BEST PRACTICES IN THE APPLICATION OF IMMUNOHISTOCHEMISTRY TO DIAGNOSTIC UROLOGIC PATHOLOGY: 

LESSONS FROM USES & ABUSES

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Toward Best Practice IHC use in routine practice

• When IHC stains exceed H&E stain
  - Complex case or
  - Lack of best practice approach
Toward Best Practice IHC use in routine practice

Surgical Pathology
- Foundation is the integration of clinical history, gross examination & microscopy
- Cornerstone is still the H&E with appropriate and judicious IHC support – IHC guides; does not dictate the diagnosis
- Practice made considerably more objective by ancillary techniques e.g. IHC

Toward Best Practice IHC use in routine practice
- Serious misdiagnoses are made by inappropriate use of IHC or incomplete knowledge of antibody/ies
  - More is not necessarily better
- IHC adjunctive method, histology key
  - If you have no idea, don’t mark it
- Start with a question based on morphology
- Apply a judiciously constructed panel based on the differential diagnosis generated by the case

Toward Best Practice IHC use in routine practice
- Panel should include expected positive and expected negatives
- There are no absolutely specific or sensitive antibodies
  - Anomalous stuff happens
  - Sensitivity and specificity is not inherent to the antibody, but to the antibody applied in a given setting
- Evaluate the stain paying attention to pattern (nuclear, cytoplasmic, membranous, etc.)
- ALWAYS evaluate the controls (positive and negative)
- Diagnose the case after review of IHC only in the context of the morphology and the clinical situation
### GOWN’S LAWS OF IMMUNOCYTOCHEMISTRY

- There is no perfect marker of any tumor
- There is no perfect fixative for all antibodies
- If everything in the tissue section appears positive, nothing is actually positive
- All that turns brown (or black, or red, etc.) on the slide is not positive
- Under inappropriate conditions, any antibody can be made to appear positive on any tissue
- In any given immunocytochemical run involving multiple slides, tissue will fall off the slide corresponding to the most critical antibody
- The diagnostic power of any immunocytochemical preparation is no greater than the knowledge and wisdom of the pathologist interpreting it

### Best “Special Studies” in Surgical Pathology

- Good thin section and well stained H&E slides
- Additional sections, recuts and levels
- A phone call to the clinician (or reviewing the electronic medical records)
- Another trust-worthy pair of eyes (colleague)
- Placing the diagnostic dilemma in context of the clinical situation and management considerations
- Having a best practice approach immunohistochemistry

### SELECT BEST PRACTICE IHC APPLICATIONS IN UROLOGIC PATHOLOGY

- **Bladder:**
  - Proving origin/differentiation in unusual primary or at a metastatic site
  - IHC in flat intraepithelial lesions
- **Prostate:**
  - Proving origin at a metastatic site
  - Issues related to triple cocktail use in prostate biopsies
- **Kidney:**
  - Proving renal origin at a metastatic site
- **Testis:**
  - Screening panels for tumors involving testis – primary or metastatic sites
  - Characterizing the various germ cell components
PROVING UROTHELIAL DIFFERENTIATION

Carcinoma of unknown origin or patient with history of bladder/renal cancer:
- Lymph node
- Lung
- Liver
- Bone
- Prostate

“Unusual carcinoma” in the bladder

Metastatic tumors to the bladder:
- Melanoma
- Prostate
- Colorectal
- Cervix
- Ovary
- Renal

Primary urothelial carcinoma:
- UCa with small tubules
- Plasmacytoid
- Micropapillary
- Etc

Challenges:
- Poorly differentiated carcinoma
- “Characterless”: solid, nested & trabecular architecture

Hallmarks:
- Frequent squamous and / or glandular diff.
- Cells with nuclear grooves
- Nuclear atypia obvious +/- anaplasia

Approach
- Clinical history (invasive, usually high stage carcinoma)
- Compare with primary
- Judicious IHC: ? Best markers
**URINARY BLADDER - IHC**

- **Diagnosis of metastatic urothelial cancer**
  - CK7 (+) (>90%)
  - CK20 (+) (40-70%)
  - p63 (+) (60-90%)
  - High molecular weight cytokeratin 34BE12 (+) (60-90%)
  - GATA3 (60-70%)
  - Uroplakin II (+) (50-80%)
  - S100P (70-80%)
  - Uroplakin III (+) (20-50%)
  - Thrombomodulin (+) (60-75%)
  - CEA, Leu-M1 (±) (minimal value)

