Current Concepts in Gynecologic Pathology

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October 15, 2011
Educational Objectives

**Ovarian Pathology**
1. New Approach to Ovarian Carcinogenesis
2. Ovarian Borderline Tumors; Diagnostic Challenges

**Endometrial Pathology**
3. Immunophenotypic Approach to High-Grade Endometrial Carcinomas
4. Histologic Basis of Biological Behavior of Selected Endometrial & Mixed Tumors

**Cervical Pathology**
5. High Grade Cervical Intraepithelial Lesions: How to Avoid Over- & Underdiagnosis in Everyday Practice
Abbreviations

- Epithelial ovarian cancer – EOC
- Carcinoma – CA
- Serous carcinoma – SC
- Mucinous carcinoma – MUC
- Endometrioid carcinoma – EMC
- Clear cell carcinoma – CCC
- Transitional cell carcinoma – TCC
- Malignant mixed müllerian tumor – MMMT
- Carcinosarcoma – CS
- Undifferentiated carcinoma – UDCA
- Dedifferentiated carcinoma – DDCA
- Cystadenofibroma – CAF
- Low malignant potential – LMP
- Borderline tumor – BT
- Atypical proliferative tumor – APT
- Micropapillary serous ca – MPSC
- Intraepithelial carcinoma – IEC
1. New Approach to Ovarian Carcinogenesis

Dualistic Model
Operative (Limited) Paradigm of Ovarian Carcinogenesis

- **EOC regarded as a single disease**
  - composed of several different types, but majority HG SC
  - differences between other types obscured
- **Regarded as ovarian in origin**
  - CAs in the pelvis tend to involve the ovary
  - often as dominant ovarian mass
- **Classification based on histomorphologic findings** (WHO 1973)
  - provided basis for performing clinicopathologic studies
  - molecular tools to study pathogenesis not available
  - failure to identify the precursor of EOC
  - limited understanding of ovarian carcinogenesis
- **Empirical management**
  - no change in overall survival in >50 yrs
    - (despite advances in radical surgery and cytotoxic CTX)
  - early detection strategy (last 2 decades) failed to provide survival benefit

Serov, Scully, Sobin 1973 (WHO); Kurman, Shih 2011
Serous Carcinoma: Evolution of Concepts

- Traditionally graded as WD/ MD/ PD
- Spectrum of same disease - WD SC (LG) progression to PD (HG)

- Advances in molecular biology & tissue sampling methodology
- Recent data based on combination of morphologic, IHC, molecular genetic studies
- Culminate in proposal of a dualistic model of ovarian carcinogenesis
- Linking APST (typical SBT) to APMT (M-SBT) to LG SC
- **LG SC** - distinct entity different from **HG SC** (2-tier grading system)

*Malpica 2004; Shih, Kurman 2004*
Precursors of LG SC

- **APST - APMT - LG SC:**
  - morphologic continuum
  - supported by shared distinctive mutational & genetic alterations, & similar DNA methylation profiles (different from HG SC)*
  - LG SC appears to develop in a stepwise fashion from SBT

- **APMT (Non-invasive MPSC), (Micropapillary SBT):**
  - closer molecularly to invasive LG SC than to APST**

• **APT/ BT/ LMP** – acceptable terms

  “**BT**” preferred term
  “**Tumor of LMP**” accepted synonym *(WHO, 2003)*
  “**APT**” first used by *Russell* in the 1970s,
  gaining more support recently *(Kurman group, etc)*

• Most common types: **APST & APMT**

  *Seidman 2000, Lee 2003*
Dualistic Model of Ovarian Carcinogenesis

• Based on distinctive clinicopathologic & molecular genetic features
• Provides new insights into the molecular pathogenesis of EOC
• Accommodates & confirms the heterogeneous nature of EOC
• Places the major histologic types into 2 groups (types I & II)
• Links specific histologic types with their putative precursors

• Emerging concepts are novel and highly provocative
• Awaiting confirmation, modification, or revision
Dualistic Model of Carcinogenesis

- **Type I tumors:**
  - LG-SC
  - LG-EMC
  - CCC *(mixed features)*
  - MUC
  - Malignant Brenner tumor

- **Type II tumors:**
  - HG-SC
  - HG-EMC
  - CS (MMMT)
  - UDCA

*Kurman, Shih 2011*
Dualistic Model

• **Type I tumors:**
  - generally indolent
  - large cystic masses/ unilateral (bilateral-MPSC, APMT-seromuc)
  - lower stage: Ia (confined to ovary)
  - display *frequent somatic mutations* of genes participating in signal transduction pathways (SC 75%, mutually exclusive)
    - *KRAS/BRAF/ERBB2, PTEN/PIK3CA, CTNNB1, ARID1A, PPP2R1A*
  - relatively stable genetically
  - favorable prognosis

• **Type II tumors:**
  - highly aggressive
  - small size/ solid/ bilateral
  - advanced stage: II-IV (>75%)
    - extraovarian spread
  - harbor *TP53* mutations (>95%)**
  - inactivation of *BRCA 1/2* exp (~50%)
  - chromosomally highly unstable:
    - diffuse & high levels of DNA copy number gains or losses at loci
      - *CCNE1 (cyclin E1), NOTCH3, AKT2, RSF1, PIK3CA*
      (compared to Type I)

*Singer 2003, **Senturk 2010*
Prevalence of Histologic Types of EOC & Associated Molecular Genetic Changes

Type I
- ARID1A
- CTNNB1
- PTEN
- PIK3CA
- PPP2R1A

Type II
- TP53 mutation
- Chromosomal instability

Inactivation of BRCA 1/2
(Mutation or hypermethylation)

Endometrioid
Clear cell
Low-grade serous
High-grade serous
Mucinous
Cardinal molecular genetic changes include somatic mutations in KRAS, BRAF, & occasionally ERBB2 & PIK3CA. Mutated gene products activate the signaling pathways that regulate cellular proliferation & survival, and promote tumor initiation & progression.
Pathway Alterations Involved in the Development of Other EOC

- **LG EMC:**
  - mutations that deregulate \textit{PI3K/ PTEN} signaling pathway
  - mutations of \textit{CTNNB1} gene (encodes b-catenin)

- **CCC:**
  - activating mutation of \textit{PIK3CA}
  - somatic inactivity mutation of \textit{ARID1A} (tumor suppressor gene)

- **MUC:**
  - mutation in \textit{KRAS} gene
Do LG & HG SC Coexist?

- **210 SC** retrieved & reviewed from pathology archival files at Johns Hopkins Hospital
- **3 HG SC associated w/ APST** (typical SBT)
- **3 HG SC w/ LG invasive MPSC**
- Mutational analysis for **KRAS, BRAF & p53** genes performed

**RESULTS:**
- *Identical KRAS mutations* in both HG SC & APST components (2)
- remaining cases were negative for KRAS mutations (4) (only 2 cases informative from a molecular genetic standpoint)
- none of 6 cases had *p53 mutation* in both LG & HG components

*Dehari 2007*
Can LG SC Progress to HG SC?

- Rarely (~2%) LG and HG serous tumors do coexist. Presence of identical KRAS mutations in both LG & HG components indicates a common lineage/clonality.
- HG SC presumably develops along the LG (type 1) pathway & have different molecular pathogenesis compared w/ conventional HG SC (type II pathway).
- The possibility of independent synchronous tumors highly unlikely. Lack of p53 mutation in all 6 cases. Additional studies needed to determine the validity of this hypothesis.

