NEW DEVELOPMENTS IN IMMUNOHISTOCHEMISTRY IN GYNAECOLOGICAL PATHOLOGY

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TOPICS TO DISCUSS

- Typing of ovarian carcinoma
- Typing of uterine carcinoma
- Endometrial versus cervical adenocarcinoma
- Cervical neuroendocrine carcinomas
- TTF1 in gynaecological neoplasms
- HMGA2 in vulvovaginal mesenchymal neoplasms
- New markers of ovarian sex cord-stromal tumours
- New markers of yolk sac tumour
TYPING OF OVARIAN CARCINOMA

At present of limited therapeutic significance
Treatment more dependent on stage and grade
A NEW ERA OF TARGETTED THERAPY FOR OVARIAN CANCER

Ongoing trials regarding alternative therapeutic agents in clear cell, low grade serous and mucinous carcinoma (chemoresistant neoplasms)

Will be important to accurately type on small biopsy
REPRODUCIBILITY OF TYPING OF OVARIAN CANCER

- Historically significant interobserver variability, especially for poorly differentiated tumours
- Main problem is SEROUS/ENDOMETRIOID
- Other problem is CLEAR CELL (true clear cell/clear cell change in serous or endometrioid)
USEFUL MARKERS

Å WT1- most serous carcinomas positive
Å p53 and p16- most high grade serous positive
Å hepatocyte nuclear factor 1β- marker of clear cell carcinoma
HIGH GRADE SEROUS VERSUS ENDOMETRIOID

serous

endometrioid
WT1

- A good marker of ovarian, tubal, peritoneal serous carcinomas (85-90% positive)
- A endometrioid, clear cell, mucinous rarely diffusely positive
- A useful in distinction between serous and endometrioid or serous and clear cell
- A most ovarian undifferentiated and transitional carcinoma also positive (suggests that most are variants of high grade serous)
CLEAR CELL VERSUS CLEAR CELL AREAS IN OTHER TUMOURS

- Classic areas of serous or endometrioid
- True clear cell - solid, papillary, tubulocystic patterns, hobnail cells, eosinophilic stromal hyalinisation
- WT1, p16, p53 (serous +ve; true clear cell -ve)
- ER (clear cell almost always -ve; serous often +ve, endometrioid usually +ve)
- Hepatocyte nuclear factor 1 beta (useful marker of clear cell carcinoma)
CLEAR CELL CARCINOMA
SEROUS WITH CLEAR CELLS
POST -CHEMOTHERAPY

- Increasing tendency to neoadjuvant chemotherapy followed by surgery
- Post-chemotherapy serous carcinomas may have clear cytoplasm and mimic clear cell carcinoma
- Maintain characteristic immunophenotype
- Need pre-chemotherapy biopsy
POST CHEMOTHERAPY

WT1

p16
HEPATOCYTE NUCLEAR FACTOR 1 BETA

A good marker of ovarian (and uterine) clear cell carcinoma (discovered from gene expression studies)

A endometriosis (associated and not associated with clear cell carcinoma) may be positive

A occasionally other neoplasms positive

A need more studies (and new monoclonal antibody)
OVARIAN CARCINOMAS

Â true mixed tumours extremely uncommon
Â using modern diagnostic criteria with or without markers, excellent interobserver agreement in typing is possible (Kobel et al. AJSP 2010;34;984-993)
UTERINE SEROUS VERSUS ENDOMETRIOID CARCINOMA

• papillary variants of endometrioid (including usual type, villoglandular and with small non-villous papillae)
• glandular variants of serous
• morphologically ambiguous and mixed tumours exist
• typing of uterine carcinomas much more problematic than ovarian carcinomas
PAPILLARY VARIANTS OF ENDOMETRIOID CARCINOMA
SEROUS ADENOCARCINOMA

Å NOT papillary serous adenocarcinoma
Å many cases have predominant or exclusive glandular architecture
DISTINCTION BETWEEN SEROUS AND ENDOMETRIOID

• important
• surgical operation may differ
• adjuvant therapy may differ
• ultimate prognosis will differ
MORPHOLOGY
IMMUNOHISTOCHEMISTRY-SEROUS VERSUS ENDOMETRIOID

Å use a panel (ER, p53, p16) (others may include PTEN, IMP-3)
Å interpret along with morphology
Å overlap in significant number of cases
CLASSIC IMMUNOPHENOTYPE