- **Histogenesis-associated markers**
  - Plasmacytoid U Ca
Plasmacytoid U.Ca - CK20

S100P

GATA3

Nuclear staining

lower sensitivity but higher specificity than S100P for urothelium
GATA3 – Wide Range of Expression

- Positive in
  - Breast, trophoblastic tumors, paragangliomas, salivary gland neoplasms, squamous carcinomas, basal cell carcinomas, yolk sac tumors, pancreatic ductal adenocarcinomas


Uroplakins – II and III

- Protein constituents of the urothelial plaques in vesicles of urothelium
- Vital role in expansion and contraction through vesicle cycling
- Subunits uroplakins Ia, Ib, II, and IIIa
- Unique and characteristic feature of urothelium
- Previous data for UP3, new data for UP2

Uroplakin 2 versus Uroplakin 3

- Among UC metastases, UP2 showed greater intensity and proportion, (both p<0.001), with higher sensitivity (73% vs 37%, respectively, p=0.001).

Smith et al. Histopathology. In press
Uroplakin 2 versus Uroplakin 3

Villoglandular variant simulates colorectal carcinoma

Smith et al. Histopathology. In press
IMMUNOHISTOCHEMISTRY IN FLAT LESIONS OF THE BLADDER

Panel: p53, CD44 (standard isoform), CK20

Indications:
- Marked denudation – residual basal cells vs “clinging” CIS
- Distinction between reactive atypia and CIS (large cell non-pleomorphic or “small” cell)
- Pathologist favors CIS but has reservations making diagnosis
- CIS with unusual morphology – Pagetoid, undermining, etc.

Caveats:
- Not applicable for dysplasia vs CIS
- Greater caution while evaluating post-treatment biopsies
p53: 55-80% of CIS

CD44: 96-100% of CIS

CK20:
CK20 (+) : 50-100% of CIS

p53

CD44(-)
CD44

UROTHELIAL ASSOCIATED-MARKERS

Prostate vs. Urothelial Carcinoma
- Often in bladder neck specimens
- Therapeutically critical differential

- PSA
- PSAP
- NNX1.3
- Prostein (P501S)
- ERG-TMPRSS2
- PSMA

- CK20
- P63 or MWCK
- GATA3
- Uroplakin 2
- S100p
- Uroplakin 3

CAUTION: Both may coexist!

?Urothelial Carcinoma vs. ?Prostatic Carcinoma
Urothelial Carcinoma vs. Prostatic Carcinoma

UCa

PCa

GATA3

CK5/6

S100P

P501S

PSMA

NKX3.1
ERG IHC

Concurrent PCa & UCa

Virtually any tumor from the body can spread to the bladder on occasion. Problem areas:

Enteric morphology: Colon and appendiceal primary vs. bladder primary

- Morphologically identical
- May have a surface well-differentiated “villous adenoma” surface component
- Helpful features: - Clinical history of high-stage colon cancer
- Absence of intestinal metaplasia
- Immunohistochemistry (CK7, CK20, CDX2) not helpful (β-catenin, nuclear positivity, limited role)
Nephrogenic adenoma
Clear cell adenoCa of bladder
Urothelial Ca with glandular morphology
Prostatic adenoCa

Pax2/8
90% 10-20% 0% 0%

AMACR
100% 75% Frequently positive 70-100%

S100A1 94% 10% 0% 0%

Ki67 % + nuclei
2-5% 40-50% 30-40% 2-25%

PSA 0-2% 0 0 70-100%

Spindle cell lesions
Benign (PMP) vs. Malignant - H&E diagnosis

- PMP / PSFMT
  keratin (+/-), SMA (+), desmin (+/-), p63 (-), Alk-1 (+)

- Sarc. Ca
  keratin (+/-), SMA (-), desmin (-), p63 (+/-), Alk-1 (-), HMCK & CK5/6 (+)

- LMS
  keratin (-/+), SMA (+), desmin (+), Alk1 (-/+), p63 (-)
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• To confirm focus as cancer
• Confirm benignity in ASAP felt to be benign
• Unusual patterns
  • Atrophic
  • Pseudohyperplastic
  • Double – layer
  • PIN-like

**Indications for IHC – Needle Biopsy**

**Atypical small cell proliferations**

- To confirm focus as cancer
- Confirm benignity in ASAP felt to be benign
- Unusual patterns
  - Atrophic
  - Pseudohyperplastic
  - Double – layer
  - PIN-like

**Atypical large acinar proliferations (intraductal patterns)**

Post – treatment setting
## IHC in Prostate Needle Bxs.

