patients as likely responders to cisplatin-based neoadjuvant chemotherapy and would thus allow for the more appropriate provision of systemic chemotherapeutics. Furthermore, miRNAs could be used in this capacity to not just dictate the utility of cisplatin-based therapy but may also identify other chemotherapeutic options for those deemed unlikely to respond to cisplatin, thus personalizing treatment and optimizing the likelihood of response.
miRNAs as prognostic indicators in bladder cancer

While there are no current prognostic calculators for bladder cancer that utilize miRNA, there is potential for miRNAs to act in this realm. Xie et al. identified five miRNAs in a systematic review and meta-analysis that could potentially be useful in prognostics, citing high levels of miR-21 and miR-222 and low levels of miR-214 were associated with low overall survival. Furthermore, they detailed high levels of miR-143 and miR-155 were associated with poor progression-free survival (105). More recently, Yin et al. proposed a 21-signature miRNA profile to determine prognosis in BC patients (106).

MiRNAs may specifically be useful in addressing the need for a prognostic indicator for risk of disease progression. This would be particularly helpful in those who have low stage/grade disease and therefore are—based on conventional indicators—stratified as having a low risk of progression. That is, those with low stage disease who are determined to be at high risk of progression would benefit from more frequent clinical surveillance, thus providing earlier detection of disease progression, and allowing for better therapeutic options and outcomes.

UPPER TRACT UROTHELIAL CARCINOMA

Upper tract urothelial carcinoma (UTUC) refers to urothelial cancer of the renal collecting system, including the renal pelvis and the ureter. While the role of miRNAs in UTUC is far less elucidated than in UCC (bladder cancer), several studies have identified miRNAs that are dysregulated in UTUC. A serum miRNA analysis by Tao et al. found significantly different expression patterns of 13 miRNAs between UTUC patients and cancer-free patients (107). Another study by Hsu et al. identified miR-145-5p as downregulated in UTUC tissue, and a modulator of cell migration and invasion through its regulation of ARF6 (108). Furthermore, rescue of miR-145-5p expression yielded a suppression of EMT markers through its correlation with an increase in E-cadherin levels and a decrease in MMP7 and N-cadherin (108). EMT was also noted to be inhibited by miR-30a-5p in a study comparing UTUC tissue to adjacent normal tissue after nephroureterectomy for UTUC (109). In addition, Browne et al. identified several miRNAs that were associated with an invasive phenotype in UTUC, including miRs-10b-5p, -26a-5p, -31-5p, and -146b-5p (110). Lastly, Ke et al. found that miR-210 was overexpressed in UTUC compared to benign urothelium and proposed miR-210 involvement in promoting UTUC carcinogenesis and tumor progression (111).

While there is a paucity of literature regarding the role of miRNAs in UTUC in comparison to that of bladder cancer, several miRNAs have been identified with potential for therapeutic and diagnostic targeting. While further research is necessary, these miRNAs show promise in bladder cancer and UTUC alike to both further advance and refine the clinical management of these malignancies.
CONCLUSION

The clinical pathways and algorithms used for diagnosing and treating cancer are in the midst of a seismic shift and continue to rapidly evolve. An emphasis on individualized care—targeted therapy—is at the forefront of these changes. The ability to increasingly characterize a tumor based on its genetics has opened the door for improved diagnosis, prognostication, and treatment. While there are many factors found in the genetic profile of a cell that may aid its transformation into a cancer, abundant research across the spectrum of cancer biology has explored miRNA and how these short, non-coding RNA segments regulate gene expression. The potential clinical applications of miRNA are vast. MiRNAs as biomarkers could revolutionize how cancer is detected, monitored, and treated. For example, reliable urine miRNA biomarkers in bladder cancer could negate the need for the multiple invasive procedures. Further, establishing a miRNA profile for a specific tumor may elucidate its chemosensitivities, and in turn, enable personalized, targeted chemotherapy. Bladder cancer is particularly deadly in its advanced and metastatic stages and using the genetics of a tumor to refine therapeutic options could greatly alleviate the mortality burden. MiRNA research, for bladder cancer and beyond, holds great promise.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this chapter.

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REFERENCES


MicroRNA in Metastatic Bladder Cancer


Abstract: Chromatin deregulation is an emerging theme in cancer pathogenesis, and bladder cancer stands out among many other cancer types with frequent mutations of genes involved in epigenetic regulation. Defects in chromatin-level regulation can be manifested at multiple levels such as changes in DNA methylation, histone methylation patterns, and non-coding RNAs. Chromatin modifiers mutated in bladder cancer, such as KDM6A, KMT2D, KMT2C, ARID1A, EP300, have been studied in bladder cell line models. Also, there are studies that mapped the active regulatory landscape of bladder cancer and histone modification profiles. Collectively, existing literature emphasizes the importance of a thorough understanding of epigenetic deregulation in bladder cancer. The epigenetic signatures of bladder cancer can be targeted via epigenetic drugs or other genome editing tools, ultimately bringing specific treatment options for this cancer.
This chapter provides an overview of the epigenetic modifications in bladder cancer, and the potential of epidrugs for the treatment of bladder cancer.

**Keywords:** chromatin modifiers in bladder cancer; epidrugs for bladder cancer; epigenetics in bladder cancer; histone methylation in bladder cancer; mutations in bladder cancer

**INTRODUCTION**

Cancer is a complex disease with many hallmarks (1). During the last decade, there has been a tremendous effort to characterize the genomic landscape and to identify molecular subgroups of diverse cancer types (2–4). All these molecular studies made it clear that epigenetic deregulation was a common theme implicated in tumorigenesis. It became apparent that proper epigenetic regulation is essential for normal cellular homeostasis and any deviation from this tightly regulated balance disrupts the cellular states and may result in tumor formation (5, 6). Among all the other cancers, bladder cancer has an exceptionally high rate of chromatin modifier mutations (7), and thus considered as a disease where epigenetic deregulatory mechanisms play a fundamental role. Bladder cancer mostly originates from the urothelium and causes over 200,000 deaths each year (8). Its main classification is done based on histopathology as non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). Recent studies characterized the mutational landscape of both MIBC and NMIBC and further identified the consensus molecular subgroups, providing fundamental insights about the pathogenesis of bladder cancer (9–12). However, there is still need for further studies to characterize the epigenetic deregulation of bladder cancer in detail and use this information for specific diagnosis and treatment of bladder cancer. This chapter mainly focuses on the chromatin modifiers frequently mutated in bladder cancer, the major regulatory mechanisms disrupted, and the potential use of epigenetic therapies in bladder cancer.

**EPIGENETIC REGULATION AND CANCER**

To understand and explain the origin and characteristics of the cancer, several theories have been proposed throughout the years. “Hallmarks of cancer” proposed by Hanahan and Weinberg conceptualizes and organizes the principles in a logical framework (1). All hallmarks and characteristics define functional properties acquired by normal cells in the way of progressive transformation from normal state to neoplastic state (6, 13). Acquisition of hallmarks depends on alterations in the genome, epigenetic reprogramming, and microenvironmental remodeling. In addition to genetic alterations, epigenetic modifications contribute to gene expression deregulation in cancer. Aberrations in epigenetic mechanisms, such as DNA methylation, histone modifications, deregulation in non-coding RNAs (miRNA, lncRNA), play an important role in tumorigenesis
Chromatin Modifier Mutations in Bladder Cancer

contributing to the different hallmarks of cancer (14, 15). These epigenetic deregulations may result in inappropriate activation or inhibition of gene expression.

DNA methylation occurs at cytosine residues at CpG dinucleotides. While CpG dinucleotides spread throughout the genome, CpG islands (CGIs) are located at 5’ regulatory regions, such as promoter of genes. Promoter DNA methylation is associated with repression of transcription (16, 17). In human cells, 3 different DNA methyltransferases (DNMT1, DNMT3A, and DMT3B) catalyze the transfer of methyl group to cytosine residue (18). Aberrations in the maintenance of the DNA methylation are critical for tumorigenesis. Global hypomethylation and the promoter hypermethylation are the characteristics of the cancer epigenome and contribute to the overexpression of protooncogenes and the silencing of tumor suppressor genes, respectively (19, 20). Oncogenic signaling pathways also direct the activity of global methyltransferases which contributes to the shift from normal to cancer-specific methylation profile (15). Alterations in the DNA methylation have been known as early event in bladder cancer development and are considered as a hallmark of cancer (21).

In a eukaryotic nucleus, DNA is wrapped around the histone octamers forming nucleosome structure. N-terminal tails of core histone protein (H2A, H2B, H3, and H4) are largely targeted for the posttranslational modifications (PTMs), such as methylation, acetylation, and phosphorylation (16). Modifications in histone tails affect the chromatin structure which is critical for the gene regulation (22). Chromatin structure is highly dynamic, and orchestrated by chromatin remodeling complexes, and histone modifying enzymes. Aberrations in histone modification caused by defects in activity of histone modifying enzymes and chromatin remodeling complexes may contribute to the neoplastic transformation (23). Mutations in histone genes or chromatin modifier proteins are frequently detected in many cancer types, resulting in impairments in gene expression programs and genomic integrity (24) (Figure 1).

CHROMATIN MODIFIERS FREQUENTLY MUTATED IN BLADDER CANCER

To advance our understanding on the molecular landscape of cancer, large-scale genome wide studies, especially the TCGA project, collected data on gene expression, transcript splice variation, protein expression, DNA copy number alterations, somatic mutation, DNA methylation, and gene fusion, and also clinicopathological data from many cancer types, including bladder cancer (11, 25). Integrated omics studies have revealed that, with five or more mutations per megabase, bladder cancer has a higher mutational burden compared to the other cancer types (10, 11, 25, 26). The most common mutations in bladder cancer occur in genes functioning in histone modification and chromatin remodeling genes. These include ARID1A (25%), KDM6A (24%), KMT2D (27%), EP300 (15%) (27). Globally, almost 80% of all bladder cancer patients have mutations in genes involved in epigenetic regulation, demonstrating the high degree of epigenetic dysregulation in this cancer (28). It is also important to the notice that chromatin modifiers
mutated in bladder cancer mostly function in active chromatin organization and activation of gene expression. In this context, it might be speculated that chromatin modifier mutations in bladder cancer results in a closed chromatin configuration, likely prohibiting the expression of genes required for urothelial differentiation while resulting in gene expression programs supporting proliferation and tumorigenesis (Figure 2).

**EPIGENETIC LANDSCAPE OF BLADDER CANCER**

As already mentioned, aberrations in the epigenetic landscape are one of the hallmarks of cancer and abnormalities in DNA methylation, chromatin modifier mutations, and altered gene expression of chromatin modifiers and non-coding RNAs result in changes in cellular characteristics and promote the unfavorable prognosis. The association between epigenetic landscape and gene expression in bladder cancer has been addressed in several studies (10, 11, 29). One study defined the genome-wide chromatin accessibility profiles and cancer-specific DNA regulatory elements across 23 cancer types from TCGA (including bladder cancer) and
identified significant correlations between gene expression and chromatin accessibility, integrating transcriptomics data and ATAC-seq (the assay for transposase-accessible chromatin using sequencing). Besides, this study demonstrated impressive similarity between ATAC-seq based clustering and previously established mRNA, miRNA, DNA methylation or copy number variation (CNV) profile-based classifications. In this study, it has been also revealed that a somatic mutation observed in one regulatory region of bladder cancer increases chromatin accessibility and changes gene expression in mutant bladder cancer (29).

To further extend the knowledge on epigenomic landscape of bladder cancer, van der Vos et al. (10) conducted a study on genome-wide histone methylation profiling of MIBC (10). Integrated analysis of the H3K27me3, a repressive histone mark, and H3K4me1 and H3K4me3 (gene-activating histone marks) ChIP-seq and RNA-seq data indicated that different enhancer regions play critical role in the characterization of luminal and basal subtypes of MIBC.

Non-coding RNAs, such as microRNAs (miRNA), long non-coding RNAs (lncRNA), circular RNAs (circRNA), piwi-interacting RNAs (piRNA), small nuclear RNA (snRNA), and small nucleolar RNAs (snoRNA), are not translated into proteins, but they have still significant functions in every cellular process. NcRNAs also contribute to the epigenetic alterations that promote bladder cancer development and progression (30).

Prognostic biomarker potential of DNA hypermethylation has been widely investigated in bladder cancer (31, 32). Yet, further investigation is necessary to obtain more sensitive and specific biomarkers. It has been identified that CpG-rich transposons, such as LINE1, are hypomethylated in bladder cancer types. This leads to retrotranspositions inducing genomic instability (33). A recent study investigated global histone acetylation levels and its prognostic value in bladder
cancer patients and reported decreased H3 acetylation level in both NMIBC and MIBC patients compared to normal urothelial control group (34).

Histone deacetylases (HDACs) are divided into different classes based on their similarity to yeast HDACs (35). Another study reported that HDAC-1, HDAC-2, and HDAC-3 expression levels are elevated in urothelial carcinoma. Notably, increased HDAC-1 and HDAC-2 levels were associated with high grade tumors. Moreover, high grade tumors with high HDAC-1 expression correlated with worse prognosis compared to low grade tumors (36). This finding supports the therapeutic target potential of HDACs. Another study identified the chromatin interactions by Hi-C, integrating it with transcriptome and enhancer profiles in luminal and basal types of bladder cancer. Even though the study implicated the association between epigenomic landscape and 3D genome structure in a subtype-specific manner, further studies are needed to comprehensively unveil the molecular basis and involved factors (37).

### FUNCTIONAL OUTCOMES OF CHROMATIN MODIFIER MUTATIONS IN BLADDER CANCER

Given the high rate of chromatin modifier mutations in bladder cancer, there have been many studies investigating the functional impact of the mutations in different model systems (Table 1) (38–46). Polycomb repressive complex 2 (PRC2)-dependent epigenetic regulation is critical for cell differentiation and proliferation in bladder urothelium (47). SWI/SNF complex acts as an antagonist of PRC2 complex promoting the expression of genes which are silenced by PRC2 (48).

<table>
<thead>
<tr>
<th>Mutation/loss of function</th>
<th>Functional outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARID1A</td>
<td>Impairments in cell cycle&lt;br&gt;Genomic stability&lt;br&gt;Induced cell proliferation</td>
<td>(38, 39)</td>
</tr>
<tr>
<td>KDM6A</td>
<td>Induced tumor immune escape&lt;br&gt;Activation of proinflammatory pathways&lt;br&gt;Induced proliferation&lt;br&gt;Deregulation in the expression of cell identity related genes</td>
<td>(40–43)</td>
</tr>
<tr>
<td>KMT2C</td>
<td>Increased chromatin instability&lt;br&gt;Impairments in DNA replication and repair&lt;br&gt;Misregulation of apoptosis, and cell cycle control</td>
<td>(44)</td>
</tr>
<tr>
<td>KMT2D</td>
<td>Impairments in DNA replication and cell cycle&lt;br&gt;Induced invasion, migration, and viability</td>
<td>(45, 68)</td>
</tr>
<tr>
<td>CBP/EP300</td>
<td>Impairments in histone acetylation&lt;br&gt;Increased anti-tumor immunity</td>
<td>(46)</td>
</tr>
</tbody>
</table>
ARID1A belongs to SWI/SNF complex proteins (49), and it is frequently mutated in primary human bladder carcinoma (25). ARID1A has role in the tumor suppressor mechanisms regulating cell cycle progression and maintaining genomic stability (38).

ARID1A protein loss is predominantly observed in high grade and high stages of bladder tumors that indicates association with poorer prognosis (39, 50). The potential functions of ARID1A have been investigated in urothelial cells of ARID1A knockout mice. It has been shown that loss-of-function mutation in ARID1A upregulates urothelial cell proliferation, emphasizing the tumor suppressor role of ARID1A in bladder cancer development (39). Additionally, findings implicated an antagonistic relationship between ARID1A and PRC2 complex in bladder (51). However, function of ARID1A might be context-dependent since different studies addressed opposing roles for ARID1A in different cellular processes and cancer types (52).

KDM6A (UTX), lysine histone demethylase, physically interacts with chromatin modifying enzymes, such as KMT2C (MLL3) and KMT2D (MLL4) (53). KDM6A protein contains tetratricopeptide repeat (TPR) domains and Jumonji C (JmjC) domain. JmjC domain catalyzes the removal of the methyl group from H3K27me2 and H3K27me3 (53, 54). TPR domain conducts interaction with components of MLL3 and MLL4 complexes (55). The function of KDM6A has been the subject of numerous studies. These studies reported that KDM6A regulates gene expression and cellular processes. As a component of the COMPASS complex, KDM6A is involved in regulation of gene activation (56–58). Loss-of-function and inactivating mutations frequently occur in several neoplasms, including bladder tumors (59–62). Reduced KDM6A expression and KDM6A mutations is correlated with poor prognosis in bladder cancer (40). Furthermore, potential roles of KDM6A in immune response have been shown via TIMER and CIBERSORT algorithms. Gene set enrichment analyses have indicated that the signaling pathways involved in immunity have been repressed in patients with mutated KDM6A. These findings imply the relationship between KDM6A mutations and anti-tumor immunity (40). In another study, Kobatake et al. showed that decreased expression of KDM6A is associated with the activation of proinflammatory pathways (41). Increased proliferation has been observed in two different KDM6A knock-out bladder cell lines (42). Notably, KDM6A has a role in safeguarding luminal gene expression program in bladder cancer cell lines (43).

Studies focused on the function of KMT2C (MLL3, histone lysine methyltransferase 2C) in normal cells defined its role in regulation of enhancer activity, focusing on the profile of H3K4me1 mark (63, 64). Independent from its H3K4me activity, the roles of KMT2C in transcription regulation have been shown in recent reports (65, 66). Tumor suppressor role of KMT2C has been reported for urothelial carcinoma. KMT2C silencing in 2 different bladder cancer cell lines has been shown to directly or indirectly affect the expression of genes involved in cell cycle control, DNA repair, DNA replication, and apoptosis (44). To investigate its further effects, genome-wide binding profile of KMT2C has been mapped via ChIP-seq (44). To evaluate the effects of KMT2C on epigenetic landscape of bladder cancer, Rampias et al. (44) also studied the changes in H3K4me3, H3K27ac, and H3K9ac histone modifications upon KMT2C silencing. Knockdown of KMT2C influences the enhancer activity in bladder cancer cell lines. In parallel with its well-established role in deposition of H3K4me1, co-localization of KMT2C with
active enhancer mark H3K27ac points out the increased enhancer activity (44). Additionally, KMT2C loss affects expression of genes critical for cell adherence, extracellular organization, and epithelial differentiation (44).

KMT2D (also known as MLL4) is one of the histone methyltransferases that may play a critical role in tumorigenesis and progression of bladder cancer (63). KMT2D regulates the activity of H3K4 methylation (67). KMT2D has high mutation rates in bladder cancer. Low levels of KMT2D are associated with lymph node metastasis (68). KMT2D mRNA and protein expression is decreased in 4 bladder cancer cell lines (T24, J82, UM-UC-3, and HTB-9) compared to normal bladder cell line. Silencing of KMT2D induces invasion in T24, and HTB-9 cell lines, while its overexpression suppresses. It has been demonstrated that KMT2D regulates level of H3K4me1 in bladder cell lines (68). Interestingly, while Sun et al (68) showed association between higher KMT2D expression and higher survival rate, Ding et al (45) implied that KMT2D mutations are associated with better prognosis in bladder tumors. Gene set enrichment analysis has indicated that KMT2D mutations are also significantly associated with cell cycle and DNA replication processes (45).

CREB-binding protein (CREBBP or CBP) and E1A binding protein (EP300 or P300) are transcriptional coactivators which also have ubiquitin ligase activity and histone acetyltransferase activity (69). CBP and EP300 are frequently mutated in a variety of human tumors (70). These inactivating alterations resulting in deregulation of acetylation and neoplastic transformation have been investigated in tumor models and bladder cancer lines (71, 72). Duex et al. (71) defined that those mutations are largely enriched at histone acetyltransferase domains of EP300 and CBP, implying potential significance of the domain activity on tumorigenesis. They also postulated that impairments in histone acetyltransferase activity are more likely to be linked with aggressive, MIBC cases (71). It was also identified that mutations in EP300 promote the signaling pathways involved in anti-tumor response in bladder cancer (46).

MANIPULATING CHROMATIN MODIFIER MUTATIONS FOR TREATMENT OF BLADDER CANCER

New strategies and options for diagnosis and treatment of bladder cancer are needed to augment pharmacological outcome. The utilization of epigenetics for diagnostic markers and therapy targets is a rapidly developing and promising area. The reversibility of epigenetic changes serves a great potential as a therapeutic target in bladder cancer. Improvement of epidrugs has great advantage for cancers or disease in which epigenetic dysregulation plays a key role (Figure 3) (73).

Inhibiting the DNMT enzymes, gene silencing can be reversed, and in turn expression of tumor suppressor genes is recovered. It has been revealed that 5-Aza-2′-Deoxycytidine (DAC), DNMT inhibitor, induces cell cycle arrest, and increases the susceptibility to chemotherapy in bladder tumors (74). 5-aza-2′- deoxycytidine and 5-azacytidine are approved for treatment of myelodysplastic syndrome and myeloid leukemia by the FDA. There are ongoing clinical trials for use in bladder cancer therapy (75).
Several HDAC inhibitors show promise in urological cancers (76). It has been demonstrated that cellular growth and proliferation is inhibited upon the treatment of bladder cancer cells with HDAC inhibitors Vorinostat, Romidepsin, and Trichostatin A (77, 78). Further analyses showed that changes in the protein expression are mostly associated with apoptosis, regulation of cell cycle, and DNA damage repair mechanisms in response to treatment with these HDAC inhibitors (78). HDAC inhibitors Romidepsin, have been approved by FDA for treatment of cutaneous T cell lymphoma (CTCL), while Belinostat and Panobinostat approved for the treatment of T cell lymphoma (79). In combination with the other chemotherapy agents, HDAC inhibitors synergistically affect the cell cycle arrest, apoptosis, and differentiation of malignant cells (30, 80). It has been shown that combination of DNMT inhibitor and HDAC inhibitors has also synergistic effect on cancer cells (76).

Increased expression levels of G9a, H3K9 methyltransferase, have been detected in bladder cancer. Inhibition of G9a in bladder cancer suppresses the proliferation inducing autophagic cell death in bladder cancer cells (81). Treatment of bladder cancer cell lines with small molecule UNC064, G9a inhibitor, decreases the cell viability, while inducing the apoptosis (82).

As a catalytic subunit of PRC2 complex, histone methyltransferase EZH2 regulates trimethylation of H3K27 (H3K27me3) (83). This histone mark is critical for repression of gene expression. An increasing number of evidence demonstrated that EZH2 dictates both development and progression of different types of tumors.
Dysregulation in EZH2 expression has been associated with increased cell proliferation, invasion, and metastasis (84). Notably, it has been reported that EZH2 is also linked with the chemotherapy resistance (85). Upregulated expression of EZH2 plays oncogenic roles in bladder cancer. Since it affects the gene expression and regulates the several cellular mechanisms, EZH2 serves a great potential as a target for treatment (86). Currently, EZH2 inhibitor Tazemetostat is being investigated in ongoing clinical trials for treatment of urothelial carcinoma, in addition to lymphomas, and other solid tumors (87–89).

Therapeutic targeting of epigenetic modifiers which are currently in clinical trials (https://www.clinicaltrials.gov/) is summarized in Table 2. In a review, Ozgun et al. (28) evaluated the combination of EZH2 inhibitors or HDAC inhibitors with retinoids in bladder carcinoma. They pointed out the potential therapeutic options with retinoic acid and its derivatives and emphasized the clinical trials investigating combinatorial use of retinoids with epidrugs (28).

Another type of epigenetic therapy is based on miRNA manipulation. Strategies basically focus on regulation of the miRNA expression and activity in cancer cells (75, 90). This manipulation is managed via mimicking the specific miRNAs (91) or administration of epidrugs, such as EZH inhibitors (92, 93).

CRISPR/Cas9 system has become widely used technology for genome targeting. Due to the technical improvements, targeted epigenome editing can be achieved using CRISPR platform (94). The fusion of dCas to chromatin-modifying domains represents a powerful tool for chromatin editing (95). Epigenome editing is achieved in human cells through CRISPR activation and inhibition systems (CRISPRa/CRISPRi). These approaches can be applied for epigenetic reprogramming both in vivo and ex vivo, disease modeling, therapeutic targeting, and cellular therapies (94). Recent clinical studies have been performed CRISPR-based epigenome editing in human hematopoietic progenitor and stem cells for the treatment of an immune disease (96). Yet, there is a need for more effort to establish routine clinical use.

<table>
<thead>
<tr>
<th>Table 2: Potential epidrugs for bladder cancer</th>
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<tbody>
<tr>
<td><strong>Epidrug</strong></td>
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<tr>
<td>5-Aza-2′-Deoxycytidine</td>
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<tr>
<td>5-azacytidine</td>
</tr>
<tr>
<td>Vorinostat</td>
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<tr>
<td>Romidepsin</td>
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<tr>
<td>Belinostat</td>
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<tr>
<td>Tazemetostat</td>
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</table>
CONCLUSION

Mutations in chromatin modifying genes are highly frequent in bladder cancer (11). There have been plenty of studies which emphasize that epigenetic deregulations are critical for understanding of bladder cancer pathogenesis, characterization of phenotype, determination of disease outcome, and also for directing the treatment options. Clinical adoption of bladder cancer epigenetics is still in a developmental phase. Expanding our limited knowledge on epigenetics of urothelial malignancies will contribute to the improvements in diagnosis, and development of more precise targeted therapies. Drugs targeting epigenetic machinery, epigenetic biomarkers for diagnosis, and tailoring the epigenome state of cancer cells are the emerging fields in personalized medicine. Yet, implementation of epidrugs and epigenome editing to clinic still needs to overcome many challenges.

Acknowledgment: This work was funded by The Scientific and Technological Research Council of Turkey (TÜBİTAK), and the EMBO Installation Grant (No:4148). Burcu Akman is a recipient of scholarship from the Scientific and Technological Research Council of Turkey (TÜBİTAK) project (#120C129).

Conflict of Interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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Abstract: Renal cell carcinoma accounts for most malignant renal cancers, with clear cell as the most common subtype. Nowadays, the typical presentation of loin pain, frank hematuria, and palpable mass of renal cell carcinoma are seen less frequently. The advancements in medical imaging, in particular abdominal imaging, have significantly increased the number of small renal masses detected incidentally. Urothelium lining of upper urinary tract starts from the calyces and run the entire length of the ureter till the vesico-ureteric junction. Urothelial carcinoma is the malignancy of this urothelium tract. Established risk factors for renal cell carcinoma, and to some extent to upper tract urothelial carcinoma, include male gender, smoking, hypertension, obesity, and end stage renal diseases. This chapter provides an overview of the etiology of renal cell carcinoma and upper tract urothelial carcinoma.

Keywords: etiology of kidney cancer; etiology of renal cell carcinoma; etiology of upper tract urothelial carcinoma; risk factors for etiology of upper tract urothelial carcinoma; risk factors for renal cell carcinoma
INTRODUCTION

Renal cell carcinoma (RCC) accounts for most malignant renal cancers. The subtypes of RCC include clear cell (most common), papillary, chromophobe, multilocular cystic, medullary, and collecting duct RCC. With the advancements in medical imaging and easily accessible abdominal imaging facilities, increasing numbers of small renal masses are being detected with majority of patients diagnosed incidentally when they are imaged for other non-urological reasons or symptoms. This has certainly led to an increasing detection rate of renal cancers, leading to earlier intervention and better treatment pathways for localized small renal cancers. This is supported by the latest Global Cancer Statistics report, as there were 431,288 new renal cancers diagnosed worldwide in 2020 alone. This incidence rate accounted for 6.1/100,000 age standardized rate (ASR) in males and 3.2/100,000 in females (1). Following early detection of incidental small renal cancers, effective treatment options have been successful in the last few decades. These treatment options range from minimally invasive percutaneous laparoscopic cryoablation and radiofrequency ablation to open or robotic assisted partial/total nephrectomy. The success of these modalities of treatment have transformed and improved the prognosis of patients with these incidental early detected small renal cancers.

The upper urinary tract urothelium is the innermost lining of the urinary tract starting from renal calyces collating towards renal pelvis and running the entire length of the ureters till its termination in the vesico-ureteric junction. Upper tract urothelial carcinoma (UTUC) is the malignant change that can occur anywhere along the entire length of the urothelium. Typical clinical presentation of UTUC include hematuria, loin pain and less commonly, palpable mass. Clinical outcome usually depends on the clinical stage at presentation, grade, and presence of muscle invasion of the urothelial carcinoma. UTUC affects the older population with a peak incidence between 70 and 90 years of age (2). Approximately 40–50% of patients present with non-muscle invasive UTUC, 50–60% present with muscle invasive, locally advanced stage, and 25% present with metastases (3). This chapter provides a snapshot of the etiological factors of RCC and UTUC.

RENAL CELL CARCINOMA

The incidence of renal cancers worldwide has an age standardized rate of 4.6 per 100,000 with a morality rate of 1.8 per 100,000 in 2020 (4). Incidence rates vary regionally, largely due to healthcare accessibility for the local population and data acquisition in terms of data reporting. The highest incidence of renal cancers in 2020 was recorded in Northern America (ASR 12.2 per 100,000) with the lowest incidence observed in Middle Africa (ASR 1 per 100,000) (4). Clearly this high incidence detected in Northern America compared to the rest of the world reflects the early detection of incidental renal cancers due to easy access to radiological abdominal imaging facilities in their healthcare system coupled with robust clinical disease reporting. In Europe, the ASR is 8.3–10.3 per 100,000 persons, depending on the different regions of Europe, comparable with Australia and New Zealand (ASR 10.3). In Asia, the range of ASR is 1.4–4.1 (4).
Certainly, demographic factors account for the incidence rates throughout the world and this also reflects the Human Development Index (HDI) of the countries, with the incidence and mortality rates increasing with increasing HDI status. HDI reflects the standard of living, health and knowledge aspects of a country population, with positive correlation towards incidence of cancers.

