Immunohistochemistry in the diagnosis of bladder cancer

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- Always interpret immunohistochemistry findings in the clinical/radiological and morphological (macroscopic and microscopic) context of the case, never in isolation
- Do not use a single marker in isolation: use a panel
- Immunohistochemistry must be used with knowledge of the sensitivity and specificity of each marker
- Morphology is of primary importance in diagnosis; immunohistochemistry may add nothing, yield results that are confusing or, in some cases be very helpful!

Overview

- Flat lesions
- Tumour variants
- Involvement by tumours other than of bladder origin
- Recognition of bladder cancer at distant sites
- Spindle cell lesions
- Mimics
- Staging

Immunohistochemistry aids in assessment of urothelial dysplasia/CIS

- Harnden P *et al.* Cytokeratin 20 as an objective marker of urothelial dysplasia. Br J Urol 1996;78:870-75.
- McKenney JK *et al*. Discriminating immunohistochemical staining of urothelial carcinoma in situ and non-neoplastic urothelium. An analysis of cytokeratin 20, p53 and CD44 antigens. Am J Surg Pathol 2001;25:1074-78
- Mallofré C *et al*. Imunohistochemical expression of CK20, p53 and Ki-67 as objective markers of urothelial dysplasia. Mod Pathol 2003;16:187-191
- Hodges KB *et al*. Urothelial dysplasia and other flat lesions of the urinary bladder: clinicopathologic and molecular features. Hum Pathol 2010;41:155-162 [review]

Reactive atypia versus dysplasia/CIS

	CK20	CD44s	p53	MIB-1
Normal	Umbrella cells	Basal/ parabasal	Focal/-ve	<10% basal
Reactive	Umbrella cells	Full thickness	Focal	Slight increase
Dysplasia/ CIS[1]	Increased/ full thickness[2]	Basal or -ve	Marked increase	Moderate to marked increase

[1]These are distinguished primarily by morphology, not by immunohistochemistry
[2]In approximately 85% of cases of CIS

Dysplasia versus urothelial carcinoma in situ (CIS)

- Distinguished by morphology, <u>not</u> by CK20 immunohistochemistry
- Dysplasia has less nuclear hyperchromasia and/or loss of polarity than CIS
- <u>Both</u> dysplasia and CIS may show full thickness CK20

Morphologic expressions of urothelial carcinoma in situ. A detailed evaluation of its histologic patterns with emphasis on carcinoma in situ with microinvasion

McKenney JK *et al.* *Am J Surg Pathol* 2001;25:356-362

Cytological changes (CIS)

- Nucleomegaly
- Nuclear pleomorphism
- Irregular nuclear membranes
- Hyperchromasia
- Irregular chromatin distribution
- Macronucleoli
- Round nuclear shape
- Mitoses in mid to upper urothelium

Architectural changes (CIS)

- Loss of the normal perpendicular orientation of the urothelial cells to the basement membrane (polarity)
- Nuclear crowding with overlapping of nuclei
- Irregular thickness
  - hyperplastic
  - attenuated
  - denuded

Five major patterns of urothelial carcinoma in situ

McKenney JK *et al.* 2001

- Large cell CIS with pleomorphism
- Large cell CIS without pleomorphism
- Small cell CIS*
- Clinging CIS
- Cancerisation of urothelium
  - Pagetoid
  - Undermining or overriding

*not meant to imply neuroendocrine differentiation

Other *potential* markers in differential diagnosis of flat urothelial lesions:

- P16^{ink4} Yin M *et al.* *Hum Pathol* 2008;39:527-535
- CK5/6 Belanger *et al.*; *Yu W et al.* [x 2 USCAP abstracts 2012]; Edgecombe A *et al.* Appl Immunohistochem Mol Morphol 2012;20:246-71.
- HER2 Gunia S *et al.* Anat Pathol 2011;136:881-888; Jung *et al.*[USCAP 920, 2013]
- H3 (PPH3) Gunia S *et al.* J Clin Pathol 2012;65:715-20
- IMP3 (KOC) Li L *et al.* 2008 Hum Pathol 2008;39:1205-11
- Racemase (AMACR) Aron *et al.* [USCAP abstract 2012]
- SPINK1 Khani F *et al.* [UCSCAP abstract 2012]; McDaniel et al [USCAP 2013 – lacks sensitivity]