- **Basal cell cocktail**  
  - p63 and 34βE12
- **Triple cocktail “PIN cocktail”**  
  - p63/34βE12/AMACR
- **ERG immunohistochemistry**  
  - Additional marker, only if triple not conclusive

**PSA** – to prove prostate origin – NA, Cowper’s glands

## Triple cocktail

- ** Expected reactions  
  - PCa: p63(-), HMCK(-), AMACR(+)
  - Benign small cancer mimics: p63, HMCK(+), AMACR(-)
  - HGPIN: p63, HMCK(+), AMACR(-/+)
  - Ductal cancer:  
    - Invasive component: p63, HMCK(-), AMACR(+)
    - Intraductal component: p63, HMCK(+), AMACR(+)
  - Urothelial cancer: p63, HMCK(+/-), AMACR(+)}
P63, HMWCK and AMACR cocktail
EQUIVOCAL IHC

- Results not entirely complimentary
- Unexpected basal cell layer staining
- Results supportive but all glands in an already small or difficult focus not represented in the IHC
60% of PCa harbor any ETS-rearrangement
50% of PCa – TMPRSS2-ERG
Detection by IHC or FISH
  * High concordance in hormone naïve
  * IHC detection in ~30% in needle setting
Do we need a 4th marker?
  * Helps in about 5% of cases with equivocal triple cocktail
Additional: Marker of prostate histogenesis

ERG Immunohistochemistry
IHC in a pt. with one (+) core

- Confirm bilaterality: clinical staging - almost 50% patients with prostate cancer treated with RT
- Accurate assessment of # of cores involved – Active surveillance
- Quantitation of cancer – Active surveillance (>50% may exclude)
Work-up of Atypical Foci with Definite Cancer in Other Parts

Patient with Gleason score 3+4 or higher grade cancer on at least one part.

Work up other parts with small foci of possible 3+3=6

Generally, not indicated, as additional IHC confirmation will likely not change management

Abberant expression p63 in Prostate cancer
IHC IN KIDNEY SURGICAL PATHOLOGY

- Confirming Renal origin
- *Histologic subtyping of RCC*

Metastatic sites
Primary tumors
Small biopsies and FNAS
CONFIRMING RENAL ORIGIN

Carcinoma of unknown origin or patient with history of RCC:
- Lymph node
- Lung
- Liver
- Bone
- Other

"Unusual carcinoma" in the kidney
- Epithelioid PEComa
- Urothelial Carcinoma
- Metastatic carcinoma to the kidney versus
- Poorly differentiated, high grade RCC (unclassified) versus
- Lymphoma, sarcoma, melanoma, other

APPROACH TO APPLICATION OF IHC IN RENAL TUMORS

Is the neoplasm a carcinoma?:
rule out Epi AML (PEComa), lymphoma, sarcoma, melanoma etc

Is the carcinoma a renal primary?:
rule out urothelial carcinoma, metastasis

Can you subtype the renal cell carcinoma?:
Clear cell vs papillary vs chromophobe vs oncocytoma vs translocation associated Ca …..
**RCC antigen**

Monoclonal antibody against brush border of healthy PCT

<table>
<thead>
<tr>
<th>RCC types</th>
<th>Other tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell RCC (85%)</td>
<td>Breast ca</td>
</tr>
<tr>
<td>Papillary RCC (95%)</td>
<td>Parathyroid ca</td>
</tr>
<tr>
<td>Oncocytoma &amp; Chromophobe (-/+</td>
<td>Embryonal ca, testis</td>
</tr>
<tr>
<td>Collecting duct Ca (-/+</td>
<td>Lung</td>
</tr>
</tbody>
</table>