Parker 2004, Dehari 2007
Dualistic Model

- **Type I tumors:**
  - develop slowly in a stepwise fashion
  - ass. w/ well recognized precursors:
    - cystadeno(fibro)ma/BT- **LG SC/ MUC**
    - endometriosis(ma) - **LG EMC/ CCC**

  **SR:**
  - Stage I: >95% (MUC), 70% (CCC)
  - Stage I: 100% (APST & MPSC)
    - 99% (APMT-GI)
  - Stage II-III: 70% (APST)
    - 50% (MPSC, APMT-GI)

- **Type II tumors:**
  - rapidly growing
  - not ass. w/ morphologically recognizable precursor lesions
  - develop “de novo” from ovarian inclusion cysts

  **SR:**
  - Stage I: >90%
  - Stage II-III: 20-30%

*Riopel 1999, Seidman 2000, Kurman 2008*
CCC

- Exhibit features of both type I & type II tumors

- **Similarity to Type I:**
  - develop from well characterized precursors
  - large cystic tumors
  - Stage I at presentation (70%) (*Veras 2009*)

- **Similarity to Type II:**
  - typically HG, very aggressive at higher stage (30%)

- **So where does CCC belong?**
  - *chromosomal instability index* very similar to that of LG SC
  - frequent *PIK3CA* activating & rare *TP53* mutations (*Kuo 2009*)
  - strongly support classifying CCC as type I tumor from both clinicopathologic & molecular perspectives (*Shih 2010*)
Is It Feasible to Detect HG SC at Stage I?

- **Advanced stage disease 75-80% vs 20-25% Stage I**

- **Detection while still Stage I is highly unlikely**
  - small at presentation, even when extraovarian disease
  - rapid spread to pelvic/abdominal cavities
  - cannot be detected by pelvic exam or transvaginal US

- **Perspectives**
  - goal of screening: detection of low-volume disease (even if outside ovary)
  - developing a panel of sensitive & specific molecular biomarkers expressed early in ovarian carcinogenesis (preceding development of morphologically recognizable precursors)
  - Tx instituted based on marker detection only
Origin of Ovarian Tumors: Current Hypothesis

- **SC (LG/HG):**
  FT epithelium (benign or malignant) that implants on the ovary (vs conventional view of ovarian surface epithelium or cortical inclusion cyst)

- **EMC & CCC:**
  Endometriosis (thought to develop from retrograde menstruation)

- **MUC & BT:**
  Not well established (possible origin from transitional epithelial nests in paraovarian locations at the tubo-peritoneal junction)
Spread of STIC from the Fimbria to the Ovarian Surface

Development of Cortical Inclusion Cyst from Tubal Epithelium

Downloaded from Kurman, Shih 2010 (Amer J Surg Pathol)
STIL - Serous Tubal Intraepithelial Lesion
STIC - Serous Tubal Intraepithelial CA

(precursor of occult invasive HG SC in FT closely resembling ovarian HG SC; gene expression profile more closely related to FT) (Marquez 2005)

Downloaded from Kurman, Shih 2011 (Hum Pathol)
Fimbria with STIC & Associated Invasive HG SC

Downloaded from Kurman, Shih 2011 (Hum Pathol)
Development of LG EMC/CCC from Endometriosis

Downloaded from Kurman, Shih 2010 (Amer J Surg Pathol)
Ovarian Carcinogenesis: Emerging Concepts in Progress

- Type I & II ovarian tumors develop independently along different molecular pathways.
- Both types originate outside the ovary and involve it secondarily.
- Only true primary ovarian neoplasms: gonadal stromal & germ cell tumors (analogous to testicular tumors).
- Shifting the early events of ovarian carcinogenesis to the FT & endometrium (instead of ovary).

Clinical Implications & Prevention Approaches:
- Salpingectomy (ovarian conservation/hormonal f-n & fertility) (SC)
- Tubal ligation (EMC & CCC)
2. Ovarian Borderline Tumors: Diagnostic Challenges
Ovarian Epithelial Tumor Categories

- Cystadeno(fibro)ma
- Atypical proliferative tumor (BT, LMP)
- Intraepithelial carcinoma (non-invasive)
- Microinvasion/ Microinvasive carcinoma
- Invasive Carcinoma
BORDERLINE OVARIAN TUMORS: A WEB-BASED ATLAS

NCI Borderline Ovarian Tumor Workshop, Bethesda, MD, August 27-28, 2003

Introduction

It is said that a picture is worth more than a thousand words. Pathologists believe that a picture with a few descriptive words is ...priceless. The purpose of this web page is to offer practicing surgical pathologists and pathology residents didactic samples of ovarian borderline tumors (also called atypical proliferative tumors or tumors of LMP) and the tumors that enter into their differential Dx.
“One of the most difficult areas in gynecological pathology is the spectrum of diseases that fall between the categories of clear cut benign epithelial lesion and clear cut invasive carcinomas. Pathologists sometimes disagree on terminology, even on the definition of apparently self-explanatory terms such as "destructive invasion", "severe nuclear atypia" and "microinvasion". For this reason, the National Institute of Health convened pathologists, clinical & surgical oncologists, epidemiologists and basic scientists interested in this field for the Borderline Ovarian Tumor Workshop".
DISCLAIMER:

This selection of images is for educational purposes only. The views are those of the contributors and do not reflect endorsement by the NIH or the University of Illinois at Chicago. The images contain statements related to rendered diagnostic opinions. In view of the wide range of opinions regarding the tumors under discussion, none of these statements is intended as, or should be interpreted as representing the "standard of care."
APT: Common Features

- **Gross:**
  - large, cystic, unilateral

- **Histology:**
  - epithelial proliferations w/ architectural complexity
  - nuclear stratification
  - mild to moderate nuclear atypia
  - mitotic activity (not atypical)
  - absence of stromal invasion

- **Survival:**
  Stage I – virtually 100%
SBT Category

- Heterogeneous group of tumors
- Includes 2 groups (stages in development of invasive LG SC)
- APST considered precursor of MPSC

**Atypical proliferative serous tumors (APST) (typical SBT)**
- predominant form of SBTs (~74%)
- behave in a benign fashion
- associated with non-invasive implants
- relatively favorable outcome (even w/ extraovarian disease)

**Micropapillary serous CA (MPSC) (micropapillary type SBT)**
- smaller group (~26%)
- behave like a LG SC
- marker for invasive implants, thus for invasive CA
- significantly more unfavorable outcome

Noninvasive MPSC: *(Kurman 2008)*
aka “SBT w/ Micropapillary Features” *(WHO)*

- Commonly both patterns coexist

- **Criteria** (for classification as MPSC to be clinically significant) *(Bell 2004)*
  - involvement of **at least one confluent area** (w/o intervening APST)
  - measuring **at least 5 mm** of micropapillary or cribriform pattern, or both

- **MPSC**: proposed as immediate precursor of LG SC: *(Seidman 2000)*
  - >75% of invasive MPSC associated w/ SBTs (>90% of which display micropapillary pattern)
  - associated invasive implants represent metastatic ca
  - tumors w/ invasive implants best considered advanced stage ovarian CAs
    (vs non-invasive implants)
“SBT”: Should It Still Be Used?

• With the division of **APST & MPSC**, the “SBT” category vanishes (created decades ago and served a very useful purpose by delineating & isolating the low end proliferative serous tumors from typical HG SC)

• Category of SBT (in regard to particular subtype) is now obsolete

• Continued use of the term “borderline” prevents an appropriate clinical Mx & obscures the important research questions that await further investigation

*Seidman 2000*
APST (SBT, typical type)

• **Histomorphology** *(Bell 2004)*
  - variably sized (large & small) fibrous papillae w/ irregular contours
  - exhibit a **hierarchical branching pattern** (of progressively smaller papillae ending in epithelial tufts)
  - extensive tufting with small detached clusters of similar cells
  - glandular structures containing papillae embedded in stroma
  - papillae lined w/ mildly to moderately atypical columnar, polygonal, or “hobnail-shaped” cells; ciliated cells are often seen
  - rare mitoses can be seen
Web-based Atlas (Soslow)
MPSC (SBT, Micropapillary/Cribriform type)

**Micropapillary type:**
- endophytic or exophytic long slender delicate (filiform) “micropapillae” w/ minimal or absent fibrovascular core, and smooth contours
- emanate directly in a nonhierarchichal fashion from cyst walls or circumferentially from large broad fibrous/edematous cores (“medusa”)
- filiform papillae at least 5x as long as wide to qualify *
- lined by round cuboidal cells with scant cytoplasm, high N:C ratio, mildly to moderately atypical round nuclei (G2), rare small nucleoli, & no cilia
- easily identifiable mitotic figures can be seen

**Cribriform type:**
- papillae lined by cribriform nests of moderately atypical cuboidal to columnar epithelial cells

Web-based Atlas (Soslow)
Small Foci of Tumor w/ Borderline Morphology within an Otherwise Benign CAF:

**Possible approaches** *(Borderline Ovarian Tumor Workshop, 2003):*

1) Ignoring it
2) Diagnosing the entire tumor as borderline
3) Diagnosing the tumor as benign & focally borderline, no further comment
4) Diagnosing as in (3), but with a comment suggesting that the lesion is unlikely to behave aggressively
5) Diagnosing the tumor as benign and mentioning the presence of the borderline focus/foci only in the comment

**This uncertainty reflects the lack of consensus**
- unclear whether transition to BT should occur at 5%, **10%**, 15% or >
- even 100% BT (Stage I) extremely unlikely to behave aggressively
- certainly smaller proportions should be at least equally benign
- universal agreement with 1% or 2% involvement
SBT with Microinvasion: Does It Matter?