P16^{ink4} immunoreactivity is a reliable marker for urothelial carcinoma in situ Yin M *et al.* *Hum Pathol* 2008;39:527-535

- Normal urothelium showed weak cytoplastic p16^{ink4} expression
- Reactive atypia showed loss of expression in 38% cases
- Strong p16^{ink4} was seen in 100% of cases of urothelial carcinoma in situ (nuclear and/or cytoplasmic +ve)
- FISH demonstrated frequent loss of chromosome 9p21 or polysomy of chromosome 9 in malignant cells in carcinoma in situ
- Increased p16 expression was also seen in most invasive urothelial carcinoma but did not correlate with grade or stage

Cytokeratin 5/6 distinguishes reactive urothelial atypia from carcinoma in situ and non-invasive urothelial carcinoma

Belanger EC *et al.* *Mod Pathol* 2011;24 (Suppl 1) 190A

- Diffuse strong CK5/6 positivity was seen in all 20 cases of reactive atypia (these cases were p16 and CK20 –ve except for umbrella cell staining for CK20)
- CK5/6 showed no or only basal layer staining in CIS
- CK20 was strongly +ve (full thickness) in 85% of 20 cases of CIS
- p16 was strongly positive in 90% cases of CIS

Scoring the percentage of Ki-67 positive nuclei is superior to mitotic count and the mitosis marker H3 (PHH3) in terms of differentiating flat lesions of the bladder mucosa

Gunia S *et al.* J Clin Pathol 2012;65:715-20

- 32 CIS and 31 (dyplasia or reactive atypia)
- 16% or more Ki67+ve nuclei using MIB1 favoured CIS
- Less than 15% favoured dysplasia or reactive atypia
- Should be used as part of a panel rather than in isolation

Ketamine cystitis as a mimic of carcinoma *in situ*

Oxley JD *et al* *Histopathology* 2009;55:705-708

- Ketamine is an anaesthetic agent which is also in use as a recreational drug
- Produces cystitis with ulceration, eosinophils and cytological atypia mimicking urothelial CIS
- p53 and MIB-1 may be increased but CK20 always negative
- Suspect possibility in a young individual and enquire about history

Urothelial carcinoma - immunophenotype

- Uroplakin III -/+
- Uroplakin II +/-
- GATA3 +
- CK20 -/+
- S100P +
- Thrombomodulin +/-
- p63 +/-
- CK7 +/-
- 34ßE12 +/-
- CK5/6 +/-
- [PAX8 -/+]

A newly developed anti-uroplakin II antibody with increased sensitivity in urothelial carcinoma of the bladder [Hoang L *et al.* USCAP abstract 901; 2013]

- 78% urothelial carcinoma cases +ve across all grades (compared with 56% +ve for uroplakin III)
- Specific when compared with various tissues, incl. prostate and renal carcinoma

Immunohistochemical panel to identify the primary site of invasive micropapillary carcinoma

Lotan TL *et al. Am J Surg Pathol* 2009;33:1037-41

- Panel of 11 markers tested for ability to distinguish micropapillary carcinoma of bladder, lung, ovary and breast
- Best panel was uroplakin (+ve only in bladder), CK20 (+ve in bladder and some lung), ER (+ve in breast and/or ovary), WT1 and PAX-8 (both found only in ovarian in this study) Note that PAX8 positivity reported in 12-20% of urothelial carcinoma of upper tract, not selected for micropapillary type (Hughes J; Schwartz J x2 USCAP abstracts 2012)
- Pan uroplakin was positive in 92% of bladder micropapillary carcinoma