Despite the increasing trend of early detection of renal cancers leading to earlier treatment options, the mortality rates are still high at a steady rate. In fact, there were 179,368 deaths in 2020 with an ASR of 2.5 in 100,000 males and 1.2 in 100,000 females based on the GLOBOCAN 2020 report (1). This is due to the fact that RCC is still a lethal disease when diagnosed at a locally advanced stage and unfortunately a significant proportion of patients (17%) still present late in the metastatic stage (5). Mortality rates were noted to be higher in Europe (ASR range 2.4–3.4) compared to Asia (ASR 0.8–1.8) while lowest were recorded for regions of Africa (ASR 0.7–1.2) (4).

**Age and gender**

The risk of developing renal cancer increases with age, with a peak incidence of 60–70 years, an ASR of 0.5 in those below 40 years old and rising to 35 in those who are more than 75 years of age (5). However, there is a rising trend in the detection of incidental small renal cancers in the younger age groups as there is increased public awareness and easier access to routine abdominal imaging. In the latest GLOBOCAN 2020 report, the risk of developing renal cancer in males is close to double (ratio of 1.9 :1) compared to females with an ASR of 6.1 and 3.2 respectively (1). This rate has remained the same across all age groups, signifying the male predominance. In fact, men are more likely to have larger tumors, more aggressive histological types and grades, present at later advanced stages, and poorer prognosis (6). Numerous theories have been proposed for this gender propensity – androgenic hormonal cancer promotion (7) and biomolecular pathways with influences from inflammatory and immune mediated genes (8).

**Genetic factors**

The alterations in the genetic landscape for the development of RCC have been extensively studied in the last few decades. Perhaps the most influential genetic aberration in clear cell RCC (ccRCC) is the tumor suppressor gene von Hippel Lindau (vHL). The inactivation of vHL gene leads to the upregulation of hypoxia inducible factors leading to tumor neo-angiogenesis and proliferation (9). Numerous other genetic alterations have also been shown to contribute along with inactivation of vHL gene in the development of ccRCC. These are polybromol (PBRM1), BRCA1 associated protein-1, SET domain containing 2 (SETD2) and lysine K-specific demethylase 6A (KDM6A) (10). Hereditary RCC syndromes have been also well documented. The common familial RCC syndromes include von Hippel Lindau syndrome (affected gene vHL, autosomal dominant disease with risk of pheochromocytoma, pancreatic tumors and central nervous system tumors), Birt-Hogg-Dube syndrome (affected gene folliculin, autosomal dominant disease with risk of chromophobe RCC and papillary RCC) and hereditary leiomyomatosis renal cell cancers (affected gene fumarate hydratase, autosomal dominant disease with risk of papillary RCC) (11).
Smoking

Positive history of smoking has long been associated with the risk of developing RCC. Numerous carcinogens from active or passive smoking have been linked to development of cancers including renal cancers. These carcinogens include multiple chemical classes, including polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, aromatic amines, aldehydes, volatile organic hydrocarbons, and metals. The carcinogenic pathways starts with inhalation of carcinogens from smoking leading to covalent bond formations between carcinogens and DNA resulting in somatic mutations in critical oncogenes and tumor suppressor genes (12). The risk of renal cancers increases with increasing exposure to smoking leading to higher grade diseases, while cessation of smoking led to decrease in relative risk. A meta-analysis by Cumberbatch et al. confirmed pooled relative risk of RCC incidence of 1.31 for all smokers, 1.36 for current smokers, and 1.16 for ex-smokers (13).

Hypertension

Another modifiable risk factor in the development of renal cancers include history of hypertension. Previous studies have concluded the positive association of hypertension as an independent risk factor for renal cancers (14, 15). This association was further confirmed by a meta-analysis of 18 prospective studies which showed a 67% increased risk with history of hypertension and with every 10 mmHg increase in blood pressure led to a 10–22% increased risk of developing RCC (16). Possible explanations for this association include chronic renal hypoxia and lipid peroxidation leading to development of hypoxia-inducible factors which promotes malignant angiogenesis and tumor cell proliferation (17).

Obesity

Obesity has been commonly linked to risk of developing various cancers including renal cancers. A study on 77,620 participants by Macleod et al. revealed a significant association of obesity and RCC risk with a 1.7 hazard ratio (95% CI 1.06–2.79) in those with body mass index greater or equal to 35 kg/m² versus those with less than 25 kg/m² (18). With 5 kg increases in weight, there was a relative risk increase in RCC risk of 25% in males and 35% in females (19). Theories supporting the proposed association of obesity and RCC center around circulating sex-hormones, insulin-like growth factors and adipokines (leptin, adinopectin) and cellular events relating to chronic inflammation (19, 20). This led to the proposal of use of statins to decrease RCC risk, but a meta-analysis revealed no association between statins usage and RCC risk (21). However, increased levels of physical activity may decrease the risk of RCC by reducing obesity and improving blood pressure. In the same meta-analysis, Zhang et al. showed inverse association of RCC risk with physical activity (relative risk 0.88) (21).

Diet

A healthy diet combined with active lifestyle will prevent obesity and decrease the risk of RCC. Diet consisting of high fruit and vegetable intake, particularly
cruciferous vegetables, have been associated with decreased risk of developing RCC (22). However, the large EPIC study of 375,851 participants revealed no significant association between fruit and vegetable intake with RCC risk (23). In terms of alcohol intake and association with RCC risk, various studies have found mixed results with some studies showing a reduction in risk with alcohol intake while others showed no association (18, 24).

Concomitant diseases

A meta-analysis which studied the association of renal stones with RCC risk showed that there was pooled relative risk of 1.76 (95% CI 1.24–2.49) in patients with RCC and renal stones. A further subgroup analysis noted a significantly increased risk of RCC only in males and not in females with renal stones (25). End stage renal failure often leads to pathological degenerative cystic changes which is termed as acquired cystic kidney disease. These end stage kidney diseases have a positive correlation with RCC risk, with at least a ten-fold increased risk of developing RCC in their lifetime (26).

Analgesia use

Regular use of analgesia, particularly nonsteroidal anti-inflammatory drugs (NSAIDS), has been long studied in regard to its association with development of RCC risk. A meta-analysis revealed that there was an increased risk of RCC with regular use of acetaminophen and non-aspirin NSAIDS (pooled RR 1.51) (27).

UPPER TRACT UROTHELIAL CARCINOMA

The incidence of UTUC has been difficult to quantify and validate as most UTUC that arise in the renal calyces or pelvis get collated together with the rest of renal cancers. UTUC is relatively uncommon as it represents only 5% of urothelial cancers and less than 10% of renal tumors (3). Data from Western countries estimate the incidence of UTUC at 2 new cases per 100,000 person-years (2). The last few decades have seen improvement in detection of UTUC due to better imaging modalities in the form of computed tomogram and magnetic resonance urography and smaller flexible ureteroscopes that allow better visualization and subsequent tissue biopsy opportunities. This has led to early detection and thus influenced the incidence of UTUC. Furthermore, the advancements in bladder cancer surveillance and treatment have also influenced the diagnosis and earlier treatment of UTUC.

Gender

UTUC is more common in males compared to females, with studies showing that men are affected 2–3 times more than women (28, 29). This higher risk of UTUC in men is somewhat similar to higher bladder cancer risks in men too. Mortality and survival rates of UTUC have not been shown to be
dependent on gender. A study by Shariat et al. on patients who underwent radical nephroureterectomy for UTUC did not show any association between gender of patients with pathologic features, prognosis, recurrence of disease or cancer-specific mortality (28).

Smoking

Smoking is a well-known risk factor for urothelial cancers including UTUC. The estimated relative risk of developing UTUC from smoking is 2.5 to 7-fold (30). Another study involving 864 patients who underwent radical nephroureterectomy for UTUC revealed that current and long-term heavy smokers had more aggressive disease, presented at advanced later stages, were prone to disease recurrences, and had a higher cancer specific mortality rate. It was also shown in that same study, patients who stopped smoking >10 years prior to surgery for UTUC had a better oncologic outcome compared to patients who were still smoking or ceased <10 years prior to surgery (31).

Other risks

A significant risk factor for the development of UTUC is hereditary non-polyposis colon cancer (Lynch syndrome). This is an autosomal dominant disease characterized by a spectrum of malignancies (colon cancer, endometrial cancer, UTUC, ovarian cancer, gastric, and hepatobiliary cancers which occur in younger age groups). The main genetic problem arises from the germline mutation of DNA mismatch repair genes (MMR genes) or in the hEPCAM genes, which result in microsatellite instability in regions of DNA (32). The reported lifetime risk of developing UTUC in Lynch syndrome patients is between 0.4 and 20%, which translates to about 22 times higher risk than the average population (33, 34)

The risk of Balkan endemic nephropathy is another disease entity noted to increase the risk of developing UTUC which was initially discovered to be endemic in rural areas of Southeastern Europe near the Balkan peninsula. It is a tubulointerstitial disease that leads to chronic end stage kidney disease with high risk of developing UTUC. This is due to the phytotoxin of aristolochic acid (AA) which are in the common plant *Aristolochia clematitis* that grow alongside wheat fields, and therefore consumed in homemade bread (35). A meta-analysis showed that exposure to AA led to overall increased risk of urothelial tract cancers and RCC (OR 6.085) (36).

Kidney stones in renal calyces and renal pelvis can lead to chronic irritation leading to a cascade of chronic inflammation and have been shown to be associated with increased risk of development of UTUC. A Netherlands Cohort Study showed that history of kidney stones was significantly associated with an increased risk of UTUC (HR 1.6) (37).

Association with bladder cancers

Concomitant urothelial carcinoma of the bladder with UTUC is about 8–17% (2). Three popular theories have been widely recognized for this phenomenon—monoclonality, intraluminal seeding from upper tract tumor into bladder (38) and
field cancerization change. In monoclonality theory, a single genetically abnormal cell has spread through the length of urothelium; in field change theory, there is independent development of synchronous nonrelated tumors in different parts of the urothelium (39, 40). It is a well-accepted diagnostic algorithm that patients presenting with hematuria will usually require upper tract imaging to rule out renal cancers and UTUC, and a flexible cystoscopy to rule out urothelial carcinoma of bladder. Similarly, post treatment for bladder urothelial carcinoma or UTUC, there is a regime of surveillance to rule out recurrence and metachronous urothelial carcinoma. The incidence of metachronous UTUC following diagnosis of urothelial carcinoma of bladder is 0.7–1.7% with a median of 4.1 years (41), whereas the incidence of metachronous bladder urothelial carcinoma following UTUC is higher at approximately 15–50% —usually 1–2 years after diagnosis of UTUC (42).

CONCLUSION

RCC and UTUC are important cancers of the upper urinary tract. Both disease entities still carry a high mortality rate particularly if presented at later stages. There exist some common risk factors for the development of both cancers. Over the last few decades, improvement in imaging techniques, enhanced biopsy techniques, and robotics instrumentations have led to earlier detection of such tumors and earlier treatment pathways. Further understanding of the etiology of these RCC, UTUC, and increasing public awareness can lead to prevention strategies in hope of reducing the development of these cancers.

Conflict of Interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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REFERENCES


Abstract: MicroRNAs are short noncoding RNAs that regulate post-transcriptional protein expression. Aberrant microRNA expression has been widely implicated in cancer biology with various effects depending on the affected downstream target(s). In renal cell carcinoma, microRNAs have been shown to influence metastasis by targeting oncogenes or tumor suppressors in complex regulatory networks - leading them to be coined “metastamiRs.” This chapter aims to identify the microRNAs responsible for metastasis in renal cell carcinoma, review their molecular function and oncologic outcome, and discuss their potential roles for diagnosis, prognosis, and therapy.
Keywords: lncrna in kidney cancer; metastamiRs in renal cell carcinoma; metastatic kidney cancer; microRNAs in kidney cancer; non-clear cell renal cell carcinoma

INTRODUCTION

MicroRNAs (miRNA) belong to a class of short noncoding RNAs that regulate post-transcriptional gene expression. They preferentially bind to a complementary sequence typically on the 3’ untranslated region (UTR) of their respective messenger RNA (mRNA) to directly repress translation and/or target the mRNA for degradation (1). A single miRNA can target several different mRNA; conversely, a mRNA transcript can be regulated by several different miRNAs in a complex network of interwoven biological processes (2). More recently, miRNA dysregulation with respect to cancer biogenesis and progression has become an increasing topic of interest. Numerous in silico and in vitro studies have elucidated the regulatory pro- and anti-cancer mechanisms by which these miRNAs act - termed “metastamiRs” (3, 4). MetastamiRs are ubiquitous in their involvement of metastasis, including cancer cell proliferation and colonization, angiogenesis, cell adhesion and migration, apoptosis, and the epithelial-to-mesenchymal transition (EMT). MetastamiRs can be categorized as metastasis-promoting or metastasis-suppressing miRNA. This chapter focuses on metastamiRs in renal cell carcinoma (RCC) and their role in metastatic progression, diagnosis, and prognosis.

RCC, while not among the most common tumors, comprises 2.2% of all diagnosed malignancies, with a majority of cases (75%) being the clear cell subtype (5, 6). While the 5-year survival rate for localized RCC is greater than 93%, metastatic disease is not uncommon in patients diagnosed with RCC (7). An estimated 18% of patients with RCC are thought to have metastatic disease at the time of diagnosis (synchronous metastases), and 20–50% of patients with RCC are believed to develop subsequent metastatic disease during follow-up after surgical extirpation, such as partial or radical nephrectomy (8–10). The likelihood of developing metastatic RCC (mRCC) is correlated strongly with clinical staging (which in itself is based on tumor size and invasion) as well as tumor grade and histologic findings. Currently there does not exist a reliable method for predicting metastases of RCC. In light of this clinical need, there has been ongoing research into miRNAs as potential non-invasive diagnostic biomarkers, predictors of metastases, and likely therapeutic targets. This chapter aims to consolidate current perspectives on various miRNA implicated in mRCC, primarily focusing on clear cell RCC (ccRCC).

Early studies in the identification of metastamiRs implicated in RCC utilized microarrays and qPCRs to compare miRNA profiles between primary tumors that did and did not metastasize. Heinzelmann et al. were among the first to assert specific miRNA (miR-451, miR-221, miR-30a, miR-10b, and miR-29a) as signatures that would distinguish between metastatic and nonmetastatic ccRCC (11). Subsequent studies have identified more metastamiR candidates, with many studies focusing on elucidating the molecular mechanism behind
the tumor suppressive or oncogenic effects of these respective metastamiRs as well as their prognostic potential (5, 6, 12). While each new study continues to highlight the complex and variegated nature of miRNA regulation of ccRCC, we are slowly improving our understanding of the role of miRNAs in the metastatic process.

METASTASIS-PROMOTING miRNAS IN CCRCC

MiRNA that support oncogenesis are nearly always upregulated in mRCC and promote cancer cell viability, proliferation, invasion, and migration. Some mechanisms by which these miRNAs contribute to metastasis involve downregulation of genes involved in cell adhesion (E-cadherin) to facilitate EMT and inhibition of apoptotic proteins. Targets of metastasis-promoting miRNAs include tumor suppressors such as PTEN and APC. In RCC, they have also been demonstrated to inhibit long non-coding RNA (lncRNA) with tumor suppressor activities, such as miR-7 (9). In vitro studies of metastasis-promoting miRNAs have shown that their overexpression can worsen chemoresistance, as is the case with miR-221 (13, 14). A list of metastasis-promoting miRNAs and their respective targets, functions, and associated references can be found in Table 1 (13–58).

### TABLE 1

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target/Regulator</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-7</td>
<td>MEG3</td>
<td>Inhibits IncRNA MEG3 to downregulate RASL11b, resulting in increased cell proliferation, migration, invasion.</td>
<td>13, 15</td>
</tr>
<tr>
<td>miR-21-5p</td>
<td>SOX5, TIMP3, PDCD4, CASC2, PTEN</td>
<td>Downregulates PDCD4/c-Jun pathway to promote cell transformation, proliferation, and metastasis. Reduces chemosensitivity to various drugs. Inhibits specific tumor-suppressive lncRNA. Mediates metformin growth inhibition via PTEN/ Akt/mTOR pathway.</td>
<td>16–25</td>
</tr>
<tr>
<td>miR-92a-3p</td>
<td>FBXW7</td>
<td>Promotes RCC proliferation and cell colony formation.</td>
<td>26</td>
</tr>
<tr>
<td>miR-106b/5p</td>
<td>LZTFL1, SERP1, DKK2, SETD2, Capicua</td>
<td>Facilitates cell aggressiveness and stem-cell like phenotype via Wnt/β-catenin signaling. Promotes cell proliferation and invasion via MAPK signaling. Inhibits apoptosis.</td>
<td>27–31</td>
</tr>
</tbody>
</table>

Table continued on following page
<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target/Regulator</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-122</td>
<td>Dicer, occludin,</td>
<td>Promotes cell proliferation, migration, invasion, EMT. Downregulates Dicer and its subsequent downstream miR-200 tumor suppressor families.</td>
<td>32–35</td>
</tr>
<tr>
<td></td>
<td>Sprouty2, FOXO3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-125b</td>
<td>VDR</td>
<td>Promotes cell proliferation, migration, inhibits apoptosis.</td>
<td>36–38</td>
</tr>
<tr>
<td>miR-155-5p</td>
<td>FOXO3a, E2F2, PEG3,</td>
<td>Promotes cell proliferation, migration, invasion, EMT, inhibits apoptosis. Associated with sunitinib chemoresistance and decreased time to cancer progression.</td>
<td>39–44</td>
</tr>
<tr>
<td></td>
<td>AIF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-193a-3p</td>
<td>ST3GalIV, PTEN</td>
<td>Promotes cell growth, migration via PI3k/Akt pathway.</td>
<td>45, 46</td>
</tr>
<tr>
<td>miR-221</td>
<td>VEGFR2, TIMP2</td>
<td>Promotes cell proliferation, migration, invasion. Increases sunitinib chemoresistance by downregulating VEGFR2.</td>
<td>14, 47, 48</td>
</tr>
<tr>
<td>miR-223-3p</td>
<td>FBXW7, SLC4A4,</td>
<td>Promotes cell proliferation, metastasis.</td>
<td>49–51</td>
</tr>
<tr>
<td></td>
<td>hZIP1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-592</td>
<td>SPRY2</td>
<td>Promotes cell proliferation, migration, invasion.</td>
<td>52</td>
</tr>
<tr>
<td>miR-630</td>
<td>OCT2</td>
<td>Promotes cell proliferation, migration, invasion.</td>
<td>53–55</td>
</tr>
<tr>
<td>miR-671-5p</td>
<td>APC</td>
<td>Promotes cell migration and invasion via Wnt signaling.</td>
<td>56</td>
</tr>
<tr>
<td>miR-720</td>
<td>E-cadherin, beta-catenin</td>
<td>Promotes cell proliferation, migration, invasion.</td>
<td>57</td>
</tr>
<tr>
<td>miR-1293</td>
<td>HAO2</td>
<td>Increases cell viability, promotes cell migration, invasion.</td>
<td>58</td>
</tr>
</tbody>
</table>

**METASTASIS-SUPPRESSING MIRNA IN CCRCC**

Conversely to metastasis-promoting miRNA, miRNAs that suppress metastasis in mRCC tend to be downregulated in tumor cells. They generally function to inhibit cell proliferation, migration, promote apoptosis, and are associated with increased overall survival. For example, metastasis-suppressing miRNAs target oncogenes including AKT, VEGFA, and mTOR to downregulate known cellular proliferative pathways. Some miRNA such as the miR-101, miR-126, and miR-200 families are associated with responses to specific chemotherapy regimens; in these cases, downregulation of these miRNAs has been shown to lead to increased resistance to chemotherapy (59–61). A list of tumor suppressor miRNA and their respective targets, functions, and associated references can be found in Table 2 (11, 59–183).
<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target/Regulator</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Let-7b-5p, 7c-5p</td>
<td>AKT2</td>
<td>Downregulates AKT2 and increases sensitivity of cancer cells to 5-fluorouracil.</td>
<td>11, 62, 63</td>
</tr>
<tr>
<td>miR-10a-5p</td>
<td>SKA1, BDNF</td>
<td>Downregulates BDNF to inhibit invasion and EMT of cancer cells; inhibition of SKA1 suppresses tumor invasion and migration and improves overall survival.</td>
<td>11, 16, 63–67</td>
</tr>
<tr>
<td>miR-10b-5p</td>
<td>HOXA3, CREB1</td>
<td>Suppresses HOXA3 to inhibit cell proliferation, migration, invasion (via the FAK/YAP pathway).</td>
<td>68–71</td>
</tr>
<tr>
<td>miR-26a-5p</td>
<td>OGT, LOXL2, PLOD2, PTEN, E2F7</td>
<td>Affects a variety of cell-signaling pathways via downregulation of the aforementioned genes to control cancer cell proliferation, migration, invasion.</td>
<td>63, 72–79</td>
</tr>
<tr>
<td>miR-29a</td>
<td>LOXL2</td>
<td>Downregulates LOXL2 to inhibit cancer cell migration and invasion.</td>
<td>11, 78</td>
</tr>
<tr>
<td>miR-29c-3p</td>
<td>DUXAP8, DUXAP9, COL1A1, COL1A2, LOXL2</td>
<td>Downregulates DUXAP8/P9, pseudogenes implicated in tumor growth and associated with poorer disease prognosis.</td>
<td>63, 78, 80</td>
</tr>
<tr>
<td>miR-30a/-5p/-3p</td>
<td>ZEB2, GALNT7, GRP78, ATG12, WNT2, RUNX2, IGF-1R, ADAM9, LRP6, DLL4</td>
<td>Targets a myriad of genes involved in cell proliferation, migration, known tumorigenesis pathways (i.e., HIF2a).</td>
<td>11, 81–90</td>
</tr>
<tr>
<td>miR-30c-5p</td>
<td>HSPA5, MTA1</td>
<td>Downregulates proteins involved in EMT, inhibits cell invasion, and enhances sensitivity of cells to anticancer drugs.</td>
<td>11, 91–93</td>
</tr>
<tr>
<td>miR-30e-3p</td>
<td>Snail1</td>
<td>Inhibits cell invasion and migration in ccRCC.</td>
<td>94</td>
</tr>
<tr>
<td>miR-99a-3p</td>
<td>mTOR, RRM2</td>
<td>Induces G1 cell-cycle arrest via inhibition of mTOR; effects apoptosis via inhibition of RRM2 in sunitinib-resistant RCC.</td>
<td>95, 96</td>
</tr>
<tr>
<td>miR-101-5p/-3p</td>
<td>DONSON, UHRF1, EZH2</td>
<td>Downregulates EZH2 (histone methyltransferase) and DONSON (overexpressed in sunitinib-resistance) to decrease cell proliferation and improve survival. Suppresses the UHRF1 pathway (nucleotide excision and base repair), which plays a role in sunitinib-resistant RCC.</td>
<td>59, 97–99</td>
</tr>
</tbody>
</table>

Table continued on following page
<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target/Regulator</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-106a-5p/-3p</td>
<td>IRS-2, VEGFA, PAK5</td>
<td>Downregulates VEGFA, inhibits cell proliferation (via cell cycle arrest at S-G2 phase) by silencing PAK5 (-5p). Inhibits RCC proliferation via downregulation of IRS-2 (-3p).</td>
<td>100–102</td>
</tr>
<tr>
<td>miR-126</td>
<td>ROCK1, EGFL7, SERPINE1, SLC7A5</td>
<td>Inhibits cell proliferation, migration, tumor angiogenesis (via EGFL7 and ROCK1). Deactivation leads to a pseudohypoxic state due to increased HIF1α, resulting in increased cell motility and drug resistance.</td>
<td>60, 103, 104</td>
</tr>
<tr>
<td>miR-129-3p</td>
<td>TRPM7, SOX4, FAK, MMP-2/9</td>
<td>Impairs cell migration and invasion via direct targeting of multiple oncogenes.</td>
<td>105, 106</td>
</tr>
<tr>
<td>miR-133b</td>
<td>MMP9</td>
<td>Inhibits cell proliferation, invasion, induces apoptosis, and improves chemosensitivity (via ERK pathway).</td>
<td>107, 108</td>
</tr>
<tr>
<td>miR-135a</td>
<td>c-myc</td>
<td>Inhibits cell proliferation, induces G0/G1 arrest.</td>
<td>109, 110</td>
</tr>
<tr>
<td>miR-138</td>
<td>SOX4/9, TMEM40, EZH2, vimentin, HIF1α</td>
<td>Attenuates EMT, induces senescence, suppresses cell migration, invasion, and pseudohypoxic state</td>
<td>111–116</td>
</tr>
<tr>
<td>miR-141-3p</td>
<td>EAPP, HS6ST2, LOX, TGFB2, EphA2, NEK6</td>
<td>Downregulates EMT, focal adhesion, ErbB signaling pathways.</td>
<td>117–120</td>
</tr>
<tr>
<td>miR-143</td>
<td>HK2, ABL2</td>
<td>Inhibits cell proliferation, adhesion, migration, EMT.</td>
<td>121, 122</td>
</tr>
<tr>
<td>miR-145-5p</td>
<td>HK2, ADAM17, HS6ST2, LOX</td>
<td>Synergistic tumor-suppressive effects with miR-141-3p, miR-143. Involved in VHL-independent downregulation of HIF2α.</td>
<td>119, 122–125</td>
</tr>
<tr>
<td>miR-149</td>
<td>FOXM1</td>
<td>Suppresses cell migration, invasion, promotes apoptosis.</td>
<td>126, 127</td>
</tr>
<tr>
<td>miR-182-5p</td>
<td>MALAT1, IGF1R, FLOT1</td>
<td>Impairs cell proliferation (via G1 and S phase cell cycle arrest), migration, and invasion.</td>
<td>128–130</td>
</tr>
<tr>
<td>miR-186</td>
<td>E-cadherin, CDK6, SP1</td>
<td>Inhibits cell proliferation, migration, invasion.</td>
<td>131–133</td>
</tr>
<tr>
<td>mir-199a-5p/-3p</td>
<td>GSK-3β, ROCK1, TGFB1, JunB</td>
<td>Inhibits cell proliferation, migration, invasion, promotes apoptosis.</td>
<td>134–137</td>
</tr>
</tbody>
</table>

Table continued on following page
### TABLE 2

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target/Regulator</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mir-200a/b/c</td>
<td>CAV1, FLOT1, HO-1</td>
<td>All members of miR-200 family found to be downregulated in RCC. Inhibit cell proliferation and invasion, regulate EMT, ErbB pathway (200a), local adhesion (200b/c). Sensitizes cancer cells to sorafenib and imatinib via targeting HO-1 (200c).</td>
<td>61, 138–140</td>
</tr>
<tr>
<td>miR-203</td>
<td>CAV1, HOTAIR, ZEB2, FGF2</td>
<td>Inhibits EMT, migration, invasion via inactivation of PI3K/AKT pathway.</td>
<td>141–145</td>
</tr>
<tr>
<td>miR-206</td>
<td>CDK4/6/9, CCND1, VEGFA, GAK</td>
<td>Regulates cell cycle, causes mitotic arrest at G0/G1, suppresses cell proliferation, invasion, migration.</td>
<td>146–150</td>
</tr>
<tr>
<td>miR-212-5p</td>
<td>TRX15, FOXA1, XIAP</td>
<td>Inhibits cell proliferation, invasion, migration, promotes apoptosis.</td>
<td>151–153</td>
</tr>
<tr>
<td>miR-214</td>
<td>LIVIN</td>
<td>Inhibits cell proliferation, promotes chemosensitivity of cells.</td>
<td>154, 155</td>
</tr>
<tr>
<td>miR-215</td>
<td>SIP1/ZEB2,</td>
<td>Decreases cell invasion and inhibits proliferation.</td>
<td>156</td>
</tr>
<tr>
<td>miR-218</td>
<td>CAV2, GAB2, BCL9, CIP2A</td>
<td>Inhibits cell invasion, proliferation, migration via focal adhesion, inhibits tumor angiogenesis.</td>
<td>157–160</td>
</tr>
<tr>
<td>miR-362-3p</td>
<td>NLK, SP1, G3BP1</td>
<td>Attenuates sunitinib resistance, suppresses cell proliferation, invasion via AKT/FOXO3 signaling.</td>
<td>109, 161, 162</td>
</tr>
<tr>
<td>miR-363</td>
<td>S1PR1, Twist1, CREB1, Snail1</td>
<td>Inhibits cell proliferation, migration, invasion, EMT, promotes apoptosis.</td>
<td>71, 163–165</td>
</tr>
<tr>
<td>miR-372</td>
<td>ATAD2, IGF2BP1</td>
<td>Inhibits cell invasion, migration, EMT.</td>
<td>166, 167</td>
</tr>
<tr>
<td>miR-375</td>
<td>YWHAZ, YAP1</td>
<td>Inhibits cell proliferation, migration, invasion.</td>
<td>168, 169</td>
</tr>
<tr>
<td>miR-429</td>
<td>CRKL, VEGF, AKT1, Sp1</td>
<td>Inhibits cell proliferation, migration, invasion, EMT (via SOS1/MEK/ERK/MMP pathway).</td>
<td>170–174</td>
</tr>
<tr>
<td>miR-451</td>
<td>PSMB8</td>
<td>Inhibits cell proliferation and invasion.</td>
<td>11, 175</td>
</tr>
<tr>
<td>miR-492</td>
<td>–</td>
<td>Decreases cell proliferation, suppresses EMT, promotes apoptosis.</td>
<td>176</td>
</tr>
<tr>
<td>miR-497</td>
<td>VEGFR2, PD-L1</td>
<td>Inhibits cell proliferation, migration, invasion, immunomodulation (downregulates PD-L1), improves chemosensitivity to sorafenib.</td>
<td>177–180</td>
</tr>
</tbody>
</table>

Table continued on following page
**TABLE 2** Summary of metastasis-suppressing miRNAs involved in ccRCC with impacted pathways, targets, and resulting oncologic outcome (Continued)

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target/Regulator</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-532</td>
<td>AQP9</td>
<td>Attenuates cell proliferation, invasion, migration.</td>
<td>63, 181</td>
</tr>
<tr>
<td>miR-765</td>
<td>PLP2</td>
<td>Inhibits cell proliferation, invasion; eliminates accumulation of abnormal lipids involved in cancer cell metabolism.</td>
<td>182</td>
</tr>
<tr>
<td>miR-1285</td>
<td>TGM2</td>
<td>Inhibits cell proliferation, invasion, migration.</td>
<td>183</td>
</tr>
</tbody>
</table>

**CONTRADICTORY METASTAMIRS**

In light of the complexity behind miRNA regulation of ccRCC, it is not uncommon in the literature for there to be conflicting reports of a specified metastamiR. For example, in some studies, miR-15a was found to be downregulated in ccRCC tissues and found to inhibit cell proliferation and invasion (63, 74, 184). However, several other studies have reported miR-15a was overexpressed in ccRCC tissue samples and cell lines and that it enhanced cell proliferation, invasion, and was associated with a poorer prognosis (185–187). Interestingly enough, miR-15a was shown to inhibit both eIF4E, a downstream effector of mTOR, as well as BTG2, a known antiproliferative protein that affects the PI3K/Akt/mTOR pathway downstream (180, 183). In the case of miR-22, Gong et al. showed that miR-22 was overexpressed in RCC cell lines and tissues, demonstrated to enhance cell invasion in vitro, and was correlated with a worse overall prognosis and survival (188). However, two other studies had shown miR-22 to have tumor suppressive traits and demonstrated in vitro that miR-22 overexpression could inhibit cell migration, proliferation, invasion, and reverse oncogenic effects via direct targeting of Erb-B2 and PTEN (189, 190). A more detailed description of conflicting metastamiRs in RCC can be found in Table 3.

