The Double-Edged Sword of Immunostains in Diagnostic Breast Pathology

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The Double-Edged Sword of Immunostains in Diagnosis of Breast Pathology

You mean you want to talk about why IHC will kill you both ways?

Use of IHC in Diagnosis of Breast Pathology

- Distinction of noninvasive from invasive lesions
- Measurement of biomarkers
- Assessment of ductal proliferative and papillary lesions
- Differentiation between ductal and lobular CIS
- Workup of spindle cell lesions
- Diagnosis of metastatic tumors in the breast
- Evaluation of sentinel lymph nodes
### Markers Staining Myoepithelial Cells (MEC)

<table>
<thead>
<tr>
<th>Nuclear</th>
<th>Cytoplasmic (+ membranous)</th>
<th>Cytoplasmic/nuclear</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p63</strong></td>
<td>SMA</td>
<td>Calponin</td>
</tr>
<tr>
<td></td>
<td>SMM</td>
<td>basal CKs</td>
</tr>
<tr>
<td></td>
<td>CD10</td>
<td>D2-40</td>
</tr>
<tr>
<td></td>
<td>h-caldesmon</td>
<td>P-cadherin</td>
</tr>
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<td></td>
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</tr>
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<td></td>
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<td>Nestin</td>
</tr>
<tr>
<td></td>
<td>p75</td>
<td>CD109</td>
</tr>
<tr>
<td></td>
<td>Stratifin</td>
<td>CD44s</td>
</tr>
<tr>
<td></td>
<td>Muscle-specific actin</td>
<td></td>
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<tr>
<td></td>
<td>Caveolin 1 and 2</td>
<td></td>
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<tr>
<td></td>
<td>Metallothionein</td>
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</table>

### Comparison of Reactivity by MEC Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Myoepi. cells</th>
<th>Myofibroblasts</th>
<th>Vessels</th>
<th>Carcinoma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>Rare +</td>
</tr>
<tr>
<td>Calponin</td>
<td>+++ to ++++</td>
<td>++</td>
<td>+++</td>
<td>Rare +</td>
</tr>
<tr>
<td>SMMHC (SMM)</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>Rare +</td>
</tr>
<tr>
<td>p63</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>Occasional +</td>
</tr>
<tr>
<td>CK5/6 (other HMW CK)</td>
<td>+++ to ++++</td>
<td>-</td>
<td>-</td>
<td>Occasional +</td>
</tr>
</tbody>
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*P63 positivity in ~100% adenoid cystic carcinoma and majority of metaplastic carcinoma
CK5/6 positivity more likely to be seen in high grade IDC and DCIS

### Markers staining myoepithelial cells (MEC)

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Panel of at least two markers--

**p63 + cytoplasmic marker (SMM or calponin)**

### Pitfalls in Interpreting MEC Markers

- Stromal (myofibroblast and vessel) staining
- Tumor cell staining
- Biology of the lesions
- Artifact in interpretation
Myofibroblast Staining Mimicking ME Cells

- SMA > calponin > SMM
- Not seen with p63 or CK5/6
**Myofibroblast Staining Mimicking ME Cells**

Myofibroblasts around inv. gland

ME cells around DCIS

**SMM stain**

**Tumor Cell Staining from MEC Markers**

- More common with p63 and CK5/6
- Rarely with SMM and calponin

**Pitfall of Tumor Cell Staining**

- Location and shape of positive nuclei
- Intensity of staining
Pitfall of Tumor Cell Staining--

Pitfalls in Interpreting MEC Markers

- Stromal (myofibroblast and vessel) staining
- Tumor cell staining
- Biology of the lesions
  - Phenotypic alterations in DCIS-associated ME cells
  - Phenotypic alterations in ME cells-associated with benign sclerosing lesions
  - Non-invasive lesions without expression of MEC markers
  - Invasive carcinomas with expression of MEC markers
- Artefact in interpretation

Phenotypic Alterations in DCIS-associated ME Cells

- Reduced expression to focal absence of one or more MEC markers in DCIS-associated ME cells
- Incidence of attenuated expression
  - Overall: SMM (77%) > CK5/6 (30%) > calponin (17%) > p63 (13%) > SMA (1%)
  - However, variable in each case

Reduced MEC Marker Expression in DCIS

Papillary DCIS often with attenuated MEC expression around the ducts.