HER2 gene amplification occurs frequently in the micropapillary variant of urothelial carcinoma: analysis by dual color *in situ* hybridisation

Ching CB *et al.* *Mod Pathol* 2011;24:1111-19

- 68% of 19 cases of micropapillary urothelial carcinoma had 2+ or 3+ immunohistochemistry for HER2 protein
- Gene amplification was present in 42% of 19 cases with 100% correlation with 2+ or 3+ protein expression
- 53% of samples had aneusomy of chromosome 17 (*HER2* is at 17q11-21)
- Previous investigations on conventional urothelial carcinoma found an inconsistent and often low frequency of *HER2* gene amplification with no strong correlation between protein expression and gene amplification

Her2 amplification distinguishes a subset of non-muscle-invasive bladder cancers with a high risk of progression Chen PC-H *et al.* *J Clin Pathol* 2013;66:113-119

- No PUNLMPs or Ta/T1low grade urothelial carcinomas has Her-2 amplification
- 9% of Ta/T1 high grade urothelial carcinomas had Her-2 amplification
- Recurrence and progression were significantly higher in high grade cases with amplification compared with those without

GATA3 and placental S100 (S100P)

Higgins *et al. Am J Surg Pathol* 2007;31:673-80

GATA3

- Positive (nuclear) in 67% urothelial carcinoma (n=300)
- Negative in all cases of prostatic adenocarcinoma (n=256) and in all renal cell carcinoma (n=133) [positive in basal cells in benign prostate]
- Positive in many breast cancers
- Positive in normal T lymphocytes (regulator of T cell development)

S100P

- Positive in 78% urothelial carcinoma (cytoplasmic +/- nuclear)
- Positive in 2% prostatic adenocarcinoma; positive in 1% of renal cell carcinoma; positive in pancreaticobiliary adenocarcinoma

Combined S100P and GATA3

- 95% urothelial carcinoma were positive

Utility of GATA3 immunohistochemistry in differentiating urothelial carcinoma from prostate adenocarcinoma and squamous cell carcinomas of the uterine cervix, anus and lung

Chang A *et al* Am J Surg Pathol 2012;36:1472-6.

- Positivity in invasive high grade urothelial carcinoma in 80% cases and mets to lung, typically strong and diffuse
- All high grade prostatic adenocarcinomas were negative
- Weak/focal positivity in 10% and 19% anal and cervical squamous carcinomas, typically weak/focal
- All primary lung squamous carcinoma negative

A study of immunohistochemical differential expression in pulmonary and mammary carcinomas

Yang M, Nonaka D. *Mod Pathol* 2010;23:654-61.

- GATA3 was diffusely positive in 72% breast carcinoma (n=133)
- All lung carcinoma (which included 39 squamous carcinoma) were GATA3 negative (n=197)

Selective immunohistochemical markers to distinguish between metastatic high grade urothelial carcinoma and primary poorly differentiated squamous carcinoma of the lung

Gruver AM *et al. Arch Pathol Lab Med* 2012;136:1339-46

- GATA 3 was positive in 78% urothelial carcinoma and in this study in 23% primary squamous carcinoma of lung
- Desmoglein-3 was expressed in 11% of urothelial carcinoma and 87% lung squamous carcinoma
- Uroplakin III positive in 14% urothelial carcinoma and 0% of lung squamous carcinoma

GATA3 expression in paraganglioma: a pitfall that could lead to a misdiagnosis of urothelial carcinoma

[So JS and Epstein JI, USCAP abstract 1041, 2013]

- 12 paragangliomas of bladder and 20 paragangliomas of other sites examined
- At least 2+ positivity in 85% bladder cases and 65% of extravesical paragangliomas