It is difficult to surmise how and why certain miRNA were found to have conflicting expression levels in RCC. While clearly further experiments are needed to elucidate these mechanisms, these findings also reflect how multifaceted and heterogeneous RCC can be. An interesting notion could be that some dysregulated miRNAs exhibit different expression levels with regards to metastatic and non-metastatic tumors. For example, while miR-146a-5p was demonstrated to be upregulated in primary renal cell tumors, it was shown to be downregulated in metastatic renal cell tumors, and perhaps could be implicated in the transition between primary tumor and metastasis (223). Examples such as miR-146a-5p exemplify the complexity of the network of miRNA regulation in carcinogenesis and highlights how miRNA regulatory function is oftentimes both cancer- and target-specific.
<table>
<thead>
<tr>
<th>miRNA</th>
<th>Conflicting Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-15a</td>
<td>Promotes cell migration, invasion, and proliferation via inhibition of BTG2; associated with worse survival. Underexpressed in small renal masses; suppresses cell proliferation and invasion via inhibition of eIF4E and OGT.</td>
<td>185–187</td>
</tr>
<tr>
<td>miR-22</td>
<td>Promotes cell invasion, predicts worse prognosis and overall survival. Suppresses cell proliferation, invasion, promotes apoptosis (via Erb-B2, PTEN).</td>
<td>188</td>
</tr>
<tr>
<td>miR-23b/-3p</td>
<td>Has oncogenic properties via inhibition of PTEN; higher expression correlated with worse survival. Inhibits cell proliferation, migration, and invasion; increased expression correlates with improved survival.</td>
<td>191</td>
</tr>
<tr>
<td>miR-28-5p</td>
<td>Promotes chromosomal instability via Mad2 inhibition in VHL-associated RCC. Suppresses cell migration and invasion via targeting RAP1B.</td>
<td>195</td>
</tr>
<tr>
<td>miR-29b</td>
<td>Promotes cell invasion and proliferation by targeting KIF1B. Inhibits tumor cell migration and invasion via LOXL2 expression.</td>
<td>197</td>
</tr>
<tr>
<td>miR-34a</td>
<td>Overexpressed in RCC; inhibition can rescue tumor suppressive functions (p53-DAPK). Downregulated in RCC in patient serum and tissue; inhibits cell proliferation by targeting Notch1.</td>
<td>198–201</td>
</tr>
<tr>
<td>miR-139-5p</td>
<td>Upregulated in mRCC. Lower expressions correlated with worse survival, increased risk of RCC recurrence.</td>
<td>204</td>
</tr>
<tr>
<td>miR-144/-3p</td>
<td>Promotes cell migration, invasion, sunitinib resistance via downregulating ARID1A. Inhibits cell proliferation and invasion via targeting mTOR, MAP3K8.</td>
<td>208</td>
</tr>
<tr>
<td>miR-204-5p</td>
<td>Upregulated in mRCC. Suppresses tumor growth via inhibition of autophagy; downregulation promotes tumorigenesis; inhibits proliferation and invasion via RAB22A inhibition.</td>
<td>209, 210</td>
</tr>
<tr>
<td>miR-210-3p</td>
<td>Upregulated in all types of RCC, potential biomarker for mRCC. Suppression of tumorigenesis and EMT via inhibition of TWIST1; reduced expression leads to chemotherapy resistance via increased ABCC1, MDR-1 levels.</td>
<td>214–216</td>
</tr>
<tr>
<td>miR-224</td>
<td>Upregulated in tissue and exosome samples; promotes invasion in ccRCC via OCLN; associated with upregulated PD-L1 on cancer cells. Decreased expression (via ceRNA LINC01094) promotes ccRCC development.</td>
<td>46, 219–221</td>
</tr>
</tbody>
</table>
NON-CCRCC METASTAMIRES

As ccRCC makes up an overwhelming majority of all cases of RCC (80–90%), it stands as no surprise that much of the research in metastamiRs is regarding ccRCC. Few studies of metastamiRs implicated in other RCC subtypes exist in literature currently. While papillary RCC (pRCC) is the second-most common subtype of RCC, it is still rare in comparison to ccRCC and its pathogenesis is not nearly as understood as its counterpart (224). Wala et al. utilized an integrated genomic analysis to identify miR-199a-3p as a likely tumor suppressor in pRCC by preventing dysregulation of genes in the focal adhesion pathway and maintaining integrity of the extracellular matrix (224). This finding is not unique to pRCC and it is consistent with other studies (Table 1) demonstrating miR-199a's role as a tumor suppressor in ccRCC. Likewise, Samaan et al. demonstrated miR-210 as a potential prognostic marker in ccRCC, but the expression levels were more attenuated in other subtypes of RCC including papillary, chromophobe, and benign oncocytoma (216). Several studies have also looked at differing miRNA signatures in being able to uniquely identify the subtypes of RCC (225, 226). Given the paucity of miRNA studies exclusive to pRCC or chromophobe RCC, it remains difficult at this time to draw conclusions of metastamiRs in non-ccRCC tumors.

ROLE OF LNCRNA IN METASTAMIR REGULATION IN RCC

Recently, emerging studies have shown lncRNA plays an important regulatory role alongside metastamiRs in RCC. Sun et al. showed how the lncRNA XIST directly interacts with oncomiR miR-106b-5p to silence its effects on downstream genes, resulting in tumor suppression activity (227). Other studies have demonstrated how certain lncRNA, such as MALAT1, act as a “sponge” that can silence tumor suppressor miRNA, resulting in increased cell proliferation and invasion; experiments have subsequently shown how knocking down these lncRNA can rescue tumor suppressive miRNAs and their respective functions (128, 145, 228). The relationship between lncRNA and metastamiRs presents a novel development that, with further studies, may also portend future directions in prognosis and treatment.

MIRNAS AS BIOMARKERS OR THERAPY IN RCC

As shown in the above tables, several metastamiRs have been postulated to be useful as potential biomarkers of prognosis, disease progression, or metastasis. Several metastamiRs could also serve as potential predictors for responsiveness to chemotherapeutic regimens (229). One of the more promising prognosticators for disease progression is the oncomiR miR-21, as several studies have commented on its potential utility as a ccRCC-specific miRNA signature of disease progression (24, 25, 135, 193). Similarly, miR-10b and the miR-200 family are tumor suppressors that have been demonstrated to be downregulated in mRCC and are
quantitatively associated with worse prognosis (68, 135, 138, 139). While several more metastamiRs have been proposed as predictors of disease progression, conflicting reports of their function in literature ultimately makes their utility inconclusive at this time (see Table 3). Thus, no metastamiR has currently supplanted existing calculators and nomograms for RCC prognosis, namely MSKCC, UCLA, and SSIGN (229). Furthermore, there are no clinical trials or practices to date of utilizing miRNA-targeted therapies for patients with mRCC. More research is needed to strengthen existing conclusions and clarify conflicting findings of metastamiRs in the treatment, diagnosis, and prognosis of RCC.

CONCLUSION

MiRNAs play a paramount role in cancer biogenesis, and in the case of RCC, miRNA expression can either promote or suppress the metastatic process by affecting cell proliferation, migration, invasion, and viability. Several miRNAs have also been associated with increased resistance to standard chemotherapeutic regimens (i.e., sunitinib) for RCC. While many of the metastamiRs discussed concerning RCC can be categorized broadly as metastasis-promoting or metastasis-suppressing miRNA, there remain a significant number of miRNA with seemingly contradicting properties - a testament to the complexity of miRNA regulation underpinning mRCC. In addition to directly inhibiting downstream mRNA, some metastamiRs have been postulated to interact with IncRNA and act as a “sponge” to prevent their oncogenic or tumor suppressive abilities. In terms of impending development, miRNAs have potential to function as prognostic indicators for predicting patient response to treatment or patient survival, though no current nomogram or prognostic calculator for RCC survival currently incorporates miRNA, and their role in targeted therapy and diagnosis remain to be seen. Future research of miRNA in RCC will likely see further investigation into elucidating the molecular underpinnings behind the contradictory metastamiRs as well as the possibility of their role in patient-centered targeted treatment and prediction of metastatic disease.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this chapter.

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New Trends in Robotic Retroperitoneal Partial Nephrectomy

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers-robotic-nephrectomy

Abstract: Robotic technology and new surgical adjuncts are continually evolving to aid the operating surgeon and improve patient outcomes. Retroperitoneal access in renal surgery has clear benefits over traditional transperitoneal surgery with robotics augmenting the surgeon’s ability to operate in this anatomically confined space. Traditionally, the retroperitoneal approach was reserved for patients with posterior or laterally located tumors, or in patients with hostile abdomens; however, more streamlined surgical robots, improvements in port placement and increased utilization of the retroperitoneal approach has meant that the vast majority of small renal masses can be safely accessed via the retroperitoneum. This chapter aims to explore this paradigm shift further, while also exploring the use of added technologies and variations in surgical techniques.

Keywords: nephron sparing surgery for small renal mass; retroperitoneal approach to nephrectomy; robotic assisted partial nephrectomy; robotic retroperitoneal partial nephrectomy; transperitoneal approach to nephrectomy
INTRODUCTION

Representing 2–3% of cancers, kidney cancer is in the top ten most prevalent cancers in the Western Society (1), with an annual increase of 2% in incidence worldwide (2). Most of these renal cancers are found incidentally, with this rise in incidence partly due to the increased use of cross-sectional imaging (1). Renal cell carcinoma is the most common solid lesion within the kidney comprising approximately 90% of all kidney malignancies. Robotic assisted partial nephrectomy (RAPN) is increasingly becoming the gold standard for the treatment of the small renal mass. With an ageing general population and increases in Chronic Kidney Disease (CKD), nephron sparing surgery will become increasingly important in the management of renal tumors (3). RAPN has historically been performed via the transperitoneal approach (T-RAPN), however the use of a retroperitoneal approach may especially aid the treatment of posterior and laterally placed tumors without compromising oncological or patient outcomes. Here we aim to review the evolution of nephron sparing surgery and describe the recent consensus and updates surrounding the use of a retroperitoneal approach in the context of evolving robotic technology and innovative surgical techniques.

THE SMALL RENAL MASS: EVOLUTION OF TREATMENTS

Small renal mass (SRM) is defined as a solid enhancing renal tumor of less than 4 cm in maximal diameter. Small renal masses comprise >40% of new renal cancer diagnoses (4). Approximately 80% of small renal masses are malignant, while the other 20% usually represent benign masses with the rate of malignancy increasing with increasing tumor size (3). The majority of SRMs exhibit a slow growth rate and possess low metastatic potential. The most common solid lesion in the kidney is renal cell carcinoma and this makes up over 90% of all kidney cancers. Historically, the gold standard of treatment for any solid renal lesions was surgery with a radical nephrectomy, which resulted in patients losing a large portion of nephrons and resultant negative impact upon renal function.

With evolving technology and research, we now know that the cancer specific outcomes for partial and radical nephrectomy are equivalent. Partial nephrectomy has the added benefit of preservation of renal function and potentially limiting the incidence of cardiovascular disease and its health implications (5). Open partial nephrectomy is a very morbid operation with a prolonged length of hospital stay, large incision, and complications that are associated with major open abdominal surgery. With the evolution of technology with laparoscopy, and now robotic assisted, partial nephrectomy has become the gold standard surgical treatment for most SRMs. The evidence favors partial nephrectomy for T1 tumors; however, there is limited evidence on the optimal surgical treatment for patients with large renal masses. Partial nephrectomies have been reported on much larger tumors, however the feasibility is dependent on tumor factors such as size, location, proximity to renal hilum or collecting system and patient factors including tissue types, body habitus, previous interventions (5). With increasing experience we can perform increasingly complex partial nephrectomies, preserving renal function whilst still not compromising cancer outcomes (6).
With advancements in early diagnosis and development of minimally invasive procedures, there has been a paradigm shift in the management of SRMs to favor nephron sparing surgery. Some institutions have coined terms such as the “trifecta”—relating to negative surgical margins, and nephron sparing procedure with no post-op urological complications (7), and more recently the evolution towards a “pentafecta” to also include ischemia time <25 minutes and return of renal function to within 90% of pre-operative levels with no upstaging of CKD (8). All of these factors are achievable with traditional nephron sparing surgery in the form of an open partial nephrectomy; however, the morbidity of a large flank incision is a difficult to justify to patients when removing a T1a, localized low risk disease.

Progression of nephron sparing surgery

In the last two decades, the preferred techniques for partial nephrectomy have transitioned from open to minimally invasive. Because conventional laparoscopic partial nephrectomy remains a technically challenging procedure, the increased accessibility to robotics has emerged as an alternate minimally invasive option for surgeons willing to adopt a new technique. Compared to traditional laparoscopy, robotics has a comparable learning curve, but increased dexterity, improved vision, and enhanced surgical precision. With regards to partial nephrectomy, this allows operators to both dissect and reconstruct with more precision and speed. Controversy regarding the optimal surgical approach for achieving the “trifecta” in minimally invasive partial nephrectomy still exists. This chapter aims to highlight the retroperitoneal approach and the issues/ benefits this technique delivers.

Partial nephrectomy remains the standard of care for T1 tumors, with its utility still being explored in the management of T2 tumors (6). Increased utilization of laparoscopic and robotic nephron sparing surgeries allow for a minimally invasive surgery, however, require an additional level of surgical training and experience to achieve equivalent oncological outcomes when compared to open surgery. In the laparoscopic era, partial nephrectomy was a difficult surgical procedure due to the difficulties associated with laparoscopic suturing, however robotic assisted surgery has revolutionized this and is now the standard of care for small renal masses in institutions where robotic platforms are available. Recent studies have shown that oncological outcomes are comparable between open and minimally invasive partial nephrectomy (9, 10); however, variability in intra-operative ischemic time and post-operative complications were often proportional to the operating surgeon’s experience (11). Interestingly, in patients who were found to have positive surgical margins post nephron sparing surgery, salvage nephrectomy often does not reveal residual carcinoma in the final specimen (12), raising questions as to what degree of positive margin should be respected.

There has since been a shift towards enucleation of tumors, which aims to further preserve normal renal parenchyma without compromising oncological outcomes. Tumor enucleation is defined as the dissection along the peritumoral pseudocapsule without additional renal parenchyma (13, 14). Enucleation of renal tumors during partial nephrectomy can be performed without negatively impacting disease recurrence or long term survival (15). A study by Ishiyama et al in 2021 compared the outcomes for 704 patients with T1 renal tumors who underwent RAPN either using the enucleation or standard resection technique. Their data showed that enucleation contributed to early preservation of renal
function as measured by eGFR, without compromising oncological margins or patient outcomes (16). Interestingly, trifecta attainment was emphasized in patients with more complex renal tumors, whereby preservation of renal functions when compared to standard resection was most pronounced in the complex tumor group (16).

Transperitoneal vs. retroperitoneal approach

Traditionally the transperitoneal approach to laparoscopic kidney surgery is taught. The advantages of increased working space and more familiar landmarks makes this technique accessible. Due to the approach vector to the kidney, this technique still remains the standard for very anterior and medial tumors (17, 18). Retroperitoneal access is familiar to Urologists, however, development of the retroperitoneal space remains a meticulous and crucial step that requires surgeon familiarity with anatomical landmarks and an ability to maximize retroperitoneal working space without breaching the peritoneum (18, 19). Once gained, retroperitoneal access gives visualization of posterior, lateral and a significant proportion of anterior renal lesions and provides direct access to the renal hilum and reduces the risk of renal pedicle injury during its isolation (20).

Recently, a large multi-institutional Italian cohort study named the RECORD 2 Project compared perioperative outcomes of transperitoneal and retroperitoneal approaches in minimally invasive partial nephrectomy patients (21). Overall, 1669 patients were sampled, and included both laparoscopic and robotic techniques, with the majority (1256) being transperitoneal approach. In this study, the transperitoneal approach resulted in shorter operative time, on average by 35 minutes, however exhibited a modest increase in both intraoperative overall complications (3%) and intraoperative surgical complications (3.6%) when compared to the retroperitoneal approach. Postoperatively, the trifecta outcomes were comparable between the groups, with the retroperitoneal group showing an added reduction in both length of surgical drain time and length of stay (21). Similar results have been reported previously by Fan et al (22), Ren et al (23), and Xia et al (24).

Benefits of retroperitoneal approach

Retroperitoneal access permits direct access to the hilum and renal artery without the need for colon mobilization and gives optimal visualization of posteriorly and laterally located tumors (25). It also allows for direct access and isolation of the artery within minutes of commencing the dissection. Not entering the peritoneal cavity avoids bowel mobilization and provides a virgin approach in patients who have had previous abdominal surgery. This reduces the risk of iatrogenic damage, ileus, peritoneal irritation by surgical procedure (26), and intraoperative tumor spillage throughout the peritoneum. Some surgeons feel more comfortable with a transperitoneal approach due to familiarity and increased working space, however more standardized methods to gaining retroperitoneal access, and improved retroperitoneal dilatation techniques are making this approach less challenging (27). Previous multicenter comparison studies have reported that the retroperitoneal approach was often performed in higher volume centers, in units which were highly motivated to perform nephron sparing surgery (21).
Traditionally, RAPN continues to gain popularity as the minimally invasive surgical technique of choice for T1 renal tumors. Traditional RAPN has been taught and performed in a transperitoneal fashion, however the retroperitoneal approach provides an attractive alternative with practical advantages. Retroperitoneal approach is particularly advantageous for posterior tumors or peri-hilar tumors due to the anatomical relations to this approach (28) and is particularly attractive in patients who have had previous abdominal surgery (25). Furthermore, high volume units may employ the retroperitoneal approach for all but the most anterior and hilar of tumors. A recent study by Malki et al. compared the outcomes of 127 patients with a body mass index (BMI) of >30 kg/m² who underwent a RAPN (29). Of these 127 patients, only 17 patients were treated via the transperitoneal approach due to anterior-hilar renal tumors. In this cohort, 86% of tumors were accessible via the retroperitoneal approach, including 25% of renal lesions that were located anteriorly (29). Additionally, the group reported significantly shorter operative time, fewer postoperative complications, a shorter hospital stay, less blood loss and lower rates of transfusion in this obese cohort, without oncological compromise (29). With epidemiological data suggesting an ever more obese society with higher rates of renal cancer diagnoses, the ability to be competent in R-RAPN will be paramount for future Urologists.

Using robotic assisted technology appears to augment the surgeons ability to work in this confined space (28,30), where traditional laparoscopy has limited maneuverability of the straight instruments and especially difficulty with the renorrhaphy (31). Additionally due to articulating robotic arms, the relative lack of retroperitoneal space becomes less of an issue (32). The improved technology with the DaVinci robot allows increased accessibility within the retroperitoneum with the articulating arms and the decreased need for space between the arms, thus making it more conducive to operating in the small retroperitoneal space (23, 24). With regards to operative comparison, Retroperitoneal Robotic-Assisted Partial Nephrectomy (R-RAPN) has shown reduced operative time, significantly reduced blood loss (33), and overall reduced length of stay when compared to transperitoneal surgery (28). With regards to the trifecta, there has been no difference in warm ischemia time, oncological margins or 30-day post operative complications between the two approaches (33). Additionally, the retroperitoneal approach is associated with a shorter time to normal diet, less time with an Indwelling Catheter (IDC) and reduced need for opioid medications in the recovery period (26).

So whilst R-RAPN offers comparative surgical outcomes when compared to its transperitoneal counterpart, it carries additional benefits in shorter operative time and patient length of stay (17). Shorter length of stay is largely attributed to earlier return of bowel function and drain removal (21). Laviana et al. showed that T-RAPN added $2337 in cost when incorporating disposables, extra length of stay and staffing required (21). Where increased variability in warm ischemia time and post-operative complications are documented for RAPN, they strongly correlate to the operating surgeons procedural experience (25). All this provides advantage to both patient, surgeon and healthcare institution alike, potentially meaning more cases on an operating list, i.e., greater efficiency, shorter in-patient bed occupancy and fewer complications. Although there remains no consensus on the optimal approach for RAPN, tumor location and surgeon experience with the approach should dictate the decision.
ADVANCES IN ROBOTIC ASSISTED PARTIAL NEPHRECTOMY

With equivalent oncological outcomes with improved surgical morbidity, the paradigm has shifted to favor minimally invasive nephron sparing surgery for localized kidney masses. Subsequently, increasing familiarity with these procedures means that Urologic surgeons are taking on more challenging cases. Continued advancements in robotic surgery and novel adjuncts aim to further improve patient and oncological outcomes, while minimizing risk and renal impairment. Intraoperative administration of indocyanine green (ICG) is one such advancement, which has been proposed to help surgeons assess kidney and tumor perfusion intraoperatively. Selective clamping and off-clamp partial nephrectomy is another change to the traditional partial nephrectomy procedure. The use of intraoperative ultrasound is now routinely employed for endophytic tumors and is a useful adjunct for difficult to identify tumors and for minimizing loss of normal parenchyma.

Indocyanine green and ICG-fluorescence

The use of ICG and near-infrared fluorescence (NIRF) have been proposed to help surgeons assess both tumor margins and kidney perfusion intraoperatively (34, 35). ICG acts as a fluorescent tracer which can be visualized by NIRF intraoperatively. ICG-fluorescence imaging intraoperatively allows surgeons to ensure that the arterial supply to the tumor and necessary part of the kidney are isolated prior to excision of the tumor. This allows for minimizing bleeding from missed renal arteries during warm ischemia. It also allows for selective and super-selective clamping of renal arteries, minimizing the ischemia to normal renal parenchyma. Conventional pre-operative imaging with CT angiography helps describe individual patient’s anatomy; however, it fails to describe the nuance of intrarenal vascular distribution. Modern three-dimensional image rendering and the use of holographic technologies detail the anatomy more comprehensively, but none of these modalities can confirm downstream tissue ischemia intraoperatively (36, 37). By using NIRF imaging, real-time confirmatory devascularization can be achieved. If tumor devascularization is inadequate or healthy parenchymal margins are poorly delineated, ICG can be used to help identify further segmental arterial branches to aid devascularization and help the surgeon achieve selective regional control (38).

Clamping techniques in nephron-sparing surgery

Maximizing nephron sparing during partial nephrectomy involves minimizing resection of healthy parenchyma and judicious use of renal ischemic time. Variation in arterial clamping techniques including off-clamp and selective clamping were developed in an attempt to improve post-operative renal function following nephron sparing surgery. Early studies suggest that selective and super-selective clamping, enabled by NIRF-ICG more precisely defines the surgical margin; leading to earlier recovery of renal function in the short term when compared to started whole-clamping RAPN. A systematic review by Veccia compared the renal function outcomes of 369 patients who underwent RAPN either with or without NIRF-ICG. Analysis revealed a higher overall eGFR in the short-term period of
1–3 months post RAPN plus higher split differential function on renal imaging during the same period. Interestingly, there was no statistical difference in eGFR between the groups at the time of discharge, implying that the effects of renal ischemia are delayed. Whether or not this translates into a clinical benefit for the patients in the long term is yet to be elucidated (38) Selective and super-selective arterial clamping aims to reduce global renal ischemia and continues to emerge as a technique for providing selective regional ischemia (39).

Renal function at the short and intermediate terms are described as being superior in these off clamp and selective clamp groups, however multiple studies have shown no difference in renal function at the 6 month mark (40). Antonelli et al compared the oncological and functional outcomes of 2075 patients following T-RAPN and R-RAPN undertaken either on or off-clamp. This meta-analysis revealed that renal function as measured by eGFR at 6, 12, and last available follow up were not statistically different, in the context of equivalent oncological outcomes (41). Although the off-clamp group led to higher blood loss, transfusion rates were equivalent between the groups with an added benefit of less major complications (41). Although patient’s age at the time of surgery plays a significant role in post-operative renal function (42), in patients with normal baseline renal function and healthy contralateral kidney, the impact and significance of warm ischemia time appears negligible. With this in mind, patient safety and oncological outcomes remain paramount, whereby current recommendations continue to endorse the main artery clamp technique as the gold standard for complex renal tumors (40).

**Airseal**

The addition of ‘airseal’ has revolutionized laparoscopic surgery by enabling a stable pneumoperitoneum (or retroperitoneal space) with continuous smoke evacuation and gas recirculation throughout surgery. This allows surgeons to operate in a stable environment even when suctioning is required. This enables more streamlined faster surgery and a constant environment which improves vision and space, especially beneficial in the small retroperitoneal space. Prior to this, suction of blood and smoke meant loss of the space and impaired vision of the tumor margins during excision or renorrhaphy, potentially compromising patient care and leading to an increase in operative time (43, 44). For this reason, AirSeal is often preferred, especially in complex procedures where a stable working environment is essential. This effect is likely compounded while using the retroperitoneal approach compared to transperitoneal due to the already limited space.

A recent single surgeon, prospective randomized trial conducted by Feng et al aimed to compare pneumoperitoneum related complications with standard vs valve-less insufflation systems (45). Although no difference was seen with regards to post-operative analgesia use or length of hospital stay, a significant reduction in mean pain scores at 12 hours post-operatively was seen. Furthermore, there was a significant reduction in subcutaneous emphysema, particularly amongst the AirSeal group, while operating at pressures of 12 mmHg. These findings were reflected by Desroches et al., showing comparable rates of pneumothorax with significant reduction in surgical emphysema and pneumomediastinum while using AirSeal at 12 mmHg (46).
TilePro and endoscopic ultrasound

The role of intraoperative ultrasonography in partial nephrectomy has been well described. It augments the surgeons tactile feedback and helps to localize tumors, delineate tumor margins, identify multifocal disease and, with the aid of color doppler, assess vascular supply to renal tumors including location of accessory renal arteries (45, 46). These features are useful in difficult to see, endophytic tumors or those with unclear margins or poorly defined arterial supply (47). Through the use of TilePro software, the operating surgeon can view the ultrasound picture on the robotic console screen in real time while they move the probe over the kidney. This allows for improved ability to identify tumors that are endophytic or poorly defined and thus minimizing unnecessary kidney mobilization or excess excision of normal kidney margins (48). The use of the laparoscopic ultrasound probe is more difficult in the confined space of the retroperitoneum, but it remains a useful tool.

Developments in robotic technology

The intuitive DaVinci surgical system continues to dominate the robotic surgical market with an estimated current instillation base of 5764 units worldwide as of June 2020, however the competition is increasing (investigative and clinical urology). It is an exciting time for the technology of robotic surgical devices in partial nephrectomy with multiple other new robotic platforms evolving in this sphere. This is largely due to the expiration on multiple key robotic device patents in 2019, opening the door for competing companies to develop and implement robotic technology. The CMR Surgical Versius robot has initial experience with partial nephrectomy. The feasibility of this platform for partial nephrectomy continues to be explored and the technology continues to be refined to better facilitate this procedure. Medtronics Hugo robotic surgical system, the Korean system Revo-I, and Elementall Healthcare Distalmotion Dexter, amongst many others systems, are under early evaluation and clinical investigation (investigative and clinical urology) (49).

The Intuitive DaVinci robotic surgical systems have revolutionized minimally invasive nephron sparing surgery. With the most updated technology, the Xi allows for better articulation of wrists and arms, narrower profile which allows instruments to be closer together and thus allows more precise work in the limited space of the retroperitoneum. Previous issues with difficult instrument triangulation, robotic arm clashes and limited working space may be truncated with the help of this new technology and are particularly advantageous in the retroperitoneal approach.

The use of the fourth arm is a known adjunct for transperitoneal renal surgery, however its utility in the retroperitoneal approach was previously limited due to crowding of robotic arms and difficult triangulation. Use of the fourth arm in retroperitoneal RAPN was first described by Feliciano et al in 2012 (32). The team described the use of the fourth arm as a method for keeping the renal hilum on gentle but constant traction, which in doing so, allows the operating surgeon to use both left and right arms to meticulously dissect the hilum (32). It can also be used throughout later dissection to retract perinephric fat, tissues,
or peritoneum. Improved counter-traction, target exposure and ability for the surgeon to operate with both arms can all be achieved with selective deployment of the fourth arm. Hence, during critical parts of the procedure such as hilar dissection, tumor excision and renorrhaphy, the benefits of a fourth arm appear compelling. Other studies describe the benefit of using the fourth arm, particularly for optimizing tissue exposure (33), however the benefits in operative time, blood loss and oncological outcomes using this technique have yet to be described.