- Prostate |
- Ovary |
- Melanoma |
- Epididymal cystadenoma |
- Mesothelioma

**PAX8**

Paired box transcription factor, similar to PAX2

Predominantly data from polyclonal antibody – new monoclonal

<table>
<thead>
<tr>
<th>RCC types</th>
<th>Other tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell RCC (&gt;95%)</td>
<td>Similar to Pax2</td>
</tr>
<tr>
<td>Papillary RCC (&gt;95%)</td>
<td>Thyroid neoplasms</td>
</tr>
<tr>
<td>Wilms tumor</td>
<td>Extensive GYN positivity</td>
</tr>
<tr>
<td>Metanephric (+) adenoma</td>
<td></td>
</tr>
<tr>
<td>Oncocytoma (+)</td>
<td></td>
</tr>
<tr>
<td>Chromophobe RCC (+/-)</td>
<td></td>
</tr>
<tr>
<td>Collecting duct Ca (-/+</td>
<td></td>
</tr>
<tr>
<td>Translocation assoc. Ca (-/-)</td>
<td></td>
</tr>
</tbody>
</table>
Metastatic Clear cell RCC (Bone)

85% of met RCC are PAX 8 (+)

Parathyroid Carcinoma

Positive in RCC
- Clear cell RCC (60%)
- Pap RCC (80%)
- Clear cell-pap RCC
- Oncocytoma
- Translocation assoc RCC
- Chromophobe RCC (-)

Other tumors
- Ovarian Ca (serous, clear)
- Endometrial Ca

S100A1

Among the 13 member S100 protein family.
Expressed in numerous cell types, not well studied
Carbonic anhydrase IX

*Family of zinc containing metalloproteinase that regulates cell proliferation, adhesion and metastasis*

<table>
<thead>
<tr>
<th>Kidney tumors</th>
<th>Other tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell RCC (+)</td>
<td>Most carcinomas of endometrium, stomach, lung, cervix, liver, breast etc.</td>
</tr>
<tr>
<td>Papillary RCC (-/+)</td>
<td></td>
</tr>
<tr>
<td>Chromophobe RCC (-)</td>
<td></td>
</tr>
<tr>
<td>Oncocytoma (-)</td>
<td></td>
</tr>
<tr>
<td>Urothelial Ca (+/-)</td>
<td></td>
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</tbody>
</table>

Prognostic utility of CA IX in clear cell RCC
Ksp-cadherin in distal convoluted tubules
Cathepsin K

- Expression is related to overexpression of MiTF
- PEComas: moderate to strong and diffuse cytoplasmic staining is seen in all variants
  - co-expressed with other melanocytic markers (more diffuse than HMB-45)
- MiTF-TFE3 translocation associated carcinomas
  - t(X;1): >85% cases, diffuse
  - t(X;17): 0%
  - t(6;11): 100% of cases, diffuse

Other renal tumors: Negative except nonspecific in necrotic areas

CONFIRMING RENAL ORIGIN

Is the neoplasm a carcinoma?:
- Renal “related”
  - AE1/AE3 (+)
  - EMA (+)
  - Vimentin (+)
  - CK7 (-), CK20 (-)

Is the carcinoma a renal primary?:
- Renal associated
  - “RCC marker” (80%)
  - PAX8 (>90%)
  - S100A1
  - CD10 (+) (94%)
If history of renal mass and renal histogenesis markers are negative?

- **Consider: Chromophobe carcinoma**
  - CD117 (+) and Ksp-Cadherin (+)

- **Consider: Epithelioid PEComa and translocation carcinoma**
  - Cathepsin K, MelanA/HMB45

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### Renal Clear and Papillary Tumors

<table>
<thead>
<tr>
<th>Clear cell RCC</th>
<th>Clear –Papillary RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-9 (+)</td>
<td>CK 7(+)</td>
</tr>
<tr>
<td>RCC (+)</td>
<td>Racemase (-)</td>
</tr>
<tr>
<td>Pax8 (+)</td>
<td>HMCK (+)</td>
</tr>
<tr>
<td>Vimentin (+)</td>
<td>RCC, CD10(-)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Papillary RCC</th>
<th>Metanephric adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC (+)</td>
<td>RCC (+)</td>
</tr>
<tr>
<td>CK7 (+)</td>
<td>CK7 (+)</td>
</tr>
<tr>
<td>Racemase (+)</td>
<td>Racemase (+)</td>
</tr>
</tbody>
</table>