- SBTs with small foci of intrastromal tumor cells ≤3-5 mm or <10mm²
- Often do not elicit a significant stromal reaction
- Can be found in 10-15% of SBTs

- **Eosinophilic cell pattern** (most common)
  - isolated cells/small nests w/ abundant eosinophilic cytoplasm in stroma
  - surrounded by clear spaces (retraction)
  - not ass. w/ unfavorable Px (SR 100% after 6.7 yrs follow-up)
  - does not alter clinical behavior (no clinical relevance)

- **Micropapillary pattern** (uncommon)
  - displays micropapillae (resembles LG SC)
  - may be designated also as “Microinvasive CA”
  - represents higher risk lesion w/ clinical course analogous to LG SC

SBT - Prognostic Indicators

• **Stage** of the disease

• **Nature of peritoneal implants/ extraovarian disease** (*Seidman 2000*)
  - survival data analyzed on 467 advanced stage SBTs
  - 104 had invasive implants (22% of all implants)
  - responsible for 67% of all tumor-related deaths (7-fold higher)

- distinction of invasive vs noninvasive implants may be extremely difficult
- in particular, with associated marked desmoplastic response
  (desmoplastic non-invasive implants)
- closely simulates host response of invasive CA
  (most difficult lesions to assess)
Peritoneal Implants

- Architecturally complex peritoneal proliferations ass. w/ SBTs
- Seen in 20-30% of SBTs at presentation
- Noninvasive & invasive

- **Noninvasive implants:**
  - superficial location on the surfaces of peritoneum/submesothelial/between omental lobules, along the septa
  - lack tissue invasion (micropapillary architecture within the stroma, or solid or papillary nests with a cleft)
  1) **epithelial type** - without a stromal response
  2) **desmoplastic type** - accompanied by a marked stromal reaction

McCaughey 1984, Scully 1999, Seidman 2000
Peritoneal Implants

• **Noninvasive**
  - assoc. w/ APST
  - represent reactive mesothelial hyperplasia or “true” implants from benign proliferative lesions
  - behave in a benign fashion

  - **SR:** 95.3%
    (w/ improved criteria ~100%)

• **Invasive**
  - assoc. w/ MPSC
  - represent metastatic LG SC
    (initially occur on the surface, but eventually invade; reason why some pts w/ “noninvasive implants” die of disease)
  - behave as CAs

  - **SR:** 66% (at 7.4 yrs f/u)

*Seidman 2000, Russell 2002, Kurman & Shih 2011*
Do Invasive Implants Ever Arise from APST (Typical SBT)?

- **Very unlikely!** Implants ass. w/ APST are non-invasive (Kurman 2008)

- Reported invasive implants ass. w/ APST probably contained occult areas of unsampled MPSC (adequate sampling!)

- If no areas of MPSC found, question whether implants truly invasive

- Micropapillary architecture: strong predictor of invasive implants

- **Adequate sampling** (Borderline Ovarian Tumor Workshop, 2003)*
  - at least 1 section/cm for tumors smaller than 10cm
  - 2 sections/cm for larger tumors (excluding smooth-walled cystic areas)
  - when micropapillary foci histologically, further sampling encouraged
  - grossly negative omentum sampled extensively: at least 1 section/2cm

Non-Invasive Implants: Epithelial Type

- Branching papillary proliferations w/ small epithelial tufts & buds on peritoneal surface or smoothly contoured submesothelial invaginations
  - well-defined/ frequently rounded free floating structures surrounded by reactive mesothelium
  - papillae with fibrous or hyalinized cores, +/- psammomatous Ca++
  - lined by mild to moderately atypical cuboidal to columnar cells
  - no destruction of underlying stroma

- Permeation of stroma by individual eosinophilic cells (w/o invasion of underlying tissue) should be included in non-invasive category

Web-based Atlas (Oliva)
Endosalpingiosis (D/Dx):  
- glands lined by tubal-type epithelium w/o significant atypia  
- blunt simple papillae with no significant epithelial stratification  
& no detachment of cell clusters
Non-Invasive Implants: Desmoplastic Type

• Gland-like & papillary structures, single & small nests of cells, in edematous/ inflamed fibrous tissue (“stuck on” the peritoneal surfaces)
  - circumscribed w/ sharp interface with normal tissue
  - abundant but superficial stromal reaction
  - low epithelium:stroma ratio (prevalence of stroma vs epithelium)
  - totally disorganized arrangement of bland epithelial elements, psammoma bodies, mesothelial cells and inclusions
  - complex but bland epithelial elements merging w/ adjacent stromal cells, although sometimes clefts may be seen
  - stroma with maturing/organizing granulation tissue appearance (“tissue-culture” type fibroblasts or spindled mesothelial cells)
  - typically ass. w/ inflammatory cells & recent hemorrhage

• Often both types of noninvasive implants present in same patient

Web-based Atlas (Oliva)
Invasive Implants

- Definition varies from study to study (expanded/less restrictive criteria)

- **Irregular infiltrative carcinomatous deposits** (papillae surr. by a space, angulated glands/interanastomosing, single cells) deeply penetrating stroma
  - irregular infiltrative margin of underlying tissue/fat
  - distinct from the surrounding stroma
  - higher epithelium:stroma ratio
  - more ordered radial growth/orientation
  - relatively uniform papillary or confluent cribriform aggregates
  - HG cytology

- **Micropapillary architecture** or **small solid nests or papillae** surrounded by a clear space or cleft (resembling LG SC) w/o true invasion of underlying tissue

Web-based Atlas (Oliva)
Web-based Atlas (Oliva)
Lymph Node Lesions Associated w/ SBTs

- **Endosalpingiosis** (benign tubal-type/müllerian glandular inclusions)
- **Mesothelial hyperplasia** involving lymph node sinusoids
- **Individual cells/clusters w/ abundant eosinophilic cytoplasm** in predominantly subcapsular sinuses
  - nature unclear, exfoliated mesothelial cells?
  - similar cells present at the surface of SBTs in the ovary
- **Small papillary clusters/glandular inclusions** resembling primary tumor, usually just beneath the capsule
  - majority associated w/ endosalpingiosis in the same node
  - no stromal invasion

*Clement 1996, Seidman 2000, Robboy 2002*
Lymph Node Involvement: Clinically Significant?

