Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8479 - 8504
Received 25 April 2021; Accepted 08 May 2021.

Update of Bladder Cancer Classification Using Available Immunohistochemical Markers

HebatullahAbulkhair[1]Yousria Mohamed Mohamed EL-Gohary[2],Hesham Radwan Abdel-Aziz [3], TaiseerRefaat Ibrahim[4]

[1]Assistant lecturer of pathology, Faculty of Medicine, Zagazig University, Egypt.
[2,3,4] Professor of Pathology, Faculty of Medicine, Zagazig University, Egypt.
Corresponding author:HebatullahAbulkhair Ahmed
Email:hebatullahabulkhair@gmail.com

Abstract

Background: Bladder carcinoma is the sixth-most common cancer in the USA, and the majority of these tumors are non-invasive stage Ta at first diagnosis without evidence of invasion
In 2018, there is an estimated 81,190 new bladder cancer (BC) cases diagnosed in the USA with 17,240 deaths. Three-quarters of new cases occur in men (higher in some regions and reflects smoking and occupational differences and access to healthcare), women have greater disease-specific mortality. Reasons for the disparity in gender incidence and mortality include differences in hormonal profiles (activity and levels of the sex steroid hormone pathway) and differences in the timelines of female referrals to investigation from primary care
In Egypt, bladder cancer accounts for about 30% of all cancers, and it is the most common malignancy in men and the second most common malignancy in women after breast cancer, it has been related to chronic infection with Schistosoma haematobium, causing mainly squamous cell carcinoma (SCC).In the current era of precision molecular medicine, there is a growing need for identification and validation of biomarkers that can assist in patient selection by accurately predicting treatment response. As the resources in the worldwide health care system are finite, the search for the best possible criteria of analysis is indispensable to optimize cost-benefit ratio in the treatment of cancers including the bladder cancer. Can the highly successful scenario of breast cancer be developed and implemented for the treatment of bladder cancer where the newly recognized subtypes can be reliably identified by using a panel of immunohistochemical stains.
Key words: Bladder Cancer (BC), squamous cell carcinoma (SCC), of immunohistochemical stains.

1.Introduction:

The molecular subtypes of bladder cancer have different clinical behavior and sensitivities to chemotherapy. The bladder cancers of the basal subtype were more aggressive with shorter survival when compared to luminal cancers, however they were more sensitive to cisplatinum based chemotherapy and the patients with this form of the disease appeared to gain more benefits from frontline chemotherapy when compared to luminal subtypes **(1,2).**

2.Epidemiology:

Bladder cancer (BC) is the 4th most common cancer in men and the 11th most common in women **(3).**

The gender ratio of male to female is 3 to 1 and the best-known environmental risk factor is smoking. Bladder cancer occurs mostly in people aged above 55 years **(4).**

BC risk has been observed to be lower in women with older age at menarche, parity compared to nulliparous women, and the use of estrogen and progestin therapy.

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Furthermore, women have been shown to have a higher stage at BC diagnosis, which is thought to be due to irritative lower urinary tract symptoms being more likely to receive a diagnosis of urinary tract infection. However, women have also been shown to have poorer survival outcomes when adjusted for all stages. This may represent differences in treatment efficacy, cancer biology and drug interactions **(5, 6)**.

In Egypt, bladder cancer accounts for about 30% of all cancers, and it is the most common malignancy in men and the second most common malignancy in women after breast cancer, it has been related to chronic infection with schistosoma haematobium, causing mainly squamous cell carcinoma (SCC) **(7).**

Urothelial carcinoma can be subdivided primarily into conventional and nonconventional subtypes. Conventional urothelial carcinoma comprises the vast majority (>90%) of all forms of urinary tract cancer and arises from either papillary or flat in situ lesions. In contrast, nonconventional carcinoma of the urinary tract includes squamous cell carcinoma, adenocarcinoma, and small cell carcinoma **(8).**

Squamous Cell Carcinoma (SCC) of the bladder is a rare histological type in the Western countries and North America, representing around 5% of all urinary bladder carcinomas. In Africa, the incidence is different especially in the countries located on the Nile River; because of the high incidence of schistosomal infection that leads to chronic irritation and squamous metaplasia **(9).**

Historically, the incidence of bladder SCC was known to be high in Egypt; however, recent data from Egypt show significant decrease in this incidence along the last decades **(9).**

So, the histopathological pattern of bladder cancer is changing among Egyptians. Over the last decades, the incidence of bladder SCC is declining, while bladder TCC is rising **(9).**

3. Aetiology and Risk Factors:

3.1. Tobacco smoking

Smoking is the most important risk factor for bladder cancer. Smokers are at least 3 times as likely to get bladder cancer as non-smokers. Smoking causes about half of all bladder cancers in both men and women **(10).**

Tobacco smoking is well-established risk factor for BC, causing 50-65% of male cases and 20-30% of female cases. With incidence of BC is directly related to the duration of smoking and the number of cigarettes smoked per day **(11)**.

There is increase in risk estimates for current smokers relative to never smokers may be due to changes in cigarette composition. The risk of BC was observed to decrease in those who stopped smoking, with about 40% reduction within 1-4 years of quitting smoking and 60% after 25 years of cessation. Encouraging people to stop smoking would result in decreasing the incidence of BC equally in men and women **(12, 13).**

3.2. Occupational exposure to chemicals

Occupational exposure to chemicals is a very important risk factor for BC. Work-related cases have accounted for 20-25% of all BC cases in several series.The substances involved in chemical exposure include benzene derivatives and aryl amines (2-naphthylamine, 4-ABP, 4, 4'-methylenedianiline, and o-toluidine), and occur in occupations in which dyes, rubbers, textiles,

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paints, leathers, and chemicals are used, with the risk is significantly greater after 10 years or more of exposure; the mean latency period usually exceeds 30 years **(14,15).**

The chemicals involved have contributed minimally to the current incidence of BC in Western countries because of strict regulations. Importantly, the extent and pattern of occupational exposure have been changed because awareness of safety measures **(16, 17)**.

3.3. Radiotherapy

Increased rates of secondary bladder malignancies have been reported after external-beam radiotherapy (EBRT) for gynecological malignancies, with relative risks of 2-4. It has been proposed that patients who have received radiotherapy for prostate cancer with modern modalities may have lower rates of in-field bladder secondary malignancies. As BC requires a long period to develop, patients treated with radiation and with a long life-expectancy are at a higher risk of developing BC **(18, 19).**

3.4. Dietary factors

Several dietary factors have been considered to be related to BC; however, the links remain controversial. The European Prospective Investigation into Cancer and Nutrition (EPIC) study is an on-going multicenter cohort study designed to examine the association between diet, lifestyle and environmental factors and cancer. They found no links between BC and fluid intake, red meat, vegetable and fruit consumption, and only recently they have described an inverse association between dietary intake of flavonols and lignans and the risk of BC, in particular aggressive tumors**(20).**

3.5. Gender

Although men are more likely to develop BC than women, women present with more advanced disease and have worse survival. It has been suggested that women are more likely to be older than men when diagnosed, with a direct effect on their survival. In addition, delayed diagnosis is more likely in women after hematuria is observed, as the differential diagnosis in women includes diseases that are more prevalent than BC **(21,22).**

Differences in the gender prevalence of BC may be due to other factors besides tobacco and chemical exposure. In a large prospective cohort study, postmenopausal status was associated with an increase inBC risk, even after adjustment for smoking status. This result suggests that the differences in oestrogen and androgen levels between men and women may be responsible for some of the difference in the gender prevalence of BC. Previous study showed that cancer-specific mortality was higher in female patients **(23,24).**

3.6. Genetic factors

There is evidence that genetic susceptibility factors and family associations may influence the incidence of BC. It was found that family history of cancer in first-degree relatives was associated with an increased risk of BC; with the association being stronger among younger patients **(25).**

Genome-wide association studies (GWAS) of BC identified several susceptibility loci associated with BC risk. Polymorphisms in two carcinogen-metabolizing genes, NATS and GSTM1, have been related to BC risk, and furthermore they have demonstrated, together with UGT1A6, confer additional risk to exposure of carcinogens such as tobacco smoking **(25,26).**

3.7. Personal history of bladder or other urothelial cancer

Urothelial carcinomas can sometimes form in different areas in the bladder, as well as in the lining of the kidney, the ureters, and urethra. Having cancer in the lining of any part of the urinary tract puts the patient at higher risk of having another cancer, either in the same spot as before, or in

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another part of the urinary tract. This is true even when the first tumor is removed completely. People who have had bladder cancer need careful follow-up to look for new cancers (**27).**

3.8. Bladder schistosomiasis and chronic urinary tract infection

Bladder schistosomiasis (bilharzia) is the second most common parasitic infection after malaria, with about 600 million people exposed to infection in Africa, Asia, South America, and the Caribbean. There is a well-established relationship between schistosomiasis and squamous cell carcinoma of the bladder, although a better control of the disease is decreasing the incidence of squamous carcinoma of the bladder in endemic zones such as Egypt. Similarly, invasive squamous cell carcinoma has been linked to the presence of chronic urinary tract infection (UTI) distinct from schistosomiasis. A direct association between BC and UTIs has been observed in several case-control studies, recurrent UTIs in some series **(28:30).**

