Tumours of the Prostate and Testis – update on WHO classification, IHC and molecular Morphology

Glen Kristiansen

Professor and Chairman
Institute of Pathology,
University Hospital Bonn
WHO Classification of Tumours of the Urinary System and Male Genital Organs

Edited by Holger Moch, Peter A. Humphrey, Thomas M. Ulbright, Victor E. Reuter
WHO GU-Pathology Working Group,
Zurich, March 2015
Today's Topics

- WHO 2016 Prostate
- Diagnostic Immunohistochemistry in Prostate
- Prognostic Immunohistochemistry in Prostate Cancer
- WHO 2016 Testis
- Diagnostic Immunohistochemistry in Testicular Tumors
Histological Entities and Variants of PCa

**Acinar**
- Atrophic
- Pseudohyperplastic
- Microcystic
- Foamy
- Mucinous
- Signet ring cell-like
- Pleomorphic-giant cell
- Sarcomatoid

**Ductal**
- cribriform
- papillary
- solid
The Classics...

Acinar (>90%), GP 1-5

Ductal (3-5%), GP 4-5

Note: A **ductal** tumor component ought to be mentioned and quantified in your report.
Atrophic Variant

- Signs of atrophy
- *De novo* oder following (anti-androgenic Tx)
- Usually accompanied by ordinary acinar PCa
- usually GP 3
Pseudohyperplastic Variant

- Pitfall: do not mix up with BPH or HGPIN
- GP 3
Microcystic Variant

• Cytic, often with atrophic epithelium
• In approx. 10% of RP specimens
• GP 3
Mucinous Variant

- > 25% extracellular mucin
- Can only be diagnosed on RP (use term „mucinous features“ in Bx)
- GP 3-4 (ignore mucin, architecture counts)
Mucinous Variant

- > 25% extracellular mucin
- Can only be diagnosed on RP (use term „mucinous features“ in Bx)
- GP 3-4 (ignore mucin, architecture counts)
Pleomorphic Giant Cell Variant

• Extremely rare
• Usually associated with conventional high grade PCa
• Pitfall: can be mixed up with urothelial carcinoma

Associated acinar PCa
Intraductal Ca of the Prostate (IDC-P)
M8500/2
Intraductal Ca of the Prostate (IDC-P)
M8500/2
"The fate of old tubuli entrapped in cancer is twofold. Firstly - and by far most commonly – they vanish and obliterate, or secondly, their lumen will be filled by intra-alveolarly growing carcinoma."
**WHO 2016 Definition of IDC-P:**

**Table 3.07  **Histological features of intraductal carcinoma of the prostate

<table>
<thead>
<tr>
<th>Malignant epithelial cells filling large acini and prostatic ducts, with preservation of basal cells, and either:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A solid or dense cribriform pattern or</td>
</tr>
<tr>
<td>A loose cribriform or micropapillary pattern with either:</td>
</tr>
<tr>
<td>Marked nuclear atypia (i.e. nuclear size 6× normal or larger) or</td>
</tr>
<tr>
<td>Comedonecrosis</td>
</tr>
</tbody>
</table>
Differential Diagnosis of IDC-P

- Cribriform Hyperplasia
- HGPIN
- Ductal Carcinoma
- Invasive cribriform Pca
- Urothelial Carcinoma/CIS
- Colon Cancer
Ductal Carcinoma
PIN-like ductal adenocarcinoma
## DD HGPIN vs. IDC-P

<table>
<thead>
<tr>
<th>Diagnostic criteria</th>
<th>HGPIN</th>
<th>IDC-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cells</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cytologic atypia</td>
<td>+</td>
<td>+ (+)</td>
</tr>
<tr>
<td>Luminal bridging</td>
<td>-(+)</td>
<td>+</td>
</tr>
<tr>
<td>Solid growth</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dense cribriform</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Comedonecrosis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Enlarged nuclei (6x)</td>
<td>-</td>
<td>-(+)</td>
</tr>
</tbody>
</table>