- **Regional** lymph node involvement:
  - not associated with adverse prognosis in patients with peritoneal implants (SR 98% after 6.5 yrs follow-up)
  - do not represent functional metastatic CA (reported w/o primary tumor)*
  - proposed independent origin within the lymph node (transition from müllerian inclusions)

- **Distant** lymph node involvement:
  - may rarely be seen at presentation (more commonly w/ tumor recurrence)**

Endosalpingiosis

SBT w/ Endosalpingiosis

Endocervicosis

Mesothelial cells in subcapsular sinuses

Web-based Atlas (Soslow)
CASE 1

44 y/o F with bilateral cystic ovarian masses (23 cm & 20 cm);
TAH & BSO, omentectomy, lymph node sampling

DX: Invasive LG MPSC Arising in MP SBT w/ Omental Metastasis and Lymph Node Involvement
HG vs LG (Micropapillary) SC

- Rarely, HG SC can mimic SBT (grow in non-invasive pattern)

- **HG SC:**
  - predominantly papillary (“MD”) or solid architecture (“PD”)
  - severe cytologic atypia (>3-fold nuclear atypia, frequent mitoses >12/10 HPF, prominent nucleoli)

- **LG SC:**
  - delicate micropapillary architecture
  - relatively small tumor cells with uniform nuclei & minimal atypia
  - inconspicuous nucleoli
  - less frequent mitoses

Malpica 2004, Dehari 2007
APMT- Intestinal type

- **Incidence**: 85% of APMTs

- **Histology**:
  - dilated gland/cysts with intraglandular epithelial proliferation, papillary tufts & infoldings
  - variable degree of nuclear stratification
  - columnar (goblet) cells w/ mucinous cytoplasm, low N:C ratio, round to oval basally situated nuclei w/ only mild atypia
  - mitoses may be frequent (>5/10 HPF)
  - +/- necrosis (due to mucinous gland rupture)
  - rupture of primary ovarian mucinous tumors has not been shown to lead to subsequent development of PMP

- **Survival**:
  - worse at advanced stage (~50%)
Web-based Atlas (Vang)
APMT - Endocervical type

- **Incidence:** 15% of APMT (younger age-group)

- **Classical (Müllerian) mucinous:**
  - pure endocervical-type epithelium
  - high association w/ endometriosis

- **Seromucinous:**
  - mixed serous (cuboidal/dense eosinophilic/ciliated) & endocervical-type (columnar/abundant eosinophilic cytoplasm, apical mucin)
  - cellular papillae w/ atypical (reactive) cells (resembles SBT/MSBT)
  - typical neutrophilic infiltrates within papillae, luminal mucin & stroma

- **Mixed cell-type mucinous:**
  - heterogeneous cell population including serous, endocervical-type mucinous, endometrioid, hobnail & “indifferent cells w/ abundant eosinophilic cytoplasm”

- **Survival ~100%:**
  overwhelmingly benign behavior at all stages (>Stage I, IEC, microinv CA)

*Shappell 2002*
Web-based Atlas (Soslow)
APMT with IEC

- **Histology:**
  - **severe cytologic atypia** (sole Dx criterion), regardless of architecture
    - enlarged round to oval nuclei, high N:C ratio, some degree loss of cytoplasmic mucin, irregularly distributed (vesicular) chromatin, prominent nucleoli, mitoses
  
    - complex cribriform intraglandular/intracystic growth w/ prominent epithelial tufting, detached papillae & stratification (typical of APMT)
    
      [does not qualify in the absence of severe atypia]

- **Survival:**
  - essentially 100% for Stage I (95%, *Riopel 1999*)
    - lower SR in older studies due to metastasis simulating primary ovarian MUC or primary MUC w/ unsampled destructive invasion)
Digital Atlas (Webpathology)

Web-based Atlas (Vang)
Complex intraglandular pattern, no severe nuclear atypia; intracystic vs within stroma (APMT vs confluent/expansile type MUC, if >5 mm)

(Vang) Web-based Atlas (Ronnett)
APMT with Microinvasion

- Focus/foci of stromal invasion ≤3-5 mm or <10 mm² (derived from measurement in 2 dimensions) (GI type)
- Small glands/irregular nests/single cells w/ abundant dense eosinophilic cytoplasm, round vesicular nuclei, and prominent nucleoli invading fibrous stroma**, w/ well-defined, sharply etched rounded spaces* & clefts
- **Cytologically LG** (similar to BT)
- Clinical significance of single vs multiple foci unknown)

- **Comment:** Based on the literature, tumors with up to 5 mm of microinvasion have demonstrated an excellent prognosis.***

APMT with Microinvasive CA

• Small nests of cells or micropapillae, often surrounded by clear spaces, haphazardly infiltrating fibrous or myxoid stroma, or grow in confluent pattern of back-to-back glands or micropapillae, w/ **HG cytology** (moderate-marked atypia)** and/or

• Small invasive foci arising in tumor w/ IEC

• Appear to represent transitional stage in ovarian mucinous carcinogenesis*

• Clinical significance unclear (more studies needed)

**Bell & Scully 1990, *Riopel 1999**
APMT w/ Microinvasion

Microinvasive CA

Web-based Atlas (Ronnett)
APMT w/ Microinvasion

Web-based Atlas (Vang)
CASE 2

25 y/o F w/ unilateral cystic ovarian mass (13 cm, 573 gm); Unilateral SO

DX: Mucinous Borderline Tumor (APMT), Intestinal Type, w/ Foci of IEC & Microinvasion (Microinvasive CA)
Types of Invasion in Primary MUC

- **Confluent glandular/cribriform (expansile) type**
  - common pattern (often ass. w/ APMT, which replaces ovary)
  - resembles AEH or WD EMC of endometrium
  - complex/confluent/cribriform back-to-back glandular proliferation w/o intervening stroma (interconnected labyrinthine, uninterrupted by normal ovarian stroma) and/or papillary growth
  - lacks destructive stromal invasion

- **Infiltrative (destructive) type (frankly invasive MUC)**
  - less common pattern (D/Dx: Metastatic MUC)
  - small mucinous glands/nests/individual cells w/ eosinophilic cytoplasm surrounded by clear spaces, haphazardly/ irregularly infiltrating stroma
  - foci of destructive stromal invasion >3-5 mm or >10 mm² (>5 mm = the sole feature that correlated w/ poor Px)
Primary Ovarian MUC

Infiltrative (destructive) pattern

Confluent glandular pattern

Image Gallery (Riopel 1999)
Metastatic MUC Involving Ovary

- **Bilateral, usually small** (<10-12 cm vs >15 APMT), GI type
- **Unilateral tumor ass. w/ extraovarian disease**, GI type
- Superficial cortical/surface ovarian involvement (vs stromal APMT)
- **Characteristic nodular infiltrative pattern of invasion**
  multiple small nodules w/ aggregates of small to medium-sized, well-formed and irregularly infiltrative glands clustered together & separated from adjacent nodules by preserved and compressed ovarian stroma/structures (vs confluent diffuse)
- **LVI**, particularly in the hilum
- Ass. w/ prominent **pseudomyxoma peritonei (PMP)** ~90% (vs absent/minimal in APMT)
- **Survival (5yr SR):** Poor - **11%** vs **91%** (Stage I) & **51%** (all Stages) Ovarian MUC*

*Riopel 1999*
Metastatic MUC of Pancreas

Nodular infiltrative pattern of invasion w/ superficial cortical & surface involvement

Image Gallery (Riopel1999)
Metastatic MUC Involving Ovary

**PMP:**
- presence of mucinous material on the peritoneal surface (beyond RLQ)
- scant LG adenomatous (appendiceal) or HG (colorectal) mucinous epithelium (vs abundant proliferative mucinous epithelium in APMT)

Primarily caused by *GI (appendiceal, colorectal, gastric) / Pancreaticobiliary,* rarely by *Endocervical CA,* exceedingly rare (exception) by *Teratomatous ovarian mucinous tumors*

**Diagnostic challenge:**
- occult extraovarian primary tumor
- highly differentiated areas simulating ovarian precursor mucinous tumor (cystadenoma & APMT)
- mets from *pancreaticobiliary* (*Dpc4* neg ~50%) & *endocervical* (*p16/HPV* +) origin may resemble ovarian histomorphology
- “synchronous” appendiceal and ovarian mucinous tumors not uncommon

*Riopel 1999*
Krukenberg Tumor
D/Dx: Mucinous Tumors

• **Primary Appendiceal/Colorectal**
  
  CK7 (-)
  CK20 (+)
  CDX-2 (+)
  ER/PR (-)

• **Primary ovarian**
  
  CK7 (+)
  CK20 (+/-) (~75%)
  CDX-2 (+/-)
  ER/PR (-)

• **Ovarian mucinous tumor arising in teratoma**
pelvic tumor

CK20

CDX2

CK7

CEA

CA125
Primary Ovarian MUC, confluent glandular/expansile type

Ovarian Mucinous Tumor ass. w/ PMP (secondary involvement) [simulates APMT]