- **Oncoctyoma**
- **Chromophobe RCC**
**Renal Oncocytic Tumors**

<table>
<thead>
<tr>
<th>Oncocytoma</th>
<th>Chromophobe RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK 7 (- / +)</td>
<td>CK 7 (+ / -)</td>
</tr>
<tr>
<td>S100 A1 (+)</td>
<td>S100A1 (-)</td>
</tr>
<tr>
<td>Barttin (cytoplasmic)</td>
<td>Barttin (membranous)</td>
</tr>
</tbody>
</table>

*Not adequately studied: preliminary data*
*Not tested in hybrid oncocytic tumors*

Amylase 1A (AMY1A), EPCAM, Claudin and Caveolin 1
*Investigational*

**HLRCC-RCC**

<table>
<thead>
<tr>
<th>Renal medullary ca.</th>
<th>Urothelial carcinoma</th>
</tr>
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</table>

**IHC FOR HIGH GRADE DISTAL NEPHRON CA**

<table>
<thead>
<tr>
<th>Renal Cell CA incl. CDC</th>
<th>Renal Medullary CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX8</td>
<td>OCT3/4 (+)</td>
</tr>
<tr>
<td>RCC</td>
<td>INI1 lost (-)</td>
</tr>
<tr>
<td>S100 A1</td>
<td>PAX8</td>
</tr>
<tr>
<td>CK 7 &amp; 20 (-)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Urothelial CA</th>
<th>HLRCC-RCC/FH deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATA 3</td>
<td>FH lost (-)</td>
</tr>
<tr>
<td>S100P</td>
<td>2SC positive</td>
</tr>
<tr>
<td>HMCK</td>
<td></td>
</tr>
<tr>
<td>P63</td>
<td></td>
</tr>
<tr>
<td>Uroplakin 2</td>
<td></td>
</tr>
<tr>
<td>CK 7 &amp; 20 (+)</td>
<td></td>
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</tbody>
</table>

CAIX and Vimentin immunoreactivity can be seen in UCa
### TESTIS IHC: Screening panels

<table>
<thead>
<tr>
<th>Germ cell tumors</th>
<th>Sex cord tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT 3/4</td>
<td>SF1</td>
</tr>
<tr>
<td>SALL4</td>
<td>Melan A</td>
</tr>
<tr>
<td>PLAP</td>
<td>Inhibin</td>
</tr>
<tr>
<td>EMA(-)</td>
<td>Calretinin</td>
</tr>
<tr>
<td>Vimentin (-)</td>
<td>CD99</td>
</tr>
<tr>
<td></td>
<td>Synaptophysin</td>
</tr>
<tr>
<td></td>
<td>S-100</td>
</tr>
<tr>
<td></td>
<td>FOXL2</td>
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- **Lymphoma:** CD-45, CD3, L26
- **Visceral malignancy:** EMA (+), vimentin (±)

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### LEYDIG CELL TUMOR

**INHIBIN**

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### SERTOLI CELL TUMOR

**CALRETININ**
IHC in characterizing the different germ cell components

- There is no substitute to well (overnight) fixed sections
- Adequate sampling is key - the # of IHCs should *NEVER* exceed the H&E slides
- Remember what matters in germ cell tumors

GERM CELL TUMOR – What really matters?

One does not necessarily have to characterize every morphologically different focus

- Pure classic Seminoma vs. non-seminomatous components
- Mixed germ cell tumor
  - Specify components (as accurately as you can)
  - >80% or pure embryonal carcinoma (↑)
  - >50% teratoma (↑)

Vascular-lymphatic invasion – pathologic stage
Margin status
IHC IN GERM CELL TUMORS

• **GCNIS**: Oct3/4, c-kit, SALL4, Podoplanin, PLAP - all (+)
• **Seminoma**: Oct3/4, c-kit, Podoplanin – all (+)
• **Embryonal Ca**: Oct3/4, CD30, SOX2, Keratin weak, – all (+)
• **YST**: Glypican, AFP, Keratin strong
• **CC**: HPL, βHCG, Glypican-syncytiotrophoblasts
• **SS**: CD117, SAL4 (weak)

*Cytokeratin AE1/AE3*: E Ca, YST, T, CC
*Oct 3/4*: Seminoma, E Ca
*PLAP*: Minimal / no value – except in GCNIS
OCT3/4

Glypican

THANK YOU