### IHC:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ERG</td>
<td>neg.</td>
<td>50% pos.</td>
</tr>
<tr>
<td>PTEN</td>
<td>pos.</td>
<td>60% negative.</td>
</tr>
</tbody>
</table>
## Epidemiology of IDC-P

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Cohort size</th>
<th>Specimen Type</th>
<th>% IDC-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kovi et al.</td>
<td>1985</td>
<td>139</td>
<td>RPE</td>
<td>48</td>
</tr>
<tr>
<td>McNeal et al.</td>
<td>1986</td>
<td>64</td>
<td>RPE</td>
<td>26.5</td>
</tr>
<tr>
<td>McNeal et al.</td>
<td>1995</td>
<td>476</td>
<td>RPE</td>
<td>29.8</td>
</tr>
<tr>
<td>Wilcox et al.</td>
<td>1998</td>
<td>252</td>
<td>RPE, pT3N0</td>
<td>42.8</td>
</tr>
<tr>
<td>Rubin et al.</td>
<td>1998</td>
<td>114</td>
<td>RPE</td>
<td>37.7</td>
</tr>
<tr>
<td>Shah et al.</td>
<td>2010</td>
<td>117</td>
<td>RPE</td>
<td>17.9</td>
</tr>
<tr>
<td>Miyai et al.</td>
<td>2014</td>
<td>901</td>
<td>RPE</td>
<td>17.2</td>
</tr>
<tr>
<td>Watts et al.</td>
<td>2013</td>
<td>1176</td>
<td>Bx</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Clinical Implications of IDC-P in RPE

Presence of IDC-P shows strong associations...

- with higher Gleason Scores
- larger tumors
- positive margins
- extraprostatic extension (EPE)
- pN+
### Clinical Implications of IDC-P in RPE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>95% confidence interval (CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% LC/IDC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0%</td>
<td>2.98</td>
<td>1.69</td>
<td>5.28</td>
</tr>
<tr>
<td>=0%</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>1.42</td>
<td>0.73</td>
<td>2.79</td>
</tr>
<tr>
<td>≥8</td>
<td>3.77</td>
<td>1.55</td>
<td>9.17</td>
</tr>
<tr>
<td>T-stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T3A</td>
<td>1.58</td>
<td>0.85</td>
<td>2.95</td>
</tr>
<tr>
<td>T3B</td>
<td>1.59</td>
<td>0.77</td>
<td>3.25</td>
</tr>
<tr>
<td>Surgical margins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive</td>
<td>1.80</td>
<td>1.04</td>
<td>3.13</td>
</tr>
</tbody>
</table>

LC = large cribriform. IDC = intraductal carcinoma of prostate.

European Journal of Cancer (2014) **50**, 1610–1616
Should I grade IDC-P?

e.g. "Low grade"

"high grade"
Should I grade IDC-P?

Current WHO & ISUP 2014 recommendation:
Stick to: **HGPIN**, („Borderline Atypical“), **IDC-P**
Intraductal carcinoma of the prostate: interobserver reproducibility survey of 39 urologic pathologists

Kenneth A. Iczkowski, MD a,* Lars Egevad, MD b, Jun Ma, MD c, Nicholas Harding-Jackson, MD a, Ferran Algaba, MD d, Athanase Billis, MD e, Philippe Camparo, MD f, Liang Cheng, MD g, David Clouston, MD h, Eva M. Comperat, MD i, Milton W. Datta, MD j, Andrew G. Evans, MD k, David F. Griffiths, MD l, Charles C. Guo, MD m, Seife Hailemariam, MD n, Wei Huang, MD o, Peter A. Humphrey, MD p, Zhong Jiang, MD q, Hillel Kahane, MD r, Glen Kristiansen, MD s, Francisco G. La Rosa, MD t, Antonio Lopez-Beltran, MD u, Gregory T. MacLennan, MD v, Cristina Magi-Galluzzi, MD w, Jennifer Merrimen, MD x, Rodolfo Montironi, MD y, Adeboye O. Osunkoya, MD z, Maria M. Picken, MD aa, Nagarjun Rao, MD a, Rajal B. Shah, MD ab, Jonathan H. Shanks, MD ac, Steven S. Shen, MD ad, Ossama W. Tawfik, MD ae, Lawrence D. True, MD af, Theodorus Van der Kwast, MD kg, Murali Varma, MD l, Thomas M. Wheeler, MD mg, Debra L. Zynger, MD nh, Natasha Sahr, MD a, David G. Bostwick, MD c