- endometrioid- ER+ve, p53-ve/focal, p16-ve/focal
- serous- ER-ve, p53+ve, p16+ve

BUT SIGNIFICANT OVERLAP (recent study- 20 of 34 USC ER+ve; 8 of 34 p53 negative)
Problems

• grade 3 endometrioid carcinomas
• mixed tumours
• morphologically ambiguous tumours
• interpretation of p53 staining
• overlapping immunophenotypes (serous may be ER+ve and p53-ve) (endometrioid may be p16 positive)
p53 (all or nothing staining of importance)

- Only consider positive if diffuse strong nuclear immunoreactivity (75% cells suggested) (closely associated with missence mutation)
- p53 null (completely negative) consistent with serous carcinoma (non-sense mutation or deletion)
- Focal, weak, heterogenous (wild type) staining of no diagnostic importance
- Also of relevance in other areas of pathology (e.g., Barrett’s oesophagus)
p53
HMGA2

A marker of vulvovaginal aggressive angiomyoma

A recent studies- ovarian serous carcinomas and serous TIC also positive
HMGA2 IN OVARIAN SEROUS CARCINOMA
HMGA2 IN UTERINE SEROUS CARCINOMA

Å 30 of 33 (91%) USC positive (usually 3+ or 4+ staining)
Å 14 of 38 (37%) endometrioid carcinomas positive (usually 1+ or 2+ staining)
Å serous EIC positive
Å useful marker in distinction between uterine serous and endometrioid carcinoma (part of panel)
HMGA2 IN USC AND SEROUS EIC
Distinction Between Endometrial and Endocervical Adenocarcinoma

- primary treatment may differ (radiology often doesn’t help)
- adjuvant therapy may differ
Panel of Markers

- ER
- vimentin
- monoclonal CEA
- p16
Endometrial Adenocarcinoma of Endometrioid Type

- ER diffusely positive
- Vimentin diffusely positive
- CEA negative (squamoid areas may be positive) or focally positive
- p16 negative, focal or diffuse (but patchy) positivity (squamous areas may be positive) (VERY FEW CASES TOTALLY NEGATIVE)
vimentin
p16 in endometrioid ca
Usual Type Endocervical Adenocarcinoma

- ER negative or weakly positive
- Vimentin negative
- CEA usually positive
- p16 diffusely positive
**p16 ASSOCIATION WITH HPV IN CERVICAL ADENOCARCINOMA**

- usual type diffusely p16 positive in 42 of 43 cases (78% HPV positive)
- unusual morphological types diffusely p16 positive in 6 of 20 cases (some others focally positive) (only 1 was HPV positive)
- DIFFUSE p16 POSITIVITY IN CERVICAL ADENOCARCINOMA NOT ALWAYS DUE TO PRESENCE OF HIGH RISK HPV
Pitfalls in Immunohistochemical Panel

- only useful for well differentiated tumours (low grade endometrioid versus usual endocervical)
- small biopsies
- some endometrioid carcinomas are diffusely p16 positive (but still patchy)
- uterine serous carcinoma may be diffusely p16 positive (often also ER negative)-SIGNIFICANT PITFALL- VALUE OF HPV STUDIES
- mucinous carcinoma of endometrium (or mucinous areas in endometrioid) has inconsistent immunophenotype
vimentin
CERVICAL NEUROENDOCRINE CARCINOMAS

Å SCNEC/LCNEC
Å important to make diagnosis since aggressive and require specific management
Å SCNEC- may be overlap with small cell squamous
Å LCNEC- may be overlap with poorly differentiated squamous or undifferentiated carcinoma
Å primary versus secondary (especially from lung)
STUDY-SCNEC (n=13), LCNEC (n=8)

MARKERS
- chromogranin
- CD56
- synaptophysin
- PGP9.5
- TTF1
- p63
- CK7/20
- neurofilament
- CD99
NEUROENDOCRINE MARKERS

- 52% chromogranin positive
- 90% CD56 positive
- 90% synaptophysin positive
- 43% PGP9.5 positive
CHROMOGRANIN
TTF1

71% positive
85% SCNEC
50% LCNEC
Immunoreactivity often diffuse
TTF1

• high percentage of cervical neuroendocrine carcinomas positive
• of no value in distinction from a pulmonary metastasis
• ? useful marker of neuroendocrine carcinoma
• different clones (SPT24 versus 8G7G3/1)
p63

