



Micropapillary urothelial carcinoma: is molecular hair-splitting on target?

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Urothelial carcinoma (UC) of the bladder has been well investigated in terms of pathogenesis pathways, natural history and tumor biology. Clinically relevant biomarkers including diagnostic, prognostic and predictive molecular markers have been defined in phenotype and genotype analysis beginning with the Cancer Genome atlas (TCGA) study reported in year 2014 (1). The recent 2016 WHO Classification of Tumors of the Urinary System and Male Genital Organs enumerates several histological variants such as the micropapillary, nested, microcystic, plasmacytoid, lymphoepithelioma-like, lipoid cell, clear cell, sarcomatoid and poorly differentiated types (2). Micropapillary urothelial carcinoma (MPUC), first described by Amin *et al.* in 1994 has generated considerable interest (3). This aggressive variant of UC has a characteristic morphology, aggressive clinical behavior, high propensity for metastasis to regional lymph nodes and distant organs resulting in shorter survival. Recent analysis of molecular, phenotype and microRNA (miRNA) profiles of this variant define unique features which may assist in early recognition and timely treatment (4).

MPUC has a characteristic microscopic morphology with tight small clusters of neoplastic cells in lacunar spaces lacking fibrovascular cores. Nuclei show prominent atypia, large nucleoli, eosinophilic cytoplasm and reverse nuclear polarization in micropapillary clusters with basal secretion of MUC1 (5). Ninety five percent of MPUC tumors have evidence of lymphovascular invasion (6). Heterogeneous morphology and mixed phenotypes exist. Micropapillary genotype and behavior manifests even if a small amount of micropapillary histology (>10%) is present relative to conventional UC (7). Keratin profile in micropapillary

carcinoma is similar to conventional UC (Table 1). They are more likely to express cancer antigen 125 (CA 125) indicating glandular differentiation (7). Micropapillary carcinoma also shows positive immunostaining for epithelial membrane antigen, CK7 and CK20, and CD15 (5,17). Metastases are common at the time of initial diagnosis (18). The main differential diagnosis is metastatic serous micropapillary ovarian carcinoma in women or mesothelioma. Fifty six percent of MPUC harbor human epidermal growth factor receptor 2 (HER2) gene amplification which is significantly associated with poor cancer-specific survival rates in patients (14).

Several gene profiling studies have reported different sub-categorization of UC. The current accepted grouping reported by Choi *et al.* 2014, subtypes UC by the use of molecular markers into basal and luminal types in a pattern similar to molecular subtypes of breast carcinoma. The basal subtype is characterized by high expression levels of the markers CD44, KRT5, KRT6B, KRT14. The luminal subtype is enriched for fibroblast growth factor receptor 3 (FGFR3), KRT20, HER2, FOXA1, GATA3, TRIM24, CD24, XBP1, peroxisome proliferator-activated receptor γ (PPAR γ) (19). Alterations of the Rb pathway have been noted mainly in the basal type, while the luminal subtype is characterized by *FGFR3* and *TSC1* mutations and copy number changes (20). The luminal subtype is associated with better prognosis compared to the basal subtype; however a more aggressive p53-like subset exists within the luminal signature and shows overexpression of p53 (19). MPUC variant shows 98% luminal type molecular profile (4). This variant is consistently positive for expression of

Table 1 Comparison of demographic, clinical, histological, genetic and prognostic parameters in conventional *vs.* micropapillary UC