The mechanism by which schistosomiasis causes BC remains unknown, two hypotheses have been proposed. First, the chronic inflammatory proliferation that allows and promotes genetic alterations, which ultimately can lead to higher cancer incidence. And on the other hand, it has been suggested that in urine of patients infected with schistosomiasis, there is a production of carcinogenic substances as nitrosamines, concretely N-butyl-N (4-hydroxybutyl) nitrosamine. Importantly associated to that inflammatory reaction, there is a conversion of the transitional urothelium toward squamous epithelium; this is the cause for 70% of patients developing cancer because of bilharziasis, present with squamous cell carcinoma, rather than transitional cell carcinoma, as is usual in other geographic sites **(31).**

Bilharzial association dropped from 82.4% to 55.3% and there was a significant increase of transitional cell carcinoma from 16% to 65%, while squamous cell carcinoma was less frequent, from 76% to 28%. Intimately related to this, there was an increase in the median age of patients from 47 to 60 years. The decline in the frequency of BC is related to a decline in bilharzias egg positivity in the specimen and this suggests a better control of the endemic disease in rural population **(29).**

4.Staging and Grading of Urothelial Carcinoma:

Urothelial cell carcinoma patients are stratified by pathologic stage and grade; the basis of clinical decision-making. The stage classification differentiates between non muscle invasive (NMI; Tis, Ta, and T1) and muscle-invasive tumors (T2, T3, and T4) according to the invasion depth. Ta tumors are restricted to the urothelium; T1 tumors have invaded the lamina propria; and T2, T3, and T4 tumors have invaded the superficial muscle, perivesical fat, and surrounding organs, respectively **(31).**

This is poorly understood and believed to be a precursor of muscle-invasive tumors. The majority of patients, 70%, initially present with NMI tumors, however, up to 70% of these develop local recurrences, and patients may have several recurrences.

Roughly 25% of NMI patients progress to muscle-invasive tumors disease with a potential to develop metastasis. One problem in day-to-day clinical practice is that pathologic assessment is reported to be fairly uncertain **(31).**

Table (1): TNM classification of urinary bladder cancer **(32)**

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	T - Primary Tumor
Tx	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma in situ: "flat tumour"
T1	Tumour invades lamina propria
T2	Tumour invades muscle
T2a	Tumour invades superficial muscle (inner half)
T2b	Tumour invades deep muscle (outer half)
T3	Tumour invades perivesical tissue
T3a	Microscopically
T3b	Macroscopically (extravesical mass)
T4	Tumour invades any of the following: prostate stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall
T4a	Tumour invades prostate stroma, seminal vesicles, uterus, or vagina
T4b	Tumour invades pelvic wall or abdominal wall
	N - Regional Lymph Nodes
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph-node metastasis
N1	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N2	Metastasis in multiple lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N3	Metastasis in common iliac lymph node (s)
	M - Distant Metastasis
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

The cellular morphology of urothelial carcinoma is graded according to the degree of cellular differentiation. The grading consists of well-differentiated (Grade 1: In G1 tumours, the papillary urothelial carcinomas consist of orderly-arranged, nearly-normal, urothelial cells. The tumours display slender papillae with minimal abnormality. Mitosis and necrosis are globally absent. Nuclear pleomorphism is absent, with regular chromatin distribution. If present, nucleoli are small. Cell borders are well defined.), poorly differentiated (Grade 3: In G3 tumours, architectural and nucleocytoplasmic heterogeneity is a typical finding. Mitosis can be distributed all over the urothelium, even in superficial layers, and necrosis may be present. There is increased cellularity, nuclear crowding, and a lack of normal differentiation.

The nuclei display irregular membranes and shape and sometimes neoplastic giant cells. Chromatin distribution is granular or coarse and prominent nucleoli are common), and moderately

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differentiated (Grade 2: The architectural and nucleocytoplasmic atypia in G2 cover a broad spectrum of lesions between those seen in grades G1 and G3) tumors. Grading of cell morphology in non-muscle invasive bladder cancer (NMIBC) is important for establishing prognosis because grade 3 tumors are the most aggressive and the most likely to become invasive **(34,35).**

But nowadays for more definition of grades, urothelial carcinoma is divided into low grade (LG) and high grade (HG) carcinoma **(35)**

CIS lesions are comprised of severely dysplastic urothelium, and in older series were often categorized as "severe dysplasia." Disorderly histology with nuclear atypia characteristic of high-grade malignancy is microscopically diagnostic. Denudement of some or all of the mucosa due to loss of cellular cohesion is often identified. Most pathologists consider mild versions of dysplasia or atypia to be benign. However, lesions interpreted as severe dysplasia or severe atypia are regarded as being the same entity as CIS **(36).**

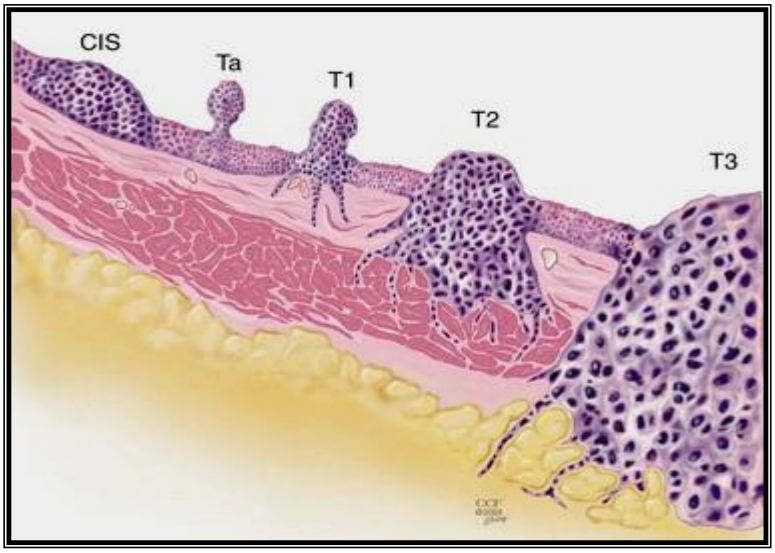

Figure (1): Steps of development of high gradeurothelial carcinoma from carcinoma in situ **(36).**

With respect to histologic patterns and microinvasion (invasion into the lamina propria to a depth of less than 2 mm), Five major patterns of CIS, have been recognized Common to each pattern was the presence of high-grade cytologic atypia, the definitional feature. The patterns include:

1. **Large cell CIS with pleomorphism**, in which the cells had abundant cytoplasm and nuclear pleomorphism.
2. **Large cell CIS without nuclear pleomorphism.**
3. **Small cell CIS**, in which the cytoplasm was relatively, scant and pleomorphism was usually minimal.
4. **Clinging CIS**, in which the urothelium was denuded with a patchy, usually single layer of atypical cell.
5. **Cancerization of urothelium** with either pagetoid spread (clusters or isolated single cells) or undermining or overriding of the normal urothelium**(37)**.

Carcinoma in situ with microinvasion into the lamina propria was evident as invasive cells with retraction artifact mimicking vascular invasion; nests, irregular cords, and strands, or isolated single cells with desmoplasia, or absent stromal response **(38)**.

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NMIBCs (Ta and T1) include low- and high-grade papillary tumors, Muscle invasive bladder cancer, hereafter termed “invasive bladder cancer," is usually high grade and characterized by a high risk of metastases to regional pelvic lymph nodes and visceral sites **(39).**

Bladder cancer progresses along two distinct pathways that pose distinct challenges for clinical management **(40).**

Low-grade non-muscle invasive (‘‘superficial’’) (NMIBCs) cancers, which account for 70% of tumor incidence, are not immediatelylife-threatening, but they have a propensity for recurrence thatnecessitates costly lifelong surveillance **(41).**

Non-muscle invasive bladder cancer (NMIBC) includes diverse clinical phenotypes ranging from Ta (non-invasive) low grade (LG) or high-grade (HG) cancer to carcinoma in situ (CIS), a high-grade intraepithelial cancer, and to an invasive high-grade phenotype (T1). A binary stratification of LG versus HG or Ta versus T1 with or without CIS informs a risk-stratified treatment decision tree regarding use of intravesical chemotherapy or immunotherapy **(42).**

In contrast, high-grade muscle-invasive bladder cancers (MIBCs) progress rapidly to become metastatic and generate the bulk of patient mortality. Radical cystectomy with perioperative cisplatin-based combination chemotherapy is the current standard of care for high-risk MIBC **(43).**

Muscle-invasive bladder cancers (MIBCs) are highly heterogeneous tumors with variable clinical courses and responses to conventional cisplatin-based combination chemotherapy (gemcitabine and cisplatin or methotrexate, vinblastine, doxorubicin hydrochloride (Adriamycin), and cisplatin [MVAC] **(43).**

Treatment selection depends heavily on clinico-pathologic features, but current staging systems are inaccurate and result in an unacceptably high rate of clinical understaging and consequently inadequate treatment **(44).**

Urothelial carcinoma can vary in appearance, but is generally characterized by nests or sheets of atypical urothelial cells surrounded by retraction artifact occasionally, individual cells may be present. Carcinoma cells are usually medium to large in size and the cytoplasm is often pale to eosinophilic in color. Nuclear atypia varies from minimal to marked, with some tumors displaying prominent pleomorphism. Nucleoli are generally rare. Angiolymphatic invasion may be present in some cases **(45).**