**CONCLUSION**

With increasing diagnosis of T1 renal tumors and the concurrent issues of ageing general population, obesity, and record rates of CKD, the need for nephron sparing surgery in the future will continue to rise. The transition from open to minimally invasive surgery has paved the way for robotic technologies to become the gold standard of care. Equivalent oncological outcomes with significantly reduced patient morbidity makes minimally invasive renal surgery the obvious choice. However, the optimal surgical approach including port placements is still being developed. This current review advocates for the use of robotic assisted partial nephrectomy, and the retroperitoneal approach for patients with posterior or laterally located tumours, or in patients with hostile abdomens from previous intraperitoneal surgery.

Ongoing advancements in robotic technology aim to negate the impact of the confined retroperitoneal space; however, further development is required. The use of adjuncts such as ICG-NIRF and Airseal systems continues to improve the procedure and anecdotally improve patient outcomes; however, their lack of routine use means their impact is yet to be elucidated on a larger scale in the literature. The use of selective renal clamping technique and tumor enucleation appear to preserve renal function in the short-term; however, the long-term benefits of this are yet to be proven in trials. Overall, the use of a retroperitoneal approach needs to be considered in the context of the patient’s disease and the surgeon’s experience with this approach. In appropriately selected patients and confident operators, retroperitoneal robotic assisted partial nephrectomy provides an oncologically equivalent surgical approach for treating small renal tumors whilst reducing patient length of stay and overall morbidity. In our experience, more than 90% of patients undergoing RAPN can benefit from these advantages by the use of the retroperitoneal approach.

**Conflict of Interest:** The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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Abstract: Testicular cancer is the most common neoplasm among young men aged 15–40 years. Overall, it is a rare malignancy and represents about 1% of the adult neoplasms and 5% of urological tumors. In 2020, the International Agency for Research of Cancer (IARC) recorded 74,458 new cases worldwide. Incidences vary greatly across the globe, ranging from 3 to 12 new cases per 100,000 males/per year in Western societies. In contrast, figures are very low in Asian and African countries. European White men seems to be more affected overall, independently of the country of residence and migration compared to other ethnicities. Incidence is increasing worldwide, and some countries, such as Slovenia and the Netherlands, registered a doubling of testicular cancer cases in the last two decades. Reasons are still unclear. Cryptorchidism (undescended testis), which increases the chances of developing testicular cancer by 3.7–7.5 times compared to the general male population, is the only risk factor unanimously recognized. Despite the increase in incidence, testicular cancer remains a relatively indolent disease with mortality figures substantially unchanged for over three decades.

Keywords: age-standardized rates of testicular cancer; cryptorchidism and testicular cancer; epidemiology of testicular cancer; incidence of testicular cancer; mortality from testicular cancer
INTRODUCTION

Testicular cancer is classified into two main histopathological groups: germ cell and non-germ cell tumors. Germ cell tumor represents the vast majority with 90–95% of the total cases (1). For this reason, we often find that testicular germ cell tumor and testicular cancer are used as synonyms. Germ cell tumors can be grouped histologically into seminomas, nonseminomas, and spermatocytic seminomas and mixed (2). The peak ages of occurrence of nonseminomas are 25–29 years, and 35–39 years for seminomas (3). In contrast to other type germ cell malignancies, spermatocytic seminomas are generally less aggressive and do not appear to share common risk factors with seminomas and nonseminomas. They also have an older peak age of occurrence (50–54 years). Spermatocytic seminomas are extremely rare, comprising only 0.6% of all germ cell tumors, while seminomas comprise 56% and nonseminomas, 43% (1). The small percentage (2%) of testicular cancers that are not germ cell tumors include stromal tumors, such as Leydig cell and Sertoli cell tumors, as well other rare or poorly defined histologic sub-types.

Over the last five decades the incidence of testicular cancer has been increasing in the developed world, while mortality rates since 1970 have declined owing to major improvements in chemotherapeutic regimes (4). The latest data from SEER (Surveillance, Epidemiology, and End Results) Program recorded an overall survival of 95%. Survivals figures range from 99% for localized disease to 73% for those affected by distant metastasis. In case of local involvement of nearby structures or lymph nodes, the survival rate is 96% (5).

RISK FACTORS

At present the prevailing hypothesis about testicular cancer is that the risk is mostly or solely determined prenatally or in utero. The only unanimously recognized risk factor is a congenital anomaly, Cryptorchidism (undescended testis), which increases the chances of developing testicular cancer by 3.7–7.5 times compared to the general male population (6). The other factors associated with increased risk are prior unilateral testicular cancer, family history of testicular cancer, and increased adult height. Of these, the highest relative risk is conferred by having a brother with testicular cancer, which increases an individual’s risk by approximately 10-fold (7).

Multiple genomic studies have been performed with the aim of identifying genetic loci likely related to testicular cancer. These studies have identified six loci on four chromosomes that seem to be correlated to testicular cancer: 9q24 (DMRT1), 5q31 (SPRY4), 12p13 (ATF7IP), 6p21 (BAKI), 5p15 (TERT, CLPTM1L) and 12q21 (KITLG). The strongest association has been identified in a single-nucleotide polymorphisms in the 12q21 locus, which confer an increase in risk of cancer of approximately three-fold per affected allele. However, even among first-degree relatives of men with testicular cancer, these risk loci are estimated to account for a very minor 11% of the risk of developing testicular cancer in brothers and 16% of the risk in sons (8).

It is still unclear the cause or combination of factors causing the progressive increase in testicular cancer among mainly white men of European origins.
Few studies have in fact determined that this ethnicity is more likely to develop testicular cancer than black or Asian men living in the same geographic region (9). Moreover, white men have experienced the greatest increases in incidence throughout the late twentieth century than any other ethnic group. In a number of countries, analyses of testicular cancer incidence trends have found them to be more consistent with a birth-cohort effect than with a calendar-period effect (3). No environmental factors or diet habits have been identified as risk factor at present.

TESTICULAR CANCER AROUND THE GLOBE

Testicular cancer is the most common type of neoplasm among young men (aged 15–40 years) in many parts of the world (10). Overall, it represents 1% of adult neoplasms and 5% of urological tumors, with incidence ranging from 3 to 11 new cases per 100,000 males/per year in Western societies (1). In 2020, the highest incidence rates were recorded in the European Area with Norway, Slovenia, and Denmark occupying the first three positions (11). In contrast, incidence rates are very low in Asian and African countries. Testicular cancer is a rare disease. In 2020, the International Agency for Research of Cancer (IARC), recorded 74,458 new cases worldwide. Age standardized rates (ASR) vary significantly across the globe with the highest figures recorded in industrialized countries such as, Europe, North America, and Australia which collectively account for 49.6% (36852 cases) of the total cases (Figure 1). Numbers are particularly high in Europe, and it is worth noting that the Top 10 countries with highest cancer incidence are all European.

Even though it is the most common cancer diagnosed in men aged 15 to 35 years, the ASR peaks in men aged between 25 and 29, and 30 to 43 years (14.5 and 13.7 per 100,000 men from 2008 to 2012, respectively), with lower rates in older and younger age groups. However, testicular cancer can be still diagnosed at any age. Its incidence varies by ethnic group, with white men having higher age-adjusted incidence rates when compared with Afro-Americans and Hispanic population, 6.7 vs 1.5 vs 4.9 per 100,000 men respectively (5). Incidence has been increasing over the past decades in the United States and other Western countries for reasons that are still unknown. Turning our attention to cancer specific mortality, the SEER Program data show a very high 5-year overall survival rates of 95.0%. for all stage cancers and 99.2% for localized to testis cancers (5).

North America

Testicular cancer is the most common cancer diagnosed in men between 15 and 35 years in the USA. In 2020, 10617 new cases of testicular cancer were recorded, which represented 14.3% of the total cases diagnosed worldwide (11). Its incidence seems to constantly increase. In 2015, ASR was reported to be 5.6 cases for 100,000 (11) while by 2019, it increased to 5.9 (5). A review from Khem et al (12) published in 2019 showed that between 1975 and 2015, there has been an annual percent change (APC) a of +1.69 for localized cancers in men aged between 25 and 39 years. Previous studies using the USA SEER database have reported that the incidence of testicular germ cell tumors increased by 51% between 1973 and 1995 (13, 14). Those figures correlate with the IARC database which recorded
Figure 1. World map of estimated number of new testicular cancer cases in 2020 (11).
220 cases in 1975, which increased to 414 in 1985, 497 in 1995, 533 in 2005 and 618 by 2016 (11) (Figure 2).

A study from Nigam et al. showed that over the period 1992–2009, 18,037 men were diagnosed with testicular cancer, of those, 10,661 (59%) were seminomas and 7376 (41%) were non-seminomas. Overall, ASR was the highest among White men (8.3 cases per 100,000 men), followed by Hispanic men (4.6 cases per 100,000 men), Asia/Pacific Islander men (API) (2.3 cases per 100,000 men), and Black men (1.5 cases per 100,000 men).

If we stratify the data in seminomas vs non-seminomas, we notice that for seminomas, the ASR for White men (5.0 cases per 100,000 men) was twice the Hispanic men (2.5 cases per 100,000 men), followed by Black men (1.0 cases per 100,000 men). In non-seminomas, the ASR for White men (3.3 cases per 100,000 men) was just slightly higher than in Hispanics (2.1 cases per 100,000 men) but almost 7 times higher than in Black men (0.5 cases per 100,000 men). Turning to mortality figures, according to SEER, by the end of 2022, 460 deaths are to be expected due to testicular cancer, which will represent the 0.1% of all cancer deaths. Trend in mortality has remained unchanged over the period 1992–2019 with a value of 0.3 death per 100,000. Over the period 2012–2018, the 5-year related survival was 95% for all cancer, and 99.2% for localized disease (5).

South America and the Caribbeans

Unfortunately, there is no comprehensive epidemiological literature on testicular cancer covering the South American countries. According to the IARC, Latin America and the Caribbeans recorded 13653 cases in 2020, which represented 18.3% of the total cases recorded worldwide. Brazil, Mexico, Argentina, and Colombia are the top four countries per incidence with 3388 (24.8%), 3337
(24.4%), 2047 (15%), and 1369 (10%) cases, respectively. Looking at the ASR, Argentina occupies the top of the list with 8.7 cases per 100,000 men followed by Uruguay and Chile with an ASR of 8.1 and 7.6 cases per 100,000 men respectively. Those figures are among the highest worldwide. From the few publications available, the figures seem to be increasing over the last few decades. As shown by Shanmugalingam et al., the annual testicular cancer ASR in Colombia varied significantly during the period studied (1983–2002) with fluctuations in incidence observed from 1 to 3 per 100,000 men. This was mirrored in the changes in annual percent change +29.1% [1989–1992] and +10.8% [1995–2002]) although these were not significant. Overall, an annual increase in incidence rates of +2.3% was observed (1983–2002) (15).

Europe

With 25,058 new cancers in 2020, which represents the 33.7% of cases registered worldwide (11), Europe accounts for the highest testicular cancer figures recorded worldwide. Western Europe, with an ASR of 8.7 per 100,000 men, leads, followed by Northern Europe (7.2), Southern and Central, and Eastern Europe (5.9 and 3.2 cases per 100,000 men respectively). Looking at specific countries, Norway, Slovenia, and Denmark occupy the top three position in Europe (and worldwide) for incidence rates with 11.8, 10.8, and 10.4 cases per 100,000 men respectively. In Table 1 are shown the 10 countries with the highest incidence in Europe. A study published in 2014, that tried to predict what would be the ASR in Europe by 2025, reported almost 23,000 new cases of testicular cancer in Europe per year, a rise of almost 24% from the estimated 18,400 cases in 2005. As can be seen at the beginning of the paragraph these

<table>
<thead>
<tr>
<th>Country</th>
<th>Cases*</th>
<th>ASR (Cases per 100,000 men)</th>
<th>Mortality (Cases per 100,000 men)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norway</td>
<td>343</td>
<td>11.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Slovenia</td>
<td>114</td>
<td>10.8</td>
<td>0.22</td>
</tr>
<tr>
<td>Denmark</td>
<td>300</td>
<td>10.4</td>
<td>0.22</td>
</tr>
<tr>
<td>Germany</td>
<td>4503</td>
<td>10.0</td>
<td>0.27</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>830</td>
<td>9.9</td>
<td>0.19</td>
</tr>
<tr>
<td>Croatia</td>
<td>192</td>
<td>9.6</td>
<td>0.58</td>
</tr>
<tr>
<td>Slovakia</td>
<td>263</td>
<td>9.5</td>
<td>0.71</td>
</tr>
<tr>
<td>Hungary</td>
<td>476</td>
<td>9.5</td>
<td>0.68</td>
</tr>
<tr>
<td>Switzerland</td>
<td>429</td>
<td>9.4</td>
<td>0.26</td>
</tr>
<tr>
<td>France</td>
<td>2752</td>
<td>9.0</td>
<td>0.34</td>
</tr>
</tbody>
</table>

GLOBOCAN 2020 (11). *Data are referred to 2020.
Epidemiology of Testicular Cancer

Figures were significantly underestimated, considering that by 2020, we surpassed 25,000 confirmed cases. Figure 3 shows trends in ASR and mortality in some of the European Countries with the highest testicular cancer rates over a period of 18 years. As can be seen, nations such as Slovenia and the Netherlands have more than doubled their cancer incidence. The IARC Website does not allow comprehensive analysis of data that are more recent than 2016 (depending on country) but as we can see from Figure 3, by 2020, these figures increased further, reaching rates of almost 12 cases per 100,000 men in Denmark and almost 11 in Slovenia. In contrast, mortality, overall, seems either stable or decreasing as noted by Park et al. in 2018 (1).

Africa

The incidence of testicular cancer in Africa is among the lowest worldwide; however, it is highly likely to be underreported. It ranges between 0.3 and 0.6 cases per 100,000 (16). According to IARC, the African continent accounted for 3302 cases which represented the 4.4% of the total in 2020. Data from GLOBOCAN 2008 show relatively high mortality rates in Sub-Saharan countries like Mali, Ethiopia, Niger, and Malawi. Mortality rate has shown a reverse trend to its incidence with higher rates in low- and middle-income countries (0.5 per 100,000) than in high-income countries. However, in the absence of a national cancer registry, it is difficult to achieve the true incidence at a national level (16).
Middle East and Asia

We have scanty data from Eastern countries. In 2020, 20651 cases of testicular cancer were reported in the whole of Asia and Middle East. The five countries with the highest figures are India, China, Japan, Turkey, and Indonesia with respectively 4638 (22.7%), 4502 (21.8%), 2458 (11.9%), 1605 (7.8%) and 1497 (7.2%) cases (11). ASR is generally low; Turkey leads with a reported ASR of 4 cases per 100,000 men followed by Japan and China with respectively 2.9 and 1.6 cases per 100,000 men recorded last in 2012 and 2010. China IARC figures seems to be higher than those reported by Pang et al. (17). Data were obtained from NCCR of China 2015 annual report. The overall incidence of testicular cancer was 0.46 cases per 100,000 men. Testicular cancer incidence were 0.53 cases per 100,000 men in urban and 0.39 cases per 100,000 men in rural areas (17). Trends seem to be following the rest of the globe with a progressive and steady increase of the cases year after year, with just few exceptions. Since 1990, countries such as Turkey, Japan, and China witnessed their cases doubling. In contrast, India has figures substantially unchanged (11) (Figure 4).

CONCLUSION

Testicular cancer is a rare malignancy representing only the 5% of all urological cancers. However, its incidence increases dramatically in specific age groups. It is the commonest cancer in young men between the ages of 15 and 40 years.
European white men seem to be the most affected and the overall incidence has been steadily increasing over the last 2–3 decades. Some countries such as Slovenia and the Netherlands witnessed their cases doubling over the same period of time. Unfortunately, the causes are still unclear. Very few risk factors have been identified at present. The only unanimously recognized risk factor is Cryptorchidism (undescended testis), which can increase the chances of developing testicular cancer by 3.7–7.5 times compared to the average population. Fortunately, despite the increase in incidence, testicular cancer remains a relatively indolent disease with mortality figures substantially unchanged for over three decades.

**Conflict of Interest:** The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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Organ Sparing Surgery in Testicular Cancer

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers-testicular-cancer

Abstract: Testicular cancer is the most common cancer amongst young adult men. The gold standard of treatment for a testicular tumor is a radical orchidectomy, where the testis and spermatic cord are removed, however up to 50% of testicular pathology is benign and these patients are being overtreated. Organ-sparing surgery can be an alternative for patients with small, indeterminate testicular lesions and normal tumor markers. It can also be considered as an option for patients with tumors in a solitary testis, or where bilateral tumors are present. Combined with frozen section examination, tumors can safely be removed, and any residual disease identified intraoperatively. Organ-sparing surgery has safe oncological outcomes, with low recurrence rates on follow up data. It also provides a beneficial effect on the fertility and the hormonal profile of these patients. As these patients have a >95% survival rate, providing a high quality of life should be prioritized.

Keywords: guidelines for organ-sparing surgery of testicular cancer; organ-sparing surgery in testicular cancer; partial orchidectomy; risk factors for testicular cancer; testicular sparing surgery
INTRODUCTION

Testicular cancer is the most common cancer amongst young adult men (1), though overall in the population, it is a relatively rare cancer. The incidence has however been increasing over the past 40 years, affecting mostly the Caucasian population in Western countries (1). The majority of testicular cancers are germ cell tumors, either seminomas or non-seminomatosus subtypes, and other benign and malignant tumors also exist. Testicular cancer is commonly associated with cryptorchidism, or maldescent of the testes, which carries a two-to-four-fold increase in the risk of developing a malignancy (2). Other risk factors include previous testicular tumor, high levels of maternal estrogen in utero, advanced maternal age, the presence of carcinoma in situ (2) or a testicular tumor in a first degree relative (3). Tumors are diagnosed with an ultrasound scan, which is easily available and quick to perform. With improving imaging modalities, ultrasound scans can detect smaller lesions within the testes including those which are impalpable, with a pick-up rate up to 7.4% in lesions of 10–15mm (4). Contrast-enhanced ultrasound has also improved the characterization of small testicular masses (5), though it is unable to definitively identify whether a lesion is benign with a standalone test. Tumor markers (alpha fetoprotein, human chorionic gonadotrophin, and lactate dehydrogenase) are used alongside imaging to aid the diagnosis of testicular tumors. They also guide disease management after surgery, and are used for cancer surveillance, as elevated tumor markers levels post-surgery are indicative of residual, metastatic disease, even if it is not evident on imaging (6).

The current gold standard of treatment for a testicular tumor is a radical orchidectomy, where the spermatic cord and testis is removed to the level of the internal inguinal ring. However, 10-50% of testicular histology is benign (3, 6), therefore radical surgery may be overtreatment for these patients, especially those with small testicular masses. Radical orchidectomy can result in subfertility, necessitate lifelong androgen replacement therapy, and have a severe psychological impact on these young patients. Organ sparing surgery (OSS) has been well practiced in urology, most notably in renal cancer patients with partial nephrectomies. The first OSS for testicular cancer was performed by Seppelt in 1982, in a patient with a testicular tumor who had previously undergone a radical orchidectomy (7). Unfortunately, the testes had to be removed six weeks later due to infection, but no residual cancer was present in the specimen (7) which proved OSS was feasible. In 1984, Richie performed OSS in a patient with bilateral testicular tumors, in a procedure he called ‘unorthodox’, and the patient was disease-free at 2.5 year follow up (8). Since then, OSS has been considered an option for testicular tumors to preserve testicular endocrine and exocrine functions (3). However, there is no clear consensus on which patients are eligible for this surgery, though recommendations do exist in many urological guidelines. Specific patient and tumor factors influence whether OSS can be considered for a particular individual. Aside from patient factors, consideration also needs to be given to where this surgery is performed as surgeons require sufficient operative experience and need appropriate availability of additional intraoperative diagnostic tools to perform a partial orchidectomy. Patients with testicular cancer have >95% five-year survival rate (6), so the long-term morbidity implications of radical surgery need to be considered; however, this needs to be in balance with providing safe oncological outcomes. The surgical management of testicular cancer should reflect the longevity of these survivors and
should consider the impact on quality of life, potential effects of the hormonal profile of the testes, and the impact on fertility. This chapter describes which patients are eligible for OSS, summarizes the current testicular cancer guidelines, explains the surgical technique of a partial orchidectomy, presents the outcomes of OSS, and finally provides key take home messages for urologists.

**PATIENT ELIGIBILITY**

There is a lack of consensus regarding which patients are eligible to be considered for OSS. Testicular tumors may be malignant or benign in nature; however, it is only possible to determine this for certain with histology once the tumor has been removed. Percutaneous testicular biopsies are not routinely performed due to the risk of cancer seeding as a result of scrotal violation (9). Palpable tumors have been shown to be malignant in up to 90% of cases (10). If there is a confirmed malignant germ cell tumor present, in almost all cases, the adjacent tissue will contain carcinoma in situ (CIS) (10), also known as tubular intraepithelial neoplasia. CIS is a precursor to testicular cancer, which has 70% risk of transforming to a testicular tumor within 7 years (11). Patients should therefore only undergo OSS for a malignant germ cell tumor in specific circumstances. These patients should be recommended low dose adjuvant radiotherapy, close surveillance, or radical orchidectomy if CIS is present (11). Radiotherapy and a radical orchidectomy will affect any potential of fatherhood, so if the patient wants to preserve fertility, this can be delayed, but close observation with ultrasound is mandatory (11). Epidermoid tumors, which are a subtype of germ cell tumors, are benign and are not surrounded by CIS (12) and therefore, OSS is a safe option for these patients.

There has been increased detection of impalpable, small testicular masses in recent years due to advancements in imaging, which are predominantly benign (80%) (10), with Leydig cell tumors being the most common (13). A multicenter randomized controlled trial from France collated all cases of Leydig cell tumors between 1986–2014 and randomized 56 patients to radical orchidectomy or OSS. They found no difference in disease free survival between groups, demonstrating that OSS is a viable option for these patients (14). As well as tumor factors, there are also patient factors that need to be taken into account. For patients to be considered for OSS, they need to have normal preoperative testosterone levels and the tumor volume needs to be less than 30% of the testis (10), so that a sufficient amount of the testicular parenchyma can be preserved for endocrine and exocrine function for the surgery to be beneficial.

The major urological committees, including the American, European, and Canadian associations, have all produced guidelines for testicular cancer and these include recommendations for patient selection for OSS. A summary of these guidelines is shown in Table 1.

**American Urology Association guidelines for OSS**

The American guidelines do not recommend OSS for a suspected malignant tumor. They specify strict criteria for which OSS may be considered, these include: a mass <2cm, indeterminate findings on ultrasound, negative tumor markers
TABLE 1  Summary of patient eligibility criteria for organ-sparing surgery for testicular cancer according to Urological Association Guidelines (European, American, and Canadian)

<table>
<thead>
<tr>
<th>Patient factors</th>
<th>Tumor factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal levels of testosterone pre-operatively</td>
<td>Size &lt;2cm</td>
</tr>
<tr>
<td>Solitary testes</td>
<td>Bilateral synchronous tumor</td>
</tr>
<tr>
<td></td>
<td>Indeterminate findings on ultrasound</td>
</tr>
<tr>
<td></td>
<td>Normal tumor markers</td>
</tr>
</tbody>
</table>

(alpha fetoprotein and human chorionic gonadotrophin), solitary testis, or bilateral synchronous tumors. If OSS is used, they state that patients should be informed of: a higher risk of local recurrence, strict monitoring with ultrasound and clinical examination, the need for radiotherapy to reduce the risk of local recurrence, the impact of radiotherapy on sperm and testosterone production and the risk of testicular atrophy, altered fertility and potential need for testosterone therapy (15). It is also recommended that when the tumor is removed, multiple biopsies of the surrounding tissue should be taken for histology with an experienced pathologist (15) to assess the presence of residual disease.

**European Association of Urology guidelines for OSS**

The European Association of Urology guidelines state that testicular sparing surgery is an acceptable treatment option in patients with suspected benign tumors, or indeterminate masses with negative tumor markers. They maintain that a radical orchidectomy is the gold standard of care in patients with a likely malignant testicular tumor. However, testicular sparing surgery can be considered in patients with synchronous bilateral tumors or tumors in a solitary testis, but it should only be offered in combination with frozen section examination. Patients should be aware that limited data exists on the safety of OSS oncologically, and local recurrence rates are up to 26% (16). Patients should also be informed of the risks of requiring radiotherapy should CIS be discovered. They also state that OSS should only be carried out in experienced centers (11).

**Canadian Urology Association guidelines for OSS**

The Canadian guidelines are very clear that a radical orchidectomy should be performed for patients with a testicular tumor. They do state that in ‘very rare cases’, where there is the possibility that the tumor is benign, an excisional biopsy of the tumor can be performed with the use of frozen section. They recommend considering OSS in ‘very select patients’, who have bilateral synchronous tumors or solitary testis, with normal testosterone levels. If OSS is performed, it should be done by an experienced surgeon and patients should be informed about the risk of requiring radiotherapy should CIS be discovered. Patients should also be counselled on the need for testosterone replacement and the effect on fertility (17).
OSS for malignant germ cell tumors

It can be summarized from the guidelines that the use of OSS in malignant cases should be reserved for patients with bilateral synchronous tumors, or a solitary testis. OSS is therefore not recommended for patients with a ≤2cm likely malignant tumor, with a normal contralateral testis. The largest series to-date of patients who underwent OSS with malignant germ cell tumors is from the German testicular cancer study group. They performed OSS in 101 patients with bilateral tumors, or in a solitary testis. The procedure was performed in eight high-volume centers and the average size of the tumor removed was 15mm (5–30mm). They found concurrent CIS in 84% of the patients, and 79% of these patients underwent radiotherapy, the others underwent surveillance. The main consideration for patients considering OSS is the presence of CIS, which will progress to invasive disease without treatment, hence detailed patient involvement and pre-operative counselling is paramount.

OSS in the pediatric patient

All recommendations mentioned thus far are for the adult population. However, pediatric patients are also at risk of developing testicular tumors, though they are rare, only accounting for 1–2% of all childhood tumors (18). The majority of these are benign, with teratoma being the most common type (19). Teratomas in pre-pubertal boys act in a benign way, unlike in the adult population and are not associated with CIS (20). The most common malignant tumor in the pre-pubertal pediatric patient is a yolk sac tumor, 90% of which will have an elevated alpha fetoprotein level which makes it a very sensitive diagnostic test for these tumors (19). The post-pubertal patients are more likely to have a malignant tumor and therefore OSS is not recommended in this cohort. As with the adult population, the gold standard for treatment is a radical orchidectomy but as the majority of tumors are benign, the implications of testicular removal in such a young patient, who is still developing, should be considered. OSS in the pediatric population is lacking long-term follow-up data, and therefore any divergence from the guidelines should be taken with caution. If OSS is performed for benign tumors, it should be done using intraoperative frozen section as this is proven to correlate well with final histology (19).

SURGICAL PROCEDURE

Testicular sparing surgery has been performed since the 1980s. It was progressively developed until 2002, when Hopps and Goldstein finalized the technique with the addition of intraoperative ultrasound and the use of a microscope to remove non-palpable tumors (21). Tissue diagnosis was confirmed intraoperatively by frozen section (21). The majority of cases reported in the literature are performed macroscopically, with the use of ultrasound for localization of the tumor (13). There is a limited description of the microsurgical technique, for the non-palpable tumors, where a microscope is used to localize the tumor (13). An added benefit of the microsurgical method allows the concurrent extraction of sperm for patients with fertility issues (13). There is no reported comparison of
the two techniques and therefore surgeons performing the procedure should choose the technique in which they are most skilled. A recommendation which was imperative in the guidelines, was that partial orchidectomy surgery should only be performed in high volume centers, by experienced surgeons. Urologists should therefore be mindful that it is an option for select patients, even if it is not offered locally and should refer their patients appropriately after multidisciplinary discussion.

Pre-operative assessment

All patients identified with a testicular tumor should have a full work-up alongside the ultrasound scan, which include tumor markers, and a CT scan of the chest, abdomen and pelvis (11). All patients should have a baseline testosterone measurement and if it is normal and the patient is appropriate, they can proceed to OSS (11). All patients should be offered sperm banking prior to OSS, as there is the potential of subfertility following surgery. Patients should be counselled thoroughly before OSS about the risk of residual disease, need for adjuvant therapy if CIS is discovered, the potential limitations of frozen section examination, and the need for close follow up and monitoring (3).

Surgical technique

Partial orchidectomy should be performed through the standard inguinal incision, in the event that a radical procedure would need to be performed and to avoid the involvement of the scrotum (3). There is no clear evidence for cord clamping in a partial orchidectomy and the majority of authors do not advocate this as there may be damage to the testis through by compromising the vascular supply (10). A series of 65 patients in Austria, by Leonhartsberger et al., showed that ‘no-clamping’ technique had disease-free results at 50-month follow up in all patients (22). The delivered testis should be placed well away from the incision to avoid contamination should the lesion be malignant (9). Some authors advocate the use of cold ischemia with the testes being placed on ice at this time (10); however there is no clear evidence that this is advantageous (22). The main consideration should be for operative time and the excision of the tumor should not take longer than 30 minutes, as there are proven morphology changes to the Sertoli cells after this duration of time (22). The tunica vaginalis should be opened to expose the tunica albuginea and the tumor located if palpable (Figure 1). The tunica albuginea should then be opened over the tumor, in a transverse incision to identify an avascular plane (23). Once exposed, the tumor should separate easily from the normal parenchyma due to the presence of a pseudocapsule covering it; if it is difficult to extract, this can indicate malignant infiltration (24). If the tumor is impalpable, an ultrasound probe or a microscope can be used to identify the lesion and a 2–5mm excision margin should be removed (10). The tumor and additional samples taken from the tumor bed, should be sent for frozen section examination to identify histology, presence of CIS and surgical margin positivity (24). If the histology shows a benign lesion, the incision site in the testis should be irrigated and then the tunica albuginea can be closed with a running suture and the testis replaced in the scrotum (25).
Role of frozen section for OSS

Frozen section examination is an integral part of OSS, and if it is unavailable intraoperatively, the surgeon should not perform a partial orchidectomy. When it was initially used, there were concerns about small, insufficient samples providing inaccurate histology results (26), but it has been shown to be a highly reliable method of analyzing testicular tumors. Of note, Elert et al. proved that in a large series of 354 patients with testicular tumors, frozen section was 100% sensitive and 100% specific, meaning all patients with malignant tumors were identified (27). A recent review article of all studies using frozen section in OSS, included 1052 patients, and they showed that <1% of malignant tumors were diagnosed as benign and required a completion orchidectomy at a later date (26). The quoted positive predictive value was 98% (26), meaning that frozen section use intraoperatively is a valuable tool in indeterminate, likely benign testicular lesions, with normal tumor markers. It can be safely used to prevent patients with small, benign tumors having unnecessary radical orchidectomies (28). The main limitation with frozen section is from interpretation of the histology and therefore an experienced urological pathologist with experience in testicular tumors is required.