Features	Micropapillary UC	Conventional UC	Reference
Prevalence estimates	0.7–2.2% of urothelial cancers	>90% of bladder cancers	Amin <i>et al.</i> (3); Lopez-Beltran <i>et al.</i> (2)
Age (mean)	70.6±9.3	69.6±10.9	Guo <i>et al.</i> (4)
Gender	5.1:1	3.05:1	Guo <i>et al.</i> (4)
Multifocality	58%	38%	Fairey <i>et al.</i> (8)
Carcinoma <i>in situ</i>	67%	62%	Fairey <i>et al.</i> (8)
pT stage			Wang <i>et al.</i> (9)
≤pT1	12%	34%	
pT2	21%	31%	
pT3/4	66%	35%	
Extravesical disease	66%	35%	Wang <i>et al.</i> (9)
Progression of NMIUC to MIUC	67%	<5%	Kamat <i>et al.</i> (10)
Grade			Fairey <i>et al.</i> (8)
Low grade	3%	17%	
High grade	97%	83%	
Lymph node involvement	50%	10%	Wang <i>et al.</i> (9)
Lymphovascular invasion	73%	24%	Wang <i>et al.</i> (9)
Molecular subtype	Luminal (98%)	Luminal (53%); basal (47%)	Guo <i>et al.</i> (4)
mRNA signature	GATA 3, S100P, uroplakins, ERBB2, CD24, FOXA1, XBP1, thrombomodulin, MUC1, CA125	KRT20, GATA3, uroplakins, ERBB2, ERBB3; activated wild-type <i>p53</i> gene, expression signature; CD44, CDH3, KRT5, KRT6, KRT14	Lopez-Beltran <i>et al.</i> (2); Guo <i>et al.</i> (4); Solomon <i>et al.</i> (11)
IHC profile	PPAR γ , GATA3 and uroplakin 2, CK7, CK 20, p63, HMWCK	PPAR γ , GATA3 and uroplakin 2; CK20, CK5/6, p63	Paner <i>et al.</i> (12).
Genetic profile (hierarchical cluster analysis: upregulated genes)	<i>RBM38, MRPL4, ERF, C20ORF96, NR4A1, EFNB1, TRIM29, KRT5, LY6D, IRF2</i>	<i>KCNF1, TRPV6, IGDCC3, SLC30A2, PROM1, MUC1, GDPD3, ARRB1, CLDN3, MESP1</i>	Guo <i>et al.</i> (4)
<i>TERT</i> gene	100%	66% muscle invasive; 74% non-muscle invasive	Nguyen <i>et al.</i> (13)
HER2 over-expression	56%	36%	Behzatoğlu <i>et al.</i> (14)
miRNA signature	Downregulation of miR-296 Upregulation of RUVBL1	miR-31 and 64 miR-149 (tumour progression) miR-149 (cancer-specific survival)	Guo <i>et al.</i> (4); Izquierdo <i>et al.</i> (15)
Therapy	Intravesical therapy is ineffective; p53 variant is more resistant to chemotherapy; early radical cystectomy is the treatment of choice for NMIUC and MIUC	Intravesical therapy with BCG for NMIC; neoadjuvant methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) or platinum based chemotherapy followed by cystectomy for MIUC	Knollman <i>et al.</i> (16); Guo <i>et al.</i> (4)
Median survival (months)	35.4	20.8	Guo <i>et al.</i> (4)
10-year CSS			Wang <i>et al.</i> (9)
Unmatched	31%	53% (P=0.001)	
Stage-matched	31%	40% (P=0.41)	

UC, urothelial carcinoma; NMIUC, non-muscle invasive urothelial cancers; MIUC, muscle-invasive urothelial carcinoma; BCG, Bacillus Calmette-Guérin.

markers of terminal luminal differentiation such as GATA3 and uroplakin 2, as well as PPAR γ (4).

Preliminary subtypes of UCs were first identified in TCGA study (19) in unsupervised clustering by non-negative matrix factorization of mutations and focal somatic copy number alterations (SCNAs) which identified three groups: group A, highly enriched in focal SCNAs in several genes, as well as mutations in *MLL2*. Group B, the “papillary *CDKN2A*-deficient *FGFR3* mutant,” enriched in papillary histology with loss of *CDKN2A*, and 1 or more alterations in *FGFR3* and group C, “*p53*/cell-cycle mutant,” which has *p53* mutations in nearly all samples, and enrichment for *Rb1* mutations, and amplifications of *E2F3* and *CCNE1* (20). Clusters I and II both express high HER2 levels and have an elevated estrogen receptor beta signaling signature, suggesting a relationship to HER2-positive breast cancers as well as those of luminal A breast cancer and has high expression of GATA3, FOXA1 and uroplakins. Cluster II differs from cluster I in the absence of papillary morphology or *FGFR3* events. In contrast, cluster III (“basal/squamous-like”) is similar in some respects to both basal-like breast cancer which express high levels of keratins 5, 6 and 14 (19,21). These groups are clinically relevant with basal bladder cancers carrying the poorest prognosis and shortest disease-specific survival (19,20). Pathway analysis has led to identification of Stat-3, nuclear factor- κ B, HIF-1, and p63 as probable transcriptional drivers of basal gene expression (19) and correspondingly, PPAR- γ and estrogen receptor as drivers of the luminal gene expression pattern.