Web-based Atlas (Ronnett)
Metastatic Colonic CA
(deceptive pattern of invasion/w/o destructive stromal)
[simulates APMT]

Metastatic Colonic CA
(marked nuclear atypia)
[simulates APMT w/ IEC]
Endometrioid Tumors

- **APET:**
  - marked glandular crowding with budding and branching glands
  - mild to moderate cytologic atypia

- **APET w/ IEC:**
  - marked cytologic atypia
    (nuclear enlargement, vesicular chromatin, prominent nucleoli)

- **APET w/ microinvasion (<5mm or ≤10mm²):**
  - *infiltrative pattern* (more common)
  - haphazardly arranged glands/nests ass. w/ an altered stroma
    *confluent pattern* (less common)
  - cribriform architecture within glands as opposed to within stroma
    (not universally accepted as pattern of invasion)

- **Small foci of WD CA arising within an endometriotic cyst (2 mm)**

- **Implants:** very rare and poorly documented

*Seidman 1996*
Endometrioid Cystadenoma

APET w/ Squamous MP

Digital Atlas (Webpathology)
WD EMC (Villoglandular type)  
WD EMC (Sqaumous MP)

Digital Atlas (Webpathology)
## D/Dx: Endometrioid Tumors

<table>
<thead>
<tr>
<th>Colorectal</th>
<th>Primary ovarian</th>
<th>Endocervical</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK7 (-)</td>
<td>CK7 (+)</td>
<td>CK7 (+)</td>
</tr>
<tr>
<td>CK20 (+)</td>
<td>CK20 (-)</td>
<td>CK20 (-)</td>
</tr>
<tr>
<td>CDX-2 (+)</td>
<td>CDX-2 (-)</td>
<td>CDX-2 (-)</td>
</tr>
<tr>
<td>ER/PR (-)</td>
<td>ER/PR (+)</td>
<td>ER/PR (-)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p16 (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPV-ISH (+)</td>
</tr>
</tbody>
</table>
Clear Cell Tumors

- AFs and APTs very uncommon
- Almost all tumors in CC category are CAs
- AF to AF-like patterns of CCC represent morphologic continuum*
- No uniformly accepted diagnostic criteria:
  - AF vs APT
  - AF/APT w/ cytologic atypia vs APT with IEC vs AF-like patterns of CCC w/ subtle cytologic atypia
- Thresholds vary between GYN pathologists

Yamamoto 2007, Vang 2008
Clear Cell AF vs APT

• **Both resemble one another:**
  - round simple glands within a fibromatous stroma
  - round, dense, and *eosinophilic intraluminal secretions*
  - epithelium often flattened (obvious CC diff. may not be apparent)
  - at least focally: hobnail cells

• **Clear Cell AF:**
  - glands widely spaced apart by fibroblastic stroma
  - lined by 1-2 layers of *w/o stratification*
  - cuboidal or flattened cells w/ abundant clear cytoplasm
  - no nuclear atypia

• **Clear Cell AF – Borderline (APCCT):**
  - marked glandular crowding +/- small solid nests
  - more irregular gland contours & *stratification*
  - some degree of atypia
Clear Cell AF

Digital Atlas (Webpathology)
CC AF (borderline) w/ focus of microinvasion

Digital Atlas (Webpathology)
Marked Cytologic Atypia in CC Tumors

- exact level of atypia sufficient for IEC or CCC not well-defined
- degree of nuclear atypia in AF/APT can overlap w/ CCC
- some CCC can have deceptively bland nuclei

- **APCCT w/ IEC** *(Tavassoli 2003; Kurman 2008)*
  - marked nuclear atypia w/o stromal invasion
  - criteria not well-defined

- **APCCT w/ microinvasion** *(Bell 1985)*
  - focus <3 mm w/ glands, small solid nests/single cells w/ malignant nuclei
  - haphazardly arranged within desmoplastic, myxoid, or edematous stroma
  - 2/3 patients were alive at 4 & 6 years, 1 patient recurred at 3 years

- **Adenofibroma-like pattern of CCC**
  - at least focal areas w/ combination of glandular crowding, marked nuclear atypia & epithelial stratification beyond the level expected for APCCT

- **Implants:**
  - extra-ovarian implants at the time of surgery have not been described
  - frequent “implants of endometriosis” elsewhere in the pelvis *(Veras 2009)*
CCC

- **General rule** for the distinction of APT from CA *(WHO Classification)*
  - presence of stromal invasion

- **CCC** – an exception to this rule
  1) *infiltrating or confluent patterns* (obviously invasive)
  2) *intracystic proliferative pattern* (appear to proliferate within a cyst)
     - may not have apparent stromal invasion of the traditional type
     - conventionally diagnosed as CA (generally aggressive behavior)

- **CCC** – *typically not graded* (unlike other histologic types)
  - grade has not correlated with Px (in several studies)
  - present at Stage I in 70% *(Veras 2009)*
  - generally less responsive to CTX than SC
CCC: Different Pathways of Pathogenesis

- **CCC arising in a AF/APT** (less common)
  - progression from benign precursors, more LG/ often at advanced stage
  - more frequent tubulocystic architecture
  - less frequent endometriosis (not ipsilateral)

- **CCC ass. w/ endometriosis** (more common)
  - evolution from endometriosis, higher histologic grade/ often at Stage I
  - more frequent papillary architecture
  - more frequent endometriosis (ipsilateral)

**CC IEC arising within an endometriotic cyst** (lowest level of atypia in lining cells required for Dx/ not well-defined; overlap with “atypical endometriosis”)

- **CCC arising de novo**
  - indeterminate (not related to above)

CCC (Tubulocystic & Papillary)
(clear or oxyphilic cytoplasm)

Signet-ring cell appearance
(prominent vacuoles)

Digital Atlas (Webpathology)

PD/HG
(Inflammatory BG)
D/Dx

• CCC
  - HNF-1b (+)
  - WT-1 (-)
  - ER/PR (-)

• LG SC
  - HNF-1b (-)
  - WT-1 (+)
  - ER/PR (+)

Sangoi 2007, Yamamoto 2007
Transitional Cell Tumors

- **Benign Brenner tumor** (majority)
  - small solid nests of transitional cells w/ sharply defined outlines
  - in dense fibroblastic stroma
  - bland cells w/ pale cytoplasm & oval nuclei w/ longitudinal grooves, and small inconspicuous nucleoli
  - ass. w/ mucinous lesions (juxtaposed/abrupt transition/hybrid) (~25%)

- **APBT/ Borderline Brenner tumor** (rare)
  - crowded complex nests & large papillary fronds
  - exuberant proliferation w/ tongues protruding into luminal spaces
  - some degree of cytologic atypia, mitoses (~ LG Urothelial CA)

- **IEC and microinvasion** (not specifically defined)

- **Malignant Brenner tumor** (rare)
  - HG nuclei (~HG Uroth CA), invasion/desmoplastic stroma, Ca++
  - BG component of precursor lesions (BBT/APBT)

- **TCC** (not uncommon), often bilateral
  - HG invasive CA in the absence of precursor lesions
  - mixed w/ HG CA: SC, EMC, MUC, CCC
Benign Brenner Tumor
Brenner Tumor Ass. w/ Mucinous Cystadenoma

Digital Atlas (Webpathology)
Borderline Brenner Tumor
Malignant Brenner Tumor

Digital Atlas (Webpathology)
CASE 3

81 y/o F with unilateral ovarian mass (3.6 cm);
TAH & BSO

**DX:** Malignant Brenner Tumor Arising in a BG
of Atypical Proliferative Brenner Tumor &
Associated Mucinous Cystadenoma
TCC: Histologic Patterns

• **Resembles HG Urothelial CA (TTC)**
  - large blunt elongated papillae w/ broad or fine vascular cores
  - papillae often project into cystic spaces
  - elongated cells/oval nuclei with longitudinal grooves
    (“coffee bean nuclei”; can be lost in HG tumors)
  - rounded or flattened cells, especially in the superficial half
    (areas of squamous differentiation)

• **Solid papillary pattern**
  - papillary stromal vascular cores and septa maintained with
    coalescence of large papillae lined by
  - thick bands of undulating stratified transitional epithelium
  - flat luminal border

• **Solid nested pattern**

• **Other**: diffuse, insular, trabecular, microcystic

*Eichhorn 2004*
TCC (solid papillary pattern)
CASE 4

46 y/o F with unilateral ovarian mass (9 cm), multicystic with lobulated solid areas; TAH & BSO

**DX:** Transitional Cell Carcinoma
TCC (solid nested pattern)
<table>
<thead>
<tr>
<th>D/Dx</th>
<th>TCC, primary ovarian</th>
<th>TCC, metastatic bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT1</td>
<td>(+)</td>
<td>WT1 (-)</td>
</tr>
<tr>
<td>CK20</td>
<td>(-)</td>
<td>CK20 (+)</td>
</tr>
<tr>
<td>CK7</td>
<td>(+)</td>
<td>Thrombomodulin (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CK7 (+)</td>
</tr>
</tbody>
</table>

Logani 2003
Kentucky Farm House
3. Histologic Basis of Biological Behavior: Selected Endometrial, & Mixed Tumors

Current Concepts
HG Endometrial Carcinoma w/ Ambiguous Morphology

HG EMC or SC?