Complications of OSS

Patients who undergo OSS have different risks and complications from those undergoing a radical orchidectomy. There can be concerns regarding the viability of the
testis after OSS and reported rates of postoperative testicular atrophy can occur in 3–5% of patients (25). This can consequently affect the endocrine function of the testes and account for late onset hypogonadism (25); however, the cases reported in the literature are attributed to poor selection of patients and unfavorable surgical technique (11). There is also some suggestion that handling of the vas deferens can lead to inflammation and chronic obstruction of the vas, which can contribute subfertility (22). In patients where a malignant tumor has been removed, there is a high chance of residual CIS and therefore it is recommended that patients undergo radiotherapy if they wish to preserve the testis. However, the patients should be aware that in a solitary testis, this will result in infertility and patients may choose to postpone their treatment until their family is complete (29). There is also evidence that local radiotherapy can impair Leydig cell function and up to 40% of patient will require exogenous testosterone supplementation, regardless of the radiation dose (29).

OUTCOMES OF ORGAN-SPARING SURGERY

Radical orchidectomy can be regarded as over-treatment of small testicular tumors and therefore OSS should be considered as an alternative for all appropriate patients. Testicular cancer survivors have a long life expectancy and providing a high quality of life for these patients should be prioritized. Testicular tissue preservation can maintain the endocrine function of the testes, so that patients do not require testosterone supplementation, and there are also benefits from a fertility perspective. However, there are concerns regarding the oncological outcomes of this surgery, due to the presence of residual CIS. The data is promising, as survival rates of OSS for testicular cancer are reported in the literature between 80–100%, with the vast majority being 100%, and recurrence rates between 0–15.9% (25). The current literature on this is summarized below, and the take home message for urologists is summarized in Table 2.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Take home messages for urologists</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organ sparing surgery for testicular cancer</strong></td>
<td></td>
</tr>
<tr>
<td>• OSS is not advocated for testicular tumors which are suspected to be malignant, except in patients with a solitary testis or bilateral tumors</td>
<td></td>
</tr>
<tr>
<td>• OSS is an option for patients with lesions &lt;2cm with normal tumor markers, and indeterminate findings on ultrasound who wish to preserve testicular tissue</td>
<td></td>
</tr>
<tr>
<td>• Partial orchidectomy should be carried out alongside frozen section examination by an experienced surgeon and pathologist</td>
<td></td>
</tr>
<tr>
<td>• Patients who undergo OSS should be aware of the need to undergo radiotherapy to prevent disease recurrence should residual CIS be present and of the implications of this on their fertility and hormonal status</td>
<td></td>
</tr>
<tr>
<td>• Survival rates of OSS for testicular cancer are reported in the literature between 80–100%, with the vast majority being 100%, and recurrence rates between 0–15.9%</td>
<td></td>
</tr>
<tr>
<td>• Fertility can be altered by the presence of a testicular tumor alone and therefore all patients undergoing OSS should still perform sperm banking preoperatively</td>
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</tr>
<tr>
<td>• OSS performed for benign lesions does not affect patient’s testosterone levels</td>
<td></td>
</tr>
</tbody>
</table>
Organ Sparing Surgery in Testicular Cancer

Oncological outcomes

OSS is only recommended for malignant tumors in a solitary testis, or if the patient has bilateral testicular tumors, which is a relatively small patient subset. Therefore, there are only small numbers of patients included in studies who have undergone OSS for a malignant tumor. A meta-analysis in 2020 collated 201 patients with germ cell tumors who had OSS and quoted a local recurrence rate of 7.5% and positive margin rate of 1.4% and concluded that OSS is a safe option in malignant tumors (30). The largest series of patients with germ cell tumors were the 101 patients from the German testicular cancer study group. Their results showed that after a median of 80 months follow up (4–191 months), 99% of patients were disease free. There were 67 patients who underwent radiation for CIS and two (3%) of patients developed disease recurrence and were successfully salvaged with radical orchidectomy (31). Hiedenreich et al. performed OSS on 73 patients with tumors bilaterally and in a solitary testis, with follow up over a period of 91 months. They found 98.6% of patients had no evidence of disease and one patient died of tumor progression (32). Steiner et al. had a series of 30 patients, 11 of whom had germ cell tumors removed by OSS. Only one of these patients developed disease recurrence, who chose to avoid radiotherapy, and required a radical orchidectomy at a later date (33). All patients were disease free at 46.3 months follow up (31). Bojanic et al. performed 26 OSS on 24 patients with germ cell tumors and found that seven patients developed disease recurrence after an average of 20 months (34). The majority of these patients (71%) underwent radical orchidectomy, however the remaining patients had repeat OSS due to small tumor sizes (5mm and 6mm) and there was a 100% survival rate of all patients (34). However, as mentioned, these studies only include very small patient numbers and there is a lack of evidence on long term follow up of OSS patients with malignant tumors. CIS is shown to recur after several years, but current limited follow up data is only available so longer term follow up will aid in the validation of OSS for these patients.

Fertility outcomes

Fertility should be given careful consideration for testicular cancer patients, as this cohort is the prime age to start having children. It has been noted that for a patient undergoing a radical orchidectomy, there is a significant decrease in spermatogenesis, with some patients being azoospermic after surgery (35). There is likely a genetic association between testicular cancer and impaired spermatogenesis (35) and most men with tumors have abnormal semen analysis preoperatively (13) meaning it is even more vital to preserve as much testicular tissue as possible. There is however limited data available on fertility after OSS (13) and the largest trial to date to record semen parameters after OSS for benign lesions, found no significant decline postoperatively (35). There is no direct comparison of radical surgery and OSS yet conducted and only a small proportion of the current literature reports on postoperative semen parameters. Sexual function can also be altered by body image. In a survey of 234 testicular cancer patients, 96% responded that it was important for self-confidence to have two testes present in the scrotum (37), which OSS can maintain. Preoperative sperm banking should be undertaken in all patients with testicular cancer. If OSS is performed to aid with
fertility, surgeons must be wary of the 30 minute dissection time to preserve Sertoli cell function and postpone radiotherapy if CIS is present, with close monitoring until their family is complete (35).

**Hormonal outcomes**

Changes in reproductive hormones are well documented after orchidectomy. This should be an important consideration in testicular cancer patients as they are young patients, with long term survival rates. As hypogonadism can cause a decrease in lean muscle mass, poor libido, altered glycemic control and lipid control (38), OSS should be favored where possible. Hormonal changes after OSS are reported in 22 studies to date (13), the largest being the German study group with 101 patients: 9.7% of which had low testosterone levels after 80 months (31). However, most (79%) of these men had radiotherapy and it should be noted that patients who underwent OSS for benign lesions, who do not require additional therapy, did not have any testosterone deficiency (13). Results from the meta-analysis of germ cell tumor patients showed that 2.8% patients had postoperative testicular atrophy and 7.8% patients required testosterone replacement after their OSS (30). An assimilation of case reports showed the majority of OSS patients after 93 months follow up did not require testosterone supplementation and they reported satisfactory sexual function (10). An analysis of hormonal function and bone metabolism in testicular cancer patients found that 19.5% of men who underwent a radical orchidectomy displayed low serum testosterone and 50.6% demonstrated evidence of bone damage (osteopenia and/or osteoporosis) on DEXA scans (39). This again highlights the importance of preserving testicular tissue to maintain normal hormone levels for the patient’s long-term health benefit. The cardiovascular health of testicular cancer survivors has also been shown to be negatively impacted and is thought to be due to hormonal alterations of surgery. A study of over 2500 patients over a period of 18 years in the Netherlands, showed that testicular cancer survivors had double the risk of myocardial infarction compared to the general population (40).

**CONCLUSION**

OSS is a safe option for patients with indeterminate, small testicular lesions and, should also be considered an alternative for patients with bilateral tumors, or tumors in a solitary testis. OSS provides the patient an option in the management of testicular cancer, where radical treatment would leave them infertile and dependent on exogenous testosterone replacement therapy. OSS can be a safe alternative, combined with frozen section and adjuvant treatment with radiotherapy in the presence of CIS, which shows excellent oncological control of testicular cancer.

**Conflict of Interest:** The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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Abstract: Penile cancer is an uncommon type of malignancy. In 2020, globally, 36,068 new cases were diagnosed according to the International Agency for Research on Cancer. The majority (over 95%) of penile cancers are squamous cell carcinoma. Penile cancer generally affects men from low socio-economic groups with poor hygienic standards. The highest figures have been recorded in countries such as South America, Africa, and India. Human papilloma virus (HPV16-18) infection, phimosis, and smoking have been found to be the strongest risk factors for penile cancer, and they can significantly increase its incidence. Penile cancer usually affects patients late in life, with the highest incidence recorded in the >60-year age group.

Keywords: epidemiology of penile cancer; human papilloma virus and penile cancer; incidence of penile cancer; penile cancer around the globe; risk factors for penile cancer
INTRODUCTION

Penile cancer is a rare tumor. With 36068 cases, it occupies 30th position on the list of the most common cancers recorded worldwide (1). The vast majority (over 95%) of penile cancer are squamous cell carcinoma. The most common are Grade 1–2 tumors (2). Penile cancer is typical of non-industrialized countries with low hygiene and education standards (3–6). Its incidence varies greatly from country-to-country. The highest rates are reported in South America, South Africa, and India. The incidence increases with age, being the highest in the >60 year age group (7). This chapter provides an overview of the global incidence and risk factors of penile cancer.

RISK FACTORS

Several risk factors, such as Phimosis, human papilloma virus (HPV), smoking, and low social economic status have been found to associated to penile cancer (5).

Phimosis

Phimosis is strongly correlated to penile cancer and should not be considered a mere physiologic after the age of 6 years (8). According to the available literature, Phimosis is among the strongest risk factors for penile cancer (9). In 1986, Hellberg et al. reported a relative risk of 64.6 for penile cancer among men with phimosis (10). A meta-analysis showed an odds ratio of 21.1 (95% CI 5.6–26.2) (11). In a study in Washington state by Daling and co-workers, phimosis was associated with a significant 11.4-fold increase in risk of invasive penile cancer and 3.8-fold increased risk of carcinoma in situ (12).

Circumcision and hygiene

A recent consensus meeting in Brazil by The Brazilian Urology, Clinical Oncology, Radiation Oncology, and Pathology Societies (5) found evidence that circumcision of newborns (13) reduces the risk of penile cancer, in particular, the most invasive type (13). The European Urology guidelines support this evidence stating, “Neonatal circumcision reduces the incidence of penile cancer; however, it does not seem to reduce the risk of PeIN (Penile intra-epithelial neoplasia)” (14). This though do not apply to circumcision in adulthood (5). Circumcision does seem, however, to protect against penile HPV infection in adults, especially in HIV-positive patients (15) and helps to maintain adequate genital hygiene, which is also essential in reducing the risk of malignancy (16).

Human papilloma virus

There is a rich documentation available about penile cancer and HPV (17). According to De Martel (18), of the 26000 cases of penile cancer recorded by
GLOBOCAN in 2012, 13000 cases (50%) were attributable to HPV. Of these, 9100 cases (73%) were caused by the HPV 16/18 strains. Chaux et al. found that patients with a sexual history of >10 lifetime female partners were more prone to contract HPV-positive tumors compared to those with <6 lifetime partners (4). HPV vaccination reduces the risk of penile cancer, as it results in a significant decrease in genital, precancerous, and malignant lesions (19).

Smoking

Smoking is a direct, independent, dose-related risk factor for penile cancer; multiple studies have shown an association between tobacco smoking and penile cancer. Tsen et al., in their case-control study, showed a 2.4-fold risk increase in those who have ever smoked and an even higher incidence (OR 3.1) in current smokers (20). A 230-case Brazilian study found that over 50% of penile cancer patients with squamous cell carcinoma were smokers (21). In Chaux’s study, those figures were even higher (76%) (4). A study with age-matched controls in India reported a dose response association between penile cancer and various forms of tobacco use (smoking, chewed tobacco, and even snuffed tobacco) (22).

Education and socio-economic status

It has been demonstrated in multiple studies that penile cancer generally affects patients belonging to low socio-economic groups (23). Chaux et al found that most of the patients affected by penile cancer, lived in rural or suburban regions and received only elementary education (<6 years of school). The gross family income was below the minimum wage in most cases. About 50% of patients in Brazil present at an advanced stage at diagnosis (24). Moreover, one of the main causes of delay is the lack of knowledge and embarrassment (25). Educational campaigns for penile-lesion identification might improve the early diagnosis of penile cancer and should be encouraged as penile cancer has an easily recognizable slow-growing pattern.

PENILE CANCER AROUND THE GLOBE

Penile cancer is a rare cancer. With 36,068 cases and 13,211 deaths in 2020, it occupies the 30th position for incidence and the 31st place for number of deaths among all cancer (1). Its age standardized rate per 100,000 inhabitants (ASR) is around 0.8 and vary greatly around the globe (1, 3). Penile cancer generally affects populations from developing countries such as South America, South Africa, and South-Central Asia (Table 1). In North America and Europe, it ranges from 0.51 to 0.94, while certain African and South American countries show a two or threefold increased incidence (Table 2). In this regard, the ASR of penile carcinoma in Uganda, Botswana, and Paraguay are among the highest worldwide, with a reported values of 4.6, 4.4, and 3.4, respectively, according to latest available data from the International Agency for Research on Cancer (IARC) 2020.
### TABLE 1  
Cases of penile cancer worldwide in 2020

<table>
<thead>
<tr>
<th>Continent</th>
<th>Crude numbers</th>
<th>ASR (World)</th>
<th>Cum. risk</th>
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</thead>
<tbody>
<tr>
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<td>0.21</td>
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<tr>
<td>Latin America and the Caribbean</td>
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</tr>
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<td>6762</td>
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<tr>
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<td>0.18</td>
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<tr>
<td>Oceania</td>
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<td>0.17</td>
</tr>
<tr>
<td>Africa</td>
<td>2060</td>
<td>0.53</td>
<td>0.13</td>
</tr>
<tr>
<td>Northern America</td>
<td>1741</td>
<td>0.51</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Stratified per geographical area and ordered by ASR (11).

### TABLE 2  
Cases of penile cancer recorded in South America

<table>
<thead>
<tr>
<th>Country</th>
<th>Crude numbers</th>
<th>ASR (World)</th>
<th>Cum. risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latin America and the Caribbean</td>
<td>4988</td>
<td>1.3</td>
<td>0.41</td>
</tr>
<tr>
<td>Saint Lucia</td>
<td>5</td>
<td>3.9</td>
<td>1.24</td>
</tr>
<tr>
<td>Paraguay</td>
<td>115</td>
<td>3.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Bolivia, Plurinational State of</td>
<td>159</td>
<td>2.0</td>
<td>1.61</td>
</tr>
<tr>
<td>Colombia</td>
<td>550</td>
<td>1.9</td>
<td>0.54</td>
</tr>
<tr>
<td>Honduras</td>
<td>70</td>
<td>1.9</td>
<td>0.46</td>
</tr>
<tr>
<td>Venezuela, Bolivarian Republic of</td>
<td>270</td>
<td>1.8</td>
<td>0.51</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>101</td>
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<td>Argentina</td>
<td>407</td>
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<tr>
<td>Suriname</td>
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<tr>
<td>Peru</td>
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<td>0.56</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>34</td>
<td>1.3</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Stratified per geographical area and ordered by ASR (1).
North America

North America has historically a low incidence of penile cancer with an ASR of 0.51 cases per 100,000 men (1). The incidence of penile cancer among all races combined was found to be higher in the South (0.442 per 100,000) and significantly lower in the West (0.328 per 100,000) than in the other regions of the United States. The lowest incidence was found among Asia-Pacific Islander men in the West (0.184 per 100,000), whereas the highest rate was found in White men in the North-East and West (0.381 and 0.342 per 100,000 respectively). In contrast, Black men had the highest rates in the South (0.477 per 100,000) and Midwest (0.417 per 100,000). Overall, the highest rate of penile cancer was found among Black men in the South (0.477 per 100,000) (26). In terms of time trends, Goodman et al. found significant (P = 0.0002) decrease of –1.2% in the average annual incidence rate between 1973 and 2003. The drop was more accentuated for Black than White men: the average percent change in incidence was –1.9% for Black men (P = 0.004) and –1.1% for Whites (P = 0.0009) during the 31-year time period analyzed (26). In terms of age of diagnosis, a comprehensive epidemiological study, showed that median age at diagnosis of penile squamous cell carcinoma was 68 years, although considerable variation by race and ethnicity was found. Afro-Caribbean ethnicities were diagnosed at younger ages (median 62 years) compared with whites (median 68 years) (P < .00001) and Asians/Pacific Islanders (median 68 years). Hispanics were diagnosed at substantially younger ages (median 58 years) compared with non-Hispanics (median 69 years) (P < .00001) (27).

South America

South America is one of the areas with the highest number of penile cancer cases in the World with a total of 4988 in 2021 and ASR of 1.3 case per 100,000 men (1) (Table 2). In 2021, Brazil accounted for the highest number of cases (1658 cases and ASR of 1.3) but not the highest incidence. Saint Lucia and Paraguay have ASR almost three times higher, 3.9 and 3.4, respectively, followed by Bolivia, Colombia and Venezuela. Unfortunately, large epidemiological studies are currently not available in South America. A prospective study in Paraguay showed that the average patient age was 62 years old and that the majority of them lived in rural or suburban regions with only elementary education (4). In Brazil, a similar work showed similar results with 39.2% of the patients above the age of 66 and only 21.04% under the age of 46 (24). Similarly, to the Paraguayan study, it was found that most of the cases (149 cases - 53.02%) were identified in the North and North-East regions of the country, where the human development index is the lowest.

Europe

According to the IARC, the ASR of penile cancer in Europe is 0.94 cases per 100,000 men (Table 2). It usually affects patients in their 60s or older with only a
few cases before age 50 (28). The highest incidence is recorded in Macedonia, 1.7. Moreover, there are a few countries such as Poland, United Kingdom and Germany that exceed the European average figure with an ASR of 1.3, 1.2, and 1.1 cases per 100,000 males, respectively. A recent review from Arya et al. showed that ASR in England rates have increased from 1.10 in 1979 to 1.33 cases in 100,000 men in 2009. The rise in ASR was more marked from 2000 to 2009. The crude number of cases diagnosed each year has increased from 1979 to 2009 by 70.1 % (241 cases compared to 410 cases). By comparison, in the Netherlands, the 3-year moving average incidence rate also showed an increase from 1.4 to 1.5 per 100,000 men-year during 1989–2006 (29), although these figures also included data on the incidence of carcinoma in situ. In Saxony, Germany, a similar trend has been documented with an ASR increase from 1.2 per 100,000 in 1961 to 1.8 per 100,000 in 2012. During the period 2003 to 2012, the incidence rate (ESR) increased statistically significantly from 0.9 per 100,000 in 2003 to 1.8 per 100,000 in 2012 (28). In contrast, studies from Finland and Denmark have demonstrated a decrease in incidence rates (16, 30).

Africa

The African region accounts for a relatively low number of penile cancers; 2060 cases were recorded in 2020 (1). However, it hosts few of the countries with the highest incidence in the world, such as Eswatini and Uganda, which have an ASR of respectively 7 and 4.6 cases per 100,000 men. There is a generalized lack of information about the epidemiology of this disease, as many others in this region of the world, which highly likely to be due to the lack of funding and the poor quality of the healthcare systems. True figures are likely to be higher, considering the endemicity of HPV infection and sexually transmitted diseases. As noted in other areas of the world, the incidence of penile cancer is higher in low- and middle-income countries (31) which account for about 10% of penile cancers in certain regions of Africa (32).

Middle East, Asia, and Oceania

According to the latest figures provided by the IARC, the Asian area accounts for the highest number of penile cancer cases in the world, whereas Oceania occupy the last position. However, if we look at the age standardized rates, they both sit well below the world average rate (0.8 cases per 100,000) with 0.74 and 0.64 cases per 100,000 respectively. In 2020, 20315 cases were recorded in Asia, which represents 56.3% of the total (1), whilst only 202 cases were reported in Oceania (1.8%). India is the leading country in Asia per incidence rate; with 1.6 cases per 100,000 is second only to Nepal (1.7). In 2020, 10677 cases were recorded, which represented 52.5% of the total Asian cases (20315) and 29.6% globally (36068) (1). Despite the lack of accurate information, the incidence in the area might be reducing. A comprehensive review done by Cardona et al. in 2017 showed an ASR of 1.81 cases per 100,000 men when referring to the 1980–1989 period (3). China, with 4628 cases and an ASR of 0.42 per 100,000 men, is one of the countries with the lowest rate of penile cancer, not only in Asia, but worldwide (1).
CONCLUSION

Penile cancer is a rare malignancy. With 36068 cases recorded in 2020, it occupies the 30th position of the commonest cancers and the 31st per mortality (13211 cases). Over 95% of penile cancer diagnosed are squamous cell carcinoma type, and the majority are Grade 1 and 2. It has been observed that penile cancer is more common in non- or low-industrialized countries, in particular in those where hygienic standards and income are the lowest. South America, Africa, and India are the area where the incident is the highest. The population affected is generally old, with the highest figures recorded in the over 60-year age groups. Multiple risks factors have been identified with HPV infection as strongest factor. It is believed that over 50% of the penile cancer are attributable to HPV, and of these, 73% caused by the HPV 16/18 strains. Phimosis and smoking increase the risk of penile cancer—11.4 and 2.4 times, respectively. Strong emphasis will have to be given to prevention, awareness, and HPV vaccination programs.

Conflict of Interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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REFERENCES


Prostate Cancer Diagnosis: Biopsy Approaches

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers-prostate-cancer-biopsy

Abstract: Prostate cancer is a common and increasing malignancy in men. Tissue is generally obtained using prostate biopsy for diagnosis and risk stratification. There are many prostate biopsy techniques. Historically, the transrectal approach has been the most adopted. In many centers, however, there has been a shift towards transperineal prostate biopsies, increasingly performed under local anesthetic. The transperineal approach has proven advantages, including better sampling of the anterior area of the prostate and lower infection rates. Biopsies are typically performed using a combination of a systematic and targeted approach. Targeting of lesions identified by magnetic resonance imaging can be performed cognitively, assisted by a fused imaging approach with the transrectal ultrasound, or directly within the magnetic resonance imaging scanner. There are several novel developments in the field, which include robotic techniques to guide biopsy needles based on fusion images or directly targeting lesions robotically during in-bore magnetic resonance imaging.
Keywords: image-guided prostate biopsy; magnetic resonance imaging for prostate biopsy; prostate biopsy approaches; transperineal prostate biopsy; transrectal prostate biopsy

INTRODUCTION

Prostate cancer is common. In 2020, it was the second most frequent malignancy in men worldwide. The highest incidence is found in Northern and Western Europe, the Caribbean, and Australasia; the lowest is in Asia and Northern Africa (1). Non modifiable risk factors include advancing age, ethnicity, and family history with a link to the BRCA gene mutation (1, 2). Numerous modifiable risk factors have also been identified and include smoking, levels of physical activity, metabolic syndrome, and sexual activity/ejaculatory frequency (1–5). Various dietary factors have been attributed to prostate cancer development and include well-done meat, sugar-sweetened beverages, dairy products, and processed foods (1, 2, 6–8).

Prostate cancer is often initially detected opportunistically by blood tests examining levels of prostate specific antigen (PSA) or by digital rectal examination (DRE). The latter examination relies on user experience and has been found to have a low sensitivity of around 50% in a primary care setting (9). PSA is a protease secreted by the prostate gland that has been utilized as a biomarker for prostate cancer since the late 1980s (10). It is not only useful in diagnosis but also for risk stratification and, following treatment, as a marker of recurrence (11). Widespread screening for prostate cancer using PSA, however, is a contentious issue. Evidence suggests that PSA screening can identify additional cancers; however the majority are low grade with no improvement seen in overall survival as a result (12). As such, international guidance focuses on individualized risk and shared decision making with risk-benefit discussion so that patients are informed of the potential for false positives and over-investigation/treatment before undertaking a PSA test (11, 13, 14).

Where there is suspicion of localized prostate cancer, such as abnormal DRE or raised PSA, further investigation is usually with multiparametric magnetic resonance imaging (mpMRI) of the prostate (11, 15, 16). mpMRI should be reported using the Likert or Prostate Imaging-Reporting and Data System (PI-RADS) scoring systems which standardize interpretation. Both systems score the investigation on a scale of 1 to 5 whereby 1 suggests that clinically significant disease is highly unlikely to be present and 5 suggests it is highly likely to be present (17, 18).

Biopsies are usually performed for abnormal DRE or mpMRI result suggestive of clinically significant disease, though the decision for biopsy should always be made in clinical context and in discussion with the patient. The use of prostate cancer risk calculators can assist decision making and are advocated by guidelines (11, 15). Prostate biopsies aim to confirm the diagnosis and assess the histological architecture using the Gleason grade, which is used to create a Gleason score, or more recently ISUP grade (19, 20). The Gleason score or ISUP grade is then used alongside mpMRI staging and PSA to stratify locally advanced prostate cancer risk. Risk groups vary depending on the guideline typically utilizing a 3-tier
system of low, intermediate, and high risk (11, 21). In the United Kingdom (UK) however the National Institute for Health and Care Excellence (NICE) have recently adopted the Cambridge Prognostic Criteria (CPG) which is a 5-tier system (22). There is evidence that suggests that the CPG system may allow for more accurate prognostication and therefore more specific and appropriate treatment (22, 23). Once risk-stratified, patients can then be counselled on treatment options appropriate for their prostate cancer, with active surveillance usually offered to the lower risk groups and radical treatment for those at higher risk (11, 21, 22). This underlines the importance of accurate histological information obtained from biopsy.

PROSTATE BIOPSY PRINCIPLES

Prostate biopsy was originally performed by targeting abnormalities felt on DRE with a biopsy needle (24). Whilst biopsy is still commonly undertaken by a transrectal (TR) approach, the methodology and improvements in targeting abnormalities have improved considerably. Furthermore, there has been a shift toward a transperineal approach. The practicality and merits of different approaches and techniques are discussed herein.

Prostate anatomy

Anatomically, the prostate is divided into glandular zones—comprising of peripheral, central and transitional zones—and non-glandular anterior fibromuscular zone, all of which are contained within the prostatic capsule (Figure 1) (25). Additionally, the peri-urethral zone is a thin layer of tissue around the urethra consisting of small ducts which can give rise to the median lobe in benign prostate hypertrophy (BPH) (26).

Figure 1. Zonal anatomy of the prostate. Parasagittal (left) and transverse (right) sections of the prostate, where dotted lines represent their intersection. The diagram illustrates the zonal anatomy, anterior fibromuscular zone in orange, peripheral zone in green, transitional zone in purple and central zone in blue.
Transrectal ultrasound

As a diagnostic modality, transrectal ultrasound (TRUS) has been found to have a low accuracy for detecting prostate cancer (27, 28). As a result, TRUS is primarily used to visualize and guide biopsy needles during the procedure (29).

Procedural analgesia

Prostate biopsy is recognized to be an uncomfortable procedure. Pain caused by TR biopsy is multifactorial, initially arising from the insertion of the probe, followed by piercing of the rectal mucosa and prostatic capsule (30). Insertion of the probe stimulates rectal stretch receptors, and some evidence suggests that those with a low anorectal compliance find biopsies more uncomfortable (31). Rectal mucosa is considered to be insensate proximal to the dentate line, however, apical biopsies may traverse this boundary due to the acute angle required and are considered among the most painful location to biopsy by the TR approach (30, 32). Prostatic innervation is predominately from within the capsule, pain appears to increase with the number cores taken with a cumulative effect, increasing after each consecutive biopsy (30, 33, 34). It has also been observed that younger patients report a higher rate of discomfort (31, 35, 36).

Analgesia is therefore an important component of the procedure, as whilst many men are able to tolerate the procedure without, in those who find it particularly uncomfortable it can limit the procedure and lead to refusal of re-biopsies, if required (32, 37, 38). Periprostatic nerve blockage (PNB) has been found to be an effective method of reducing pain during the procedure (39, 40). The extra injections required for a PNB do not appear to confer additional risk, including that of infection, although operators need to be mindful of local anesthetic (LA) toxicity which can occur if it is injected directly into the prostatic venous plexus (39–42). PNB is performed using a spinal needle under ultrasound guidance, 1% lidocaine is typically the agent of choice and between 5–10 ml of this is utilized, with evidence suggesting 10 ml to be an optimal dose (43). There are a multitude of approaches, the most common is the basal block whereby LA is infiltrated in the space between the seminal vesicle and prostate on either side, this area is identifiable as a hyperechoic pyramid on ultrasound termed “Mount Everest” sign (44, 45). Apical blocks have also been described alone or in combination with the basal technique, though there is conflicting evidence with some studies concluding equivocal efficacy and others suggesting apical approach may be superior (43, 46–50). Discomfort caused by probe insertion is not ameliorated by PNB and is part of the procedure that some men find the most uncomfortable (49). Additional topical anesthetic, usually in the form of intrarectal lidocaine gel, is therefore commonly used in combination with PNB and can safely reduce pain associated with probe insertion (39, 40). A less commonly used alternative to intrarectal lidocaine is topical glyceryl trinitrate (GTN) which has also been shown to be effective (51, 52).