Annals of Diagnostic Pathology 18:333-342; 2014

Microphotographs circulated
PIN – IDCP - Invasive

43% agreement for IDCP
Gleason Grading of Prostate Cancer

Original

ISUP (2005)

ISUP(2014)/WHO (2016)

1
2
3
4
5

✓ Dr. Donald Gleason
✓ 1966
✓ 5 Pattern (1-5)
✓ Gleason score=1°+2°

✓ International Society of Urological Pathology (ISUP)
✓ 2005/2014
Grading Cribriform Glands

GP 3 ?

GP 4?

Is the outer contour decisive?
Grading Cribiform Glands

GP 3 ?

...or inner values ?

GP 4?
All cribriform glands are reported as GP 4

ISUP2014/WHO2016
ISUP2014/WHO2016
All glomeruloid glands are reported as GP4 (beginning of Cribriform growth)
Grade groups:

<table>
<thead>
<tr>
<th>Gleason score</th>
<th>Grade Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-6</td>
<td>1</td>
</tr>
<tr>
<td>3+4=7</td>
<td>2</td>
</tr>
<tr>
<td>4+3=7</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>9-10</td>
<td>5</td>
</tr>
</tbody>
</table>
What is the correct term for these grades?

• Epstein grade?
• ISUP grade?
• WHO grade?

• ISUP/WHO:
  Grade Groups!

Note: this was meant as a provisional term.
Problem: Gleason Grading on RPE

Gleason score „classic“: 4 + 3 = 7

Gleason score ISUP 2005: 4+3 = 7 (5)

Gleason Score ISUP 2014: ??? (4+3=7 (50% GP4; tert. 5), Grade Group 3)

Gleason Score WHO: 4+5=9, Grade Group 5
IHC in Prostate Diagnostics
Typical diagnostic problem: carcinoma vs. atrophy/reactive changes?

Table 1. Evolution of immunohistochemical basal cell markers in diagnosis of prostate disease

<table>
<thead>
<tr>
<th>Basal cell marker</th>
<th>Year first described</th>
<th>Routine use</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin 903 (34ßE12)</td>
<td>1985</td>
<td>Yes</td>
<td>23–25</td>
</tr>
<tr>
<td>P-Cadherin</td>
<td>1997</td>
<td>No</td>
<td>26</td>
</tr>
<tr>
<td>CK 5/6</td>
<td>2002</td>
<td>Yes</td>
<td>27, 28</td>
</tr>
<tr>
<td>p63</td>
<td>2000</td>
<td>Yes</td>
<td>16, 29, 4</td>
</tr>
<tr>
<td>bcl-2</td>
<td>2006</td>
<td>No</td>
<td>30</td>
</tr>
<tr>
<td>CD109</td>
<td>2007</td>
<td>No</td>
<td>31</td>
</tr>
<tr>
<td>D2-40</td>
<td>2010</td>
<td>No</td>
<td>32</td>
</tr>
</tbody>
</table>
Cytokeratins or p63/p40?

CK5/6

p63
New tools – new pitfalls...

p63-positive PCa!
Prevalence: 1%.
p63 or p40?

Comparison of p40 (ΔNp63) and p63 in tissues – which one is the superior marker for basal cells?