5.Histopathology of conventional urothelial carcinoma:

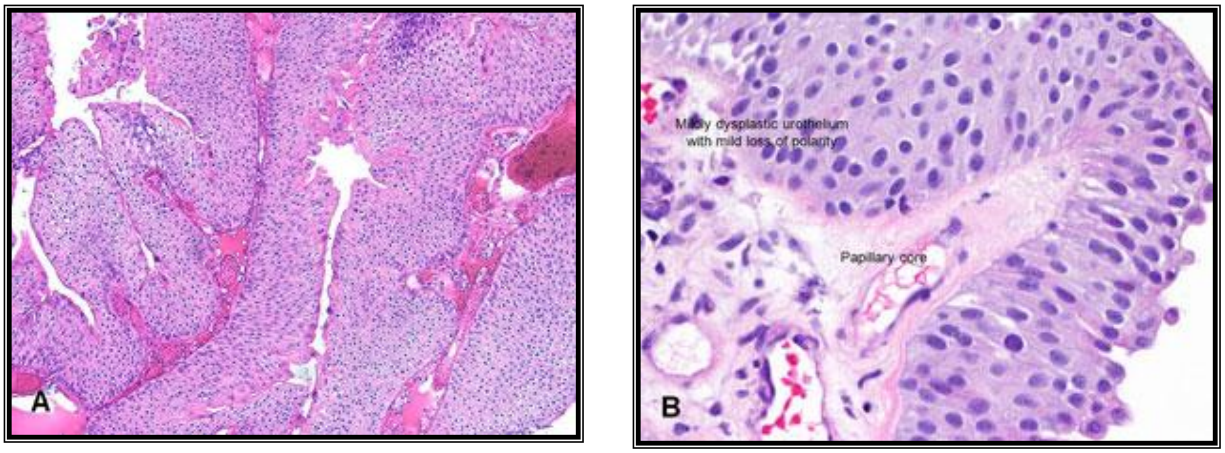

Figure (2): Low grade papillary urothelial carcinoma **(45)**

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- **Histopathology:** Papillae (with fibrovascular core) may variably exhibit branching and fusion, Mild loss of cellular polarity, Nuclear rounding, slight size variation and mildly abnormal chromatin (image A)Xmag.100 & (image B) Xmag.400

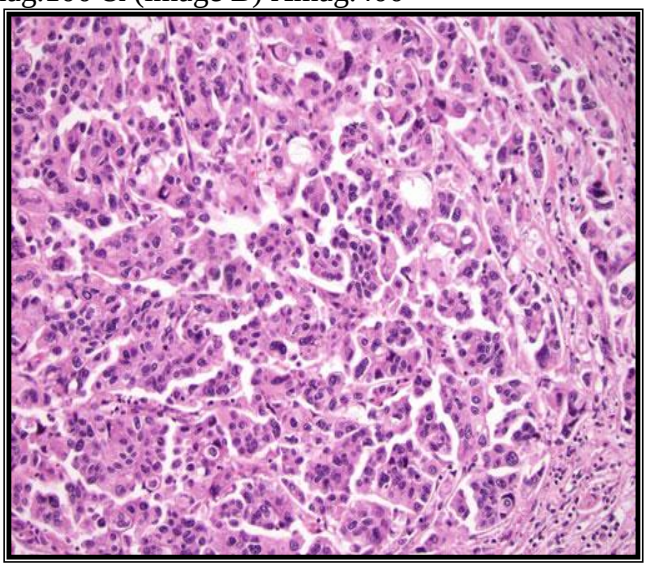

Figure (3):High grade urothelial carcinoma: Nests or sheets of atypical urothelial cells surrounded by retraction artifact occasionally, individual cells may be present. Carcinoma cells are usually medium to large in size and the cytoplasm is often pale to eosinophilic in color. Nuclear atypia varies from minimal to marked, with some tumors displaying prominent pleomorphism. Nucleoli are generally rare. Magnification x100 **(45).**

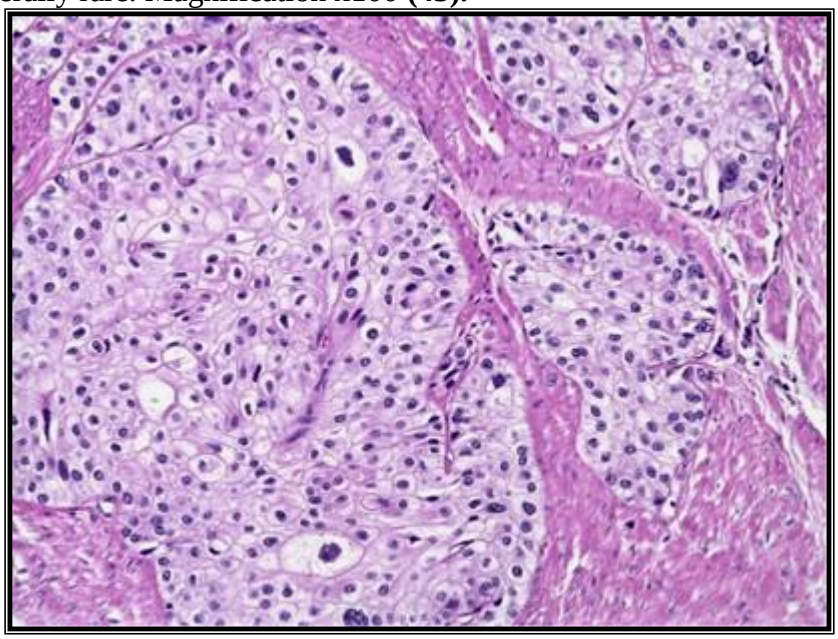

Figure (4):Invasive urothelial carcinoma. This is an example of the typical or garden variety case. Note some of the nuclei have nuclear grooves **(8)**.

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6.Variants of Urothelial Carcinoma:Invasive urothelial carcinoma with divergent differentiation:

This refers to tumors of usual type urothelial carcinoma is present along with other morphologies. Mostly in association with high-grade and locally advanced disease. This is often associated with more aggressive behaviour and the amount does not have to be extensive but should be recorded

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in the pathology report. Common divergent differentiation includes along squamous, glandular, small cell and even trophoblastic lines. **(50).**

Squamous differentiation is the most common variant in UC that is identified in up to 30% of high-grade and/or stage disease **(8,46).**

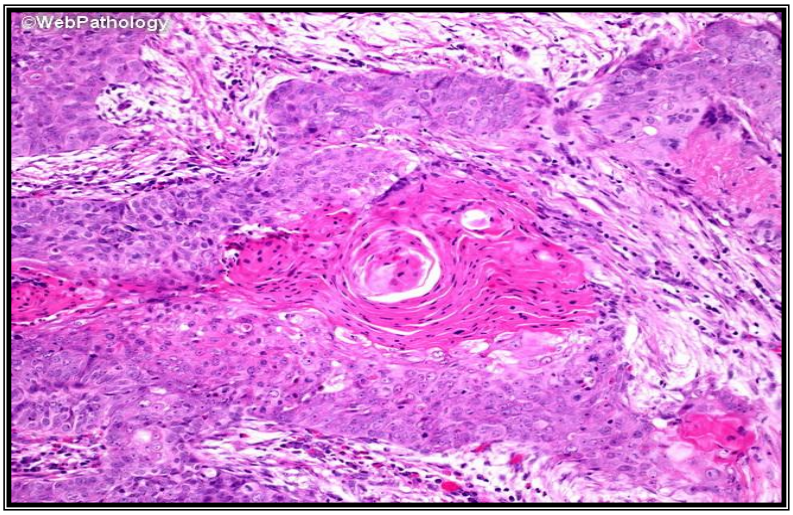

Figure (5): Squamoid differentiation in urothelial carcinoma **(46)**.

The presence of keratin pearls and copious keratin production is unequivocal for squamous differentiation in this urothelial carcinoma. Such foci comprised approximately 5% of the tumor. The presence of focal squamous differentiation in a urothelial carcinoma does not appear to have any prognostic significance**(46).**

Glandular differentiation is less common in UC and the reported incidence is variable in different studies, ranging from 8% to 18%. These tumors are characterized by the presence of high rates of Telomerase reverse transcriptase (TERT) promoter mutations. **(43, 47)**.