Round cribriform glands, negative p63, SMM and calponin.

Attenuated MEC staining in cribriform DCIS or invasive cancer?

Cribriform DCIS or Invasive Cribriform carcinoma?

Phenotypic Alterations in ME Cells Associated with Benign Sclerosing Lesions of the Breast

- Reduced expression to focal absence of one or more MEC markers in ME cells associated with benign sclerosing lesions

- Incidence of attenuated expression
  - Overall: CK5/6 (32%) > SMM (21%) > p63 (9%) > calponin (6%) > SMA (0%)
  - However, variable in each case

Patchy Attenuated MEC Staining in RSL

Reduced MEC Marker Expression in Radial Sclerosing Lesion
Almost complete absence of staining for p63

Variably Reduced MEC Marker Expression in Radial Sclerosing Lesion

Microglandular Adenosis (MGA)--
A Noninvasive Glandular Lesion Without Expression of MEC Markers
Microglandular Adenosis--
Haphazard distribution

Microglandular Adenosis--
Hypocellular collagenous stroma

Microglandular Adenosis--
Uniform small glands, open lumen, eosinophilic secretion
PAS stain

Microglandular Adenosis

p63

SMM

ER
Microglandular Adenosis

- Red flag: ER/PR negative “well-differentiated invasive ductal carcinoma”

- Characteristic H&E morphologic features
  - Uniform small round glands with open lumen and PAS+ eosinophilic secretion
  - Hypocellular collagenous/fatty stroma

- S100 diffusely and strongly +

Invasive Carcinomas Expressing MEC Markers-- Pitfall in Interpreting MEC Markers

- Carcinomas with myoepithelial differentiation
  - Adenoid cystic carcinoma (AdCC)
  - Low grade adenosquamous carcinoma (LGASC)

- Neoplastic MEC: Variable and patchy expression of individual MEC markers, typically p63 positive
  - Misleading peripheral staining (esp. p63)
  - Patchy and variable staining (SMM, calponin)
  - Multi-layering of MEC marker-positive cells (p63)

Low-grade Adenosquamous Carcinoma (LGASC)
Low-grade Adenosquamous Carcinoma (LGASC)--Invasive Carcinoma with positive MEC Markers

- Patchy MEC marker expression
- Multi-layering of p63 positive cells

- Low-grade Adenosquamous CA (LGASC)
  - p63 positive and variable expression for SMM, calponin
  - Characteristic morphologic features
    - Infiltrative
    - Spindle cellular stroma, prominent lymphoid reaction
    - Glands (long, irregular) and solid squamous nests (comma shaped extension), ± squamous cysts
    - Bland cytology
  - ER/PR/HER2 triple negative
Adenoid Cystic Carcinoma (AdCC)

- **Architectural patterns**
  - Cribriform, tubular/trabecular, solid; solid basalo id variant
- **Dual epithelial and myoepithelial cell types**
- **ER/PR/HER2 triple negative**
- **t(6;9) MYB-NFIB or t(8;9) MYBL1-NFIB translocation**
  - MYB overexpression in 80 to 100% AdCC
- **DDx depending on the growth patterns**
  - Tubular pattern: mimic benign sclerosing lesion, well-diff. IDC
  - Myoepithelial type cells: variable expression of MEC markers, usually p63 +, SMA +/-, and SMM/calponin +/-
  - Myoepithelial differentiation: pitfall in interpretation of MEC markers

Tubular AdCC-- Biphasic Epi-Myoepithelial Diff.
May mimic IDC or benign sclerosing lesion

AdCC-- Variable MEC expression and negative ER

AdCC-- Variable MEC expression and positive MYB
MYB IHC as a diagnostic adjunct in AdCC

- Tests based on *MYB-NFIB* translocation
- FISH: *MYB* rearrangement
  - 50% to 90%
- MYB IHC: diffuse, moderate to strong nuclear expression
  - 80 to 100%
- IHC more sensitive and specific assay than FISH for dx of AdCC


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Epithelial Displacement--
Pitfall in Using MEC Markers