Verena Sailer, Carsten Stephan, Nicolas Werr, Manfred Dietel, Glen Kristiansen

- 98.6% of PCa p40 & p63
- 73% totally identical
  - minor differences:
    - 20% p63 > p40
    - 7% p40 > p63

  p63 positive PCa: 1.4% (!)
  p40 positive PCa: 0.6%

ΔNp63 (p40) expression in prostatic adenocarcinoma with diffuse p63 positivity

Katsunori Uchida MD, Hillary Ross MD, Tamara Lotan MD, PhD, Jean-Christophe Pignon PhD, Sabina Signoretti MD, Jonathan I. Epstein MD, Peter B. Illei MD

Table: p63/p40 staining in 31 cores of prostate adenocarcinoma with aberrant p63 expression, 125 cores of conventional prostate adenocarcinoma, and 157 cores of benign prostatic tissue

<table>
<thead>
<tr>
<th></th>
<th>p63 (clone 4A4)</th>
<th>p40 (polyclonal ΔNp63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p63 expressing prostate adenocarcinoma</td>
<td>31/31</td>
<td>29/31</td>
</tr>
<tr>
<td>Conventional prostate adenocarcinoma</td>
<td>0/125</td>
<td>0/125</td>
</tr>
<tr>
<td>Benign prostate (basal cells)</td>
<td>157/157</td>
<td>157/157</td>
</tr>
</tbody>
</table>

"From a diagnostic perspective, the detection of ΔNp63 with use of p40 antibody provides only a slight advantage over the currently in use p63 antibody."

Positive Markers of Malignancy: AMACR

AMACR-Immunoreactivity in Prostate Cancer:

- 95% of cases positive (T>N)
- Heterogenous in appr. 50% of cases
- Dependent on fixation and processing

- Important marker in small lesions
- Pitfall: nephrogenic adenoma +++
- Pitfall: adenosis/partial atrophy in 20%-30% positive (but rarely strong)
AMACR in benign glands
AMACR in Partial Atrophy
Alternatives to AMACR: 1. FASN

Comparison of the diagnostic value of fatty acid synthase (FASN) with alpha-methylacyl-CoA racemase (AMACR) as prostatic cancer tissue marker

- 93% of cases + (T>N)
- In 91% of AMACR-neg. cases positive

Alternatives to AMACR: 2. GOLM1

GOLPH2 protein expression as a novel tissue biomarker for prostate cancer: implications for tissue-based diagnostics

G Kristiansen, F R Fritzsche, K Wassermann, C Jäger, A Tölle, M Lein, C Stephan, K Jung, C Pilarsky, M Dietel and H Moch
Alternatives to AMACR: 3. GSTpi

Glutathione S-transferase-pi protein expression in prostate cancer—not always a useful diagnostic tool

- **Problem:** 25% of benign glands are negative, which limits the value of this marker
Alternatives to AMACR: 4. ERG

positive in only 50% of PCa
## Confirmation of Prostatic Origin

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>PCA</th>
<th>pN1</th>
<th>M1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PSA</strong></td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td><strong>PSAP</strong></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
<tr>
<td><strong>AR</strong></td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
</tbody>
</table>

- **PSA**: Highly specific, tends to get lost with progression.
- **PSAP**: Highly specific, tends to get lost with progression.
- **AR**: Relatively specific, increases with progression.

### Confirmation of Prostatic Origin

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>PCA</th>
<th>pN1</th>
<th>M1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSMA</td>
<td><img src="m.png" alt="Image" /></td>
<td><img src="n.png" alt="Image" /></td>
<td><img src="o.png" alt="Image" /></td>
<td><img src="p.png" alt="Image" /></td>
</tr>
<tr>
<td>Prostein</td>
<td><img src="q.png" alt="Image" /></td>
<td><img src="r.png" alt="Image" /></td>
<td><img src="s.png" alt="Image" /></td>
<td><img src="t.png" alt="Image" /></td>
</tr>
<tr>
<td>ERG</td>
<td><img src="u.png" alt="Image" /></td>
<td><img src="v.png" alt="Image" /></td>
<td><img src="w.png" alt="Image" /></td>
<td><img src="x.png" alt="Image" /></td>
</tr>
</tbody>
</table>

- **PSMA**
  - Unspecific, but typically increases with progression.