• p53 homologue
• only nuclear immunoreactivity important
• in cervix, useful marker of squamous carcinoma
• most adenocarcinomas and neuroendocrine carcinomas negative
• useful in distinction between SCNEC and small cell squamous and between LCNEC and poorly differentiated squamous
p63

9 of 21 (7 SCNEC, 2 LCNEC) positive
5 with diffuse nuclear positivity
Occasional cases with non-specific cytoplasmic positivity
CK7/CK20/neurofilament

Å CK7- 10 cases positive (6 SCNEC, 4 LCNEC)
Å CK20- 4 cases positive (3 SCNEC, 1 LCNEC)
Å neurofilament- 7 cases positive (3 SCNEC, 4 LCNEC)
Evidence of Merkel Cell Immunophenotype

- No case positive for both CK20 and NFT
- CK20 positivity described in small cell neuroendocrine carcinomas in other organs (?
  of prognostic significance)
- Merkel cell polyomavirus negative (CM2B4)
- CK20 positivity in small cell neuroendocrine carcinoma of unknown origin not diagnostic of Merkel cell carcinoma
CD99

• marker of Ewing family of tumours
• 6 cases with membranous immunoreactivity (4 SCNEC, 2 LCNEC)
• 2 cases diffusely positive
• illustrates overlap with Ewing family of tumours
ASSOCIATION BETWEEN CERVICAL NEUROENDOCRINE CARCINOMA AND CGIN

- CGIN found in several cases
- Chromogranin positive cells in some cases (and some cases of pure CGIN)
- ? Neuroendocrine cells in CGIN are origin of some neuroendocrine carcinomas
chromogranin
TTF1 IN GYNAECOLOGICAL NEOPLASMS

- unusual cases of struma ovarii (use with thyroglobulin)
- neuroendocrine neoplasms
- metastasis from lung/thyroid
- some gynaecological adenocarcinomas positive (some diffusely so)
TTF1 IN GYNAECOLOGICAL ADENOCARCINOMAS

Å 7 of 19 ovarian serous carcinomas
Å 1 of 28 cervical adenocarcinomas
Å 6 of 31 uterine endometrioid adenocarcinomas
Å 3 of 13 uterine serous carcinomas
HMGA2

A translocation involving chromosome 12q15 found in aggressive angiomyxoma

A affects HMGA2 gene and results in expression of HMGA2 protein

A translocation and protein expression found in other mesenchymal lesions, for example uterine leiomyomas

A no protein expression in normal tissues
RESULTS

- 11 of 13 aggressive angiomyxomas positive
- 10 of 23 smooth muscle neoplasms positive
- Most, but not all, other lesions negative
HMGA2
MARKERS OF OVARIAN SEX CORD-STROMAL TUMOURS

Â inhibin, calretinin, CD56 are 3 most commonly used and are usually positive - some cases may be negative (should not doubt diagnosis)
NEW MARKERS OF OVARIAN SEX CORD-STROMAL TUMOURS

- Steroidogenic factor-1 (SF-1)- extremely sensitive marker of sex cord tumour but need more studies of mimics (endometrioid carcinoma and carcinoid negative)-nuclear staining

- FOXL2- possible marker of sex cord-stromal tumours- need more studies (recent paper- many (80%) sex cord-stromal tumours positive, including many not associated with mutation; FATWOs, UTROSCTs occasionally positive; epithelial neoplasms negative; 99% specific)-nuclear staining
GLYPICAN 3 AND SALL4 IN YOLK SAC TUMOURS

- yolk sac tumour may be difficult to diagnose
- small areas of yolk sac tumour may be difficult to identify in germ cell tumour (embryonal carcinoma, dysgerminoma, immature teratoma may mimic)
- AFP is only 60% (55-100% in various studies) positive, often focal, lot of background (blood, necrosis, serum)
- ? GLYPICAN 3 AND SALL4 SHOULD NOW BE MARKERS OF CHOICE FOR YOLK SAC TUMOUR
ADVANTAGES OF GLYPICAN 3 OVER AFP

Å greater sensitivity
Å more diffuse staining
Å more intense
Å all patterns of yolk sac positive
Å specific (embryonal carcinoma, dysgermininoma, immature teratoma negative)
Å less background staining
ADVANTAGES OF SALL4 OVER AFP

- greater sensitivity (also more sensitive than glypican 3)
- more diffuse staining
- nuclear marker
- all patterns of yolk sac positive
- less background staining
- EMBRYONAL CARCINOMA AND DYSGERMINOMA ALSO POSITIVE