63 y F with a left breast mass who underwent a core biopsy followed by excision

Epithelial Displacement after Prior Needle Biopsy

Breast triple stain
Epithelial Displacement after Prior Needle Biopsy

- Common with papillary lesions
- IHC often misleading
- H&E morphology most helpful
  - Within biopsy tracts
  - Associated granulation tissue, foamy macrophages, hemosiderin
  - Linear arrangement of glands/nests

Use of IHC in Diagnosis of Breast Pathology

- Distinction of noninvasive from invasive lesions
- Measurement of biomarkers
- Assessment of ductal proliferative and papillary lesions
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- Diagnosis of metastatic tumors in the breast
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Papillary Lesions of the Breast

(WHO 2012)

- Intraductal papilloma
  - with various benign alterations
  - with ADH involving papilloma (atypical papilloma)
  - with DCIS involving papilloma (DCIS arising in a papilloma)
- Intraductal papillary carcinoma (Papillary DCIS)
- Encapsulated (intracystic) papillary carcinoma
- Solid papillary carcinoma
- Invasive papillary carcinoma

Papillary Lesions: Challenging Morphologic Spectrum
MEC markers, CK5/6 and ER--

IHC markers useful in distinguishing papilloma from papillary carcinoma

Benign papilloma retains a continuous layer of ME cells along the fibrovascular cores

Papillary carcinoma lacks ME cells along the fibrovascular cores

Benign Papilloma--
- CK5/6 positive
- ER patchy and variable
Papillary Carcinoma--
- CK5/6 negative
- ER diffuse and strong

60 y F with a 1.5 cm breast mass--
- Multiple solid nodules
- Papillary architecture

Solid papillary architecture with reverse polarization--
- Tall columnar cells with nuclei at the apical aspect
Solid papillary carcinoma with reverse polarity

Solid Papillary Carcinoma with Reverse Polarity (SPCRP)

- Synonyms: Breast tumor resembling the tall cell variant of papillary thyroid carcinoma, tall cell variant of papillary breast carcinoma
- Characteristic H&E morphology
  - Multiple expansile nodules of papillary structures
  - Tall columnar cells, abundant eosinophilic cytoplasm, round to oval nuclei often with grooves and intranuclear inclusions
  - Apical location of nuclei (reverse polarity)
- IHC Profile
  - No myoepithelial cells (negative p63, SMM, calponin)
  - Strong expression of HMWK (CK5/6, 34βE12)
  - Triple negative or low ER/PR
  - Focal/patchy mammagloblin, GCDFP-15 and GATA3

Genetic Aberrations in SPCRP (I)

- 77% (10/13): hotspot mutation at R172 of IDH2 gene
- 80% (8/10): concurrent pathogenic mutations affecting PIK3CA or PIK3R1
- IDH2 mutation
  - Common in gliomas and AML
  - 1/971 IDC and ILC in TCGA data

Genetic Aberrations in SPCRP (II)

- R172 mutation of IDH2 gene: gain of function mutation
  - Genome-wide hypermethylation profile
  - Hypermethylation blocks cellular differentiation
- Functional studies: IDH2 and PIK3CA mutations drivers of SPCRP, with reversed nuclear polarization phenotype
- Detection of IDH2 and PIK3CA mutations: diagnosis and therapeutic target for SPCRP


(Chiang et al: Cancer Research 2016)
Differential Diagnosis for SPCRP

- Metastatic thyroid papillary carcinoma
- Papilloma with sclerosis and UDH or complex sclerosing lesion
- Papillary breast carcinoma of other types
  - Solid papillary carcinoma
  - Papillary DCIS and encapsulated papillary carcinoma

Solid papillary carcinoma with reverse polarity--
Positive breast markers, negative thyroid markers

Papilloma with UDH--

- CK5/6 positive (mosaic)
- Patchy ER
- ME markers positive along fibrovascular cores and around nodules

Solid papillary ca--

- CK5/6 negative
- ER diffuse and strong
- ME markers +/- along fibrovascular cores and around nodules
Clinical Features for SPCRP

- Overall, favorable outcome
  - 2/26 with LN or bone met
  - No met in the series (13 pts) by Chiang et al
- Prognostic markers unknown
- Best management: not well-defined
  - TN phenotype, low Ki67 index (<5%)
  - Surgery
  - Role of radiotherapy and chemotherapy unknown