- **Prostein**
  - Highly specific, tends to get lost with progression.

- **ERG**
  - Highly specific in ERG-positive cases (30-50%).

Confirmation of Prostatic Origin: NKX3.1

**NKX3.1 as a Marker of Prostatic Origin in Metastatic Tumors**

Bora Gurel, MD,* Tehmina Z. Ali, MD,† Elizabeth A. Montgomery, MD,* Shahnaz Begum, PhD,* Jessica Hicks, BA,* Michael Goggins, MD,*‡ Charles G. Eberhart, MD, PhD,*‡ Douglas P. Clark, MD,*‡ Charles J. Bieberich, PhD,§ Jonathan I. Epstein, MD,*‡‖ and Angelo M. De Marzo, MD, PhD*‡‖

Am J Surg Pathol • Volume 34, Number 8, August 2010

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**TABLE 3.** The Average Percentage of Positively Stained Cells and the Calculated Staining Scores of NKX3.1, PSA and PSAP for Normal Prostate, Primary and Metastatic Prostate Carcinoma

<table>
<thead>
<tr>
<th></th>
<th>NKX3.1</th>
<th></th>
<th>PSA</th>
<th></th>
<th>PSAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Positive (Range)</td>
<td>Staining Score(SD)</td>
<td>% Positive (Range)</td>
<td>Staining Score(SD)</td>
<td>% Positive (Range)</td>
</tr>
<tr>
<td>Normal Prostate</td>
<td>92.0 (38.3-100)</td>
<td>218.3 (85.22)</td>
<td>965.0 (0-100)</td>
<td>241.7 (76.88)</td>
<td>97.6 (0-100)</td>
</tr>
<tr>
<td>Primary Ca</td>
<td>84.7 (25-100)</td>
<td>179.1 (77.84)</td>
<td>87.3 (10-100)</td>
<td>180.7 (91.36)</td>
<td>98.6 (85-100)</td>
</tr>
<tr>
<td>Lymph Node Met</td>
<td>74.2 (0-100)</td>
<td>155.4 (84.78)</td>
<td>80.1 (0-100)</td>
<td>1743.0 (99.14)</td>
<td>94.4 (0-100)</td>
</tr>
<tr>
<td>Distant Site Met</td>
<td>54.0 (0-88.3)</td>
<td>111.4 (85.36)</td>
<td>30.8 (0-100)</td>
<td>50.8 (92.83)</td>
<td>74.2 (0-100)</td>
</tr>
<tr>
<td>Average</td>
<td>80.5</td>
<td>175.9 (87.83)</td>
<td>83.1</td>
<td>186.0 (101.29)</td>
<td>95.0</td>
</tr>
</tbody>
</table>
Confirmation of Prostatic Origin: HOXB13

HOXB13 is a sensitive and specific marker of prostate cells, useful in distinguishing between carcinomas of prostatic and urothelial origin

Justine Varinot · Olivier Cussenot · Morgan Roupret · Pierre Conort · Marc-Olivier Bitker · Emmanuel Chartier-Kastler · Liang Cheng · Eva Compérat
HOXB13 vs. NKX3.1 – which one is better?

Comparison on 52 lymph node mets of PCa

HOXB13

NKX3.1
**HOXB13 vs. NKX3.1 – which one is better?**

Comparison on 52 lymph node mets of PCa

<table>
<thead>
<tr>
<th></th>
<th>HoxB13</th>
<th>NKX3.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>40%</td>
<td>2%</td>
</tr>
<tr>
<td>weak-moderate</td>
<td>33%</td>
<td>37%</td>
</tr>
<tr>
<td>strong</td>
<td>27%</td>
<td>61%</td>
</tr>
</tbody>
</table>

Kristiansen et al., Int. J. Mol. Sci. 2017, 18, 1151
How to avoid (prostatic) disasters

Don’t rush. Take your time reading slides. 10X is a fine lens!