Low-grade non-muscle invasive urothelial cancers (NMIUC) form approximately 70% of UC. They have a good survival, however they have a tendency to recur and hence require regular monitoring and follow-ups. On the other hand, high-grade muscle-invasive urothelial carcinoma (MIUC) progress rapidly to become metastatic and carry high mortality (19). In terms of invasive and non-invasive UC two divergent pathways of tumorigenesis in bladder cancer are either *FGFR3* mutation based or carry *p53* mutation. The key genes involved in the *FGFR3* pathway are *RAS*, *STAT1*, *PIK3* and *Cyclin D1*. These are associated with low grade lesions which carry a low risk of invasion, present at a lower stage have low risk of recurrence and progression and overall carry a good prognosis. The *p53* mutation pathway involves *Rb* gene, *p21*, *bax*, *bcl2* and *TSP1* and is seen in high grade UC and carries a high risk of invasion, tumors present at high stage, recur and progress early in the disease and carry an unfavorable prognosis. NMIUC tumors frequently exhibit *FGFR3* and *PIK3-kinase*

catalytic subunit A (*PIK3CA*) mutations, few chromosomal changes, and low mitotic rate and Ki67 activity. Low-grade non-invasive papillary carcinoma is often multifocal and tends to recur following resection, but rarely progresses to invasive disease. In contrast, micropapillary variant presents with muscle invasive disease in 95% cases (Table 1). Genetic pathways which form targets for therapy are also activated including the mitogen activated protein kinase (MAPK) pathway, phosphoinositide 3-kinase (PI3K) pathway, which affects downstream protein kinase B (AKT) and mammalian target of rapamycin (mTOR) pathways are activated in low grade UC. Upstream of the RAS protein is *FGFR3*, a tyrosine kinase receptor. *FGFR3* or *HRAS* mutations are present in almost 82% of non-muscle invasive bladder cancer. The more aggressive muscle-invasive tumors carry *p53* mutations, and show a high proliferative activity as well as signs of genomic instability. *Rb1* deletions and low expression of *CDKN2A* (*p16*) forms a parallel pathway in *p53* mutated cases. *p53* and *CDKN1A* gene generate p21 protein, a cyclin-dependent kinase inhibitor. A molecular signature combining multiple genes including *FGFR3*, *PIK3CA*, *KRAS*, *HRAS*, *NNRAS*, *p53*, *Rb1*, *CDKN2A* and *TSC1* correlates well with histologic categories, and can accurately predict whether a tumor fits into noninvasive low-grade papillary or high-grade *in situ* and invasive groups (22).

The difference and similarities in genotype and phenotype of high grade muscle invasive MPUC and UC have recently been detailed in an interesting study based at the MD Anderson Institute (4). The study compares muscle invasive micropapillary UC, conventional UC and areas of both types of tumors from same patients in two independent publicly available cohorts of conventional UC and micropapillary UC. Two distinct clusters obtained in hierarchical clustering include: cluster A containing UC exclusively and cluster B with mostly micropapillary tumors. MPUC is enriched with expression signatures involved in multiple important oncogenic pathways converging on transformation (mechanisms of cancer, mechanisms of glioma/glioblastoma, *RboA*, and *p53*), cell cycle regulation (cyclins, G1/S checkpoint), DNA damage repair (*BRCA1*), and signal transduction (ephrin signaling). It is interesting to note that a micropapillary expression signature is also present in the conventional components of the tumors that contained foci of micropapillary carcinoma (4). *TERT* promoter mutations are present in MPUC, UC with micropapillary areas and conventional UC. Mutations have been identified at positions-124

(C228T) (85%) and -146 (C250T) (12%) upstream of the *TERT* ATG start site. Concordant mutations have been identified in heterogeneous tumors with MPC and non-MPC areas as well as corresponding conventional UC (13). In view of the similarity in gene signatures within heterogeneous tumors it appears that there is a common oncogenesis origin of UC and its variant histology in individual cases. HER2 protein overexpression or gene amplification has been shown in urothelial bladder cancer. This could be helpful when using targeted anti-HER2 therapy on these tumors. Fifty six percent of MPUC showed HER2 overexpression (3+ staining) while conventional UC show HER2 overexpression in 36% cases and 50% in *in-situ* carcinoma. All low grade noninvasive tumors have been reported to be HER2 negative (14).