Glandular differentiation may have a spectrum of appearances, including colonic-type glands, glands with abundant mucin production or signet ring cells. Historically, carcinomas with glandular and/or squamous differentiation (also termed "divergent" differentiation) have been considered to have worse outcomes, but no difference in outcomes was found **(48).**

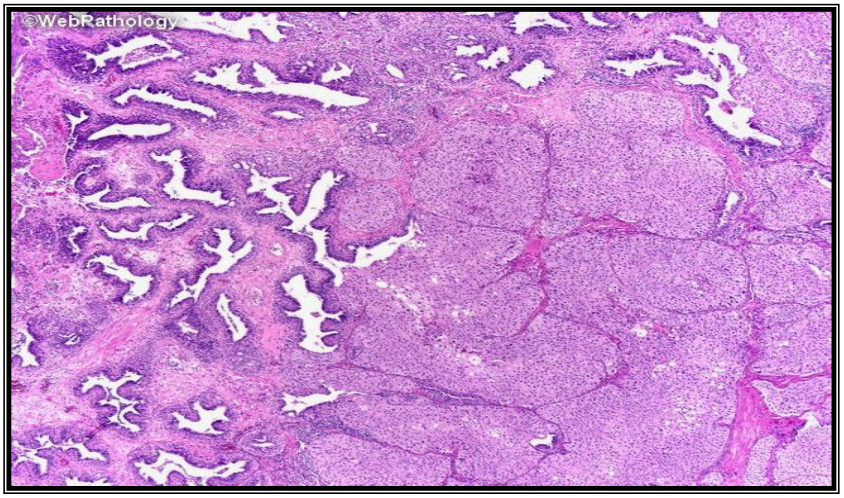

Figure (6):High-grade urothelial carcinoma with gland-like spaces. The lumina are irregular branching **(46)**

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Nested urothelial carcinoma:

This variant represents one of the most diagnostically challenging variants the carcinoma is defined by nests of extraordinarily bland urothelial cells that closely mirror normal urothelium. However, irregular nests and haphazard growth can occasionally provide clues as to the diagnosis. On superficial biopsy, this entity can closely mimic von Brunn nest proliferations. Deeper specimens often reveal an infiltrative pattern with occasional invasion into the underlying muscularis propria. Cellular atypia is also often recognized in this deeper portion of the lesion. Immunohistochemical analysis reveals similar patterns to conventional urothelial carcinoma, including strong nuclear p53 staining. Despite the small number of reported cases, this variant appears to carry a poor prognosis, with progressive disease and metastases occurring frequently **(49).**

Few molecular findings have been reported in this tumor type, the most common of which is the high rate of TERT promoter mutations that was not found in benign mimickers, as well as occasional mutations in TP53, JAK3, and CTNNB1, suggesting that this tumor likely harbors molecular alterations similar to those of UC in general **(49).**

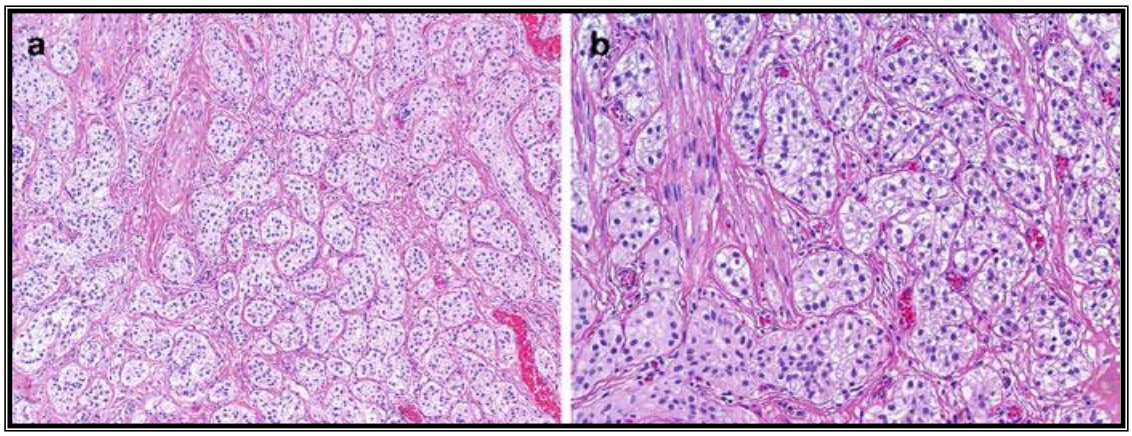

Figure (7):Nested variant urothelial carcinoma:(a, b) Nested urothelial carcinoma **(45).**

Note: the relatively uniform nuclei despite detrusor muscle invasion. This case invaded the perivesical fat and shows multiple foci of lymphovascular invasion, mag.x100 and x 200.

Small Tubular/Glandular Variant:

Some urothelial carcinomas may have an almost exclusive component of small- to medium-sized, round to elongated tubules that may be misdiagnosed as nephrogenic adenoma or cystitis glandularis.The differential diagnosis with an extension of a prostatic carcinoma is often also a consideration but easily handled by immunohistochemistry (PSA, PSAP: positive in prostate cancer; CK 20, high-molecular-weight cytokeratin; and p63: positive in more than half of urothelial carcinomas) **(8).**

The tubules of carcinoma are lined by attenuated urothelial cells in contrast to the varying admixture of cuboidal, columnar and occasionally flattened cells that line the tubules of nephrogenic adenoma. Urothelial carcinoma with small tubules may be widely invasive in spite of their deceptively bland histology. Similar to the nested variant, the chief reason for the awareness of this morphological variant of urothelial carcinoma is not to mistake it in superficial biopsies as a benign **(8).**

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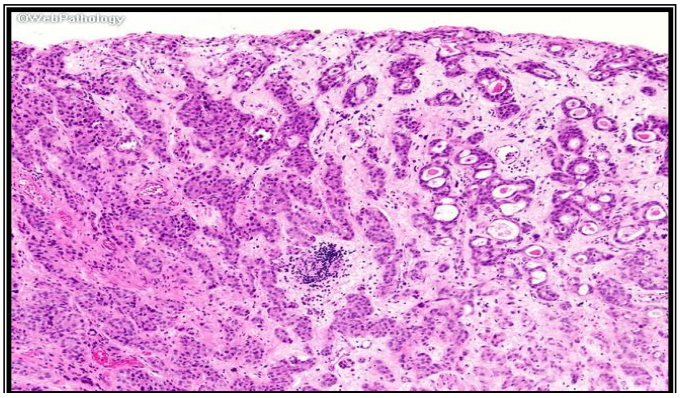

Figure (8):Urothelial carcinoma with small tubules. The left half of the image shows irregularly-shaped anastomosing nests more typical of this variant. The right half of the image shows areas of tubule formation. Some of the tubules contain eosinophilic secretion in their lumen**. (46).**

Micropapillary urothelial carcinoma:

Consist of small nests and aggregates of tumor cells where the nuclei are atypical and orientated at the periphery of the cell clusters. These tumors are commonly associated with lymphovascular invasion, present at high pathological stage and exhibit aggressive clinical behavior. It remains controversial whether this tumor should be treated differently from other high-grade locally advanced bladder cancers but most cases are treated with cystectomy as response to neo-adjuvant chemotherapy is poor **(50).**

By molecular analysis, there are consistently higher rates of erythroblastic oncogene B, a gene isolated from avian genome, also called CD340 (ERBB2) amplification reported in MPUC than in classic UC, that in some reports was additionally associated with worse outcome following radical cystectomy. In another recent study focusing on RNA expression profiling, it was reported that in MPUC there is common downregulation of miR-296 and activation of chromatin-remodeling complex RUVBL1, but it remains unclear what the exact roles are for such alterations in the development of MPUC **(50).**

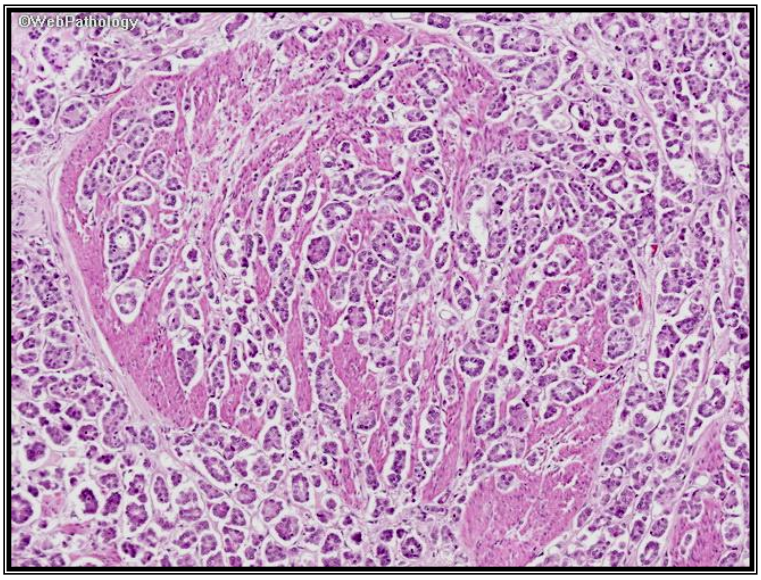

Figure (9):Micropapillary variant of urothelial carcinoma. The majority of cases of micropapillary variant present with invasion of Detrusor muscle (shown here) at the time of presentation (Stage pT2). **(46).**

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- **Microcystic Variant:**

  Comprised of small cystically dilated tubules, the microcystic variant is also diagnostically challenging and can mimic cystitis cystica. Evidence of its malignant nature is reflected in the haphazard, infiltrative pattern of the tubules and the variation in tubules size. Additional histologic features include a urothelial and glandular lining of the tubules, which may be flattened, as well as secretions that may become inspissated and appear targetoid or calcified. In general, outcomes are comparable to conventional urothelial carcinoma **(51).**