A study of gata3 and phox2b expression in tumors of the autonomic nervous system

Nonaka D *et al. Am J Surg Pathol* 2013;37:1236-41

- GATA3 +ve in 89% of 35 paragangliomas and 95% of 21 phaeochromocytomas
- GATA3 +ve in all neuroblastomas, ganglioneuroblastomas and ganglioneuromas
- +ve in parathyroid tumours and most urothelial and breast carcinomas
- +ve in a subset of squamous carcinoma
- negative in carcinoid tumours, large cell neuroendocrine carcinoma, small cell carcinoma, Merkel cell carcinoma, thyroid tumours and melanoma

p63

- Positive (nuclear) in many urothelial (and squamous) lesions. Micropapillary urothelial carcinoma consistently p63 negative, Klapper *et al*; *Zhu B et al* [x 2 USCAP abstracts 2012]
- Useful, with other markers to distinguish nephrogenic adenoma(-ve) from urothelial proliferations (+ve)
- Also useful to help distinguish urothelial carcinoma (+ve) from renal carcinoma (-ve)
- Can be useful to distinguish sarcomatoid carcinoma (at least focally +ve) from other spindle cell lesions (but not totally specific)
- Useful to identify basal cells in prostate
- Prostate cancer usually negative (but rare cases can be diffusely positive)

Rhabdomyosarcoma of the urinary bladder in adults: predilection for alveolar morphology with anaplasia and significant morphologic overlap with small cell carcinoma

Paner GP *et al.* Am J Surg Pathol 2008;32:1022-8

- Synaptophysin is expressed focally in a significant proportion of bladder rhabdomysarcomas
- This could lead to a misdiagnosis of small cell neuroendocrine carcinoma

Comparison of thyroid transcription factor-1 expression by 2 monoclonal antibodies in pulmonary and nonpulmonary primary tumors

Matoso A *et al* Appl Immunohistochem Mol Morphol 2010;18:142-9

- 5.1% of invasive urothelial carcinoma of bladder were TTF-1 positive, even with both clones for TTF-1 tested (SPT24 and 8G7G3/1)
- 5/98 urothelial carcinoma were positive; 3 had >70% positive tumour cells

Prostatic adenocarcinoma versus urothelial carcinoma

	PSA	PSAP	34BE12 and/or CK5/6	p63	Uroplakin III	GATA3	CK7 and/or Thrombo -modulin	CK20	NKX3.1	Prostein (P501S) and/or PSMA
Prostatic adenoca	+/-	+/-	-[1]	-[2]	-	-	-	-[3]	+[4]	+/-
Urothelial carcinoma	-	-	+/-	+/-	-/+	+/-	+/-	-/+	-	-

[1]May be focally positive if adenosquamous differentiation present
[2]Rare cases may be positive
[3]High grade prostatic adenocarcinoma, including some ductal adenocarcinomas are focally positive
[4]With rare exceptions

Diagnostic utility of androgen receptor expression in discriminating poorly differentiated urothelial and prostate carcinoma

Downes MR *et al.* *J Clin Pathol* 2013;66:779-786

- All poorly differentiated prostatic adenocarcinoma were strongly/diffusely AR +ve
- High grade urothelial carcinomas were either negative or had cytoplasmic staining or weak/focal nuclear staining
- In this study, strong diffuse AR positivity was not seen in urothelial carcinoma

[some other previous studies have reported AR positivity in urothelial carcinoma but may have used different antibodies/methodologies]

Immunohistochemical expression of prostatic antigens in adenocarcinoma and villous adenoma of the urinary bladder

Lane Z *et al.* *Am J Surg Pathol* 2008;32:1322-1326

- 37 cases of primary adenocarcinoma of bladder and 3 villous adenomas studied
- P501S (prostein) and PSMA (prostate specific membrane antigen) +ve in 4/37 and 7/37 respectively
- PSA and PSAP –ve in all cases [refer to paper for distributional differences from prostate cancer]

Expression of PAX8 in nephrogenic adenoma and clear cell adenocarcinoma of the urinary tract. Evidence of related histogenesis?