Studies of miRNAs in bladder cancer indicate that their specific species can be associated with bladder cancer behavior and chemosensitivity. Downregulation of miR-296 has been reported in many human cancers. It occurs in later phases of carcinogenesis and is associated with the progression to aggressive disease (23). A conclusive observation in the study by Guo *et al.* [2016] is the confirmed downregulation of miR-296 in MPUC and the over expression of *RUVBL1* (4). MiRNA-296-5p modulation was been shown to be associated with altered viability of cell lines exposed to cisplatin. This explains the chemoresistance encountered in MPUC (24). Similarly, activation of *RUVBL1* is associated with clinically aggressive disease (23). The *RUVBL1* molecule belongs to the family of AAA+ adenosine triphosphatases which are scaffolding proteins for chromatin-remodeling complexes and control diverse functions including DNA damage repair, proliferation, and invasion (23).

Outcomes of radical cystectomy for patients with MIUC are similar to those with UC when controlling for other clinical and pathologic factors (8). Conventional prognostic parameters include pathologic TNM stage, multifocality and presence of concurrent carcinoma *in situ*, lymphovascular invasion, histologic grade and adjuvant chemotherapy. Survival analysis using micropapillary gene expression signature with hierarchical clustering shows aggressive behavior is associated with micropapillary tumors as compared to conventional UC (Table 1). The so-called superficial micropapillary carcinoma, which is a high grade MPUC in stage T1, should be offered aggressive therapy instead of intravesical immunotherapy to improve long-term survival (25). The classical morphology and molecular events allow early detection of even a 10%

surface micropapillary component and hence MPUC can be detected at an early stage. Prognosis is also related to the proportion and location of the micropapillary component, with higher risk in cases with extensive micropapillary component (7). The p53-like type forms a bad prognosis group with response rates of 45% as compared to 66% in the luminal group, but the difference is statistically insignificant. A small percent of cases with a micropapillary signature exist within a genomically unstable group that overlaps with the luminal and p53-like categories (4). Bacillus Calmette-Guérin (BCG) treatment does not appear to be effective in non-muscle invasive MPUC which progresses in 67% of patients despite intravesical therapy as compared to a progression rate of less than 5% in non-muscle invasive conventional UC (10). Radical cystectomy is hence recommended by some urologic oncologists for even superficial MPUC while others have supported neoadjuvant chemotherapy followed by early cystectomy (6). However concern has been raised related to a potential poor response to cisplatin based neoadjuvant chemotherapy in MPUC (12), a fact explained at MiRNA level by the upregulation of *RUVBL1* (4). The so-called p53-ness in MPUC has also been associated with chemoresistance to cisplatin-based neoadjuvant chemotherapy (19).

In view of the key molecular pathways activated in MPUC and UC, potential therapeutic targets and drug interventions include HER2, epidermal growth factor receptor, fibroblast growth factor receptor, programmed cell death ligand 1 (PDL1) and programmed cell death protein 1 (PD1), vascular endothelial growth factor receptor (VEGFR) and vascular endothelial growth factor (VEGF). The receptor tyrosine kinase (RTK)/RAS pathway involved in cell cycle signalling is altered in 44% of tumours and tyrosine kinase inhibitors may form a treatment modality (1,22). Genes involved in regulating chromatin, the structure of DNA and proteins that makes up chromosomes, are frequently mutated and represent novel targets for bladder cancer (1,22). It seems hair splitting of UC into variants with specific molecular signatures will help define targets for therapy. It is an exciting time of translation from bench to bedside in cancer therapeutics and molecular pathologists have the potential to be the guiding hand in determining optimal treatment regimen for patients.

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