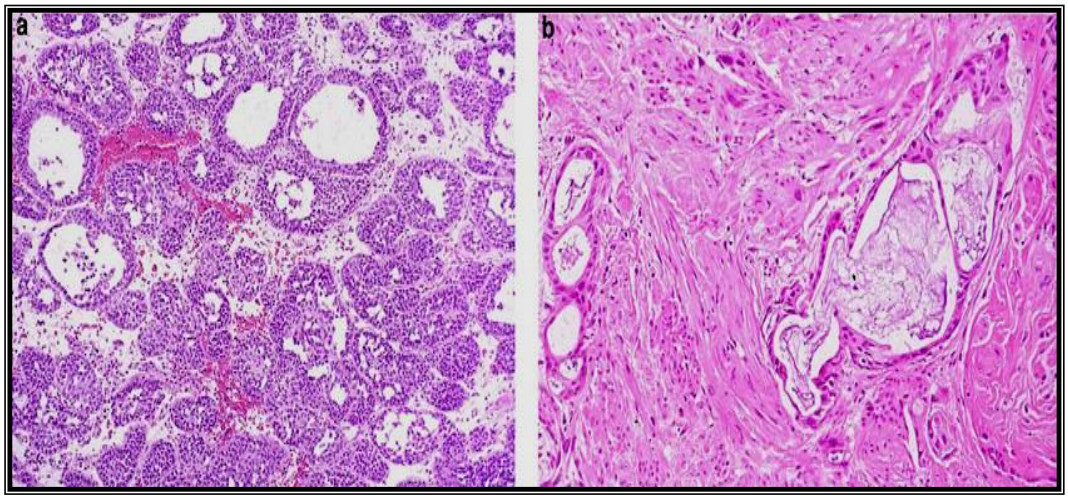

Figure (10):Microcystic urothelial carcinoma. (**a**) Urothelial carcinoma with variably sized prominent cystic change within carcinoma. (**b**) High power with 'low-grade' nuclear features and invasion into muscularis propria **(8).**

Plasmacytoid urothelial carcinoma:

A rare and aggressive variant of UC, plasmacytoid urothelial carcinoma (PUC) exhibits a diffuse and infiltrating pattern of discohesive, individual or small clusters of cells, generally with minimal stromal reaction. Tumor cells contain eccentrically located nuclei resembling plasma cells and in the vast majority of cases, tumor cells contain intracytoplasmic vacuoles that give the appearance of signet ring cells **(52).**

Clinically, PUC is characterized by advanced stage at presentation, high mortality rate, and high propensity for relapse and frequent peritoneal carcinomatosis despite sometimes the apparent initial response to chemotherapy. Recent analysis by next-generation sequencing identified the presence of CDH1 truncating mutations, and less frequently CDH1 promoter hypermethylation, as the defining feature of PUC **(53).**

Truncating somatic CDH1 mutations were identified in 84% of PUC and were specific to this histologic variant. The CDH1 wild-type PUC tumors were associated with loss of E-cadherin expression and all but one was associated with hypermethylation of CDH1 promoter. Aside from CDH1 alterations, the genomic landscape of PUC was generally similar to that of UC, not otherwise specified, with frequent mutations in genes involved in chromatin modification, cell cycle regulation, and PI3 kinase pathway **(54).**

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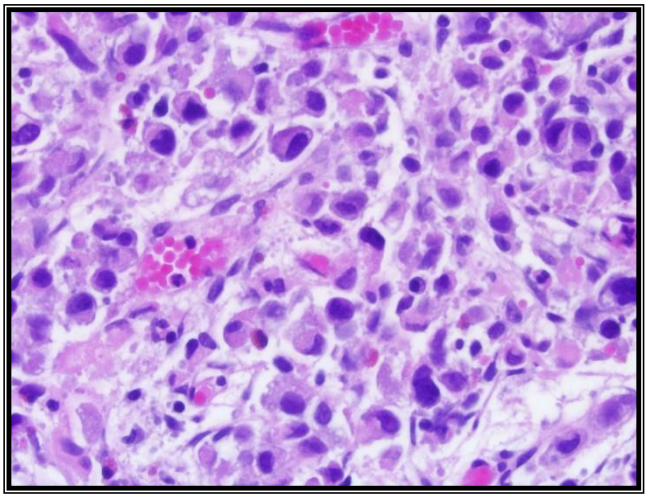

Figure (11):Plasmacytoid morphology (abundant gray cytoplasm; eccentrically placed (usually round) nucleus), +/-mucous (common), usually with conventional urothelial carcinoma **(46)**

Sarcomatoid urothelial carcinoma:

Was originally termed carcinosarcoma because of the combination of both epithelial and mesenchymal-type components. This is a rare form of bladder cancer that is typically associated with advanced stage and overall poor prognosis, in which a component of the tumor exhibits mesenchymal phenotype, most commonly in the form of spindle cell proliferation but can also be of myxoid, pseudo angiosarcomatous, and undifferentiated pleomorphic sarcoma-like morphology. The sarcomatous component can also be true heterologous elements in the form of cartilaginous, osseous, rhabdomyoblastic, or other elements **(55).**

It has been reported that the sarcomatous and urothelial components within the same tumor share common clonal origin. More recently, it has been shown that sarcomatoid UC is enriched with mutations in TP53, RB1, and PIK3CA and is associated with dysregulation of epithelial-mesenchymal transition network and overexpression of epithelial-mesenchymal transition markers **(56).**

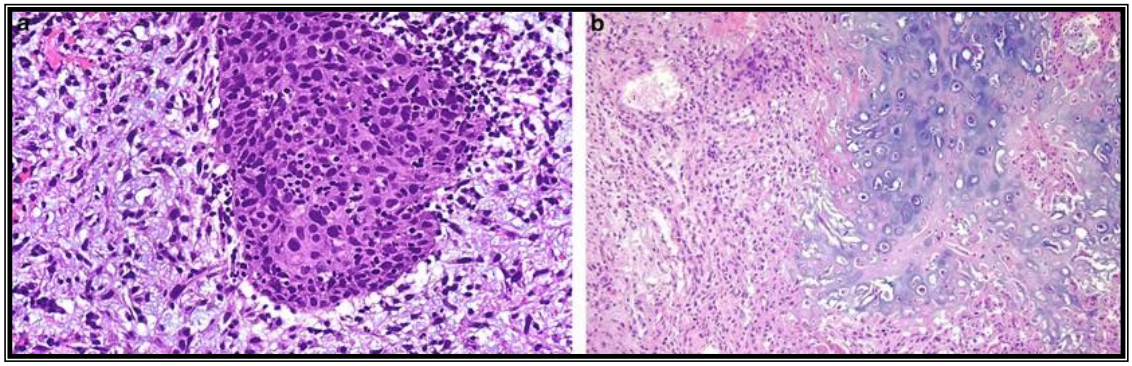

Figure (12):Sarcomatoid carcinoma of the urinary bladder. (**a**) Urothelial carcinoma and high-grade spindle cell morphology of sarcomatoid component. (**b**) Heterologous cartilaginous differentiation **(8).**

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Small cell carcinoma/ Neuroendocrine carcinoma (SmCC) of the urinary bladder:

Is a rare, aggressive tumour often present with conventional UC component (invasive or in situ) or other divergent differentiation such as squamous, glandular, and sarcomatoid, with or without heterologous element in more than 50% of cases. Its presents similarly to UC but metastasis is common and prognosis is poor. Small cell carcinoma of the urinary bladder is similar morphologically with SmCC lung but immunohistochemistry for conventional neuroendocrine markers can be variable **(8).**

Recent reports on the molecular characteristics of bladder SmCC provide significant insights into the molecular characteristics of this disease and highlight similarities and differences between SmCC of the bladder and that of the lung as well as between small cell and urothelial components within the same bladder tumors **(57).**

One of the most common findings is the near universal presence of loss of function co-alterations of TP53 and RB1, which in one study, was identified in 90% and 87% of cases, respectively (80% of tumors displayed coalterations of both genes). And even in tumors without RB1 loss of function mutations, there was loss of RB expression by immunohistochemistry, suggesting an alternative mechanism for RB loss, such as epigenetic silencing. Moreover, genes that were commonly mutated in UC were also found mutated in bladder SmCC, including TERT promoter mutations (95%) and truncating alterations in genes involved in chromatin modification such as CREBBP, EP300, ARID1A, and KMT2D in ~75% of samples. Unlike UC, there was near absence of KDM6A truncating mutations, CDKN2A deletion, and CCND1 amplifications in bladder SmCC**(57).**

