Immunohistochemical classification using urothelial differentiation markers can stratify prognosis in patients with muscle invasive bladder cancer

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ABSTRACT

Background: Recent genomic studies have revealed that muscle invasive bladder cancer (MIBC) can be classified into intrinsic molecular subtypes. In breast cancer, immunohistochemical (IHC) determination of subtypes is used to guide clinical decision making. Here, we studied IHC classification of MIBC using urothelial differentiation markers.

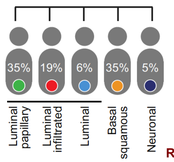
Methods: The study population consisted of 115 patients with MIBC treated with radical cystectomy without neoadjuvant chemotherapy and 10 samples of normal urothelium. The whole sections of MIBC were stained for Uroplakin3 (UPK3), GATA3 and cytokeratin5/6 (CK5/6) by IHC, which were representative molecular markers for the intrinsic subtypes. The definitions of positivity were any cancer cell staining for UPK3, over 20% of cancer cell staining for GATA3, all layers staining for CK5/6.

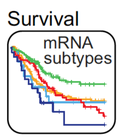
Results: In normal urothelium, UPK3 expression was observed only in umbrella cells, while CK5/6 expression was detected only in the basal layer. GATA3 expression was detected in almost all layers but its expression was higher in intermediate cells. Positive staining for UPK3, GATA3, CK5/6 was detected in 30 (26%), 92 (80%) and 37 (32%) of all MIBC cases, respectively. The positive rate for UPK3/GATA3/CK5/6 was 53/100/9% in papillary morphology (n=34), 11/67/46% in nodular morphology (n=70), 31/88/23% in pure urothelial carcinoma (UC; n=93), and 6/39/83% in UC with squamous differentiation (n=18), respectively. Patients with UPK3- (P=0.0024), GATA3- (P<0.0001) and CK5/6+ (P=0.0008) and had significantly worse prognosis than those with UPK3+, GATA3+, CK5/6-, respectively. 29/30 (97%) cases of UPK3+ were GATA3+, and UPK3+ and GATA3+ were correlated with CK5/6- (P=0.0027, P<0.0001), suggesting that GATA3+ had overlap with UPK3+ and was nearly mutually exclusive for CK5/6+. From these results, we classified three groups; differentiated (UPK3+), intermediate (UPK3-/GATA3+) and basal (GATA3-/CK5/6/+). The rate of histological grade 3, stage 3-4 and CSS at 5 years in differentiated, intermediate and basal group were 44/58/79% (P=0.065), 30/46/79% (P=0.0041), 96/65/25% (P<0.0001), respectively. In multivariate analysis, IHC classification, stage and tumor morphology were independent prognostic factors for poor prognosis.

Conclusion: Simple IHC classification using urothelial differentiation markers can improve stratification of prognosis in MIBC, which may be useful in routine clinical practice.

BACKGROUND

- Updated TCGA molecular characterization of MIBCs suggested 5 subtypes.





Robertson et al., 2017, Cell

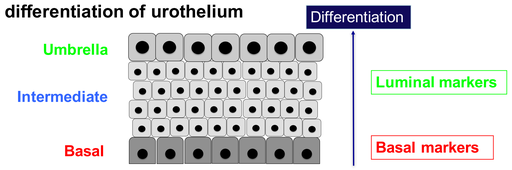
5 subtypes:
- Different clinical and pathological characteristics
- Different prognosis
- Different potential treatments

- IHC classification of intrinsic molecular subtypes in Breast cancer

Subtype	IHC criteria	Treatments	Differentiation
Luminal A	ER+,PGR+,Ki67-	Endocrine therapy	Luminal
Luminal B	ER+,PGR+,Ki67+	Endocrine + chemotherapy	Luminal
HER2 Amplification	HER2+	Anti HER2 therapy	Luminal
Basal like	ER-,PGR-,HER2-	Chemotherapy	Basal

Subtypes are shared with normal breast epithelial cells at different stages of differentiation and therapeutic strategy

- The differentiation of urothelium



Normal urothelium has three levels of differentiation

MATERIALS & METHODS

- 115 patients with MIBC treated with radical cystectomy without neoadjuvant chemotherapy
- Immunohistochemistry (IHC) on whole sections in radical cystectomy specimens

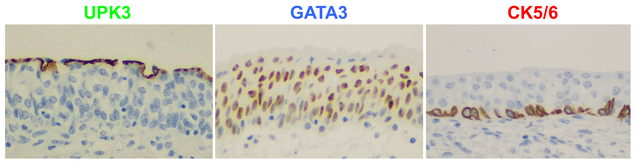
Antibody	Dilution	Pretreatment	Definition of positive stain
Uroplakin 3 (UPK3)	1:1	Proteinase K	Any staining cancer cells
GATA3	1:200	Citrate buffer	>20% staining of cancer cells
Cytokeratin 5/6 (CK5/6)	1:200	Citrate buffer	Staining of all layer

OBJECTIVES

To establish simple IHC classification, we examined the expression and localization of the common IHC markers of urothelial differentiation, Uroplakin 3 (UPK3), GATA3 and Cytokeratin5/6 (CK5/6) on whole sections of MIBCs treated with radical cystectomies.