Transperineal (TP) biopsies were initially performed under a general anesthetic (GA) but increasingly are undertaken as a LA procedure. In these cases, LA is infiltrated into the perineal skin prior to a PNB performed as described above but via the perineum (53, 54).
Equipment

An endorectal ultrasound transducer with a frequency in the range of 6–12 MHz is commonly used. Ideally, transducers should be biplane, allowing visualization of transverse and longitudinal sections simultaneously (55). When utilizing the probe for TR biopsies, two main types exist: (i) end-firing with a biopsy needle which runs parallel to the probe with a curved transducer at the tip allowing for biopsies to be taken in the sagittal or transverse plane (56, 57); and (ii) side-firing probes wherein the biopsy needle traverses the probe and a longitudinal transducer allows for biopsies to be visualized in the sagittal plane (56, 57). Initially retrospective studies suggested better prostate cancer detection with the end-firing probe because of its ability to sample apical and lateral regions of the prostate; however, subsequent randomized control trials have shown no difference between the techniques (56, 58–61).

Biopsies were originally performed using hand-driven needles but have universally shifted to the use of a spring-loaded biopsy needle gun due to gun-driven biopsies providing better tissue yields (62, 63). There are multiple biopsy guns available on the market, the majority utilize 18-gauge needles that are between 20–25 cm long. The mechanism consists of a double trocar where an inner trocar is fired into tissue, followed by an outer trocar around this to cut the tissue core; the outer trocar then retracts and the tissue can be retrieved via the tissue tray, a windowed aspect of the inner trocar (64).

Depending on the ultrasound machine, markers are typically superimposed onto the image at 5 mm intervals to allow estimate of where the gun will fire, the needle typically fires 25 mm into tissue with the windowed aspect present in the middle 15 mm. It was initially reported that tissue obtained in the first 5 mm would not be contained within the biopsy and that position should be adjusted as such; however, this has subsequently been shown not to be the case (64, 65).

Biopsy core length improves quality of tissue and sensitivity for diagnosis with suggestion that 12 mm is the minimum length required, whereas needle diameter does not appear to improve prostate cancer detection rates (66–71). Most biopsy guns are designed to obtain standard core lengths of 20 mm, some devices have been designed to take longer cores, though longer needles have an increased risk of deflection and therefore potentially lower sampling accuracy (72, 73).

Procedure

Transrectal biopsies are typically performed in the left lateral decubitus position with knees and hips flexed to approximately 90 degrees. Transperineal biopsies are most commonly performed in the lithotomy position, though the left lateral decubitus position has been described (74). A DRE is carried out prior to assess the prostate and correlate with ultrasound. Palpable abnormalities should be considered for targeting at biopsy. The probe is introduced with lubricant and analgesia administered as discussed. Prostate size, specifically volume, can be estimated using the ellipsoid volume formula which can be used to calculate the PSA density, or in patients with benign prostatic hypertrophy, can be used for treatment planning. Biopsies can then be taken in either a systematic or targeted approach. The transrectal and transperineal biopsy techniques are shown in Figure 2.
Figure 2. Parasagittal view of a prostate biopsy. Top, Transrectal approach using an end-firing probe. Bottom, Transperineal approach using the double freehand technique.
One aspect this illustrates is the difficulty of sampling the anterior prostate by the transrectal approach, an area which is more readily accessed by TP biopsy (75, 76).

**SYSTEMATIC BIOPSIES**

There are several systematic biopsy protocols. Initially, when transrectal prostate biopsy was developed, it was using a sextant pattern, with 3 cores taken from each side of the prostate, and was found to be superior to targeting lesions identified using TRUS (29). However, the sextant protocol has been found to have a high false negative rate, particularly missing apical and lateral lesions (77–79).

**Transrectal extended systematic biopsy**

Several alternative biopsy protocols have been examined with studies suggesting sensitivity can be increased by increasing the number of biopsy cores taken (29, 77–80). Further evidence, including a large systematic review, has suggested that 10–12 cores seem to be an optimal number, in what is termed an extended biopsy, and which has now become the standard of care (11, 81–83). The extended biopsy utilizes the traditional sextant biopsy with additional cores taken from the more lateral aspects of the prostate which has been shown to increase diagnostic yield (77, 84). The number of cores taken should be adjusted based on the size of the prostate with smaller prostates requiring fewer cores (84). An example of core sampling locations is shown in Figure 3.

**Transrectal saturation biopsy**

Transrectal saturation biopsy was developed in response to patients with a high clinical suspicion of prostate cancer who had undergone multiple negative biopsies;
it involves taking a much higher number of cores, usually between 20–30 (85). In the original 2001 paper, a detection rate of 34% was found amongst men who on average had undergone two previous negative sextant biopsies (85). Following its development, standard practice has now changed with patients undergoing extended biopsy protocols plus targeted biopsies in most cases. However, the role for saturation biopsy remains for those patients with negative biopsy and high clinical suspicion; this is typically in the context of persistently high PSA or abnormal mpMRI.

**Transperineal template mapping biopsy**

Transperineal biopsies initially were developed using the grid and stepper technique to perform a systematic saturation biopsy of the prostate under general anesthetic. This technique utilizes the brachytherapy needle guide developed for insertion of radioactive seeds and consists of a grid punctuated with holes to pass a needle spaced 5mm apart. If sampling the whole gland, this can result in 50–70 cores being taken. This method is highly sensitive, missing only 5% of small prostatic lesions compared with 30–40% missed at TRUS biopsy (86).

**Transperineal biopsy schemes**

To reduce the number of cores taken, various biopsy schemes utilizing the grid and stepper exist. The Ginsberg scheme was defined in 2013 to standardize this (87). The number of cores sampled is dependent on prostatic size but broadly splits the prostate into three sectors on each side, anterior, mid, and posterior, with four cores taken from each, and additional cores from a basal sector used in large prostates totaling 24 or 32 cores respectively (87). The Ginsburg scheme has been shown to yield high rates of cancer detection and is illustrated in Figure 4 (88). Whilst some studies report grid biopsies performed under local anesthetic, due to

![Figure 4](image-url) **Figure 4.** Schematic demonstrating the Ginsburg protocol for a small prostate (<30 cc) in parasagittal and axial views. Sampling locations are shown across three sectors with four cores taken from each bilaterally, totalling 24 cores. Prostatic zones are as previously labelled in Figure 1: anterior fibromuscular zone in orange, peripheral zone in green, transitional zone in purple and central zone in blue. Adapted from the description of the Ginsburg Protocol by Kuru et al. (87).
the large number of needle punctures via the perineum and a wide spread of local anesthetic that is required, this technique is generally performed under general or regional anesthesia (89, 90). To better facilitate transperineal biopsies under a local anesthetic, alternative techniques have been described.

**Transperineal freehand technique**

These include the freehand technique whereby a single puncture with an introducer needle is made on either side of the perineum and the biopsy needle passed through, thereby removing the need for multiple skin punctures and allows for local anesthetic to be localized to the limited points of puncture. Typically, local anesthetic is infiltrated in the skin with a 23- or 25-gauge needle, following which a finer spinal needle can be used to infiltrate the subcutaneous tissue, muscular diaphragm and space around the prostate apex as described earlier (91). The number of cores taken using this technique vary with some authors obtaining a smaller number of cores similar to a transrectal biopsy and others following the Ginsberg protocol (53, 91–93). Similar rates of cancer detection have been seen when comparing 10 core transperineal and transrectal biopsy and a systematic review has confirmed similar diagnosis rates between the transrectal and transperineal approach (90, 94). Tolerability of this technique appears to be good with one large series of patients reporting visual analogue pain scores (VAS) of up to 3.1/10 with the most painful aspect often reported as the infiltration of local anesthetic (53). Other series report similar results with average VAS scores between 1–5 (90, 93, 95–97). With one reporting only one of 181 patients abandoning the procedure due to discomfort (96). Advantages of the freehand technique include the reduction of urinary retention rates when compared to the grid technique. In one study, this was 10% for those undergoing grid biopsies and 1% for freehand, despite a similar number of cores being taken (31 vs 28) (91).

**TARGETED PROSTATE BIOPSIES**

Whilst systematic biopsies are useful at sampling the prostate, targeting abnormal lesions directly, previously practiced in conjunction with abnormal DRE or TRUS images, has become more relevant with the increasing use of mpMRI.

**Ultrasound targeted**

Hypoechoic lesions on TRUS can often represent prostate cancer and evidence suggests that hypoechoic lesions seen during systematic biopsy are predictors for clinically significant prostate cancer (98–101). However, whether routinely targeting these lesions increases diagnostic yield is less conclusive with conflicting results. One prospective study found no higher detection rate in hypoechoic lesions compared with isoechoic areas whereas another found that 9% of cancers were only present in the cores from hypoechoic lesions and would have been missed by systematic biopsy alone (102, 103). Further studies have reported more modest results with around 3–4% of additional cancers detected solely on cores targeting hypoechoic lesions (104, 105).
MRI targeted

For those patients who have a mpMRI suspicious for cancer, there is strong evidence that there is improved cancer detection if abnormal lesions identified on mpMRI are targeted at biopsy; and reduced diagnoses of clinically insignificant cancers if systematic biopsies are omitted (106–111). Though mpMRI targeted biopsies have been shown to be non-inferior to systematic biopsies, individual studies have shown that mpMRI targeted biopsies alone miss a small proportion, 4–16%, of clinically significant cancers that would be picked up with additional systematic biopsies (112–118). Current guidance is to offer combined systematic and targeted biopsies (11). There is no standard number of cores recommended from each target but a systematic review analyzing diagnostic yield per number of cores taken from mpMRI-targeted biopsies showed incremental gains with each additional core taken, but this benefit became minor after three cores (119). It is generally accepted that in patients who have a negative mpMRI but retain a strong clinical suspicion for prostate cancer, a systematic biopsy should be undertaken (11). This is because whilst mpMRI has a sensitivity for clinically significant prostate cancer of greater than 90%, a small proportion of lesions are not visible; furthermore, mpMRI has been shown to have a lower negative predictive value for those with high PSA (120, 121). Note that a threshold for clinically significant prostate cancer is not clearly defined with a variety of thresholds throughout the literature, though the definition most often used within studies tends to be a Gleason score ≥ 7 (22).

Cognitive targeting

mpMRI-targeted biopsies can be performed in several ways, the most straightforward is cognitive targeting (also known as cognitive fusion or visual estimation) whereby the operator uses the MRI images/report to help direct biopsies at a suspicious area. In this method, the biopsy is conducted by targeting areas on the TRUS images that would correspond to the area on the MRI and can be performed using either the transrectal or transperineal approach. Cognitive targeting using the transperineal approach can be done using a ‘double freehand’ technique whereby the introducer needle placed in the perineum is separate from the TRUS probe. This is practically more difficult as it requires the operator to manually align the TRUS probe with the needle to keep it in view. To make this technique more user friendly, a selection of devices to assist the process have been introduced. These devices incorporate a guide attached to the probe to keep the needle in line, and hence in view, to enable easier targeting.

Transrectal ultrasound-mpMRI (TRUS-MR) fusion biopsies

TRUS-MR fusion biopsies involve specialized software to overlay areas of interest seen on mpMRI onto TRUS images in real-time so that they can be readily targeted. Two main methods for registering mpMRI images onto TRUS exist; rigid registration whereby images are simply overlaid, or elastic registration which uses software to manipulate the overlaid MRI images to take into account deformation of prostatic anatomy caused by the manipulation of the rectal ultrasound probe.
Whilst some evidence suggests elastic registration is more accurate, the majority of clinical studies show no difference between the two modalities, with operator experience playing a key role in accuracy regardless of technique (122–124). Certainly, inter-operator variability and expertise is a factor, particularly for cognitive and fusion biopsy where a learning curve with higher detection rates of clinically significant prostate cancer associated with experience has been demonstrated (125–127). TRUS-MR fusion biopsies can be undertaken via the transrectal or transperineal approach though evidence suggests that the transperineal approach is better at detecting clinically significant cancers and anterior tumors with a lower rate of complications compared with transrectal (76, 128, 129).

**In-bore magnetic resonance image-guided biopsies**

In-bore MRI-guided biopsy, whereby MRI is used to guide the biopsy needle directly, avoids some of these difficulties (130). For transrectal in-bore MRI-guided biopsies, patients undergo a diagnostic prostate mpMRI and then return for a guided biopsy. This is usually performed prone using a needle guide which is adjusted and re-imaged until correctly positioned, at which point the biopsy needle is inserted, re-imaged and then biopsy taken (130). MRI compatibility is a key consideration, meaning devices need to be free of ferromagnetic/electronic materials that could interfere with image capture. Whilst the majority of systems use a transrectal approach, transperineal and transgluteal techniques have been described (131, 132).

**Evidence to support targeting methodology**

The three MRI targeting techniques described above were compared in the FUTURE trial in men with previous negative systematic biopsy and PIRADS 3 or greater lesion on mpMRI, and no difference in detection rates was found between methods, though it was underpowered (133). Some evidence suggests improved detection of clinically significant prostate cancer and reduced detection of insignificant prostate cancer utilizing in-bore MRI compared with TRUS-MR Fusion (134). However, systematic reviews have shown none of the three above methods to be superior, though one did show a trend in favor of MRI-ultrasound fusion over cognitive targeting, at present there remains no clear consensus (135–137). Of note, there appears to be no additional complications seen in utilizing the fusion or in bore approach (138, 139). From a cost and logistics perspective performing in-bore MRI targeted biopsies is clearly a more costly and resource intensive procedure, requiring an MRI scanner, expertise, and compatible equipment. Cost effective analyses have shown cost of in-bore MRI biopsy is similar to general anesthesia transperineal biopsy but more than double that of a local anesthetic transrectal biopsy (140).

**Robotic biopsies: TRUS-MR fusion-guided robotic biopsy**

Robotic biopsy methods have been developed using TRUS-MR fusion. Various designs exist, though the general principle is that lesions identified on mpMRI and fused with TRUS images are targeted with the robotic arm which defines
penetration angle and depth by positioning a needle guide with stop bar (141–143). The insertion and firing of the needle gun are then performed by the surgeon at the predefined position and depth (142, 143). Though robotic fusion biopsy has been described by both a transrectal and transperineal approach, the majority utilize the latter. One advantage of this is that the system maps out the intended biopsies and correlates an appropriate pivot point to site the trocar needle thereby minimizing the need for repeated skin puncture (142, 143). Whilst the system can accurately target MRI lesions using fusion technology it can also be used to take systematic biopsies with initial studies still showing benefit of taking both targeted and systematic biopsies by this approach (142–145). Though the majority of these early studies utilize general anesthesia, there have been reports of its initial use under local anesthesia with sedation (146). To date, there is limited evidence for fusion robotic biopsy, though one retrospective study reported higher rates of detection for clinically significant cancers and lower complications with transperineal robotic biopsy compared with transperineal cognitive biopsies (147). Robotic guidance likely represents a method to reduce learning curve and standardize biopsies (147).

**Robotic biopsies: MRI-guided robotic biopsy**

Transrectal in-bore MRI robotic biopsies are performed similarly to standard in-bore MRI biopsy with patients in a prone position within the scanner. A rectal needle guide is inserted and attached to the robotic manipulator which sits between the patients’ legs (148–151). The MRI is performed, and area of interest identified, following which the robotic transrectal needle guide is positioned using specialized software with the ability to fine tune the position of the needle path in line with the area to be biopsied (148–151). The robotic arm is MRI compatible by virtue of pneumatic stepper motors powered by compressed air from outside the MRI room (148–151). Once the needle guide is accurately positioned, the patient is removed from the bore of the machine and an MRI compatible transrectal biopsy gun is used within the guide to take a biopsy from the predetermined location (148–151). The advantage of this over the non-robotic method is the speed and ease of needle positioning which otherwise has to be performed manually with the patient removed from the scanner each time. Though there is limited evidence available on this technique at present, early reports suggest a high rate of success and cancer detection (148–151).

**PROCEDURAL COMPLICATIONS**

Despite being similar procedures, the complication profile of transrectal and transperineal biopsies varies, in particular with respect to the risk of infectious complications.

**Infection in transrectal biopsies**

For the transrectal approach, infection is a greater consideration due to the passage of the needle through rectal mucosa. Rates of post biopsy sepsis/severe infection
resulting in hospitalization have been reported in around 3% of patients, though up to 10% in one Norwegian series (152–157). Those most at risk of infectious complications include those with pre-biopsy bacteriuria, urethral catheterization, and prior urogenital infection (158). Multiple comorbidities, particularly diabetes mellitus, have been found to be associated with an increased risk of hospitalization (158). Of note, neither increased number of biopsy cores nor the use of a periprostatic nerve block appear to have any bearing on the rate of infectious complications (41).

**Antibiotic prophylaxis in transrectal biopsy**

A Cochrane review in 2011 concluded that antibiotic prophylaxis is effective at reducing infectious complications in TR biopsies (159). Subsequent evidence has confirmed that antibiotic prophylaxis is more effective if a minimum of 1 day duration is given and commenced at least 24 hours prior to biopsy (160, 161). A cause for concern is the observation of increased fluoroquinolone resistance in some centers, with a baseline prevalence estimated in a meta-analysis from 2012 of around 17% (152, 162, 163). Fluoroquinolone resistance has been found at higher rates in men who have undergone previous fluoroquinolone prophylaxis, those who have undertaken international travel, particularly to areas with increased resistance and those who have had recent hospital admission (158, 162). It has also been observed at higher rates in physicians and relatives of hospital employees (158, 162). Men with fluoroquinolone resistance have been shown to be at higher risk of infectious complications (162, 164). The significance of this appears to be an increase in infectious complications and resultant hospital admissions reported across multiple centers, with one large population study in Canada showing a rise in infection related admissions from 1% to 4% in 10 years (152, 163, 165, 166).

One approach used to combat this has been routine pre-biopsy swabs to determine if a patient has fluoroquinolone resistance, with prophylaxis tailored accordingly. Evidence has shown that this targeted prophylaxis approach reduces overall infectious complications, though rates of sepsis in some studies were found to be unchanged (161, 167, 168). Augmented prophylaxis is another approach, whereby multiple antibiotic agents are used in combination. This has been shown to reduce infectious complications compared to single agent prophylaxis with the majority of studies included within the metanalysis using fluoroquinolone as one of the agents, often in conjunction with an aminoglycoside (161). Recently, there has been concern with regards to increasing recognized adverse effects of fluoroquinolone antibiotics and their use in perioperative prophylaxis has been restricted in some regions (169, 170). As a result, there has been an increased emphasis on alternative antibiotics for prophylaxis. Fosfomycin has been found to be an effective prophylaxis with low rates of resistance and less infectious complications than fluoroquinolones (161, 171–173). Aminoglycoside, piperacillin/tazobactam, and cephalosporin prophylaxis has also found to be comparable to fluoroquinolones (159, 161, 174). Whereas co-amoxiclav has been shown to be less effective (175, 176). Single doses of pre-biopsy carbapenem antibiotics have also been used with good effect and some evidence suggests its use may not select for carbapenem resistant organism (177–180).
Non-antibiotic measures in transrectal biopsy

Non-antibiotic measures to reduce infectious complications include pre-biopsy rectal enema or rectal preparation with povidone-iodine. Pre-biopsy rectal enema appears to have no impact on rates of infectious complications/hospitalization (41, 159). Rectal preparation with povidone-iodine however has been shown to reduce both infectious complications and hospitalization (41). International guidance generally recommends using targeted or augmented prophylaxis alongside povidone-iodine rectal preparation (11, 181). EAU guidance however also strongly suggests considering the transperineal approach to reduce infectious complications (11).

Antibiotic prophylaxis in transperineal biopsy

In comparison to the transrectal approach, the risk of infectious complications in transperineal biopsy appears to be much lower with multiple studies reporting low rates of infectious complications with an incidence of sepsis ranging from 0–0.11% (53, 96, 97, 182–185). Whether antibiotic prophylaxis is required is an ongoing debate with its omission in some studies maintaining a low rate of infectious complications (1.9–3.6%) and no episodes of sepsis (90, 186, 187). A systematic review on the matter reported no significant difference in infectious complications for patients given antibiotic prophylaxis than those not with a pooled rate of infectious complications in the non-antibiotic prophylaxis group of 0.31% (185). Risk factors that have been identified for infectious complications in transperineal biopsy include diabetes mellitus and history of urinary retention (186). Interestingly, one study showed that asymptomatic patients with positive urine cultures pre-biopsy did not have an increased rate of urinary tract infection (188).

Urinary retention

Urinary retention is a risk of both transrectal and transperineal biopsies though transrectal biopsy appears to have a lower risk of retention than transperineal with one large UK population study reporting rates of readmission for retention at 1.9% vs 1% for transperineal and transrectal respectively (189). The figure of 1% for urinary retention post transrectal biopsy is supported by other literature reporting transrectal complication rates (182). There is a wide range of urinary retention rates post transperineal biopsy reported in the literature, between 0.05–10% with the lowest rates reported in local anesthetic freehand biopsies compared with higher rates in grid biopsies (87, 89, 90, 92, 181, 182, 185, 188).

Bleeding

Hematuria and hematospermia are common complications of transrectal and transperineal biopsies with rectal bleeding and perineal hematoma unique complications to transrectal and transperineal biopsies respectively (89, 184). Minor hematuria is common in both cohorts but significant bleeding requiring hospital admission is rare with similar rates reported for both approaches of around 1% (96, 183, 187, 189).
Concurrent use of antiplatelet and anticoagulant therapy

Concurrent use of aspirin during transrectal biopsies has been examined and systematic reviews have concluded that whilst it may increase or prolong minor bleeding, it is safe to continue, with an increase in risk of self-limiting rectal bleeding (190–192). The evidence on warfarin use during transrectal biopsies is limited by cohort size but several smaller studies report no increase in bleeding complications as a result of warfarinization (193–195). This suggests it may not be necessary to discontinue warfarin prior to biopsy, though a survey of urologists in 2010 found that 85% would do so routinely (196). Very little evidence exists to guide the use of novel oral anticoagulants (NOACs) such as apixaban and rivaroxaban, agents that are increasingly being utilized and as such further studies are required (197). Limited evidence exists for transperineal biopsy but in one study, in patients receiving antiplatelet or anticoagulant therapy, an increase in minor bleeding was noted but no severe bleeding events observed, though of note only a minority of patients were taking NOACs (198).

Erectile dysfunction

Erectile dysfunction is a common complication of both transrectal and transperineal biopsy. Systematic reviews report that prostate biopsy results in a decrease in erectile function at 1 month post biopsy which resolves spontaneously by 3–6 months, though the effect may persist slightly longer when biopsies are undertaken by transrectal as compared with transperineal approach (199, 200).

Needle tract seeding

Post biopsy seeding of cancer to the needle tract used during biopsy is incredibly rare. A review of the literature in 2015 identified 40 cases of this, 9 of these were taken via the transrectal approach and 31 via a transperineal approach. Seeding was generally seen in high grade disease. Of note, all of the transperineal biopsies were taken prior to 2000 generally using larger bore needles and although no correlation was observed by the authors in terms of needle devices, or diameter, it is fair to say that the approach used today is quite different (201).

Mortality

Death following prostate biopsy is rare, with rates up to 0.1% reported for both transrectal and transperineal biopsies. Additionally, studies have found that 120-day mortality rates in men undergoing prostate biopsies is no higher than in the control arm of men who did not undergo biopsy (202, 203).

CONCLUSION

Prostate biopsy is a key procedure in the diagnosis and risk stratification of prostate cancer. The transperineal approach is becoming more widely adopted...
and increasingly under local anesthetic. Further developments in the field include an increase in targeting accuracy by the addition of robotic devices, however, there is a lack of evidence demonstrating the benefit of these at present.

**Conflict of Interest:** The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter

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Prostate Cancer: Advances in Radiation Oncology, Molecular Biology, and Future Treatment Strategies

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers-prostate-cancer-radiation-oncology
Abstract: Prostate cancer remains an important health problem worldwide affecting one in every six men including members of vulnerable communities. Although successful treatments have been delivered to men affected with the disease resulting in improved patient outcome, process improvements including therapy titration and augmentation are needed to optimize tumor control and limit normal tissue injury from therapy. In this chapter, we describe current management strategies for optimal patient care with radiation therapy and opportunities for improvement of care moving forward with applied science to apply therapy in a strategic manner, potentially improving care and outcome for patients treated for this disease.

Keywords: clinical process improvement for prostate cancer; modern care for the prostate patient; patient outcome in prostate cancer; radiation therapy for prostate cancer; treatment strategies for prostate cancer

INTRODUCTION

Prostate cancer is an important issue affecting a substantial number of men. Incidence of prostate cancer remained stable despite a 4–6% annual increase of advanced disease as the proportion of prostate cancer diagnosed at advanced stage increased from 3.9% to 8.2% over the past decade (1). Clinical outcomes in patients with early disease with favorable features relative to Gleason grade and prostate-specific antigen (PSA) are outstanding with current therapy including surgery and radiation therapy. Patients with intermediate risk factors have excellent outcomes with established treatment strategies when applied in the appropriate manner. Research is focused on which patients with intermediate risk require treatment in addition to radiation therapy and if therapy is needed, what should be the type and duration of therapy. Historically, Hormone therapy using Casodex and Lupron have been used with radiation therapy. Gleason grade of 7 or 8 and PSA greater than 10 will characterize patients as unfavorable intermediate disease who require additional therapy beyond radiation therapy to optimize care. Patients with high-risk features including Gleason grade 9 and 10 disease require new strategies in addition to hormone therapy which can be directed by modern translational science. In this chapter, we review process improvements in the clinical application of radiation therapy and future opportunities for additional therapies to complement radiation therapy for patients at risk for recurrence.

CLINICAL PROCESS IMPROVEMENTS: RADIATION ONCOLOGY

Process improvements in radiation oncology have demonstrated outstanding progress in the care of prostate cancer patients. Volumetric planning has provided security in radiation therapy target definition and modern imaging tools including multi-parametric magnetic resonance imaging. New metabolic agents used for
Positron emission tomography have provided more confidence that tumor targets are well defined and treated with accuracy (2–4). Intensity modulation has permitted radiation oncologists to place sharper dose gradients across normal tissue structures, including bladder and rectum, with increased dose to tumor target, permitting higher dose to tumor targets with no additional clinical morbidity. This has served to expand our role in prostate cancer to treat early metastatic disease with success (3, 5, 6). Decreased dose and sharper dose gradients to normal tissue, aided by intensity modulation, decrease radiotoxicity to the rectum, small bowel, bone structures including the acetabulum, and bladder (Figure 1).

The advances in external beam radiation therapy treatment planning and delivery have positioned radiation therapy very well in the care of patients with prostate cancer. Image guidance secures and confirms the significant impact of intensity modulation on patient care. Because of the security of daily treatment execution, radiation oncologists have been able to adjust daily treatment dose to levels securing optimal outcome (3, 7–12). The process improvements in technology have permitted investigators to compress both daily and total treatment time without accelerated risk for normal tissue injury (3, 7–11, 13). Hypofractionation protocols decreasing the duration of treatment with increased daily dose are maturing and many investigators in the radiation oncology community consider compressed treatment programs moving towards the standard of care in patients with normal and near normal prostate anatomy and genito-urinary function (8–11, 14, 15). Brachytherapy as monotherapy remains an outstanding therapy option for patients with low and early intermediate risk disease (3, 16–19). Modern real time image guidance in the development and execution of the plan has made brachytherapy an outstanding treatment option. Brachytherapy with external therapy provides excellent outcomes in patients with less favorable intermediate-risk disease and high-risk disease when anatomically appropriate (Figure 2).

In the near future, clinical protocols will include radiotherapy with or without radiopharmacy directed to sites of metastasis at presentation. With these treatments, the outcome of patients with early metastatic disease is evolving to become equivalent to patients with local disease at presentation (2, 3, 20–22). The future of radiation therapy in the treatment of locally confined and early metastatic disease is significant and will use elements of advanced technology during radiation therapy such as intensity modulation, daily image guidance, optical tracking, stereotactic therapy, radiopharmacy, and brachytherapy (2, 3, 7–11, 22). These tools have already permitted radiation oncologists to increase dose to prostate cancer targets without an increased risk of normal tissue injury. There is increased confidence that outcomes relative to both tumor control and normal tissue injury are improved. The objective for the next generation of studies is to optimize care for patients by identifying which patients need additional therapy coupled with radiation therapy and what therapy to apply. There are a growing number of agents approved by the FDA extending hormone treatment beyond the longstanding use of Lupron agonist/antagonist management and now direct therapy to additional androgen related pathways including multiple oral medications. Modern science will identify additional strategies for patient care especially for patients considered high risk and insensitive to hormone medication. How and when to apply these strategies coupled with evaluation for the duration of therapy will be vetted in the next generation of clinical trials.
Figure 1. Process improvements in radiotherapy of prostate cancer. A, Dose gradients across bladder and rectum for a traditional radiation therapy using intensity modulation. Daily image guidance allows for adjustments in positioning each day relative to target motion. B, A cone beam computer tomography image obtained pre-therapy to validate target positioning on a daily basis. The security provided by image guidance permits titration in planning target volumes which in turn decrease dose to normal tissue further. The use of volume modulated arcs permits rapid therapy delivery over a few minutes giving confidence to both physicians and patients in limiting intrafraction motion of targets further promoting security in daily treatment execution. C, Arc geometries applied to prostate cancer care. Optical tracking provides both stability and security in daily positioning and monitors external motion during therapy. D, An example of optical tracking in a prostate cancer patient. Image courtesy of the Department of Radiation Oncology, UMass Chan Medical School and UMass Memorial Health.
Radiation therapy after prostatectomy remains an important component of patient care. Although surgery remains an important option for patient care in prostate cancer management, often surgeons are confronted with more challenges than anticipated with extracapsular spread of tumor, lymph node involvement, perineural invasion, Gleason grade, and seminal vesicle invasion; all these are indicators of risk for local regional recurrence of disease. Although debate continues as to when to intervene with radiation therapy post-operatively, many in the radiation oncology community feel treatment is more efficacious earlier in the disease process (23–26). In contrast, many in the urology community prefer to defer referral of the patient to radiation oncology until there is continuous elevation in PSA (24, 27, 28). Evidence today suggests efficacy with earlier intervention than later before PSA becomes significantly elevated. Having established this point, the radiation oncology community is challenged by defining a target to treat as

Figure 2. Permanent seed brachytherapy. This image shows the application of permanent seed brachytherapy in a high stage patient with the radiation dosimetry superimposed on the image courtesy of the Department of Radiation Oncology, UMass Chan Medical School and UMass Memorial Health.
treatment is being directed to a biomarker. Radiation oncologists have traditionally targeted the urethral anastomosis, former prostate capsule, and the undersurface of the bladder as high-risk targets with nodal volume therapy treated at the discretion of the radiation oncologist on an individual basis driven by the initial pathology. Although this demonstrated success, the choice of targets was thoughtful but simultaneously arbitrary based on the perception of tissues considered at risk (29–31). Modern imaging has helped radiation oncologists pivot from this position and re-visit target definitions by optimizing targets that would be considered high risk and targets of intermediate risk with the option of dose painting to high-risk targets (Figure 3). In this case, metabolic imaging supported the identification of a bulk tumor aggregate which could be treated as a high-risk target with adjoining tissue, and tissue previously defined as high risk defined at intermediate risk, thus limiting the risk of normal tissue injury. The high dose volumes were titrated to areas of activity defined on anatomical imaging.