Be aware of benign mimickers.

Diagnose “atypia” or “suspicious for cancer” when in doubt (>5 minutes) (...but do not over-use this diagnosis)

Use and interpret IHC with respect and caution. Morphology rules!

„The good thing about IHC is, that it gives you another day…“
David Grignon
Prognostic IHC in Prostate Cancer
Lack of prognostic markers in Prostate Cancer?

on average: 10 new prognostic markers per month!
How reproducible are immunohistochemical prognostic markers?

Pubmed: identification of 30 independent prognostic markers

Validating published immunohistochemical prognostic markers:

• Validation cohort-
  238 RPE cases (Zurich) on TMAs, 1 core/case
  median follow up: 5.3 years

• Automated immunohistochemistry (Ventana/Bond)

• Supervised reading of slide by a single investigator
Staining Patterns of evaluated Prognostic Markers

ADAM9
AKT1
ALCAM
AR
BCL-2
CB1R
CD10
CD24
CD138
CRISP3
CRGA
ECAD
ERα
ERβ
EZH2
HDAC2
HSP27
Ki-67
MUC1
NCAD
NFKB
p21
p27
PSMA
SPINK1
p53
VIM

Fabian Huber
Many studies are over-optimistic and insufficiently validated: Follow REMARK-guidelines.

A retrospective observation does not necessarily allow a prospective application.

Quantitative cut off-based IHC is problematic.

Establishing a robust immunohistochemical prognostic test for patient care is difficult.

Ki-67 is a strong prognostic marker in...

- Watchful waiting cohorts
- Radical prostatectomy cohorts
- Radiation therapy cohorts

Why then is it not used in clinical practice?
### Studies endorsing Ki-67 as a Prognostic Marker in PCa:

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Cohort Type</th>
<th>Cohort Size</th>
<th>Endpoint</th>
<th>cut-off</th>
<th>p-value (univariate)</th>
<th>p-value (multivariate)</th>
<th>Hazard ratio</th>
</tr>
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- Statistics
Ki-67 in general: How can we standardize the staining?

Pre-analytics:  
- time to fixation
- time in fixative, temperature
- fixative (additives?)
- embedding
- storage conditions

Analytics:  
- sectioning
- Antibody (clone Mib-1?)
- IHC-protocol: HIER? Detection?
Is inter-Lab Variability an Issue?

Central staining

100 BrCa cases
In TMA format

8-28%

Is inter-Lab Variability an Issue?

100 BrCa cases
In TMA format

Local staining

Establishing Antibody Titrations – which one is optimal?

Tolkach et al., in preparation
Influence of Ki-67 Antibody Dilution on „Proliferation“

The dilution titrates the proliferative fraction!

Image analysis alone is not the solution...

Tolkach et al., in preparation
Testis working Group
WHO March 2015, Zurich
New Nomenclature for Precursor lesion

CIS   IGCNU   TIN
New Nomenclature for Precursor lesion

CIS | IGCNU | TIN

It is no carcinoma!
New Nomenclature for Precursor lesion

CIS IGCNU TIN

It is not intraepithelial!
Neue Nomenklatur für Vorläuferläsion

CIS IGCNU TIN

„unclassified“ or „unknown“? - sounds stupid.
New Nomenclature for Precursor lesion

CIS  IGCNU  IGCN  GCNI  GCNIS  ?
GCNIS !!!