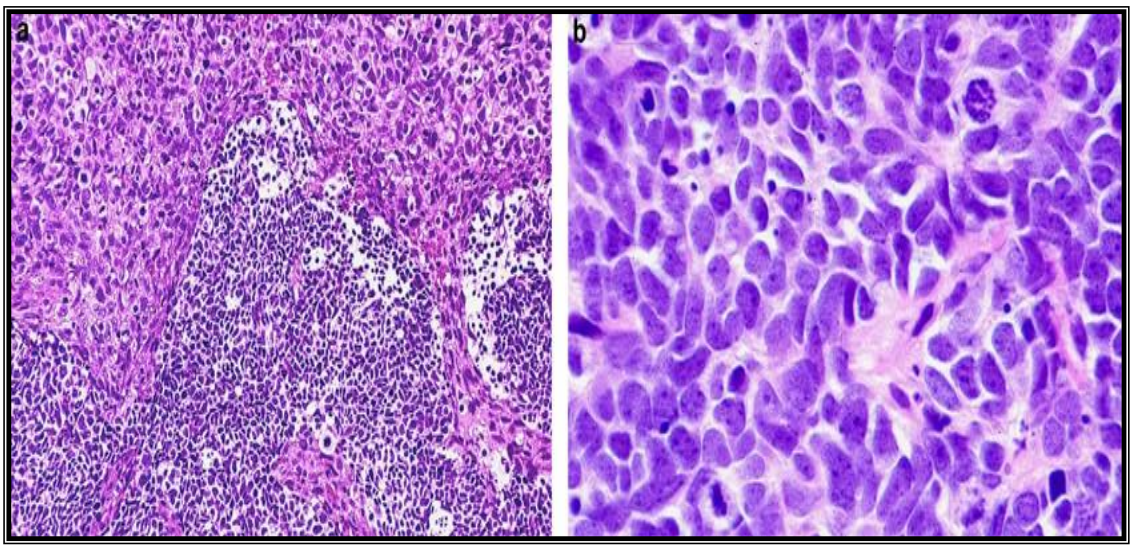

Figure (13):Small-cell carcinoma of the urinary bladder. (a) High-grade conventional urothelial carcinoma juxtaposed with small-cell carcinoma histology. (b) Typical cytological features of small cell carcinoma component **(8)**

- **Lymphoepithelioma- like carcinoma of the urinary bladder:**

Tumors with this histology are so termed because of their striking morphological resemblance to the undifferentiated nasopharyngeal carcinoma or lymphoepithelioma. The neoplastic cells are large and arranged in syncytia, with individual undifferentiated tumor cells with large, pleomorphic vesicular nuclei, prominent nucleoli and numerous mitoses; the cytoplasmic borders are most often indistinct **(58).**

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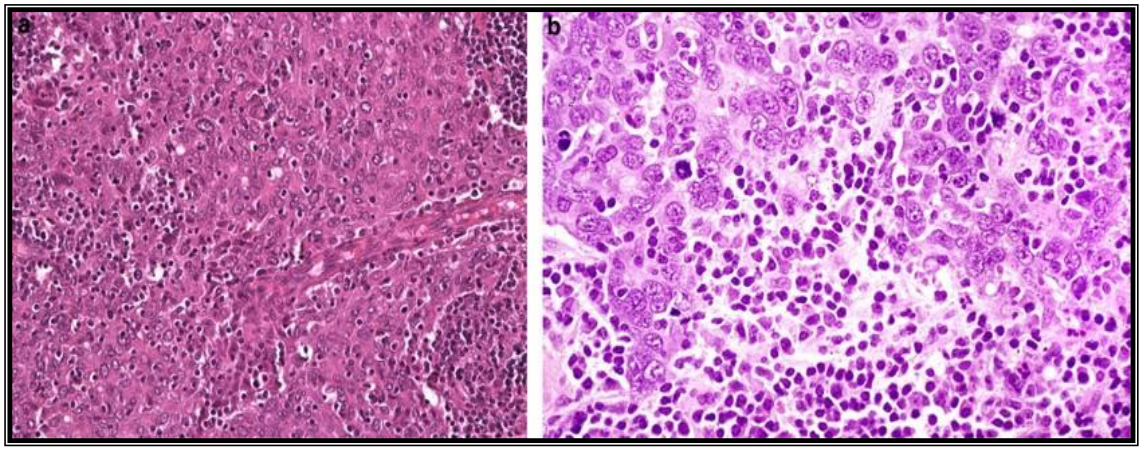

Figure (14):Lymphoepithelioma-like carcinoma of the urinary bladder. (a and b) High power of sheets of cells arranged in syncytia separated by a prominent inflammatory infiltrate. The nuclei are large and vesicular **(8).**

Urothelial carcinoma with trophoblastic differentiation:

Urothelial carcinoma with areas of trophoblastic differentiation have been reported and a small subset of these tumors had symptoms related to excess human chorionic gonadotropin production including gynecomastia. Tumors that apparently were composed solely of tissue resembling choriocarcinoma, most tumors reported in the last three decades or so have been composed of a mixture of urothelial carcinoma with trophoblastic elements **(59).**

Tumors of this type are divided into 3 categories:

1. Urothelial carcinoma with scattered syncytiotrophoblasts.
2. Urothelial carcinoma with choriocarcinomatous differentiation or pure choriocarcinoma.
3. Urothelial carcinoma with immunohistochemical expression of β-HCG, but no recognizable trophoblasts **(8).**

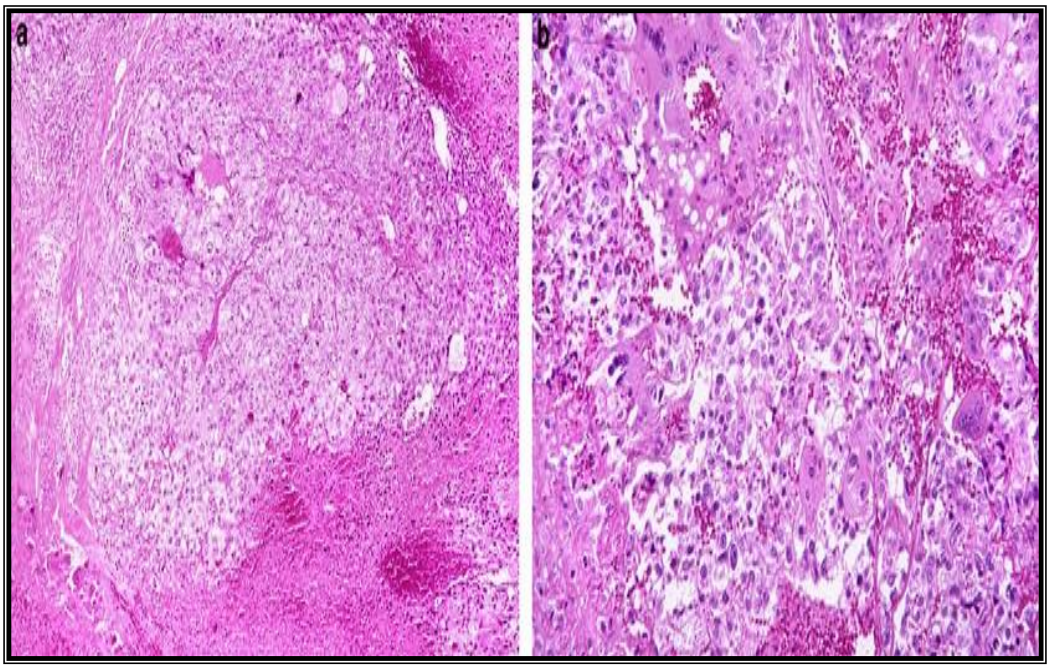

Figure (15):Urothelial carcinoma with trophoblastic differentiation. (a and b) Choriocarcinoma component with biphasic histology including syncytiotrophoblastic giant cells. (Adopted with permission from Dr Jae Y. Ro, Houston, TX, USA.) **(8)**

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- **Urothelial carcinoma with rhabdoid features:**
  Urothelial carcinomas may also rarely have focal areas showing rhabdoid features: a population of large, relatively discohesive cells with distinct cell borders, large vesicular nuclei, prominent nucleoli and eosinophilic cytoplasmic inclusions. Less than 10 cases have been reported in adults. Most cases have features of conventional urothelial carcinoma at least focally; concurrent carcinoma in situ, small-cell and sarcomatoid histology have been reported **(60).**

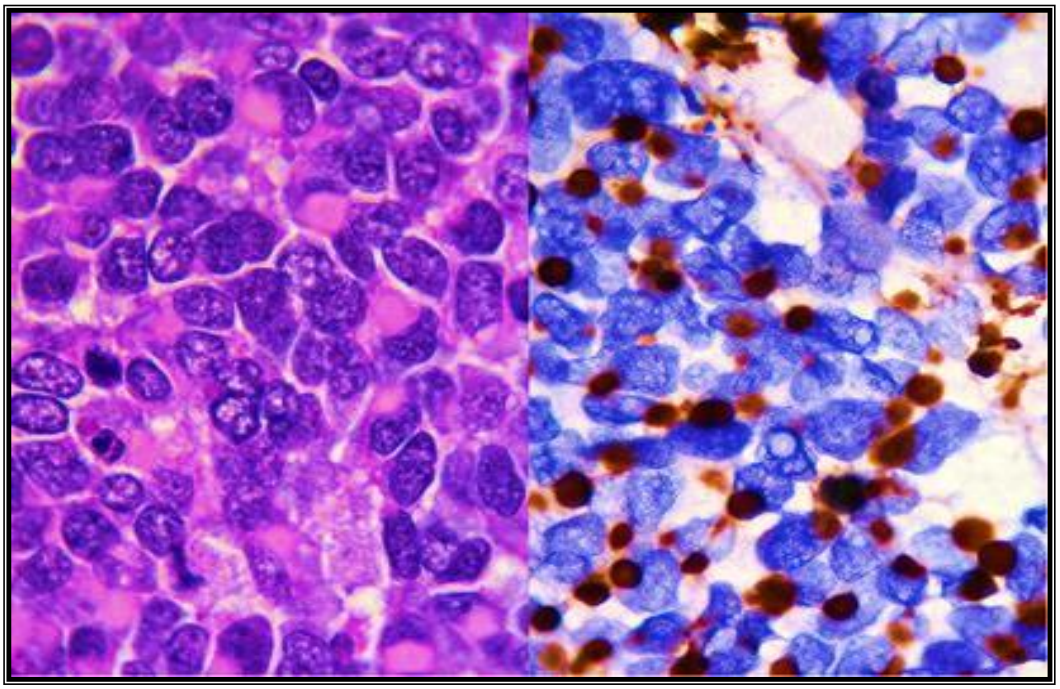

Figure (16):Urothelial carcinoma with rhabdoid features. Left: undifferentiated carcinoma with rhabdoid cells. Right: cytokeratin stain with perinuclear inclusions (Adopted with permission from Dr Anil Parwani, Pittsburgh, PA, USA**). (8)**