**IMAGING AND MODERN CARE FOR THE PROSTATE PATIENT**

The importance of the development of anatomic and metabolic imaging for patient care, especially in radiation therapy, cannot be overstated (31–33). Prior to the development of volumetric imaging, patients were planned for radiation therapy on fluoroscopic simulators with catheters and contrast material placed into the bladder and rectum. While effective, there was no optimal definition of tumor and normal tissue targets, and mega voltage imaging could not validate target position nor volume of normal tissue in the therapy fields. The advent of volumetric imaging and replacement of fluoroscopic simulators with computed tomography permanently altered the process of simulation and workflow for both the planning team and the radiation oncologist. Fusion technology has permitted multiple datasets to be integrated with radiation therapy planning and imaging and serves to optimize target definition (31–33). Four-dimensional planning
programs secured challenges imposed by motion and serve to optimize the location of bowel position during respiration. The practice of radiation oncology has become fully integrated and synergistic with modern imaging. The radiation oncologist now must be more expert than our mentors in the application of imaging to therapy. Not only do we need to define if an abnormality is present or absent, but also define the volume of interest in its entirety, including tissues of both high and intermediate risk of disease, to create a treatment plan, and define normal tissue dose volume metrics for dose delivery. The addition of magnetic resonance imaging with computed tomography has optimized the anatomy of high-risk regions and better-defined multiple structures, including the fat plane between the anterior wall of the rectum and the prostate to improve contouring of disease, thus permitting the placement of sharper dose gradients across critical normal tissues (3, 7–11).

Metabolic imaging with Axumin and prostate-specific membrane antigen targeted therapy has helped define areas of disease that might otherwise be overlooked, especially in the post prostatectomy setting with elevation in PSA including identification of patients with oligometastasis (31–33). Radiation oncologists can identify metabolically active areas as high-risk including sites of limited metastatic disease and treat these regions to full dose while titrating dose to metabolically inactive regions (31–33). These images have altered how radiation oncologists contour nodal anatomy, and image guidance is giving confidence to the radiation oncology community to titrate target volumes. These imaging tools provide opportunity to adjust volumes to high-risk targets with dose painting and radiosurgery techniques. Optimal targeting with image guidance has the potential to improve patient outcome and decrease the immediate need for additional therapy such as hormone therapy. In the future, this effort will expand and include patients with oligometastatic disease who will be treated with definitive intent. It is anticipated we can titrate high dose volume directed to areas of metabolic and anatomic disease and place areas traditionally thought at risk and treat them to a more intermediate dose. Advanced imaging tools may provide security that we are treating the appropriate volume to the optimal dose and spare normal tissue for additional therapies to be considered at a later time point if needed (23, 24). It is becoming clear the therapy community will become more aggressive in the management of patients with advanced disease at presentation and therapies beyond traditional application of hormone therapy.

Genomic and Molecular Applications: Current Clinical Use

Researchers have been evaluating newly defined roles for genomic signatures and biomarkers in assigning risk and appropriate therapy. Although traditional risk categories defined by stage, Gleason grade, and PSA have been used effectively in the past, genomic signatures have the potential of adjusting care in low, intermediate, and high-risk populations. A patient defined as low risk with favorable PSA and Gleason score but may have an unfavorable genomic biomarker supporting treatment at presentation. Signatures may define intermediate risk patients who may benefit from augmented therapy and signatures may tailor therapy as needed for high-risk patients to align with biomarker expression. Following similar pathways identified for management of breast cancer, signature molecular profiles are being defined for prostate cancer
management. In patients with prostate cancer, traditional definition of disease is related to clinical stage, Gleason grade, and PSA coupled with anatomic and possible metabolic imaging. Prolaris, Decipher, and Oncotype genomic profiling testing are available to patients to help define molecular signaling that may suggest a different disease process than implied by traditional biomarkers and tools used to assign risk. In the future, next generation sequencing may be used to complement more traditional biomarkers defined on immunohistochemical staining including markers for neuroendocrine expression (33–35).

To date, this has largely been perceived as of benefit to patients recognizing the need for continued process improvements as each signature becomes validated moving forward (2, 3, 24). There are clinical situations where profiling has identified a treatment pathway not anticipated with traditional mechanisms. Recent publication suggests that deep learning models can be used to personalize prostate cancer decision making for patient care. Clinical and pathology data from five prostate cancer clinical trials (NRG/RTOG 9202, 9408, 9413, 9910, and 0126) was re-purposed to determine if multi modal artificial intelligence models could outperform traditional established clinical risk stratification models of the National Comprehensive Cancer Network (NCCN) and D’Amico stratification. The data involved pathology samples from 5,654 trial patients with high and sufficient quality digital histopathology image data. The results confirmed that artificial intelligence model did outperform traditional clinical risk stratification for predicting outcome, therefore improvements in personalization strategies will help identify patients who could benefit from augmented therapy and potentially titrate therapy for those with favorable features (35).

Radiation therapy has a prominent role in the treatment of prostate cancer and will continue to be a primary treatment option for populations at risk for developing the disease. As our technologies have improved, our outcomes have improved as dose to tumor and sharper dose gradients across normal tissue targets, target validation, and daily imaging has served patients well by assuring security in treatment targeting. Further improvements in magnetic resonance and metabolic imaging will further improve targeting and patient outcome. Moving forward, we need to continue to evaluate which patients benefit from additional therapy and optimize integrated therapy for patient populations at risk for recurrence. This will require careful clinical trials to identify patients at risk for recurrence and how to apply additional therapies moving forward.

COMBINATION THERAPIES

There is evidence that additional therapy coupled with radiation therapy improves clinical outcome in patients with unfavorable intermediate risk and high-risk prostate cancer (36, 37). For example, multiple forms of hormone therapy coupled with radiation therapy has demonstrated improvements in clinical outcome for intermediate and high-risk patients (3, 36, 37). However, despite the advantage in patient care and outcome, the duration of hormone therapy, the impact of hormone on normal tissue, and the quality of life remain understudied. Current data show that protracted hormone therapy has demonstrable impact on cardiac, musculo-skeletal, and neurocognitive health (2, 36). Therefore, an opportunity
exists to mitigate these issues using basic science and applied molecular strategies for future clinical programs.

In the past, multiple agents have been approved for patient care in prostate cancer, many directed towards androgen-directed pathways including the androgen receptor. Abiraterone acetate (Zytiga), apalutamide (Erleada), orgovyx (Regugolix), and enzalutamide (Xtandi) are new approaches to patient care directed towards androgen inhibition (37–42). While currently used for recurrent disease, studies are now needed to determine if these medications can function as a surrogate for traditional hormone therapy in primary management with the objective of limiting the sequelae seen with Lupron therapy. An equally important objective is to determine the duration of therapy and evaluate the risk benefit ratio of maintenance therapy or whether efficacy of management is optimized during the course of radiation management. These areas remain less well defined and are of important clinical relevance to patient care and quality of life. Radium 223, sipuleucel T immunotherapy, and more traditional chemotherapy with Docetaxel have been used in patients with advanced disease with and without hormone therapy, often with limited success due in part to previous treatments and limitations in patient normal tissue reserves (37–42). Leutium 177 ligand is a novel radiopharmacy tool, FDA approved, which delivers beta particle radiation therapy to PSMA expressing cells and the immediate microenvironment. This has the potential of augmenting radiation therapy to sites of metastatic disease (22). However, to move the field forward, additional new ideas are needed from basic science to apply to patient care moving forward, especially for patients with unfavorable features at risk for progressive disease including those with unfavorable biomarkers and castrate resistant status.

**FUTURE CONTRIBUTIONS FROM THE SCIENCE OF PROSTATE CANCER**

A primary objective to move treatment from bench to bedside is to define, as best as possible, the mechanism of hormone-radiation therapy interaction and promote the survival benefit for integrated therapy and potentially titrate the current approach of protracted therapy for at risk patients. This would have the potential of decreasing the development of castrate-resistant disease and possibly limit. A better understanding of fundamental mechanism of tumor cell kill would permit evaluation of alternate therapies promoting the integration of science-directed therapies driven by biomarkers defined as high risk.

Basic science is also yielding promising results by identifying additional targets for radiation therapy. Prostate cancer cells express different adhesion molecules than normal prostate including integrins; therefore, targeting adhesion molecules in parallel with radiation therapy could provide additive cell kill in prostate cancer patients (43–50). Simon and colleagues at the University of Massachusetts demonstrated that high doses of radiation were required to suppress integrin expression (one of the adhesion molecules) in prostate cancer cells and that traditional doses were less effective, implying resistance to traditional radiation therapy and indirectly supporting the utility of higher dose daily treatment that supports an argument for radiation doses similar to modern high
dose stereotactic therapy (50). Wang and colleagues demonstrated that Casodex decreased adhesion properties and sensitized prostate cancer cells to radiation therapy. This would suggest that the addition of casodex or surrogate would enhance tumor cell kill with radiation therapy and possibly permit lower doses of radiation therapy to be used and generate similar outcomes. From these series of experiments, cells cloned after surviving radiation therapy have demonstrated resistance to radiation therapy after re-culture. These cells exhibit multiple phenotypic and molecular properties including epithelial-mesenchymal differentiation as well as features consistent with neuroendocrine differentiation (51, 52). Each of these areas have become important opportunities for study and we have pursued these pathways to determine if additional opportunities exist to apply alternate therapy to radiation treatment to increase tumor cell kill. Our group has been able to reverse therapeutic resistance with application of strategic molecular silencing therapy directed towards selected molecular targets (51, 52). Strategies directed to targets associated with survivin and poly (ADP-ribose) polymerise-1 (PARP-1) inhibition exhibit promise in further sensitizing prostate cancer cells to radiation therapy through multiple mechanisms including DNA repair (53–57). Extracellular signal related kinases (ERK 1 and ERK 2) appear to be additional targets to sensitize prostate cancer cells to radiation therapy (51–53). A series of recent experiments in our group have demonstrated interesting results relative to radiation cell kill in cells that have demonstrated resistance to therapy. Prostate cancer cell line (DU) that survived and regrew post radiation (DI) demonstrated morphologic features consistent transformation into neuroendocrine phenotype expressing neurotensin receptor 1, chromogranin B, and neuron specific enolase, unlike the parent DU cell line. In clonogenic assay, DI cells consistently demonstrate therapeutic resistance in comparison to the parent DU cell. DI cells, ERK1/2 activity is constitutively active in the resistant DI cell, less so in the DU cell. As can be seen in Figure 4, when the resistant DI cell is pre-treated with ERK 1/2 inhibitor U0126, the cells revert to the response to radiation similar to the parent DU cell. This is an

**Figure 4.** Combination therapy with radiation and ERK inhibition in neuroendocrine prostate cancer. As seen in the Western blot (a), DI (resistant) cells in a serum free medium display constitutive phosphorylation of PKC and ERK1/2, but not AKT. In figure 4b, clonogenic assay was performed with DI cells treated with and without ERK1/2 inhibitor U0126 (1 μM) 1 hr before exposed to IR with a significant improvement in cell kill when the inhibitor is applied prior to radiation therapy. (Image courtesy of the Department of Radiation Oncology, UMass Chan Medical School and UMass Memorial Health).
exciting finding as it provides an opportunity to study possible mechanisms to therapeutic resistance and pathways to provide additional therapy to mitigate this point.

Therefore, potential targets for therapy directed towards these expression products and molecular pathways are potentially helpful for patient care moving forward. Evidence suggests an important role for non-coding micro-RNA as a regulatory component to the identification of prognostic factors associated with prostate cancer, including defining altered microRNA patterns and clusters in prostate cancer. These are compounds of a limited number of nucleotides that regulate the expression level of multiple genes. These can become important for the next generation of biomarkers including prediction of malevolent behavior and tumor subtypes and have been identified both in circulation and in urine. The micro transcripts function through base pairing with messenger RNA and dysregulation of microRNA is identified in multiple malignancies. Recent literature suggests that microRNA can function in multiple capacities either initiating cancer or promoting the disease, therefore may prove be a valuable biomarker for identifying disease and a target for therapy in select patients (58–61). This will require detailed study, however coupled with additional biomarkers, may potentially influence how therapy is applied moving forward. Persistent elevation of these biomarkers post therapy may function as a surrogate for defining the duration of therapy in prostate cancer which to date remains less well understood.

CONCLUSION

In this chapter, we reviewed recent clinically important developments in radiation therapy of prostate cancer. Radiation therapy provides pathway to the care for patients with prostate cancer and plays an increasingly important role in patients including those with risk of treatment failure. Identifying agents that can increase cancer cell mortality in conjunction with radiation therapy is an important next step for progress in therapy, including situations that will require advanced radiation therapy techniques for the treatment of patients with oligometastatic disease. We have made progress, however much more is left to be discovered.

Conflict of Interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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Improving Prostate Cancer Care through Quality Assurance Programs

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers-prostate-cancer-care
Abstract: Continuous quality assurance assessment and control in healthcare is essential to provide patients with the best possible care. Quality assurance programs have been developed to improve future healthcare by thoroughly studying patient outcomes on a physician- or institutional-level. Through the continuous and cyclical process of data registration, evaluation and adaptation, opportunities are sought to improve (individual) patient outcomes. Over the past decade, quality assurance programs have been initiated within urological clinical practice, mainly focusing on the diagnosis and surgical treatment of prostate cancer. While they all share the same philosophy to improve healthcare, existing quality assurance programs differ greatly. To date, little is known about their effects on the outcomes of prostate cancer care. In this chapter, we summarize the current knowledge regarding quality assurance programs within prostate cancer care. We provide insights into how quality assurance programs can improve and assure future diagnosis and treatment of prostate cancer.

Keywords: cyclical quality assurance for prostate cancer; improving prostate cancer care; quality assurance for prostate cancer; requirements for quality assurance programs; statistical quality assurance for prostate cancer

INTRODUCTION

Quality assurance and control has rapidly become part of the everyday vocabulary in healthcare. In the early 1900s, with his landmark publication, Ernest Amory Codman was pioneering in this field (1, 2). With the End Results System, Codman advocated tracking patient outcomes in order to improve the quality of healthcare. It was his belief that high-quality care did not derive from fancy equipment, but rather from self-assessment by healthcare professionals. Although being ostracized by his colleagues for his idea, it forms the basis of many contemporary initiatives to improve the quality of healthcare worldwide.

Following in Codman’s footsteps, physicians have sought a more scholarly approach to quality assurance in healthcare by acquiring knowledge and expertise from the industrial sector (3, 4). Prospective registries, the contemporary, more advanced equivalent to Codman’s End Results Cards, have been implemented and play an important role in present-day quality assurance programs (QAPs). QAPs are structured programs in which healthcare employees critically review the outcomes of their patients and continuously analyze and discuss these results in order to improve the outcomes.

Healthcare QAPs originated in general surgery, but over the past decade they have also been initiated in the urological practice with a particular focus on the diagnosis and surgical treatment of prostate cancer (PCa) (5). The formation and structure of these QAPs have previously been described, but little is known about the effects of these QAPs on the outcomes of PCa care (6). Therefore, this chapter reviews the available literature on QAPs in PCa care, answering the following questions: (ii) what is the theory behind QAPs; (ii) which organizational requirements are necessary; and (iii) what is the available evidence on the effect of QAPs on PCa care?
CYCLICAL QUALITY ASSURANCE

QAPs use continuous and short-cycled processes of data registration, evaluation, and adaptation to improve outcomes. This ideology did not originate from healthcare, but from the production industry. In the 1950s, Dr. William Edwards Deming developed a cyclical technique to address and solve problems in production lines and thereby improve the quality of industrial/organizational processes continuously, herewith building on the work of Dr Walter Andrew Shewhart (the Plan, Do, Check, Act (PDCA)-cycle; Figure 1) (7). Deming’s philosophy revolutionized the industrial output in post-war Japan, where this philosophy is known as “kaizen”—the continuous search for opportunities for all processes to get better (3). Although Deming’s PDCA-cycle was intended for the industrial sector, it could also be applied in healthcare systems (8). The PDCA-cycle is comprised of four steps: (Plan) identifying clinical steps that require improvement; (Do) implementing interventions; (Check) evaluating clinical outcomes after these interventions; and (Act) implementing these interventions (if outcomes are favorable) in clinical practice.

After the completion of one full cycle, a new period of data collection, data analysis and evaluation ensue. The length of each cycle depends on what is being investigated at the time; a sufficient number of events must occur to detect a change in outcome. Therefore, cycle lengths can range from three cycles in 1 day to one cycle in 16 months (9). Depending on the objective to be achieved, the duration of a cycle chain (first to last cycle of one chain) may also vary enormously (1 day to 4 years). To manage the duration and analyze the quality, it is imperative to have a predetermined end date of the cycle. Therefore, before starting the PDCA-cycle, a statistical well-designed power calculation is essential.

STATISTICAL QUALITY ASSURANCE

It is essential to analyze outcomes in a correct manner to carefully target improvement efforts and assess the success of implemented pathways,

![Figure 1. The Plan, Do, Check, Act (PDCA)-cycle (or Deming’s cycle) – a continuous and cyclical technique to improve outcomes. Figure from: https://www.praxisframework.org/en/library/shewhart-cycle](https://www.praxisframework.org/en/library/shewhart-cycle)
protocols, and improvement plans (10). The use of statistical process control (SPC), developed by the aforementioned Dr. Shewhart, aids in testing the effectiveness of an intervention. SPC has found its way into healthcare systems over the last two decades (11).

In every (production) process, two types of variation can be distinguished: common cause variation and special cause variation, both affecting (product) quality (10, 12). Common cause variation is defined as variance inherent to the process itself; similar to many population characteristics that follow a Gaussian distribution with approximately 5% of measurements that fall outside of the 2 standard deviation limits. Special cause variation, on the other hand, is defined as variance that can be attributed to a specific cause (i.e., an intervention). The presence of special cause variation is a signal that the process has changed (either for better or for worse). Shewhart developed run and control charts to distinguish these types of variation within a production line process. For illustrative purposes, a control chart was created indicating the number of prostate biopsies for a hypothetical cohort of men suspected of having prostate cancer (Figure 2).

Although effective in detecting large shifts in a production line, Shewhart control charts are unable to find moderate or small shifts. This reduced sensitivity can be compensated for by augmenting Shewhart control charts with cumulative sum (CUSUM) control charts (13, 14). Unlike Shewhart control charts, CUSUM

![Figure 2. Control chart representing the average number of transperineal prostate biopsies per patient over time in a teaching hospital. Normal cause variation is present due to differences in physicians and baseline characteristics of patients; however, special cause variation was observed between July and October 2021. Special cause variation was defined as any outcome above or below 3 standard deviations (SDs) and 4 out of last 5 outcomes above or below 1 SD. This substantial rule violation was accompanied by the introduction of a new physician; a physician-in-training took significantly more prostate biopsies per patient. Through performance feedback and discussion, the number of biopsies normalized again as of November 2021.]
control charts represent information of current and previous samples at each point. Plotting the cumulative sums of deviations from the target value of current and previous samples results in greater sensitivity for detecting shifts or trends over the traditional Shewhart control charts. By way of example, a CUSUM chart was created indicating the number of positive surgical margins of one surgeon’s consecutively treated patients (Figure 3).

In addition to assessing the success of implemented interventions, comparing outcomes of physicians/hospitals is an important feature of QAPs; not to stimulate competition, but to identify variation in care processes that may be associated with outcomes (outcomes research). However, given that patient populations may differ between physicians and hospitals, assessment of and adjustment for case-mix variation is warranted. Methods have been proposed to analyze whether physicians/hospitals differ in outcome when compared to the mean risk of the case-mix subgroup (15, 16). By using multivariable regression models, the observed/expected ratio (O/E ratio) of each physician/hospital and outcome can

![Risk adjusted CUSUM plot of one surgeon’s positive surgical margins](image)

**Figure 3.** Risk-adjusted cumulative sum (CUSUM) plot of hypothetical data. The plot represents the surgical margin status of one surgeon’s consecutively treated patients. First, a prediction model is created using logistic regression with clinical variables as input and positive surgical margins (PSM) as output. This model predicts the probability of PSM for each individual patient. The probability ranges from 0 to 1. If a patient had a surgical margin (unwanted outcome), a score of 1 minus the predicted probability is added to the cumulative sum (line goes up). If a patient had a negative surgical margin (desired outcome) the predicted probability is subtracted from the cumulative sum (line goes down). The control limit is calculated using the standard deviation of the mean proportion of PSM and the group size and a weighted parameter (usually 4). If a small difference should be detected or if there are little data points, the weighted parameter can be decreased. For indicative purposes, the control limit is set at 3. The control limit is reached at the 94th patient, which indicates that the surgeon had more PSM than predicted based on the patients’ clinical variables. This could be a reason to evaluate the surgeon’s technique to improve the PSM rate.
be calculated. This is the for case-mix adjusted ratio indicating the quality of a physician/hospital. As a visual aid, case-mix adjusted funnel plots can be constructed; any physician/hospital that falls outside of the 95% confidence intervals has outcomes that significantly deviate from the average of the group. Figure 4 shows a funnel plot of hypothetical data illustrating the O/E ratios of positive surgical margins of 9 different surgeons.

**Figure 4.** A funnel plot was constructed of a hypothetical cohort of prostate cancer patients who underwent robot-assisted radical prostatectomy. The funnel plot displays the observed/expected (O/E)-ratio of positive surgical margins (PSM) of 9 different surgeons. One surgeon, highlighted as 1, is depicted above the 95% confidence interval upper control limit, which indicates that they make more PSM than expected based on the patients’ clinical characteristics when compared with the other surgeons. This could be a reason to evaluate the technique of surgeon 1 in order to improve the PSM rate.
REQUIREMENTS FOR QAPS

Properly constructing a QAP is essential for its success. Given the continuous nature of QAPs, the first and foremost requirement is motivated physicians that form the steering committee of the program. Their indispensable input identifies clinical steps or outcomes that require improvement. Subsequently, these outcomes must be properly registered in prospective (institutional) databases. Quality assurance can be performed both on a physician or institutional level. Multicenter collaborations and/or hospital networks facilitate an inter-institutional comparison. However, the accompanying data transfers between hospitals can be problematic because of technical or (patient)privacy issues. These problems may be solved by using secured internet-based, multicentric electronic data capture (EDC) systems managed by an independent data processor, who takes care of pseudonomization of patient level data before analyses are performed (17).

Before starting data collection, consensus must be reached on relevant outcomes indicative of quality of care. The International Consortium for Health Outcomes Measurement (ICHOM) has developed specific sets of relevant patient outcome measures for both localized and advanced PCa, that can be registered in a standardized way (18, 19). Confidence in data accuracy and completeness is fundamental for quality assurance and for the provision of sufficiently robust evidence on which to base changes in practice recommendations. Physician-reported data is reliable to benchmark outcomes (20). However, the data collection process should be well described and monitored in order to provide accurate and complete data that can be used for multiple purposes (i.e., research or hospital management). To prevent bias, both analyses and interpretations should be performed by independent parties. Additionally, participants put themselves in a vulnerable position in which they receive feedback (criticism) on their professional functioning, therefore data should be handled confidentially. Presenting the data in a safe environment in which the participants can freely discuss without repercussions is a prerequisite. This can be achieved by anonymizing data or limiting data access to participants of the QAPs only (21). Participating physicians/hospitals are expected to trust the data and the data collection process; if they perceive the feedback as non-credible, they may not be motivated to change their practice.

The last requirement is back-up by hospital managers. Drafting, implementing, and maintaining a QAP requires monetary investment. Therefore, the functioning of the QAP must also be evaluated: is it cost-effective? Depending on the subject, calculating quality-adjusted life years (QALYs) or the incremental cost-effectiveness ratio (ICER), defined by the difference in cost between two interventions divided by the difference in their effect, can provide insight into its cost-effectiveness. Improvement in the quality of care is associated with less comorbidity and less frequent follow-up treatment. Therefore, QAPs can lead to long-term cost reduction (22–24).
EFFECTS OF QAPS IN PCA CARE

We reviewed the literature on studies assessing the effect if QAPs in PCA care. In this, we specifically searched for studies that mention a QAP or improvement cycle according to the definitions of section 2.

The first attempts to develop QAPs for PCA care were made in Sweden. The merger of several regional databases created the National Prostate Cancer Registry (NPCR) (25). Using this database, surgeons were able to compare outcomes of their hospital to historical data of other hospitals and to the national average. In 2017, the NPCR opted for full transparency. All outcomes were made publicly available through an online dashboard in order to stimulate national quality control. With the help of this online dashboard, physicians can compare hospital-specific outcomes between Swedish hospitals. To preserve the privacy of physicians, individual surgeon-specific outcomes are only accessible to colleagues within their own department. The NPCR has already proven its worth. In 2014, an increased rate of readmissions after prostatectomy was observed, mainly due to anastomotic leaks. Videos of these patients were reviewed, and the literature was searched to identify surgical steps during the apical dissection and suturing the anastomosis. As a result, the surgical technique was changed, which led to a decrease in the readmission rate (from 10.6% to 5%) (25).

The Michigan Urology Statewide Improvement Collaboration (MUSIC) is a group of 46 urology practices and over 250 participating urologists (26). The QAP of MUSIC aims to improve the quality and cost-efficiency of PCA care, by reducing variance in practice. Within MUSIC, participating urologists submit data to a web-based clinical registry and, subsequently, receive quarterly reports in which their performance is compared to the statewide average and to other physicians. To date, they have published several papers on quality assurance in both PCA diagnosis and treatment. MUSIC underscores the positive changes that can be achieved with the collaborative QAP on PCA diagnostics. In an effort to improve data completeness, MUSIC has shown that QAPs are able to improve documentation of key variables, such as the clinical TNM-classification. By educating a dedicated urologist in each participating center on the importance of clinical TNM-classification for clinical decision-making and having them share this and their performance data with other members of their practice, documentation ultimately improved (27). Through performance feedback and education interventions, imaging appropriateness has been improved and biopsy-related complications have been reduced (27–29). Additionally, MUSIC has focused on the variation in surgeon-specific outcomes, such as erectile dysfunction, urinary incontinence, and complicated postoperative recovery. In accordance with the NPCR, MUSIC argues that objective identification of surgeons who achieve better outcomes will provide insight into specific techniques associated with those better outcomes (30–32). It has been suggested that peer reviewing of surgical videos and coaching may improve surgical skills and, hopefully, patient outcomes (33, 34).

Participating in a nationwide QAP is mandatory in Germany. In 2008, the German Cancer Society (Deutschen Krebsgesellschaft (DKG)) initiated a certification program to increase the quality of PCA care in Germany (35). To qualify for certification, centers must have established a quality management system and meet quality indicators yearly. Fifteen quality indicators (both treatment and
process related) were established based on expert opinion and clinical guidelines. Despite the efforts made, improvements in functional and oncological outcomes could not be demonstrated (36). In the meantime, the German Martini Clinic implemented a physician-initiated QAP on its own initiative. They realized that their institutional prospective database, initially started for scientific purposes, could also be utilized to aid in a QAP. This data collection contributed significantly to constant quality improvements over the years (37). For example, anesthetic regimens were adapted, which led to a decrease in intraoperative blood loss. In addition, nomograms were implemented and the NeuroSAFE technique was developed, which increased nerve-sparing procedures while keeping biochemical recurrence rates steady (38). They also noticed that one of their urologists had improved urinary continence outcomes compared to the others. After watching surgical videos, they found that the surgeon used a specific technique when dissecting the prostatic apex and urethra. Implementation of this technique by all other surgeons improved the urinary continence rate of all surgeons (39).

The London Cancer Network noticed poorer results compared to international colleagues, which motivated them to initiate a QAP. Through image-based surgical planning and monthly peer reviewing of individual surgeons’ outcomes, a high quality of care for patients undergoing radical prostatectomy was pursued (40). The implementation of such a QAP substantially improved quality of care, in terms of both oncological and functional outcomes; nerve-sparing surgery increased significantly while margin status remained static, and postoperative urinary continence and erectile function improved.

Similar to the MUSIC approach, Veerman et al. aimed to reduce catheter-related bladder discomfort (CRBD) after robotic-assisted radical prostatectomy by applying a QAP to the intra-operative anesthesia regime (41). After 8 cycles of different treatments and adapting the treatment protocol, the optimal treatment regime was identified. This regime reduced the incidence of CRBD from 70% to 36%, a relative reduction of 49%. Matulewicz et al. sought to determine the efficacy of comparative quality performance review to improve a surgeon-level measure of surgical oncologic quality. Participating surgeons were provided with confidential report cards detailing information about their patients’ clinical characteristics and positive surgical margin rates (42). These report card also contained information on their historical data, the institutional average, and the blinded results of peers. Before implementation of report cards, the positive surgical margin rate was 10.6%, while during and after the implementation, the positive surgical margin rate dropped significantly to 7.4%.