Germ Cell Neoplasia In Situ

GCNIS
WHO 2016 – Testicular Germ Cell Neoplasms

**GCNIS +**
- Seminoma
- Embryonal carcinoma
- Yolk sac tumor, postpubertal type
- Trophoblastic tumors
- Teratoma, postpubertal type
- Teratoma with somatic malignancy

**GCNIS -**
- Spermatocytic Tumor
- Teratoma, prepubertal type
  - Dermoid cyst
  - Epidermoid cyst
  - well differentiated neuroendocrine Tumor (monodermal Teratoma)
- Yolk sac tumor, pre-pubertal type
- Mixed teratoma and yolk sac tumors, pre-pubertal type
Trophoblastic Tumors

- Choriocarcinoma

- non-chorio-carcinomatous trophoblastic tumors:
  - Placental site trophoblastic tumor (PSTT)
  - Epitheloid trophoblastic tumor (ETT)
  - Cystic trophoblastic tumor (CTT)
Choriocarcinoma
Epitheloid trophoblastic tumor (ETT)

- Trophoblastic tumor, supposedly originating from intermediate trophoblastic cells of the chorionic laeve
- Squamoid monophasic trophoblast cells with ample eosinophilic cytoplasm
- Prominent cell boundaries, cytoplasmic/extracellular eosinophilic globuli
Cystic Trophoblastic Tumor
A Nonaggressive Lesion in Postchemotherapy Resections of Patients With Testicular Germ Cell Tumors

Thomas M. Ulbright, MD, *John D. Henley, MD,* Oscar W. Cummings, MD, *Richard S. Foster, MD,†
and Liang Cheng, MD*
## Immunoprofile

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<td>++</td>
<td>++</td>
<td>+/-</td>
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<tr>
<td>p63</td>
<td>--</td>
<td>++</td>
<td>-</td>
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<tr>
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Courtesy Prof. Dan Berney, London
Spermatocytic Tumour

Immunophenotype: PLAP– and Oct3/4-negative, CD117-positive

Formerly known as „Spermatocytic Seminoma“
OCT3/4 (POUF5F1)

- Transcription factor of embryonal stem cells, pluripotent
- 100% sensitivity for GCNIS, Seminoma and Embryonal Carcinoma (EC)

Useful for:
- Diagnosis of GCNIS
- Seminoma vs. Yolk Sac Tumor or Sertoli Cell Tumor
Podoplanin (D2-40)

- Transmembranous mucoprotein, expressed in fetal germ cells, lymphangio-endothelium and mesothelium
- Very high sensitivity for GCNIS and Seminoma
- Note: up to 30% of EC are focally positive (apically, membranous)

Useful for:
- Diagnosis of GCNIS
- Seminoma vs. Yolk Sac Tumor or Embryonal Ca
Placental Alkaline Phosphatase (PLAP)

- Enzyme of placental trophoblasts
- high sensitivity for GCNIS and Seminoma
- Note: up to 50% (!) positivity in EC/YST/CC
- Negative in Spermatocytic tumour and Sertoli Cell Tumor (SCT)

Useful for:
- Diagnosis of GCNIS
- Seminoma vs. Spermatocytic Tumour / SCT
Only Seminoma?
CD30 (Ki-1, Ber-H2)

- TNF-Receptor family
- Membranous/Golgi staining
- Most EC are positive (95%)
- Negative in other Germ Cell Tumors

Useful for:
- Embryonal Carcinoma vs. Seminoma
CD117 (c-KIT)

- Receptor Tyrosine Kinase
- Membranous expression in GCNIS and Seminoma
  - Note: spermatogonia may be positive, hence not recommended to diagnose GCNIS
- Variable expression in YST and Spermatocytic Tumour (SpT)
- Negative in EC and CC

Useful for:
- Seminoma vs. Embryonal Carcinoma
SOX17 (SRY-box 17)

- Transcription factor of embryonal stem cells
- Positive in GCNIS (100%), SE (95%), YST (50%), rarely Teratoma
- Note: sertoli cells positive- do not use to diagnose GCNIS!
- Negative in EC, CC & SpT

Useful for:
- Seminoma vs. Embryonal Carcinoma
SOX2 (SRY-box 2)

- Transcription factor of embryonal stem cells
- Positive in EC (95%) and < 1% SE
- Note: sertoli cells positive- do not use to diagnose GCNIS!
- Negative in CGNIS, YST and CC