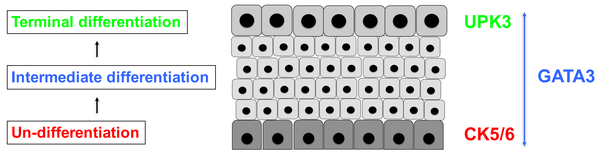
RESULTS

Markers expression in non-cancerous urothelium



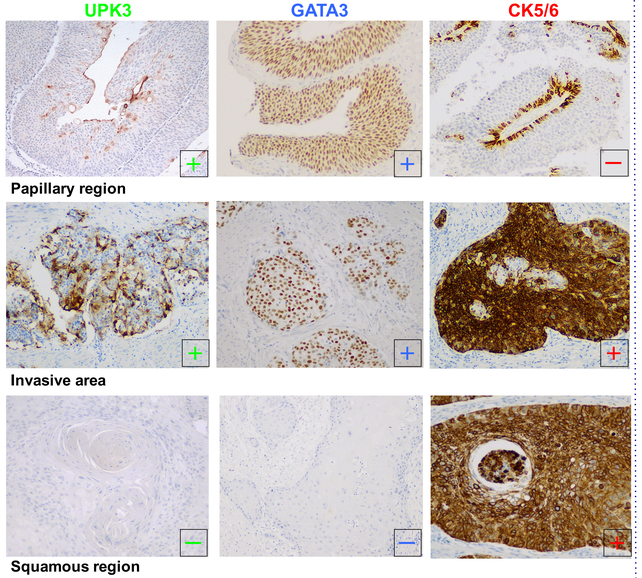
UPK3 stained only umbrella cells.
GATA3 stained almost all layers but its expression was higher in intermediate cells.
CK5/6 stained only basal cells.

The differentiation of urothelium



The expression of UPK3/GATA3/CK5/6 represents urothelial differentiation

Markers expression in non-cancerous urothelium



The definition of the positivity:

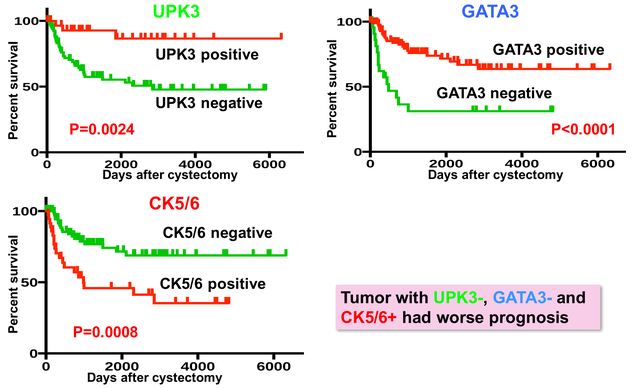
UPK3	GATA3	CK5/6
Any cancer cell staining	>20% staining of cancer cells	Staining of all layers
30/115 cases (26%)	92/115 cases (80%)	37/115 cases (32%)

The correlations between three markers

		UPK3 positive	GATA3 positive	CK5/6 positive
UPK3	positive		29/30 (97%)	3/30 (10%)
	negative		63/85 (74%)	34/85 (40%)
GATA3	positive	29/92 (32%)		18/92 (20%)
	negative	1/23 (4%)		19/23 (83%)
CK5/6	positive	3/37 (8%)	18/37 (49%)	
	negative	27/78 (35%)	74/78 (95%)	

Most UPK3+ cases were GATA3+, and both markers were almost mutually exclusive with CK5/6+

The role of markers expression in prediction of CSS



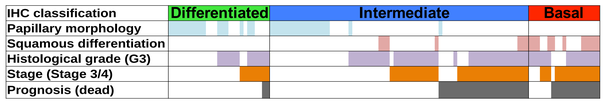
Tumor with UPK3-, GATA3- and CK5/6+ had worse prognosis

Subclassification of differentiation status

Marker	Differentiation	Subtype
UKP3+	Terminal - differentiation	Differentiated
UPK3- and GATA3+	Intermediate - differentiation	Intermediate
GATA3- and CK5/6+	Un - differentiation	Basal

