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Invasive Urothelial Carcinoma: Subtypes and Divergent Differentiation

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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

TABLE 1 **List of histologic subtypes (variants) and divergent differentiation with assigned molecular subtypes**

Subtype Histology	Proposed Molecular Subtype	Prognosis
Micropapillary	Luminal	Poor
Plasmacytoid	Luminal	Poor
Nested	Luminal or basal	Variable
Microcystic	Luminal or basal	Good
Lymphoepithelioma-like	Basal	Good
Clear cell	Luminal or basal	-
Lipid rich	Luminal	-
Giant cell	-	-
Divergent Differentiation		
Squamous	Basal	
Glandular	Luminal	
Trophoblastic	-	
Other		
Neuroendocrine	Neuronal	Poor
Sarcomatoid	Basal	Poor
Poorly differentiated	-	Poor

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

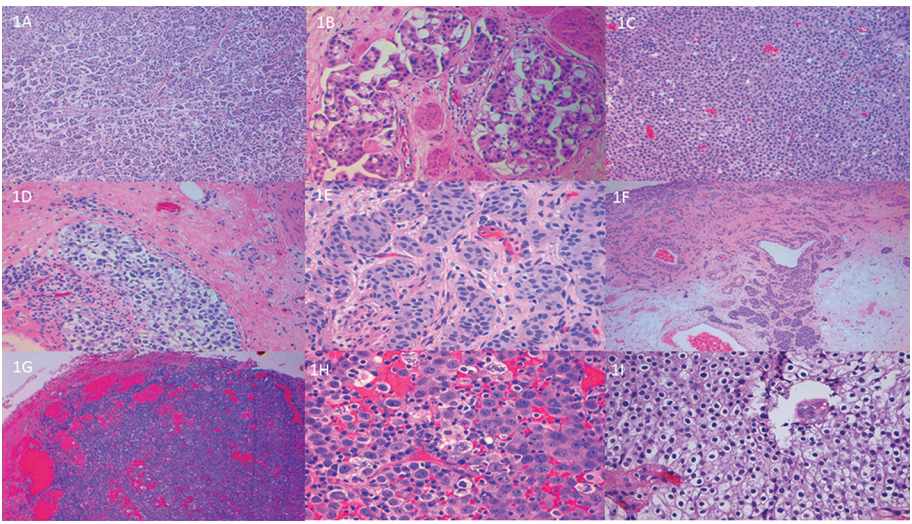

Figure 1. Patterns of urothelial carcinoma. Panel 1A low power micropapillary urothelial carcinoma with 1B highlighting cytoplasmic vacuoles and ring forms. 1C shows solid pattern of plasmacytoid urothelial carcinoma with signet forms in 1D. Small nested subtype urothelial carcinoma is present in 1E and with 1F highlighting extension deep within lamina propria. Low power (1G) and high power (1H) of lymphoepithelioma-like subtype. 1I shows medium power of clear cell subtype of urothelial carcinoma.

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

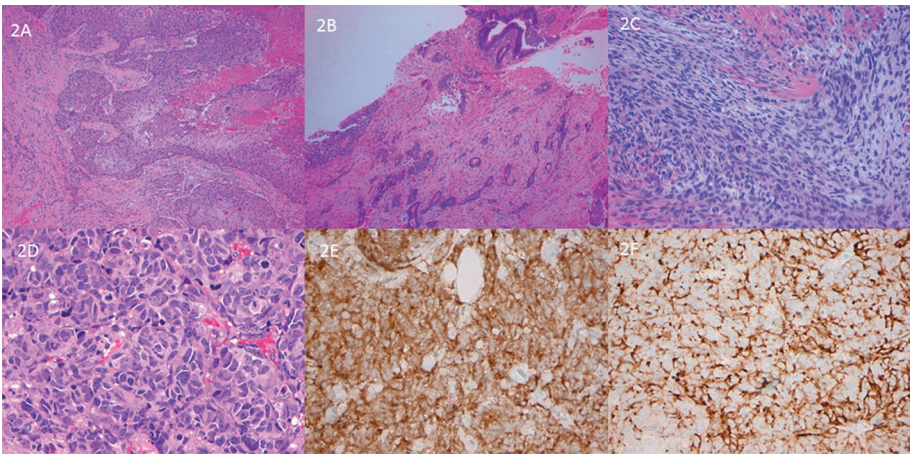

Figure 2. Divergent differentiation. Squamous differentiation (2A) and glandular differentiation (2B). Panel 2C highlights sarcomatoid features. Small cell carcinoma with nuclear moulding, hyperchromasia, mitoses (panel 2D). 2E synaptophysin and 2F chromogranin staining.

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 "drop-out" 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.

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