IHC Markers for Papillary Lesions

<table>
<thead>
<tr>
<th>Category</th>
<th>MEC markers* around space</th>
<th>MEC markers* along stalks</th>
<th>CK5/6</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilloma + UDH</td>
<td>Positive</td>
<td>Positive (continuous)</td>
<td>Positive (mosaic)</td>
<td>Variably positive</td>
</tr>
<tr>
<td>Papilloma + ADH/DCIS</td>
<td>Positive</td>
<td>Patchy to negative in ADH/DCIS</td>
<td>Negative in ADH/DCIS</td>
<td>Uniformly positive in ADH/DCIS</td>
</tr>
<tr>
<td>Papillary DCIS</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Uniformly positive</td>
</tr>
<tr>
<td>Encapsulated papillary ca</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Uniformly positive</td>
</tr>
<tr>
<td>Solid papillary ca (SPC)</td>
<td>Positive or negative</td>
<td>Negative to patchy</td>
<td>Negative</td>
<td>Uniformly positive</td>
</tr>
<tr>
<td>SPC with reverse polarity</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative to weakly pos.</td>
</tr>
</tbody>
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*MEC (myoepithelial cell) markers: p63, SMM

Use of IHC in Diagnosis of Breast Pathology

- Distinction of noninvasive from invasive lesions
- Measurement of biomarkers
- Assessment of ductal proliferative and papillary lesions
- Differentiation between ductal and lobular CIS
- Workup of spindle cell lesions
- **Diagnosis of metastatic tumors in the breast**
- Evaluation of sentinel lymph nodes

Case 1: 57 y F with a right breast mass

- Poorly-differentiated epithelioid neoplasm
- ER/PR/HER2 triple negative
- Sox10 +, S100 +

Metastatic melanoma or TNBC?
Case 2: 33 y F with an enlarged axillary LN

- Poorly-diff. epithelioid neoplasm
- ER/PR/HER2 triple negative
- Keratins (MNF116, CAM5.2 and CK18) +, Sox10 +

Metastatic melanoma or TNBC?

IHC between TN Breast Cancer and Melanoma

<table>
<thead>
<tr>
<th></th>
<th>Breast Cancer</th>
<th>Metastatic melanoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sox10</td>
<td>66% positive*</td>
<td>Positive</td>
</tr>
<tr>
<td>GATA3</td>
<td>Positive (66% TN IDC)</td>
<td>Positive</td>
</tr>
<tr>
<td>Keratins</td>
<td>Positive</td>
<td>Positive (% dependent on keratin types)*</td>
</tr>
<tr>
<td>Melan A</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>HMB45</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*Sox10 expression in 5% of luminal A, luminal B and HER2+ IDC; 0% ILC
*Positive rate for metastatic melanoma: 100% CK18, 90% MNF116, 70% CK8, 10% CK7 and CK19, 0% CK6; focal or diffuse

Case 1: 57 y F with a right breast mass

- ER/PR/HER2 triple negative
- Sox10+, S100 +
- HMB45 -, Melan A-
- Keratin +, GATA3 +

Dx: High grade TN breast cancer
Case 2: 33 y F with an enlarged axillary LN

- ER/PR/HER2 triple negative
- MNF116 +, CK18+, CAM5.2 +, CK7 -
- Sox10 +, HMB45, Melan A, S100 +
- GATA3 -

Dx: Metastatic melanoma to axillary LN

Case 3: 50 y F with a palpable right breast mass

- E-cadherin positive
- Dx: Invasive ductal carcinoma
- ER positive, HER2 negative
- Synaptophysin and chromogranin positive

Metastatic neuroendocrine tumor (NET) or
Primary mammary carcinoma with NE differentiation (NEC)?
Breast carcinoma with NE differentiation

- Increasingly being recognized, evolving entity, lacking uniform diagnostic criteria
- Morphology similar to NET of other organ systems
  - Nesting, trabecular, solid papillary, gyriform, pseudoglandular patterns
  - Plasmacytoid, spindled, finely granular cytoplasm
- ER positive, HER2 negative
- GATA3 +++, Mammaglobin and GCDFP +
- CK7 +, CK20 −, CDX2 −, TTF1 − (small cell ca: TTF1 +)