Urothelial carcinoma with clear cell features:

These carcinomas are typically high grade, so the differential diagnosis includes clear cell adenocarcinoma, metastatic renal cell carcinoma or prostatic carcinoma. Clear cell adenocarcinomas of the bladder more frequently occur in women and typically have a combination of morphological patterns within the same tumor including, solid, papillary or tubulo-cystic architectural arrangement; the papillae are lined by a single epithelial layer with a hyalinized core. The cytoplasm may be eosinophilic or clear and the cells may have a hob nail appearance. In contrast, poorly differentiated urothelial carcinomas with clear cell features have a more sheet-like growth pattern and often have more characteristic appearing areas, at least focally, of urothelial carcinoma with conventional morphology. Clear cell carcinomas may be positive for cytokeratin 7 and CA 125, and occasionally for cytokeratin 20 **(61).**

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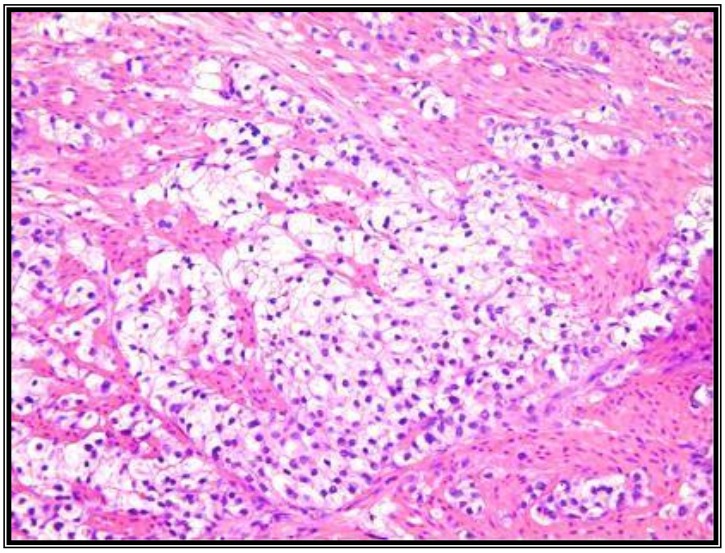

Figure (17):Urothelial carcinoma with clear cell features. Invasive carcinoma has cells with abundant clear cytoplasm. Other areas of the neoplasm had more typical morphology of urothelial carcinoma**. (8)**

Lipoid rich variant of urothelial carcinoma:

The most recent WHO classification recognizes that rare urothelial carcinomas may have large carcinoma cells with optically clear empty multivacuolated cells resembling lipoblasts. The lipid-rich cellular areas comprise from <10 to up to 50% of the tumor and, when prominent, raise the possibility of a signet ring component (glandular differentiation) or of heterologous liposarcomatous elements of a sarcomatoid carcinoma. Less than 10 cases are reported, in which the presence of lipid has not convincingly been proven by histochemistry and hence the term lipoid variant. The cells are S-100 protein-negative. The background urothelial carcinoma is invariably high grade and invasive **(51).**

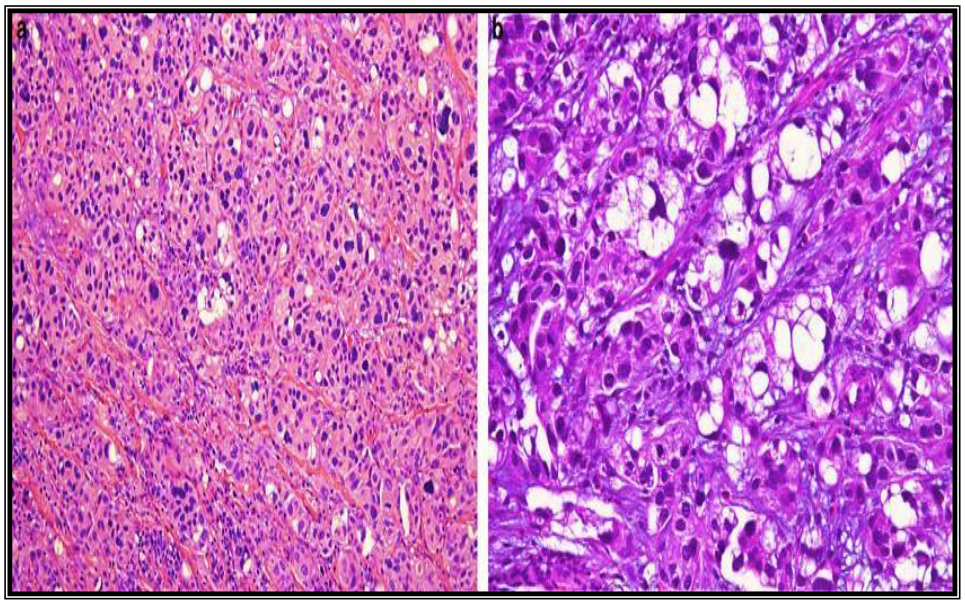

Figure (18):Lipoid-rich variant of urothelial carcinoma. (**a**) Urothelial carcinoma with cells resembling lipoblasts. (**b**) High power**. (8)**

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6.Biomarkers for Bladder Cancer Subclassification

Dadhania and colleagues performed a meta-analysis of the luminal and basal subtypes of bladder cancer in several MD Anderson and publicly available cohorts. They also validated the signature profiles of luminal and basal cancers on retrospectively collected paraffin-embedded tumor samples, as these are the types of tissue on which the standard of clinical care is based. Finally, to identify a minimal set of clinically applicable biomarkers permitting simple classification of bladder cancers into luminal and basal subtypes, they performed image-assisted analysis of selected immunohistochemical markers on parallel tissue microarrays **(62).**

The luminal cancers appeared to evolve through the papillary track while most basal forms were non papillary. The superficial papillary tumors were exclusively luminal while the invasive bladder cancers could be almost equally divided into luminal and basal types. Luminal tumors were characterized by the expression signature consisting of overexpression of E-cadherin, HER2/3, Rab-25, and Src. Basal tumors showed the expression signature similar to the basal layer of normal urothelium. **(62).**

The immunohistochemical expressions of only 2 or 3 luminal and basal markers, were enough to identify the molecular subtypes of bladder cancer with >90% accuracy. Recognition of luminal and basal/squamous types of bladder cancer by immunohistochemical staining has also been demonstrated in several other studies**(63)**

Research Group developed an immunohistochemistry (IHC)-based method for molecular subtype profiling of NMI and MI UCs. The authors also showed that molecular types such luminal and basal/squamous-like (B/SCC-like) subtypes have a major impact on progression rates **(62).**

Beyond the recognition of luminal and basal subtypes, a consensus on further sub-classification of bladder cancer has been hampered by the fact that different groups working on molecular subclassification of bladder cancer have advocated different subtypes with a variety of nomenclature **(63).**

6.1. Forkhead Box A1 (FOXA1)

FOXA1 (forkhead box A1) is the founding member of the FOX family of transcription factors that is comprised of at least 40 members. FOXA1/HNF3α (hepatocyte nuclear factor 3α), FOXA2/HNF3β and FOXA3/HNF3γ constitute the FOXA subfamily, which was originally identified for its transcriptional regulation of the genes liver-specific transthyretin (TTR) and α1-antitrypsin (Serpina1) **(63).**

FOXA1 protein is expressed in the developing and adult urothelium, whereas FOXA2 protein expression is restricted to early bladder development, where it appears to play significant role in urothelial development **(63).**

FOXA family (FOXAs) contain ~100 amino acid DNA-binding domain or FOX/winged helix domain that is highly conserved (at least 92%) within the FOXA family, and shares extremely high homology (90%) with that of its namesake, the Drosophila homologue fkh (forkhead) **(63).**

FOXA family also contains conserved nuclear localization sequences and homology in the N- and C-terminal transactivation domains. FOXAs bind as monomers to the consensus element A (A/T) TRTT (G/T) RYTY and crystallization of the DNA binding domain of FOXA3 revealed a ‘winged helix’ structure bound to DNA in a manner similar to that of linker histones **(64)**

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Unlike linker histones, FOXAs lack the basic amino acids required for chromatin compaction. Thus, FOXA binding to nucleosomes induces an open chromatin configuration enabling the recruitment of other transcriptional regulators. This function has led to FOXAs being coined as 'pioneering or 'licensing' factors. Recently, the participation of FOXA1 in chromatin remodeling has been further described, where FOXA1 binding to DNA precedes the loss of cytosine methylation and the demethylation of histone H3 lysine K4 (H3K4) during the differentiation of pluripotent P19 cell **(65).**

