**Prostate Immunohistochemistry**

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**IHC Interpretation: General Principles (1)**

Must be aware of staining pattern of antibody *in the relevant tissue*

- Nuclear/cytoplasmic/membranous
  - eg. β-Catenin used for colon cancer vs. prostate cancer
    - Nuclear positivity only in colon cancer
    - Membranous/cytoplasmic positivity in up to 88% of prostate cancer

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**IHC Interpretation: General Principles (2)**

- Not just positive or negative
  - *Quantitative aspect has to considered*
    - Immunoreactivity is a continuous variable
    - Focal positivity must be interpreted with caution
    - Negativity in limited material not diagnostic
- IHC must always be interpreted in the context of morphology

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**Literature Interpretation: Caveats**

Interpret literature data critically due to differences in:

- Staining techniques
  - Antibody type, fixation, antigen retrieval
- Characteristics of tumours studied
  - Effect of grade
    - PSA/PSAP expression lower in high-grade prostate carcinoma
    - Uroplakin expression lower in high-grade TCC

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**Literature Interpretation: Caveats (2)**

- Definition of positivity may vary between studies
  - Cut-offs used to consider CK7/CK20 as positive varies from any positivity to >10% cells positive
- Microarray techniques may overestimate specificity and underestimate sensitivity especially if staining patchy

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**Interpreting Immunohistochemistry: A Systematic Approach**

1. Check controls
   a) Appropriate tissue for control
   b) Appropriate staining reaction
2. Exclude spurious positivity (pigment, biotin etc)
3. Pattern of positivity
   a) Correlate with morphology
     - Necrosis, entrapped benign tissue etc.
   b) Nuclear/cytoplasmic/membranous
### Interpreting Immunohistochemistry: A Systematic Approach (2)

4. Semi-quantitation
   - % of cells positive; patchy; focal; diffuse
5. Amount of tumour assessed
6. Tumour characteristics
   - Subtype, grade etc
7. Immunoprofile
8. Morphological context
9. Clinical context

### Immunohistochemistry In The Diagnosis Of Prostate Cancer

**Used in two distinct settings:**

1) **Atypical prostate glands**
   - ?Benign ?low-grade prostate cancer
   - Needle bx
2) **Poorly differentiated carcinoma**
   - ?Prostate cancer ?other carcinoma
   - TURPs / mets

### Benign Prostate vs. Cancer

- Basal cells present in benign glands; absent in cancer
- H&E identification of basal cells is unreliable
  - Use immunohistochemistry in difficult cases

### HMWCK Antibodies

- **34βE12**
  - CKs 1, 5, 10, 14
- **CK 5/6**
- **LP34**
  - CKs 5, 6, 18
  - CK18 expressed by secretory cells

### p63

- p53 homologue
- Nuclear positivity
- Selectively expressed in basal cells
- Negative in secretory cells and cancer

### Basal Cell Marker Immunoreactivity in Prostate Cancer: Non-basal Cell Pattern

- **LP34**
  - CK18 in secretory cells
- **Ductal carcinoma**
  - May be diffuse
- **Microacinar**
  - Generally patchy esp. with microwave retrieval
  - More common in poorly differentiated cancers; Yang et al: 2% of metastatic Ca
Basal Cell Marker Immunoreactivity in Prostate Cancer: Basal Cell Pattern

- Ductal type prostate cancer
  - Intraductal spread of tumour
- Microacinar prostate cancer
  - Very rare
    - 1.1% cases in referral material
  - Entrapped outpouchings of PIN
  - Basal cells retained in early invasive cancer

Comparison Of Basal Cell Markers

- CK5/6 more sensitive than 34βE12
- p63 slightly more sensitive than 34βE12
- LP34 more sensitive than CK5/6 and CK14

Which Is The Best Basal Cell Marker?

- The one that works best in your lab!
- Every case is a quality control
  - Check out the background benign glands
- Consider HMWCK + p63 combination rather than HMWCK + CK5/6

Limitations Of Basal Cell Markers

- Often used as a single marker
- Cancer diagnosis based on negative staining reaction
- Benign glands are occasionally negative
- Basal cell layer fragmented in high-grade PIN and adenosis

Basal Cell Marker Negative Small Glandular Proliferations

- Prostate cancer
- Outpouchings of high-grade PIN
- Adenosis

Alpha-Methylacyl-CoA-Racemase

A revolutionary new positive marker for prostate cancer

- Identified by cDNA library subtraction and microarray techniques
  - First of many
  - Prostein (P501S): a recently described prostatic marker (benign and malignant)
- Rabbit monoclonal antibody
  - Higher affinity than conventional mouse monoclonals, especially for small epitopes
When is AMACR Immunoreactivity Considered Positive?

Only *circumferential* staining of the luminal cells that can be identified at *low (100x)* magnification with no more than weak, non-circumferential staining of adjacent benign glands.