Metastatic NET in the Breast

- Most from GI tract (ileum) and lung
- ~50% initially misdiagnosed as primary breast cancer
  - Inappropriate treatment
- (In retrospective review) architectural and cytological features of NET: increased awareness important
- IHC profile
  - NE markers positive
  - Tissue specific markers (CDX2, TTF1, PAX8) positive
  - CK7 −, ER/PR − (~10% + for ER/PR)

Metastatic NET to Breast vs Invasive mammary carcinoma with NE differentiation

<table>
<thead>
<tr>
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<th>Breast NET</th>
<th>Met NET</th>
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<tr>
<td>ER</td>
<td>Positive</td>
<td>~10% positive</td>
</tr>
<tr>
<td>GATA3</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>CK7</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Mammaglobin, GCDFP15</td>
<td>Positive (35 to 40%)</td>
<td>Lung primary may be +</td>
</tr>
<tr>
<td>CDX2, TTF1, PAX8</td>
<td>most small cell ca: TTF1 positive, regardless of primary site</td>
<td>Positive</td>
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Breast carcinoma with NE differentiation
Breast carcinoma with NE differentiation

Case 3: 50 y F with a palpable right breast mass--
Metastatic NET (from ileum) mimicking primary breast cancer

IHC markers for metastatic workup--
Breast: ER, GATA3, Mammaglobin, GCDFP15

<table>
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<tr>
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<th>Tissue-specific markers</th>
<th>Pitfalls</th>
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<tr>
<td>Breast vs metastatic melanoma</td>
<td>Sox10, HMB45, MelanA, S100</td>
<td>Breast: + Sox10, S100 Melanoma: + keratins</td>
</tr>
<tr>
<td>Breast vs lung</td>
<td>TTF1, Napsin A</td>
<td>Lung: + ER, GCDFP, GATA3 Breast: + TTF1, Napsin A (apocrine)</td>
</tr>
<tr>
<td>Breast vs ovary</td>
<td>WT1, PAX8</td>
<td>Ovary: + ER/PR Breast: + WT1 (mucinous, SPC)</td>
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<td>Breast (ca with NE diff.) vs metastatic NET</td>
<td>CDX2, TTF1, PAX8, CK7</td>
<td>Breast: + TTF1 (small cell ca) Metastatic NET: + ER/PR</td>
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IHC markers for metastatic workup--
Breast: ER, GATA3, Mammaglobin, GCDFP15

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- Presence of other tissue-specific immunoreactivity cannot by itself be used to exclude the possibility of a breast origin
- Use a panel of markers

Breast vs lung
TTF1, Napsin A
Lung: + ER, GCDFP, GATA3
Breast: + TTF1, Napsin A (apocrine)

Breast vs ovary
WT1, PAX8
Ovary: + ER/PR
Breast: + WT1 (mucinous, SPC)

Breast (ca with NE diff.) vs metastatic NET
CDX2, TTF1, PAX8, CK7
Breast: + TTF1 (small cell ca)
Metastatic NET: + ER/PR

65 y F with a 0.9 cm R breast mass on mammogram

65 y F with a 0.9 cm R breast mass on mammogram
h/o pancreatic ca with lung metastasis and enlarged thyroid with deviation of the trachea

IHC profile:
- SMM negative
- p63 focal staining
- CK5/6 negative
- ER, PR, HER2 negative

What is your diagnosis?

Metastatic thyroid carcinoma to the breast
ER negative glandular proliferation
Consider the possibility of metastasis
Distinguish metastatic from primary breast tumors

- May show similar morphologic features
  - Presence of in situ component: support breast primary
  - “Unusual” features/patterns: consider metastasis
- Tissue-specific IHC markers
  - No marker is 100% sensitive or specific
  - Panel of markers
- Clinical history
  - Review of prior slides
  - Metastasis to breast may be the first presentation
- Radiologic features
  - Most metastatic tumors in the breast: well-circumscribed nodules, mistaken for cysts or fibroadenomas

Immunostains in Diagnosis of Breast Pathology

- Understand the limitations
- Be aware of the pitfalls
- Correlate with H&E morphology, clinical history and radiologic findings

Thank you!