Immunophenotypic Approach
Morphologically Ambiguous Endometrial CA

- Distinction between SC & EMC usually achieved by histology alone
- Occasional CAs w/ overlapping serous & endometrioid features

1) Mixed morphology: some SC may arise in EMC via \( p53 \) mutation
   presence of EMC seems to have no impact on survival*
2) Subset of SC composed entirely or predominantly of glands
   and/or solid areas w/o papillary component**

- Difficult to subclassify by morphologic examination alone
- Subclassification important: significant Tx & Px implications
- \( p53 \) IHC has Px value (more aggressive clinical course)***

*Carcangiu 1992, **Darvishian 2004, **Lomo 2008, ***Garg 2010
Serous Carcinoma
Glandular Architecture & HG Nuclei
SC or HG EMC?
SC or HG EMC?
Are SC Underdiagnosed?

Study Design & Results

• 43 hysterectomy specimens from patients w/ Dx HG EA, classified as pure HG EMC (n=32), mixed HG EMC/SC (n=9), mixed HG EMC/CC (n=2)
• Representative blocks stained for p53, p16, ER (1D5), mammaglobin (MGB)
• Recorded data on clinical f/up (M=41 mos) from Tumor Registry reviewed

• Based on strong/diffuse positivity for p53 and p16
• 39% (17/43) of pure & mixed HG EMC were reclassified as SC
• All 17 tumors uniformly (-) for MGB
• ER(+) 35%
• Only 18% (3/17) patients were alive and disease free on f/up

• Re-established HG EMC p53(-) 96% & p16(-) 77%
• MGB(+) 31%
• ER(+) 73%
• 58% (15/26) patients alive and disease free
Our Study
(Poster USCAP 2009)

Serous Immunophenotype

P16

P53

MGB

ER
HG CA w/ Ambiguous Morphology: Our Study

- **HG EMC**
  - p53 (-) (<75% of cells, weak)
  - p16 (-) (<75% of cells, weak)
  - ER (+/-)
  - MGB (+) (31%)

- **SC/Immunophenotype**
  - p53 (+) (>75% of cells, strong)
  - p16 (+) (>75% of cells, strong)
  - ER (+/-)
  - MGB (-) (100%)
Conclusions

1) HG EMC & SC w/ glandular morphology are difficult to distinguish by histology alone

2) Panel of IHC stains (p53, p16, MGB) may be helpful in separating tumors with serous immunophenotype

3) Tumors with SC phenotype behave more aggressively compared to that of HG EMC

4) Accurate risk stratification is critical for tailoring optimal and effective therapeutic regimens
CASE 5

65 y/o F with abnormal uterine bleeding;
TAH & BSO

DX: HG Endometrial CA w/
Serous Immunophenotype /SC
EMC vs Endocervical CA

- **EMC**
  - Vimentin (+)
  - ER (+)
  - p16 (-) (patchy/weak)

- **Endocervical CA**
  - mCEA (+)
  - p16 (+) (diffuse/strong)
Carcinosarcoma: Homologous or Heterologous Elements

Does It Matter?
CS (MMMT)

- **WHO definition:**
  - neoplasm composed of admixed **malignant** epithelial & mesenchymal components

- **Biphasic tumor** with both **Endometrial CA & Sarcoma**
  - considered CA with “metaplastic” sarcomatoid features  
    *(McCluggage 2002)*
  - occur predominantly in postmenopausal women/ bleeding
  - biologically aggressive, often present at advanced stage
  - metastasis usually as CA or CA-predominant (intraabdominal), rarely as sarcoma (distant sites: lung, etc)
  - 5-yr SR of <35%  *(Yamada 2000)*
CS: Types of Sarcomatous Elements: Prognostically Significant?

- **HOMOLOGOUS** (tissue of origin native to uterus)
  - stromal sarcoma
  - leiomyosarcoma
  - fibrosarcoma

- **HETEROLOGOUS** (tissue of origin foreign to uterus)
  - liposarcoma
  - rhabdomyosarcoma
  - chondrosarcoma
  - osteosarcoma
CS: Prognosis

- **Disease Stage** – the most important Px factor *(Sartori 1997, Amant 2005)*
  5yr SR: Stage I >50%; advanced stage 0-25%

- **Other predictors of negative clinical outcome** (in Stage I/II disease)
  - deep myometrial invasion
  - LVI
  - histology of carcinomatous component
  - extent of sarcomatous component

- **Clinical significance of heterologous elements:**
  - significantly increased rate of recurrence & decreased SR in women w/ heterologous CS compared to homologous *(large studies, based on surgical staging: GOG, MSKCC)*
  - no prognostic significance to the presence of heterologous components *(smaller studies, based on clinical staging)*
Px Features of Surgical Stage I Uterine CS

- 47 CSs & 38 HG endometrial CAs at Surgical Stage I, at MSKCC (Ferguson 2007)

- Surgical Stage I CS w/ **homologous** sarcomatous elements have recurrence & SRs similar to high-risk endometrial CAs
  - 3yr OSR for homologous CS & HG endometrial CAs both >90%

- Profoundly poor SRs for women w/ Stage I **heterologous** CS
  - 3yr OSR 45% (heterologous) vs 93% (homologous) \( (P<0.001) \)
  (emphasizes the need for further clinical investigation of adjuvant Tx)

- Results similar to GOG Study (Major 1993)
CASE 6

61 y/o F w/ working Dx of Endometrial CA; TAH & BSO

**DX:** Carcinosarcoma (MMMT) w/ Homologous & Heterologous Elements
EMC w/
Prominent Spindle Cell Component:

Carcinosarcoma (homologous) or Metaplasia?
LG EMC w/ Spindle Cell Component

- Biphasic tumor usually of **LG** epithelial & **LG** spindled components
- **Endometrioid elements** (FIGO G1/2) often w/ squamous MP
- **Prominent spindle cell elements** (cellular, +/- mitotically active, but not markedly atypical) & hyalinized stroma
- Both elements characteristically **merge w/ one another** (“element fusion”) instead of remaining distinct (as in CS)
- Rarely may contain **bland** heterologous elements: *(Murray, Soslow)*
  - heterologous elements by themselves do not signify CS!!!
  - bland chondroid (EMC w/ chondroid metaplasia)
  - bland osteoid (EMC w/ osteoid metaplasia)

**CS = HG CA + HG Sarcoma**
(different/easily separable components/not merged)

LG EMC w/ Spindle Cell Component

Short Course #21 (USCAP 2008)
CASE 7

68 y/o w/ working Dx of Endometrial CA; TAH & BSO

**DX:** MD EMC w/ Spindle Cell Component
EMC w/
Squamous Differentiation

Does It Affect Grading?
EMC w/ Squamous Differentiation

- Common finding in EMC ~50%

- **Criteria for squamous differentiation:**
  - keratinization demonstrated w/ standard staining technique
  - intercellular bridges and/or
  - three or more of the following 4 criteria:
    a) sheet-like growth without gland formation or palisading
    b) sharp cell margins
    c) eosinophilic and thick or glassy cytoplasm
    d) decreased N/C ratio compared to foci elsewhere in the tumor