FOXA1 in Development

Identifying FOXA family as transcriptional regulators of hepatic specification led multiple groups to perform expression pattern analyses for these factors from early development throughout adulthood. FOXA2 mRNA is the first to be expressed during embryogenesis, and is observed during gastrulation in the anterior primitive streak and node with subsequent expression in the notochord, floor plate and gut. FOXA1 becomes detectable at the late primitive streak stage in the midline endoderm of mouse embryos, followed by expression in the ventral floorplate, notochord and gut. FOXA3 is the last to be activated, being expressed during hindgut differentiation. In the adult mouse, expression of FOXA1 and FOXA2 is observed within endoderm-, mesoderm- and ectoderm-derived tissues **(66).**

Through germ-line and conditional knockout approaches, the functions of FOXA1, FOXA2 and FOXA3 have been investigated both independently and, in combination with FOXA2, and each has been proven to be required during various aspects of development. FOXA1 null mice survive through embryogenesis, but are post-natally lethal due to severe hypoglycemia and dehydration as described in detail below. **(67).**

FOXA1 in Cancer:

In breast cancer, FOXA1 expression positively correlates with that of ER and another transcription factor, GATA3, both of which are necessary mediators of normal mammary gland development. **(68).**

FOXA1 is a critical transcription factor that is important in the development, terminal differentiation and oncogenesis of bladder urothelium. Evidence implicating FOXA1 in the regulation of urothelial differentiation came from studies by Varley et al**(68).**

FOXA1 is localized predominantly in the nucleus. FOXA1 binds to chromatinized DNA and opens the chromatin to allow binding of additional transcription factors. **(68).**

FOXA1 binding nucleosomes and it can bind to compacted chromatin that is inaccessible to the other factors and hence can be considered a subordinate pioneer factor **(69).**

6.2. Uroplakins

The apical surface of mammalian urothelium that is in contact with the urine is highly specialized, featuring two-dimensional (2D) crystals (urothelial plaques) of hexagonally packed 16-nm protein particles **(70).**

Normal urothelium, tissue specific differentiation products that have been well characterized both morphologically and, biochemically, i.e., urothelial plaques (diameter, 0.1 to 0.5, um), large numbers of which are present in the superficial plasma membrane of urothelial superficial (umbrella) cells. These plaques, which can already be detected in cytoplasmic vesicles, are

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characterized by a highly unusual membrane structure, i.e., the asymmetric unit membrane (AUM), the luminal leaflet of which is twice as thick (8 nm) as its cytoplasmic leaflet (4 nm**) (71)**
These plaques contain four major uroplakins (UPs): Ia (27 kD), Ib (28 kD), II (15 kD), and III (47 kD). Uroplakins Ia and Ib have four transmembrane domains, are 40% identical in sequence, and belong to the "tetraspanin" family **(72).**
Uroplakins II and III both have a single transmembrane domain, and they share a common stretch of 12 amino acid residues on the exoplasmic side of the transmembrane domain **(73).**
Within the urothelial plaques, these four major uroplakins are organized into two heterodimers consisting of Ia/II and Ib/III, as demonstrated by chemical cross-linkingand protein isolation **(74).**
Coexpression of uroplakins Ia plus II or Ib plus III permits the heterodimers to exit from the ER; these events suggest that the formation of specific heterodimers is a prerequisite for uroplakins to reach the cell surface **(75).**
Uroplakin III is an integral subunit of urothelial plaques, which contribute to the permeability barrier function of the urothelial apical surface, it plays a role in the formation of the urothelial glycocalyx and may interact, via its cytoplasmic portion, with the cytoskeleton (possibly with cytokeratin filaments **(76).**
The expression of UP transcripts in circulating and metastatic TCC cells was detected. Preservation of UP gene expression during malignant transformation may be exploited for the diagnosis and monitoring of patients with TCC. Furthermore, if expression of human uroplakin genes is restricted to urothelium, this opened the door to gene targeting strategies through exploitation of their regulatory elements **(75).**
UPIII is 1 of 4 membrane associated uroplakins synthesized in terminally differentiated, superficial urothelial cells. Uroplakins—proteins conserved over a broad range of mammalian species—form a plaque-like complex and function in cell adhesion and maintenance of impermeability. As the main differentiation-related membrane proteins of the urothelium, uroplakins would be expected to be downregulated during urothelial tumorigenesis. **(72).**
In general, about half of human muscle-invasive carcinomas retain UPIII expression. The general retention, albeit with some discontinuity, of UPIII expression in superficial cells seen here in urothelial neoplasms in rats and in other species suggests that urothelial neoplastic transformation does not always equate with significant downregulation of terminal differentiation products, even in advanced cases. Although downregulation is not a consistent feature, UPIII expression appears to be substantially perturbed, as aberrant or even increased expression appears to be common in urothelial carcinomas **(76).**
Overall, in many urothelial tumors, uroplakins may be redistributed rather than downregulated. Immunoreactivity of UPIII in carcinomas is an example of neoplastic expression of a highly specialized, tissue-specific differentiation marker that may occur even in advanced tumors **(72).**
The localization patterns of UPs in normal and malignant transitional epithelium reveal that these proteins are primarily associated with the free apical surfaces of transitional cells or their intracellular equivalents. The formation of such micro luminal structures (even) in some very advanced and metastatic transitional cell carcinomas, for which we have obtained strong evidence that they reflect some degree of true terminal urothelial differentiation, is highly noteworthy. Another example of such maintenance of a certain capacity for terminal differentiation is to be found in squamous cell carcinomas, which may focally express the keratinization-related

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cytokeratins. Given that UP is an apical marker, the finding of a peripheral (basal) localization pattern of UP Ill in some invasive and metastatic transitional cell carcinomas was surprising **(76).**

6.3. Cytokeratin 14 (CK14)

The urothelium is a specialized epithelium with the single role of sealing the luminal side of the urinary tract, forming the urine-blood barrier. It is a slowly cycling epithelium, which however, is able to regenerate within hours upon microbial or chemical injury. The function of the urothelium is executed by its apical or "umbrella" cell layer which is composed of terminally differentiated post-mitotic often bi-nucleated cells lining the inner surface of the urinary tract. Upon natural cycling or injury, these cells shed into the urine, but are replenished immediately. Each umbrella cell sits on top of multiple intermediate cells. Basal cells, in direct contact with the lamina propria, are the third urothelial subpopulation **(78).**

Cytokeratin is one of the three types of intermediate filaments that constituted the cytoskeleton of epithelial cells. CK-14 (high molecular weight keratin) is consistently expressed in basal cells of multilayered epithelia **(78).**

For many years, it has been postulated that urothelial stem cells able to regenerate all layers lie within the basal layer. However, with the use of lineage tracing experiments; the investigators were able to identify populations with stem cell properties. More specifically, basal cells characterized by Sonic Hedgehog expression (SHHC) give rise to all bladder layers upon chemical injury. Basal cells also express Keratin 5 (KRT5). However, this hypothesis has been challenged. Gandhi et al reported the existence of 2 distinct subpopulations: a basal SHHCKRT5C subpopulation responsible for the regeneration of the basal layer, and a SHHCKRT5¡ intermediate subpopulation that replenishes umbrella cells upon chemical injury **(78).**

The kinetics implies that the umbrella layer is initially repaired from intermediate cells right underneath. These intermediate cells are themselves replaced later on by basal cells, which explains why after repeated challenges, descendants of KRT5+ KRT14+ cells are located in the intermediate and umbrella layer. The ability of KRT14+ cells to support growth was confirmed in vitro with the use of explant cultures as well as clonogenic assays. Finally, an unbiased chemical carcinogenesis model revealed that, although expressed in a small minority, KRT14 marks the cells of origin in at least 50% of the cases**(79).**

Although KRT14-expressing cells have never been identified in the normal human urothelium, KRT14 marks cancer stem cells in human bladder cancer. On the other hand, KRT14 expression in human bladder cancer has been associated with squamous metaplasia **(79).**

Urothelium shows a propensity for squamous redifferentiation in inflammatory or regenerative states. The possibility of squamous differentiation in transitional cell carcinomas is well recognized and its presence is associated with poorer prognosis and lack of response to radiotherapy **(80)**

Altered or anomalous expression of the CKs is indicative of aberrant differentiation in the process of urothelial carcinogenesis **(80).**

In the transitional cell carcinomas, CK14 was positive in all areas of definite squamous differentiation identified morphologically, as well as in areas suggestive of squamous differentiation **(81).**

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Sonic hedgehog signaling has also been indicated to be indirectly linked to CK14 expression in bladder cancer, even though nothing has been confirmed yet. Hence, it will be important to define mechanisms underlying induction of CK14 expression in patients with bladder cancer. **(82).**
Kaplan Meier analyses of samples from two independent cohorts of patients revealed that patients with CK14+ bladder urothelial carcinomas demonstrated significantly reduced overall survival compared to patients with CD14– tumors. CK14 gene expression was a strong predictor of poor survival independent of tumor stage and grade. Furthermore, noninvasive Ta tumors with upregulated CK14 expression were positively associated with tumour recurrence and progression, and negatively associated with overall survival**(83**).

7.Conflict of Interest:

No conflict of interest.

8.References

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