Basal cell markers vs. AMACR

- **Basal cell markers**
  - Difference between benign glands and cancer is *qualitative*
  - No basal cells in cancer
- **AMACR**
  - Difference is *quantitative*
  - AMACR over-expressed in cancer but also expressed in benign glands
  - Hence titration of sensitivity is critical

Basal cell markers vs. AMACR Evaluation of immunoreactivity

- **AMACR**
  - Positive or negative
- **Basal cell markers**
  - Positive or negative
  - Pattern of immunoreactivity
    - “basal cell pattern”

Limitations of AMACR

- AMACR immunoreactivity in focus of cancer often *heterogeneous*
  - Initial studies: 97-100% sensitivity
  - More recent: up to 20% of *limited* cancers AMACR negative
- Commonly positive in high-grade PIN and its outpouchings
- AMACR immunoreactivity weaker in prostate cancer variants (pseudo-hyperplastic, foamy gland)

AMACR: Notes of caution

- Always use in conjunction with basal cell marker(s)
- Do not downgrade (cancer to suspicious or suspicious to benign) based on negative AMACR staining
- Do not upgrade from “suspicious outpouchings of HG-PIN” to cancer based on positive AMACR staining

Limitations of AMACR

- **Positive in**
  - Adenosis (15%)
  - Nephrogenic adenoma (50%)
  - Variety of other benign mimickers
    - Atrophy, PAH etc
  - Occasional benign glands may show generally weak positivity
Suspicious to Cancer “Based” on Racemase Positivity

1. Architecturally atypical foci with insufficient cytologic atypia
2. Foci with crush artefact
3. Too few atypical glands

AND
differential diagnosis NOT outpouchings of high-grade PIN or nephrogenic adenoma and beware of adenosis!

Sections for Immunohistochemistry

• Intervening levels must be retained on treated slides for potential immunostaining
  • Small atypical foci may not be present in deeper levels

Cardiff Protocol for Processing Prostate Biopsies

<table>
<thead>
<tr>
<th>Tissue ribbons</th>
<th>Glass slides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>Level 2</td>
</tr>
<tr>
<td>Level 3</td>
<td></td>
</tr>
</tbody>
</table>

H&E

Immuno Spares

Advantages of Cardiff Protocol

• Immunostained section corresponds more closely with H&E section
• All 3 levels immunostained
  • Basal cell marker positivity may not be present in all levels

Antibody Cocktails

• Advantages
  • Multiple markers can be performed on limited number of spares available.
  • Cost and time savings

• Disadvantages
  • Antibody dilutions cannot be optimised separately for local conditions.
  • Single retrieval method for both markers.
  • One marker may mask other if single colour detection used (AMACR + p63)

Interpreting Prostate IHC (1)

• First evaluate glands away from suspect focus
  • Quality assurance
  • Some patients have very fragmented pattern in benign glands
  • May be microscopic focus missed on H&E

• Evaluate immunostaining in focus as a whole rather than in individual glands
  • HMWCK (-) glands in adenosis morphologically identical to HMWCK (+) glands within the focus
**Interpreting Prostate IHC (2)**

**Factors to consider:**
- Morphological differential diagnosis
  - PIN Ca: AMACR not useful
  - Nephrogenic adenoma:
    - PSA/PSAP rather than HMWCK/AMACR
- Size of suspect focus
  - Small HMWCK(-): Suspicious
  - Larger HMWCK(-): Cancer
- Degree of atypia
  - Architectural
  - Cytological
- Pattern in adjacent benign glands

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**Prostate Cancer vs. TCC**

- **Prostatic markers**
  - PSA, PSAP, PSMA, Prostein (P501S), Leu7 (CD57)
- **Urothelial markers**
  - HMWCK, p63, CK7, CK20, uroplakin III

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**PSA: Sensitivity**

- Very sensitive in low-grade prostate cancer
- PSA expression lower in high-grade prostate cancer
  - 225 prostate cancers various of grades
  - All Gleason 6 tumours >50% cells PSA+
  - 26% Gleason 10 tumours <5% cells PSA+
  - PSA negativity in high-grade cancer does not exclude prostatic origin

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**PSA: Specificity**

PSA immunoreactivity reported in a variety of non-prostatic tumours
- Almost always weak and focal
- Reflection of large number of cases studied

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**PSA: Quality Control Issues**

- PSA less expressed in high-grade prostate cancer compared to benign prostate / low-grade prostate cancer
- Polyclonal anti-PSA significantly more sensitive than monoclonal anti-PSA in high-grade prostate cancer
- Use high-grade tumour for positive control and optimising dilutions

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**PSA: Sensitivity**

- Varma et al. (Am J Clin Path 2002;118:202-7)
  - 20 Gleason score 10 prostate cancers
  - Monoclonal anti-PSA: 35% <10% cells positive
  - Polyclonal PSA: 95% >50% cells +
  - *Polyclonal* anti-PSA significantly more sensitive than monoclonal anti-PSA in poorly differentiated prostate cancer
**Choice of Prostatic Marker**

- A survey of UK laboratories found that all used PSA but only about half used PSAP.
- PSA negative poorly differentiated prostate cancers may diffusely PSAP (+)
- Use of both PSA and PSAP is recommended when evaluating poorly differentiated tumours

**TCC vs. prostate Cancer**

- **Primary panel**
  - PSA, PSAP, 34βE12
- **Secondary panel**
  - Leu7, CK7, CK20, p63
- **Tertiary panel?**
  - PSMA, Prostein, Uroplakin III