*Tavassoli 2003*
EMC w/ Squamous Differentiation

- **Subtypes** (*Silverberg 1982*)
  - *Adenoacanthoma type* (benign squamous morules)
  - *Mixed adenosquamous CA type* (malignant squamous component)

- Natural history depends on histologic *(FIGO)* grade of AC component
  - **Grade 1:** <5% solid pattern
  - **Grade 2:** 5-50%
  - **Grade 3:** >50%

- Recognition of squamous/morular elements essential for grading purposes
  - should not be considered as solid component (false upgrade) (*Zaino 2009*)

- Subdivision into subtypes left to individual pathologists (*Zaino 1991*)
WD EMC w/ Squamous Differentiation:
(benign squamous metaplasia/morules)

Digital Atlas
(Webpathology)
WD w/ Squamous Differentiation:
(malignant squamous component)
CASE 8

58 y/o w/ abnormal uterine bleeding; TAH & BSO

**DX:** EMC (FIGO G1) w/ Squamous Differentiation
LG EMC Associated w/ Undifferentiated Carcinoma

A New Type of De-Differentiated Carcinoma?
Undifferentiated CA

- HG/aggressive CA of endometrium (9% of all endometrial CAs)
- Under-recognized and poorly studied (frequently Dx as G3 EMC)
- Recognition extremely important when associated w/ G1/G2 EMC
- Important to Dx solid areas as UD & avoid evaluation them as solid component of EMC (glandular elements within solid component)
- Lack of recognition of UDCA as part of mixed CA w/ ass. EMC can lead to misclassification of the tumor as EMC G2/G3:
  - UDCA + G1 EMC: FIGO G2 EMC
  - UDCA + G2 EMC: FIGO G3 EMC

- Significant difference in biologic behavior/ clinical outcome:
  - G2 EMC: excellent Px
  - G3 EMC: IM Px
  - UDCA: poor Px (irrespective of the extent of LG component)

**UDCA ass. w/ differentiated areas = DDCA**

Silva 2006, Silva 2007, Tafe 2010
Dedifferentiated CA

- **UDCA** *(Silverberg 2003, WHO classification)*:
  - neoplasm lacking any evidence of differentiation

- **DDCA** *(Silva 2007)*
  - UDCA ass. w/ differentiated areas (recently described entity)
  - UDCA related to FIGO G1/G2 EMC either in primary tumor or recurrence
  - reflects de-differentiation *(Silva 2006)*

Importance of recognition of UD component from clinical behavior standpoint

**UDCA/ DDCA**: *(Altrabulsi 2005)*
- 54% presented at advanced stage/ may pursue fulminant clinical course
- did not respond to conventional CTX regimen used for EMC
- 75% died of the disease

**FIGO G3 EMC**: 
- presented w/ advanced stage in only 30%
- 30% died of the disease
DDCA: Histomorphology

• **Gross features**: nonspecific (white & fleshy w/ foci of necrosis)

• **Microscopic features**:
  - LG (WD/MD) EMC juxtaposed with distinct UDCA
  - usually look biphassic with abrupt transition/ necrotic foci
  - solid sheets of dyshesive monotonous round to oval cells (+/- pleom)
  - enlarged nuclei, occasional prominent nucleoli, mitoses 10/10hpf
  - D/Dx: EMC G2/3 - intermixed glandular & solid/ similar cell type

• **IHC profile**:
  - inconsistent expression of epithelial markers (HG EMC: strong/diffuse)
  - heterogeneous staining for keratins: (-)/ weakly (+)/ focal (+++)
    (in 80-90% cases only 5-10% tumor cells panCK+)
  - variable expression of EMA, Vimentin, ER/PR
  - only focal (<10%) expression NE markers (40% cases)

• **Association w/ defects in DNA mismatch repair system**, including Lynch syndrome *(Garg 2011)*

CASE 9

85 y/o w/ working Dx of Endometrial CA; TAH & BSO

**DX**: De-differentiated CA
(LG EMC Associated w/ UDCA)
rhabdomyoblastic differentiation  abrupt keratinization
4. HG Cervical Intraepithelial Lesions (CIN 2+)

How to Avoid Over- & Underdiagnosis in Everyday Practice
ARE CIN2 LESIONS OVERDIAGNOSED?  
Selected Immunopanel Can Prevent Unnecessary Cervical Excisions  

R. Karabakhtsian¹, M.Cibull¹, C. DeSimone²  
Departments of ¹Pathology and ²Gynecologic Oncology  
University of KY Medical Center, Lexington, KY, USA
CIN2 Lesions of Uterine Cervix: Background

- May present diagnostic challenge
- Particularly in small cervical biopsies & focal disease
- Can be mimicked by atypical cellular changes
- Recognized interobserver variability
- Will prompt aggressive clinical management
Mimics of Cervical HG Dysplasia

- Reactive squamous atypia ass. w/ inflammation
- Atypical squamous metaplasia
- Immature squamous metaplasia
- Tubal metaplasia
- Reserve/basal cell hyperplasia
- Hormonal changes
- Atypia of atrophy
Importance of Accurate Diagnosis (Benign/CIN1 vs CIN2)

- Risk stratification for appropriate management
- Conservative approach w/ interval f/up (annual Paps or cervical biopsies) vs aggressive clinical Mx
- Prevention of unnecessary cervical excisions
- Preservation of sexual and reproductive health
- Reduction of unjustified medical cost
Aim of This Study

- Retrospectively Re-evaluate CIN2 lesions 
  (*moderate squamous dysplasia*)

  - diagnosed on cervical biopsies (bx)
  - subset of patients w/ no residual HG lesion (CIN2) in subsequent cervical excision
  - by utilizing a selected panel of three IHC stains
Study Design

- Total of 211 cervical bx from 151 patients
  (38 patients had >1 bx taken during colposcopy)
  - histologic dx of CIN2
  - performed from 2000 to 2007
  - age range 17 to 65 years (m=30), 14 (x1), 86 (x1)
  - underwent subsequent cervical excisions
    (142 LEEP, 9 Cold Knife Cone)
Histologic Findings on Subsequent Cervical Excisions

Residual CIN2 identified in 89/151 (59%) patients (130/211 bx), *group 1*

No CIN2 identified in 62/151 (41%) patients (81/211 bx), *group 2*
Study Design (cont’d)

• Tissue blocks of 72/81 cervical biopsies from 56/62 patients (group 2) were stained for three IHC markers.

• Remaining 9 biopsies from 6 patients did not have sufficient tissue for IHC work-up.

• All H&E and immunostained slides were independently reviewed by two pathologists.
Commercial Antibodies Used for IHC Work-Up

- **P16**\textsuperscript{INK4a} (CINtec\textsuperscript{R} Histology Kit, MTM Labs)
- **MIB-1** (DAKO, Carpinteria, CA, USA)
- **ProEx\textsuperscript{TM C}** (BD Diagnostics)
About Markers

- **P16\textsuperscript{INK4a} (P16)** - surrogate marker for an activated oncogene expression of HR-HPV in dysplastic cells (biomarker of cervical dysplasia) (major etiologic agent for cervical ca)

- **MIB-1 (Ki-67)** - mAB to nuclear AG Ki-67 present in proliferating cells (proliferation marker) (complimentary surrogate biomarker for HPV-related squamous dysplasia)

- **ProEx\textsuperscript{TMC}** - ABs against proteins associated w/ aberrant S-phase cell-cycle induction (MCM2 & TOP2A) (proliferation marker) (complimentary surrogate biomarker)
P16

- Cellular protein involved in cell-cycle regulation
- Expression tightly controlled (down-regulated) in normal cells
- Expressed at a very low level, non-detectable by IHC

- Strongly overexpressed in dysplastic cells due to transforming activity of viral oncoprotein E7 of all HR-HPV types
  - increased expression of E7 interferes with cell cycle control mechanism in basal cells
  - E7 interacts with pRb to cause cell proliferation and loss of differentiation
  - loss of pRb leads to p16 overexpression
Immunoreactivity/stain Interpretation for P16