Tong GX *et al. Am J Surg Pathol* 2008;32:1380-1387

- PAX8 was positive in all (100%) of 35 nephrogenic adenomas and 7/7 (100%) clear cell adenocarcinomas of urinary tract
- PAX 2 was positive in all nephrogenic adenomas and in 2/7 clear cell adenocarcinomas
- PAX8 was negative in clear cell urothelial carcinoma and all other primary bladder adenocarcinomas, prostatic adenocarcinoma and urothelial carcinomas (including variants) tested

Clear cell adenocarcinoma of the bladder and urethra: cases diffusely mimicking nephrogenic adenoma

Herawi M *et al. Hum Pathol* 2010;41:594-601

Nephrogenic adenoma
- Ki67 average 2%
- p53 typically absent
- PAX2 +ve in 89% cases

NA-like CCA
- Ki67 average 33%
- p53 average 4%
- PAX2 +ve in 30%

Differential expression of immunohistochemical markers in bladder smooth muscle and myofibroblasts, and the potential utility of desmin, smoothelin, and vimentin in staging of bladder carcinoma.

Council L, Hameed O. 2009 [see also Paner et al 2009; Miyamato et al 2010; Paner et al 2010; Bovio et al 2010 Lindh et al 2011, Hansel et al 2011]

- Smoothelin showed strong diffuse positivity in the muscularis propria
- Muscularis mucosae was weak/negative in 10/11/cases and showed moderate positivity in 1/11/cases
- Vimentin was strongly positive in muscularis mucosae but only weakly positive in muscularis propria
- Smoothelin was negative in myofibroblastic reactions to tumour

Detection of residual tumor cells in bladder biopsy specimens: pitfalls in the interpretation of cytokeratin stains

Tamas EF and Epstein JI *Am J Surg Pathol* 2007;31:390-397

- Cytokeratins may be detected in myofibroblastic cells or even smooth muscle cells in the vicinity of a biopsy/TUR site reaction and could be mistaken for residual tumour
- Morphology should be taken into account with concurrent use of α-SMA and/or desmin + h-caldesmon in interpretation
- All cases were Alk-1 -ve

Available biomarkers (cytology)

- Bladder Tumour Antigen
- Nuclear Matrix Protein 22
- ImmunoCyt/uCyt+
- Tests for aneuploidy (detected by FISH)
- Telomerase assays
- Microsatellite instability assays
- Others – cytokeratins, blood group antigens, glycoproteins, hyaluronidase, growth factors, cell adhesion molecules, FDP, cell proliferation markers, regulatory genes and proteins, survivin, aurora-A

[slide courtesy of Dr Ash Chandra, Guy's and St Thomas' Hospital, London]

FISH

- Useful in selected groups of patients as adjunct to improve sensitivity of cytology
- Follow up after intravesical therapy to increase the interval between surveillance cystoscopies
- Supportive evidence for equivocal or suspicious cytology
- Improved detection of low-grade upper tract lesions in instrumented samples

[Slide courtesy of Dr Ash Chandra]

UroVysion FISH

- Multicolour FISH developed in 2001
- Uses chromosome enumeration (centromeric) probes for chromosomes 3, 7 and 17 to look for polysomy
- Also uses a locus-specific probe to look for deletion of 9p21(p16)
- Mostly used in cytology especially in follow-up of patients with known bladder cancer

Multiprobe fluorescence in situ hybridisation: prognostic perspectives in superficial bladder cancer.

Mian C *et al J Clin Pathol* 2006;59:984-987

- 75 patients urine cytology f/u for bladder cancer
- Low risk group: FISH –ve or 9p21 loss and/or CEP3+ve
- High risk group: chromosome 7 or 17 aneuploidy
- 33% low risk cases recurred after mean 30 months; 11% progressed
- 67% high risk cases recurred after mean 17.6 months; 50% progressed