Useful for:
- Embryonal Carcinoma vs. Seminoma
Alpha Feto-Protein (AFP)

- Plasmaprotein produced by YS and fetal hepatocytes
- Positive in YST, but quite variable...
  - lack of staining does not rule out YST!
- Negative in other Germ Cell Tumors
- Note: glands in teratoma may be positive

Useful for:
- YST vs. others (less sensitive than GPC3)
Glypican 3 (GPC3)

- Membrane bound proteoglycan
- Positive in YST (80-100%), CC (80%), TE ("immature", 20%) and EC (5%)
- Negative in GCNIS and SE
- Note: syncytiotrophoblastic giant cells may be positive

Useful for:
- YST vs. others (less specific than AFP)
Glypican 3 (GPC3)
Human Chorionic Gonadotropin (HCG)

• Glycoprotein produced by placental trophoblasts and most syncytiotrophoblasts
• Positive in CC (100%)
• Note: non-diagnostic syncytiotrophoblast cells in other GCT are positive as well

Useful for:
- Confirmation of CC
GATA-3

- Member of the ZNF-transcription factor family, regulates Differentiation (T-Cells, Breast, Urothelium...)
- Positive in a variety of malignancies: breast, urothelium, basal cell carcinomas, mesothelioma, skin SCC etc.
- Trophoblast cells in CC are positive
- Syncytiotrophoblast cells usually negative

Useful for:
- Confirmation of CC
GATA-3
Immunohistochemical Workflow to Diagnose Testicular Germ Cell Tumors

**TGCT**

(+ OCT3/4/)

- **Seminoma**
  - +CD117/Podoplanin (SOX17)
  - -CD30 (AE1/AE3, SOX2)

- **Embryonal Carcinoma**
  - -CD117/Podoplanin (SOX17)
  - +CD30 (AE1/AE3, SOX2)

- **Yolk Sac Tumor**
  - +Glypican 3
    - +AFP
    - -hCG
    - +PLAP

- **Spermatocytic tumour**
  - -Glypican 3
    - -AFP
    - -hCG
    - -PLAP

(- OCT3/4)

- **Choriocarcinoma**
  - +Glypican 3
    - -AFP
    - +hCG
    - +PLAP

*ISUP 2013*
SALL4

- Member of the ZNF-transcription factor family, involved in embryogenesis
- Positive in CGNIS, SE, EC & YST. 70% CCs, 50% TE.
- Negative in non-Germ Cell Tumours.

Useful for:
- Sensitive Detection of Germ Cell Tumours
- DD of metastatic tumours of unknown origin
Inhibin

• Glycoprotein-dimer of TGF-β family, inhibits secretion of gonadotropins
• Positive in Sex-cord Stroma Tumors (SCST): 100% Leydig Cell Tumors (LCT), 90% Sertoli Cell Tumors (SCT)
• Negative in TGCT, only syncyiotrophooblasts are positive

Useful for:
- SCST vs. TGCT
Calretinin

- 29 kDa calcium-binding protein
- Positive in 100% Leydig Cell Tumors (LCT), but only few Sertoli Cell Tumors (SCT)
- Germ Cell Tumors are negative
- Note: a negative Calretin stain does not rule out sex-cord stroma tumor

Useful for:
- SCST vs. TGCT
Immunohistochemical Workflow, when SCST is in the Differential Diagnosis

**TGCT vs SCST**

- **+ SALL4**
  - Inhibin & Calretinin
    - TGCT
- **− SALL4**
  - + or − Inhibin & Calretinin
    - SCST
WHO 2016 Testis: in a Nutshell

- GCNIS: New name for Precursor Lesion
- New Classification according to GCNIS/pathogenesis of disease
- Spermatocytic Tumor: avoiding dangers of mistreatment
- Robust and growing panel of Immunohistochemistry markers for accurate diagnosis
Thank you for your attention!

The help of Prof. Dan Berney, Prof. Stefan Schweyer and Muhammad Idrees in the testis part is greatly acknowledged.