**DISCUSSION**

QAPs are increasingly implemented to achieve and maintain high quality PCa care. The currently existing QAPs differ in focus, execution, motivation, and subjects; however, they all share the same philosophy: to improve future PCa care by thoroughly studying their own retrospective data and identifying outliers. The existing literature has already described interesting results of QAPs on PCa care, such as improvement of functional outcomes, improvement of oncological outcomes and reduction of variability between physicians/hospitals.
QAPs are no stand-alone “research” projects. They are continuous cycles, incorporated into daily practice, striving for the best possible care. While the quality cycles warrant the quality of care, participating physicians should warrant the quality of the cycle. Quality assurance is achieved through the collection and analysis of reliable data and the willingness of physicians to act on these findings. Maintaining prospective registry databases alone is insufficient. Comparing one’s results with peers or the hospital average gives a good indication of performance, but to improve outcomes, discussion between peers and identifying improvement steps is essential. The willingness to improve must come from the physicians themselves; physician-initiated programs have been shown to improve both functional and oncological outcomes, whereas programs in which physicians were enforced to participate did not improve outcomes (35).

No consensus exists on the level of data transparency; opinions differ on the accessibility of quality assurance data. The Swedes have opted for full transparency and have made their data publicly available through an interactive online dashboard. Some, however, believe that patients may misinterpret the outcomes by a lack of context and lack of medical or epidemiological knowledge. Consequently, patients may avoid physicians and/or hospitals with a ‘worse’ performance. Besides, full data transparency may evoke risk-aversive behavior in physicians by not treating high-risk patients, induce registration bias, and limit physicians’ motivation. These actions are counterproductive for the progressive nature of the QAP (43, 44). On the other hand, full or partial access to quality assurance data for professionals is more accepted and even beneficial. Shared insight into the data improves physicians’ confidence in the data accuracy. Moreover, physicians can benchmark their results to the average and to their peers. Consequently, participants can identify points of improvement and they find solutions through a joint approach. In this way, the participants can learn from each other. Transparency of data within selected groups is therefore recommended to maximize the positive effects of QAP.

Criticism on the current literature on QAPs is that its focus is on improving one outcome at a time. Associations with multiple outcomes are not always taken into account. PCa care is dynamic and important outcomes (urinary incontinence, erectile functioning, positive surgical margin, etc.) are related to each other. An improvement of a specific outcome does not necessarily mean an improvement of the whole; other outcomes may be adversely affected by the intervention. The London Cancer Network and the German Martini Clinic should be commended in this respect as they reported all relevant outcomes instead of focusing on only one single quality indicator.

In order to obtain reliable results, a high volume of treated cases is desirable. High-volume centres generally have better outcomes than low-volume centres (45, 46). Additionally, improving patient outcomes through short cycles of quality improvement is easier with a higher volume of treated patients. After all, when a low volume of patients is treated, it may take years to measure a difference in outcomes after a change of practice. Collaborations between hospitals, such as networks and forming a hub-and-spoke model, can effectuate a high patient volume. The formation of hospital networks offers several other advantages. For example, centralisation is associated with increased compliance to guidelines and reduced costs. Centralisation also offers novel surgeons the opportunity to learn from expert surgeons, which may increase the quality of care in the entire network (47, 48).
Randomised controlled trials (RCTs) are the purest way to demonstrate a causal relationship between intervention and effect. However, there is a major drawback to this research method. Due to the highly selected populations used in RCTs, outcomes in daily practice may differ from RCT results. ‘Real world’ insights, gained through QAPs, can therefore be of great value in complementing evidence from RCTs (49). In the case of QAPs an unselected population is used, so outcomes are more based on daily practice and reliable for physicians. In addition, high costs are involved in carrying out RCTs, whereas QAPs are associated with minimal costs (50).

Many papers that publish on quality improvement initiatives in PCa care have positive outcomes. This may indicate a publication bias. Centres with less appealing results may be afraid to publish or struggle to find journals that accept their research. Consequently, it is harder to attribute the trend in improved results to the QAP alone. Furthermore, all centres that published on the effects of QAPs on PCa care are high-volume centres that are actively involved in the scientific community. It is possible that the effect is caused by applying the latest scientific insights, rather than learning from the best surgeon in the group. However, the results of the Dutch Institute of Clinical Auditing (DICA) counter this argument. DICA performs quality cycles regarding the treatment of several (non-urological) oncological and non-oncological diseases. All hospitals that treat a specific condition participate in the corresponding quality cycle, making it a nation-wide, population-based QAP. Results of the DICA QAPs have shown several improvements in quality of care, i.e., improved reporting, decreased between-hospital variations, decreased complication rates and improved mortality rates (51).

CONCLUSION

In conclusion, despite differences in organizational characteristics, the available literature shows positive effect of QAP (providing that motivated participants are involved). The use of QAP should therefore be recommended in urological practices. The key for success is a group of motivated physicians who lead the QAP.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this chapter.

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The Role of Family History and Germline Genetics in Prostate Cancer Disease Profile and Screening

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers-prostate-cancer-family-history-genetics

Abstract: Established risk factors for prostate cancer include age, ethnicity, a family history of prostate cancer or carrying a pathogenic germline variation in a prostate cancer predisposition gene. Approximately 10–15% of men with advanced prostate cancer have a germline genetic predisposition to the disease (i.e., BRCA2). Whilst the largest, and most well-known prostate cancer screening studies (i.e., ERSPC) have focused on the use of prostate-specific antigen as a screening tool, the incorporation of tissue and liquid genomic biomarkers alongside modern imaging modalities are being designed to individualize and improve the accuracy of both the screening and diagnostic pathway. The use of a polygenic risk scoring can now also offer a man his personalized prostate cancer risk based on a number of low-risk, common genetic variants and is currently the subject of ongoing research. The mainstreaming of genomics into the prostate cancer...
screening, diagnostic and treatment pathway will soon become embedded into routine clinical practice. This chapter aims to summarize current knowledge on the topic of men who harbor a genetic predisposition to prostate cancer, how this predisposition arises, its stratification into low-risk common variants vs. high-risk, rare variants, and its impact and incorporation into screening and diagnostic algorithms. The importance of germline genetics beyond screening and diagnostics, its role in the identification of lethal prostate cancer, and in the selection of targeted treatments for advanced disease is also discussed.

**Keywords:** familial prostate cancer; family history and prostate cancer; genetics of prostate cancer; hereditary prostate cancer; prostate cancer disease profile and screening

**INTRODUCTION**

Men with a family history of prostate cancer present a challenge in early prostate cancer detection whilst considering in parallel the well-known harms of PSA screening. The strength of a man's family history (i.e., first degree or second-degree relative) as well as the age of prostate cancer onset of his affected family members are also of importance. The literature is conflicting regarding treatment outcomes, survival, and grade/stage of disease in men with a family history, compared to those without.

Men with a family history of prostate cancer constitute an important population of men with a higher incidence of prostate cancer compared to men from the general population. Evidence suggests a spectrum of risk, with at least a two-fold increase (1) and worsening risk with the number and closeness (i.e., first degree) of relatives affected. A Swedish study reporting from a family-database of over nine million people reported a standardized incidence ratio (SIR) of 23 for men whose father and brother were affected (2). Hereditary prostate cancer (HPC) is a unique and specially defined circumstance based on a man's pedigree, with three categories described: (i) prostate cancer in three successive generations; (ii) at least two cases of prostate cancer in the family, both with an age of onset of <55 years old; and (iii) three or more first-degree relatives with prostate cancer at any age. This type of prostate cancer was first described by Carter et al in 1993 (3). It remains unclear if the biology of HPC is different to those with ‘sporadic’ (i.e., those with no family history of prostate cancer) disease but men with HPC do develop prostate cancer at an earlier age. In men with prostate cancer diagnosed at ≤55 years, HPC (as defined above) was found in up to 43% of cases. Genes implicated in HPC include BRCA1/2 and HOXB13.

**DOES PROSTATE CANCER IN MEN WITH A FAMILY HISTORY BEHAVE DIFFERENTLY COMPARED TO THOSE WITHOUT?**

Evidence for differences in disease biology between sporadic, familial, and hereditary prostate cancer is varied. Gronberg analyzed American families with familial and
HPC compared to men with sporadic prostate cancer. They showed that men with HPC were diagnosed with more aggressive prostate cancer and had an earlier age of onset (by 2 years) and had worse TNM stage (4). Poorer biochemical-free relapse rates at five-years following radical prostatectomy in men with familial prostate cancer (one first-degree relative affected with prostate cancer) compared to those without have been shown by Kupelian et al in a retrospective review of over 1,000 men. This work described family history as an independent predictor of biochemical recurrence after adjusting for age, histology, stage, and surgical pathology such as positive margins (5, 6). However in a similar analysis of 708 men undergoing radical prostatectomy published by Bova with longer follow-up (7), no differences in biochemical recurrences were seen between men with familial prostate cancer/HPC compared with men without a family history who were disease and age-matched.

In an analysis of 481,000 men in the Cancer Prevention Study II (CPS-II), men who had any family history of prostate cancer were 60% more likely to die from prostate cancer compared to those without, with a pronounced effect if the affected relative was diagnosed with prostate cancer before 65 years old (8). In an analysis of 5,519 men in the placebo arm of the Prostate Cancer Prevention Trial (PCPT), men with a family history (16% of the cohort) of prostate cancer had an odds ratio of 1.31 for harboring prostate cancer on any form of prostate biopsy undertaken during study follow-up. In the family history group, 24% who had a prostate biopsy had prostate cancer diagnosed compared with 17% of men without a family history; importantly, the investigators did not report that family history was associated with high-grade disease (9). Interrogating the Prostate, Lung, Colorectal and Ovary (PLCO) data, Liss et al found that when men with a family history underwent PSA screening, there was a significantly higher incidence of prostate cancer and prostate cancer cancer-specific mortality in those with a family history compared to those without (10).

Westerman et al. reviewed the impact of family history in a first-degree relative on clinical and mortality outcomes in a surgical population of approximately 16,000 men at the Mayo clinic undergoing radical prostatectomy from 1987–2010. Their cohort had a large incidence of family history (32.3%). They found men with a family history were significantly more likely to have organ-confined and low-risk disease and higher 10-year cancer-specific (99% vs 97%) and overall survival (92% vs 85%) compared to men without a family history (11). Overall survival has been reported as superior in men with a family history of prostate cancer in an Australian analysis of 9459 men by Ang et al (12) after adjusting for NCCN disease-risk category, age, and year of treatment. In this analysis, family history definition was a binary yes or no response relating to grandfather, father, uncle child or grandchild. Recently, Urabe et al published a meta-analysis of 8 studies with 33,027 patients reporting no impact of family history on cancer specific mortality or the risk of biochemical recurrence in patients with localized prostate cancer (13).

**HOW DOES PSA SCREENING PERFORM IN MEN WITH A FAMILY HISTORY OF PROSTATE CANCER?**

A subset analysis of European Randomised Screening Study of Prostate Cancer (ERSPC) (n=4,932) analyzed the effect of family history. The incidence of
prostate cancer differed significantly over an 11-year period between men with and without a family history (18% vs 12% respectively, HR 1.6). Family history status along with age and baseline PSA were significant predictors of prostate cancer incidence, but family history status was not an independent predictor for clinically significant prostate cancer. When men were stratified by family history status, 5.1% of men with a family history of prostate cancer were found to have clinically significant cancer compared to 4% of men without a family history (14).

When analyzing by screening arm vs non-screening arm in the PLCO screening trial, men with a family history of prostate cancer in a first-degree relative and the number of first degree relatives with a diagnosis of prostate cancer was significantly associated with prostate cancer mortality (HR 1.89) in the non-screening arm compared to the screening arm (15) suggesting a benefit to screening this group. Across both study arms, 10.5% of men without a family history were found to have prostate cancer compared with 16.5% of men with a family history. There was no difference in cancer stage, age, or PSA at diagnosis between the groups. It must be remembered however that the PLCO study was in essence (due to contamination of the trials’ screening arm), a trial of routine PSA screening vs opportunistic screening.

**SPECIFIC GERMLINE GENETIC MUTATIONS INVOLVED IN PROSTATE CANCER**

Specific prostate cancer risk genes exist, occurring rarely in the general population (0.2–0.3%) but with evidence for enrichment in cases of metastatic prostate cancer. Pritchard et al (16) highlighted the important role of DNA repair gene mutations in the biology of men presenting with advanced prostate cancer, demonstrating a relative risk (RR) of 18.6 for men with germline BRCA2 mutations and 3.1 for men with CHEK2 mutations. In their analysis of 692 men with metastatic prostate cancer, they found 11.8% of men carried a germline mutation in a DNA repair gene with 44% of all mutations found in the BRCA2 gene. These men were unselected for age at diagnosis or family history status. This differed to men with localized prostate cancer, in whom a frequency of germline mutations of 4.6% was described (17).

Pathogenic germline mutations were also found in approximately 17% of men in a cross-sectional study of 3607 men with prostate cancer, unselected for family history, age or disease stage, of which 30.7% were BRCA1/2 variants, 4.5% were due to HOXB13, 14.1% CHEK2 and 9.6% due to ATM (18). The United Kingdom Genetics Prostate Cancer Study (UKGPCS) (19) reported 7.3% of 191 prostate cancer patients with a family history of prostate cancer (with three or more cases in their family) were found to carry a pathogenic germline variant, the most commonly detected being in BRCA2 (28.57% of all pathogenic variants). Importantly, there was a significant association seen between carrying a pathogenic variant and a diagnosis of nodal or metastatic disease.
Mutations in BRCA1/2 are rare in the general population and enriched in the Ashkenazi Jews (with a frequency of approximately 2–2.5% of Ashkenazi women carrying a mutation in BRCA1/2 and 3.2–4% of Ashkenazi men with prostate cancer) (20). BRCA2 mutations confer the highest risk of prostate cancer (8.6-fold in men aged ≤65 years) (21, 22), with the effect of mutations in BRCA1 being significant (23). In an Icelandic study, BRCA1/2 mutation carriers were younger at diagnosis, (69 vs. 74 years) and presented with more advanced tumor (T) stage (T3–4: 79% vs. 36%) and histologically aggressive tumors (84% vs. 52.7%). Median cancer-specific survival (CSS) for carriers was 2.1 years compared with 12.4 years for non-carriers (24).

Poorer outcomes in carriers have also been reported. Edwards et al (13) compared overall survival (OS) after prostate cancer diagnosis in a series of BRCA2 mutation carriers and controls. BRCA2 mutation carriers had a median OS of 4.8 years vs with 8.5 years for non-carriers. Castro et al (25) reported a more aggressive prostate cancer phenotype more frequently associated with lymph node involvement and distant metastasis compared to non-carriers. An Icelandic study by Tryggvadottir et al showed a mean overall survival of approximately 2 years in men with prostate cancer who carried the specific 999del5 BRCA2 pathogenic variant compared with non-carriers (26).

The most optimal treatment strategy for men with prostate cancer who carry a high-risk genetic mutation such as BRCA2 is yet to be established, with no randomized clinical trials or large-volume series demonstrating a clear advantage of one radical treatment strategy over another. Such a trial would prove difficult due to the relative rarity of the mutation in the general population and in men with organ-confined disease undergoing radical treatment with surgery or radiotherapy. A retrospective series by Castro et al reviewed 1302 men (67 BRCA1/2 mutation carriers) with prostate cancer and found poorer metastasis-free survival and cancer specific survival after radiotherapy (27) although this was not statistically significant. The PROREPAIR-B study was a multi-center study enrolling men presenting with metastatic castrate resistant prostate cancer for germline testing for defects in 107 DNA damage repair genes. 16.2% of their population (419 men) were found to carry a germline mutation, of which BRCA2 was the most common. The investigators reported worse outcomes in men with a BRCA2 mutation receiving taxane chemotherapy as first line treatment compared to those without a BRCA2 mutation, along with a reduced median cancer-specific survival (28).

Active surveillance (AS) is now an acceptable and recommended treatment option for localized prostate cancer of favorable risk so men may avoid the risks and morbidity of radical treatment until the disease profile requires it. Carter et al (29) have demonstrated an association between the incidence of disease upgrade in men on AS with germline mutations in BRCA1/2/ATM compared with non-carriers (five-fold greater risk; adjusted HR 2.40, p=0.046).

**CHEK2, NBN, ATM**

CHEK2 mutations have been implicated in familial and hereditary prostate cancer, in particular in Slavic populations (30, 31). In a UK study of 191 men with 3 or
more cases of prostate cancer in their family, Leongamornlert et al reported CHEK2 germline mutations accounted for 14% of all germline loss of function mutations and was associated with more aggressive prostate cancer (19).

In Polish men with disease onset less than 60 years and in men with a family history of prostate cancer, frequencies of mutations in BRCA1, CHEK2 and NBN were higher than in those without. A founder mutation (675del5) in NBN has also been associated with a three-fold increase in prostate cancer incidence amongst carriers and a significant effect on overall survival after adjusting for age, stage, and tumor grade (32–34). In a UK study of aggressive prostate cancer cases, Mijuskovic et al found a protein-truncating variants in NBN was present in 5.8% of aggressive cases of prostate cancer (35). Men carrying a pathogenic variant in the ATM gene have been reported as having upwards of a four-fold increase in prostate cancer risk and were more likely to have earlier onset disease in a large case-control, European analysis by Karlsson et al (36), along with shorter survival times and younger age at death from prostate cancer (37).

**HOXB13**

Carriers of a pathogenic germline missense variant of the HOXB13 gene had a 33% risk of developing prostate cancer, compared to a 12% risk of non-carriers in a Scandinavian population of over 5,000 cases (38). An analysis of approximately 2,400 Prostate cancer families found a HOXB13 mutation in 5%, suggesting a potential role of targeted screening in men known to carry this germline variant (38, 39). A further large-scale Finnish analysis of 4,000 prostate cancer cases revealed a significantly higher carrier-rate of the specific G84E mutation amongst men with prostate cancer (3.5%) and those with a family history (8.4%) compared to controls (40). In a separate study, Ewing et al found the carrier rate of the G84E mutation was more commonly encountered in men with a diagnosis of prostate cancer at an early age and in those with a positive family history (1.4%), than those without (0.1%) (41). There was no difference in Gleason grade between carriers and non-carriers (41). Nyberg et al described age-specific risks for carriers of the pathogenic G84E variant for developing prostate cancer and stratified men by varying pedigrees. The average predicted risk of prostate cancer by age 85 was 62% for those carrying the mutation, compared with 15% for those without. In a mutation carrier with a history of prostate cancer in his father, the risk estimate ranged from 69% to 92% depending on the father’s age at prostate cancer diagnosis, and for a man with two affected first-degree relatives, the risk estimate ranged from 70% to 98% (42).

**LYNCH SYNDROME**

Lynch syndrome is a rare, inherited cancer predisposition syndrome caused by germline mutations in the miss-match repair genes; MLH1, MSH2 or MSH6. It has been estimated in a study investigating 106 men with miss-match repair mutations that the cumulative risk of prostate cancer by the age of 70 in
mutation carriers is 30%, compared with 9–12% in the general population. Of the cancers diagnosed with available histology, 5 cases (62.5%) were poorly differentiated, with a Gleason score ≥8 (42). Recent results from the first screening round of the IMPACT study described higher prostate cancer incidence in MSH2 and MSH6 mutation carriers (compared to age-matched, non-carrier controls), with results suggesting a possible benefit in targeted PSA screening in these high-risk groups (43).

**THE IMPACT OF GERMLINE GENETICS ON TREATMENT AND OUTCOMES**

Targeted therapy for men with pathogenic variants in DNA damage repair genes has been the subject of recent research. In men with metastatic castration-resistant prostate cancer with germline or somatic pathogenic variants in BRCA1/2, Olaparib has been evaluated in the UK based, Phase 2 TOPARP study (44) which recruited 92 patients with known mutations in DNA damage repair genes to receive either 300mg or 400mg of olaparib. Results showed greater radiological, PSA or circulating tumor cell response in the 400mg group, and this was greatest in those with a BRCA1/2 mutation. PARP inhibitors are now licensed in the US and Europe for men with germline mutations in DNA repair genes (BRCA1, BRCA2 and ATM) (45–47). In addition, men with advanced prostate cancer pathogenic variants in BRCA1, BRCA2 and other DNA repair genes have also demonstrated encouraging sensitivity to platinum chemotherapy (48–50).

**GERMLINE SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS)**

Large scale genome-wide-association-studies (GWAS) have led to the discovery of approximately 269 SNPs specifically associated with Prostate cancer risk (51–54) across multiple chromosomal loci. At present, 34–43% of the familial risk in prostate cancer can be explained based on these SNPs, with men in the top 1% of the risk profile having a 5.7-fold increase in risk of developing prostate cancer compared with the average risk of men in the general population (55–57).

By measuring the genetic burden for a specific disease, a polygenic risk score (PRS) provides a novel tool in identifying those at greatest or the lowest risk. A PRS is calculated by summing all detected (and weighted) risk alleles, with the effect of each allele described from published GWAS. Using PRS in addition to clinical information (i.e., age, PSA, and family history) has been shown to predict prostate cancer and also reduce the need for prostate biopsies (58, 59). Limitations include extensive underrepresentation of non-Caucasian populations in the studies have resulted in prostate cancer risk SNP discovery, though multi-ethnic analyses have now been reported by Conti et al in a recent GWAS and meta-analysis of over 107,000 cases and controls across different ethnic populations reporting 269 risk SNPs. They reported men of African ancestry having a genetic risk score (GRS) that was 2.18 times higher than that of Caucasians (57).
In a meta-analysis by Schumacher et al, men in the top 1% of the risk profile according to a 147 prostate cancer-risk SNP profile had a 5.7-fold increased risk of prostate cancer compared to men of average risk (defined as those in the 25–75th centiles of risk) (60, 61). Of note, the PRS effect increased with the presence of positive family history and in those with a prostate cancer diagnosis under the age of 55 years.

Pashayan et al. assessed the implications of using a PRS in reducing the prostate cancer over-diagnosis associated with PSA-based prostate cancer screening. They built a PRS-based on 17,000 prostate cancer cases using 66 prostate cancer risk SNPs, separating men into risk quartiles. They found that PRS-based risk stratification had the ability to lead to a 56% reduction in over-diagnosis between the lowest PRS quartile and the highest (62). The PRS described by MacInnis et al (based on 26 risk SNPs) in men specifically with familial prostate cancer (53), demonstrated the parallel effects of family history status and known prostate cancer susceptibility variants. Seibert et al reported a polygenic hazard score (PHS) using 54 prostate cancer risk SNPs. This showed the ability to predict age at prostate cancer diagnosis of any prostate cancer and aggressive prostate cancer. In this study, the positive predictive value (PPV) of PSA also increased with increasing PHS (63).

Apart from predicting risk in the general population, prostate cancer SNPs are known to modify the risk associated with \textit{BRCA1/2} mutations. Recently, the utility of a 147-prostate cancer SNP assay was investigated in approximately 1,800 Caucasian men of European ancestry from the CIMBA consortium. They reported a wide range of absolute prostate cancer risks in men with \textit{BRCA1/2} mutations, depending on where one falls on the spectrum of polygenic risk (Figure 1). These results indicate that a PRS could be clinically informative in assigning men an

![Figure 1. Risk of prostate cancer in \textit{BRCA1/2} mutation carriers according to age and polygenic risk. Reproduced from Barnes et al (64). The predicted absolute risks of developing breast cancer and prostate cancer by PRS percentile. Risks were calculated assuming the per standard deviation ratio estimates in the combined sample of \textit{BRCA1} and \textit{BRCA2} carriers. (B) absolute risk of prostate cancer in male \textit{BRCA1} carriers (C) absolute risk of prostate cancer in male \textit{BRCA2} carriers. Copyright ©2021, Oxford University Press. Figure reproduced under terms of the Creative Commons CC BY license which permits unrestricted use, distribution, and reproduction in any medium.](image)
individualized cancer risk for those carrying pathogenic \textit{BRCA1/2} variants, a small but important group of men could form part of a novel, future enhanced screening strategies for \textit{BRCA1/2} mutation carriers (64).

\section*{INCORPORATING GENETICS INTO SCREENING AND DIAGNOSTIC PATHWAYS}

The \textit{STOCKHOLM3} study (STHL3M) (65) was the first population-based prostate cancer screening study prospectively assessing a prostate cancer screening strategy incorporating genetic information. The study's screening model combined serum biomarkers (including PSA and its isomers), 232 risk SNPs and known clinical variables (e.g., age, a family history of prostate cancer, previous prostate biopsy) and compared this with a PSA alone (using a threshold of \( \geq 3.0 \text{ng/ml} \)) screening strategy. The sensitivity of the STHLM3 model for the detection of clinically significant Prostate cancer was superior (AUC 0.74 vs 0.56) when compared to PSA. The STHLME3 model also reduced the number of prostate biopsies by 32\% and avoided 44\% of negative biopsies. Given the Caucasian ethnicity of the majority of participants in the original STHLM3 screening study, the evaluation and validation of the STHLM3 model in non-Caucasian populations will be important and this is being investigated prospectively in a multi-ethnic cohort (SEPTA trial) in Chicago (NCT04583072). The STHLM3MRI study incorporated the use of prostate MRI, which combines a paired and randomized study design, the results of which have recently been published (66). When Nordstrom et al compared a strategy of PSA screening combined systematic biopsies with that of a ‘positive’ STHLM3 test combined with MRI-targeted biopsies, they found 69\% fewer low-grade cancers were diagnosed (95\% CI 52–80; 45 vs 142 per 10,000 tested men) and 52 percent fewer biopsies (95\% CI 43–58; 409 vs 853 per 10,000 tested men) were performed in the STHLM3/MRI cohort. This test combination therefore shows great promise for minimizing prostate cancer over-detection whilst maintaining the detection of clinically significant disease.

\textit{BARCODE1} (NCT03857477) will be the first prospective study to utilize a prostate cancer risk SNP profile to evaluate targeted prostate cancer screening in the general population. The investigators recruited patients via their general practitioners and offered intervention with MRI and prostate biopsy to men only falling in the top 10\% of polygenic risk. In the \textit{BARCODE1} pilot study, uptake following invitation was 26\% with 25/303 participants being identified for MRI/Biopsy invitation based on their PRS falling in the top 10\% (67). The pilot study is now complete, with the full study having completed recruitment and is ongoing.

A risk-stratified approach to refining breast cancer screening was modelled by Pashayan et al (68) in a hypothetical UK cohort of over 300,000 women comparing no screening, age-based screening and a PRS-based model where only women in the highest PRS were offered screening mammography. Reduced rates of breast cancer overdiagnosis and improved cost-effectiveness were found when women with low risk were not offered screening. The \textit{WISDOM} study is an RCT comparing personalized, risk-based screening with routine annual breast cancer screening in 100,000 women aged 40–74 in the USA. The personalized screening,
experimental arm is based on a woman's breast density, a PRS based on over 200 breast cancer risk SNPs, 9 gene-panel and ethnicity (69). A similar approach could be utilized in prostate cancer in the future.

TARGETED PROSTATE CANCER SCREENING

PSA is not a diagnostic test for prostate cancer and is unlikely to ever be deemed a satisfactory tool on its own for population screening. Given that advanced and aggressive prostate cancer can significantly affect a man's survival (70), targeting men at a high risk of cancer and a high risk of lethal prostate cancer would be the better target of a screening program. It is in this scenario where clinical and genetic risk modelling may play a large part in future targeted screening strategies.

In a prospective screening study of Israeli males with known BRCA1/2 mutations for 5 different cancers including prostate cancer, the rate of prostate cancer detection in BRCA1/2 mutation carriers was 3.8–8.6% using annual PSA screening and digital rectal examination (71). Das et al have also reported their intention to prospectively study a cohort of men with known pathogenic germline variants (BRCA1/2, HOXB13, ATM, Lynch syndrome genes), managed in a high-risk clinic which will include a PSA, DRE, SelectMDx™ and MRI based algorithm (72).

The IMPACT study (NCT00261456) is a targeted screening study enrolling over 3,000 men (BRCA1/2, MSH2, MSH6, MLH1 mutation carriers and controls) investigating the outcomes of targeted PSA screening; the screening intervention being annual PSA and a biopsy triggered with a PSA threshold of 3.0 ng/ml. Preliminary and interim results in the BRCA1/2 cohort suggested targeted screening using PSA in this population is beneficial in those with a BRCA2 mutation, with mutation carriers having with a higher rate of prostate cancer diagnosis, at a younger age and having more significant disease than non-carriers. In 2020, Segal et al reported their first round of screening combining age-stratified PSA and MRI in BRCA1/2 mutation carriers. This approach detected cancer in 8.6% of the 188 men recruited, with a significant net benefit of screening using MRI compared to PSA found in men aged 40–55 years (PSA had the highest benefit in those aged >55) (73). The early screening results of Das et al (72), the Lynch cohort of the IMPACT study (43) and interim results of the IMPACT BRCA cohort (74) have been published and the full results are awaited. Dahut et al have described a screening protocol for men with pathogenic variants in known or suspected high-penetration cancer predisposition genes where they intend on screening 500 men with prostate MRI, PSA and DRE with repeat screening interventions every two years (75).

It is yet unclear exactly what role PRS can play as a screening tool in detecting prostate cancer in asymptomatic men selected for a family history as most who will have low PSAs. The PROFILE pilot study evaluated the feasibility of recruiting men with a family history of prostate cancer to undergo up front prostate biopsy and germline SNP testing for prostate cancer risk SNPs to assign all men a PRS. No significant association between the PRS and prostate cancer diagnosis was found in 100 healthy men with a family history of prostate cancer undergoing screening prostate biopsy irrespective of PSA. However, the number of cancers
diagnosed in this group of men (mean age 53) with a low median PSA (1.3) was sizeable; 25% had prostate cancer found on screening biopsy of whom 48% had clinically significant disease. Twelve men with prostate cancer had a PSA <3 (52%). No adverse psychosocial variables were noted (76).

CONCLUSION

Germline mutations in a prostate cancer predisposition gene have emerged as important in all aspects of the prostate cancer pathway, from screening and diagnosis through to patient counselling regarding prognosis and targeted treatments. Germline analysis for prostate cancer risk SNPs is also likely to play a role in the future of prostate cancer screening and diagnostic risk-stratification pathways; identifying men who may benefit more from further diagnostic tests or reassuring those at low risk.

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Conflict of Interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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Doi: https://doi.org/10.36255/exon-publications-urologic-cancers.index
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