- Diffuse nuclear and cytoplasmic staining of cells in at least half of the epithelial thickness interpreted as positive, supporting Dx of CIN2

- Absence or only weak/focal staining of cells within the lower third of the epithelium interpreted as negative for CIN2
Immunoreactivity/stain
Interpretation for MIB-1 and ProExC

• Co-expression of nuclear staining for MIB-1 & ProExC in cells within at least half of the epithelium interpreted as supportive of CIN2

• Staining of cells confined to basal/parabasal (proliferative) layer or lower third only interpreted as negative for CIN2
CASE 1 - CIN2: H&E & P16
CASE 1- CIN2: MIB-1 & ProExC
CASE 2 - CIN2:
H&E & P16
CASE 2 - CIN2:  
MIB-1 & ProExC
CASE 3 - CIN2: H&E
CASE 3 - CIN2: P16, MIB-1 & ProExC
CASE 4 - CIN2: P16, MIB-1 & ProExC
CASE 5 - CIN2: P16, MIB-1 & ProExC
CASE 6 - CIN2: P16, MIB-1 & ProExC
## Results of IHC Stains

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<thead>
<tr>
<th></th>
<th>IHC (+)</th>
<th>IHC (+) <strong>(p16 -)</strong></th>
<th>IHC (-)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>All 3 stains *</td>
<td>Only 2 stains**</td>
<td>All 3 stains *</td>
</tr>
<tr>
<td><strong>Cervical Bx</strong> (n=72)</td>
<td>44 (61%)</td>
<td>9 (13%)</td>
<td><strong>19 (26%)</strong></td>
</tr>
<tr>
<td><strong>Patients</strong> (n=56)</td>
<td>33 (59%)</td>
<td>8 (14%)</td>
<td><strong>15 (27%)</strong></td>
</tr>
</tbody>
</table>

* All 3 stains include p16, MIB-1, ProExC
** Only 2 stains include MIB-1, ProExC
# P16 Staining in CIN2 Lesions

|                           | P16 (+) | P16 (-) *
|---------------------------|---------|----------
| Total Cervical Bx (n=72)  |         |          |
| Cervical bx w/ CIN2 (n=53)| 44 (83%)| 9 (17%)  |
| Cervical bx w/o CIN2 (n=19)| 0%      | 100%     |

*All 9 p16 (-) stains were repeated yielding similar results*
CASE 1 - CIN2: H&E (P16-)
CASE 1 - CIN2: P16, MIB-1 & ProExC
CASE 2 - CIN2: H&E (P16-)
### P16 Staining in CIN2 Lesions

<table>
<thead>
<tr>
<th>Total Cervical Bx (n=72)</th>
<th>P16 (+)</th>
<th>P16 (-) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical bx w/ CIN2 (n=53)</td>
<td>44 (83%)</td>
<td>9 (17%)</td>
</tr>
<tr>
<td>Cervical bx w/o CIN2 (n=19)</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*All 9 p16 (-) stains were repeated yielding similar results*
CASE 1 - CIN2: H&E (P16-)
CASE 1 - CIN2: P16, MIB-1 & ProExC
CASE 2 - CIN2: H&E (P16-)
CASE 2 - CIN2: P16, MIB-1 & ProExC
CASE 3 - CIN2: P16 -
CASE 4 - CIN2: *P16-*
In Situ Hybridization (ISH) for HPV

- HPV DNA probe is used
- Hybridizes to HPV
- Demonstrates reactivity only with cells containing HPV
- Probes to different HPV types:
  - 16/18
  - 31/33
  - wide spectrum (WS): 2 LR-HPV and 8 HR-HPV types (does not include at least 10 HR-types)

- **P16 (-) cases:** 4/9 no tissue left
  - 3/5 (+) by ISH (all HPV16/18)
  - 2/5 (-) by ISH
ISH

Controls

HPV 16/18 - ISH

HPV WS - ISH
CASE 1 - CIN2: $P16-$

HPV 16/18 ISH
CASE 2 - CIN2: \textit{P16-}

HPV 16/18 ISH

HPV WS ISH
CASE 3 - CIN2: *P16* -

HPV 16/18 ISH
CIN2 on Cervical Bx with Subsequent Cervical Excision

Total of 151 pts (211 bx)

LEEP/Cone (+)
89 (130)

LEEP/Cone (-)
62 (81)

-9 (no tissue)

56 (72)

IHC (+) x3
33 (44)

IHC (+) x2
8 (9)

IHC (-)
15 (19)
## CIN2 Dx by Histology & IHC

<table>
<thead>
<tr>
<th>Total</th>
<th>151pts / 211Bx</th>
<th>100%</th>
<th>CIN2 on Cervical Bx</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Excision (+)</strong> group 1</td>
<td></td>
<td></td>
<td>confirmed/present on subsequent histology</td>
</tr>
<tr>
<td>89</td>
<td>130</td>
<td>62%</td>
<td></td>
</tr>
<tr>
<td><strong>IHC (+)</strong> group 2</td>
<td></td>
<td></td>
<td>supported by IHC</td>
</tr>
<tr>
<td>41</td>
<td>53</td>
<td>25%</td>
<td>(removed or regressed)</td>
</tr>
<tr>
<td><strong>IHC (-)</strong> group 2</td>
<td></td>
<td></td>
<td>not supported by IHC</td>
</tr>
<tr>
<td>15</td>
<td>19</td>
<td>9%</td>
<td><strong>overdiagnosed</strong></td>
</tr>
<tr>
<td>No IHC</td>
<td></td>
<td></td>
<td>unknown</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>4%</td>
<td></td>
</tr>
</tbody>
</table>
CASE 1 - Overdiagnosed: CIN1 with Atypical Squamous Metaplasia

P16

MIB-1

ProExC
CASE 2 - Overdiagnosed: Atypical Squamous Metaplasia
CASE 3 - Overdiagnosed: CIN1 with Reactive Squamous Atypia
CASE 3 - Overdiagnosed: P16, MIB-1 & ProExC
CASE 4 - Overdiagnosed: Atypical Immature Metaplasia
Other Corresponding Findings in *Overdiagnosed Group*

- Colposcopic impression did not favor HG lesions in 9/15 patients
- ThinPrep did not favor high-grade lesions in 11/15 patients
Conclusions

• In **25%** of cervical bx the absence of CIN2 lesions in subsequent cervical excisions may be due to **small lesion size** with **complete removal** by colposcopic biopsy, or **regression** *(15-30% histologically confirmed CIN2-3 in cervical biopsies regress spontaneously due to local stromal immune response; Ovestad et al, 2010, Mod Pathol)*

• In **9%** cervical bx the difficult to grade lesions were **overdiagnosed as CIN2 based on histology alone**

• Combination of immunostains for **p16**, **MIB-1** and **ProExC** useful in separating CIN2 lesions from benign mimics, and prevent unnecessary cervical excisions
Conclusions

- Immunostaining patterns for proliferation markers appear to vary somewhat in distribution and intensity; **scattered & dispersed pattern for MIB-1** vs **diffuse & intense staining for ProExC**

- In rare p16 (-) cases **co-expression of MIB-1 and ProExC** can be helpful in supporting Dx of CIN2

- Caution should be exercised when interpreting **MIB-1 and ProExC** in a setting of associated inflammation, low clinical and histologic suspicion for HG dysplasia, and in the **absence of p16 reactivity** since findings may reflect reactive changes to inflammation
Conclusions

• **P16 over-expression** was present in 83%, and **absent in 17%** of CIN2 lesions in this study (*compatible with Dr. Stoler’s UVA group study at 87%, Am J Surg Pathol, August 2010*).

• 3/5 P16 (-) cases positive with **in-situ hybridization (ISH)** for HPV types 16/18.

• While not extremely sensitive for CIN2 lesions (83%), **p16** appears to be highly specific **“key” biomarker** for HR-HPV ass. cervical disease, **critical** when dealing w/ Dx challenges, **contributes greatly to accuracy of CIN2 Dx**.

• Whether **absence of p16 over-expression** in small subset of CIN2 lesions reflects **less potential for disease progression** remains to be further investigated.
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