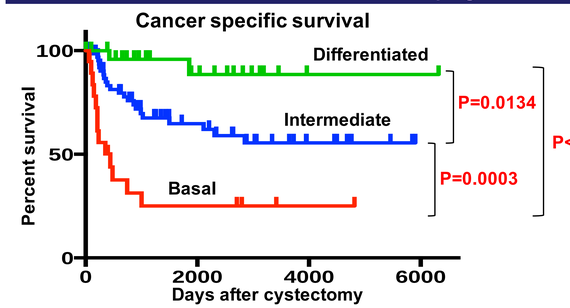
There were 3 cases with positive staining with both UPK3 and CK5/6, which demonstrated that more than 90% of cancer cells stained with CK5/6 in all layer and less than 3% of cancer cells stained with UPK3. This happened in all 3 cases, suggesting heterogeneity; therefore, we classified these 3 cases into basal group because of dominant expression of CK5/6.

	Differentiated group (n=27)	Intermediate group (n=69)	Basal group (n=19)	P value
Age				
Younger than 75 or 75	19 (70%)	37 (54%)	11 (58%)	0.3264
Older than 75	8 (30%)	32 (46%)	8 (42%)	
Gender				
Male	21 (78%)	53 (77%)	12 (63%)	0.4403
female	6 (22%)	16 (23%)	7 (37%)	
Tumor morphology				
Papillary	16 (59%)	18 (26%)	0 (0%)	<0.0001
Nodular /flat	11 (41%)	51 (74%)	19 (100%)	
Histological classification				
Urothelial carcinoma	27 (100%)	58 (84%)	8 (42%)	<0.0001
UC with squamous diff.	0 (0%)	7 (10%)	11 (58%)	
Other variants	0 (0%)	4 (6%)	0 (0%)	
Histological grade				
Grade 2	15 (56%)	29 (42%)	4 (21%)	0.065
Grade 3	12 (44%)	40 (58%)	15 (79%)	
Pathological stage				
Stage 0/1/2	19 (70%)	37 (54%)	4 (21%)	0.0041
Stage 3/4	8 (30%)	32 (46%)	15 (79%)	



Differentiated : Papillary, Pure UC, low grade, low stage
Intermediate : between differentiated and basal
Basal : Nodular, Squamous, high grade, high stage

IHC classification: characteristics and prognosis



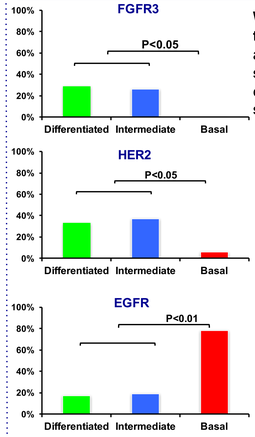
IHC classification stratified patients characteristics and prognosis

Multivariate analyses to prognosis in IHC classification

Factors	HR	95% CI	P value
IHC classification			0.0102
Basal vs Intermediate	3	1.22-7.23	0.0169
Basal vs differentiated	8.66	1.96-61.5	0.0032
Intermediate vs Luminal	2.89	0.82-18.3	0.1055
Papillary morphology	0.14	0.01-0.83	0.028
UC with squamous diff.	0.6	0.22-1.56	0.2983
Histological grade 3	0.55	0.24-1.30	0.1666
Stage 3/4	6.38	2.53-19.1	<0.0001

IHC classification was an independent prognostic factor for poor prognosis

Correlation between therapeutic target and IHC classification



We selected FGFR3, HER2 and EGFR given the fact that these are cell surface proteins and have been areas of interest in several therapeutic approaches such as small molecules, antibody and antibody-drug conjugates; and studied their expressions in different subgroups based on IHC classification

- Fibroblast Growth Factor Receptor 3 is a rational therapeutic target in bladder cancer. KM Gust and PC Black et al, Mol Cancer Ther. 2013
- Targeting HER2 with T-DM1, an Antibody-cytotoxic Drug Conjugate, is effective in HER2-overexpressing bladder cancer. T Hayashi and PC Black et al, J Urol. 2015
- Sensitivity to Epidermal Growth Factor Receptor inhibitor requires E-Cadherin expression in urothelial carcinoma cells. PC Black et al, Clin Cancer Res 2008

The expression of therapeutic targets was correlated with IHC classification

CONCLUSIONS

- IHC classification with three markers of urothelial differentiation may correlate with clinical and pathological characteristics and can be used in prediction of prognosis in patients with bladder cancer
- Although further validation is necessary to validate our findings, this simple classification has the potential to be easily adopted in routine clinical practice and helps guide physicians to decide the better treatment options

COI Disclosure Information: Tetsutaro Hayashi
I have no financial relationships to disclose