Carcinomas of Unknown Primary Site

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Metastatic Carcinoma of Unknown Primary Site

**Diagnostic Strategies**

- **Cytokeratin analyses:** low vs. high MW; CK7 and CK20; individual CKs

- **Organ-restricted markers:** PSA, thyroglobulin, GCDFP-15, etc.

- **Non-organ restricted markers:** CEA, p63, synaptophysin, ER/PR, etc.
Metastatic Carcinoma of Unknown Primary Site: Cytokeratin Analysis

- Antibodies to low and high MW cytokeratins
- Antibodies to cytokeratin 7 and cytokeratin 20
- Antibodies to individual cytokeratins (e.g., CK5, CK17)
Breast carcinoma
Lung nonsmall cell carcinoma
Ovarian carcinoma (serous)
Mesothelioma
Endometrial adenocarcinoma

Colorectal adenocarcinoma
CK7 ● CK20 ●

Transitional cell carcinoma
Ovarian carcinoma (mucinous)
Pancreatic carcinoma (subset)

CK7 ○ CK20 ○

Hepatocellular carcinoma
Renal cell carcinoma
Prostatic adenocarcinoma
Neuroendocrine carcinoma
Squamous cell carcinoma
Lung nonsmall cell carcinoma
Lung nonsmall cell carcinoma
Lung nonsmall cell carcinoma
Colonic adenocarcinoma
Colonic adenocarcinoma
Transitional cell carcinoma
Transitional cell carcinoma

CK7
Renal cell carcinoma
Renal cell carcinoma
Renal cell carcinoma

CK20
“Modal” immunophenotypes generally cited

First order approximation only

Additional tumor specific markers generally required
### Distribution of ‘Modal’ CK7 and CK20 Immunophenotypes

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>CK7</th>
<th>CK20</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal adenoCA</td>
<td>●</td>
<td>●</td>
<td>75-95%</td>
</tr>
<tr>
<td>Hepatocellular CA</td>
<td>●</td>
<td>●</td>
<td>70-90%</td>
</tr>
<tr>
<td>Lung nonsmall cell</td>
<td>●</td>
<td>●</td>
<td>90%</td>
</tr>
<tr>
<td>Lung NE carcinoma</td>
<td>●</td>
<td>●</td>
<td>60-80%</td>
</tr>
<tr>
<td>Ovarian serous CA</td>
<td>●</td>
<td>●</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>Renal cell CA</td>
<td>●</td>
<td>●</td>
<td>70-90%</td>
</tr>
<tr>
<td>Lung squam cell CA</td>
<td>●</td>
<td>●</td>
<td>50-90%</td>
</tr>
</tbody>
</table>
Metastatic Carcinoma of Unknown Primary Site

**Diagnostic Strategies**

- Cytokeratin analyses: low vs. high MW; CK7 and CK20; individual CKs

- Tumor-specific markers: PSA, thyroglobulin, GCDFP-15, etc.

- Tumor-subset markers: CEA, p63, ER/PR, etc.
Classes of Tumor-Specific Markers

- Cytoplasmic (or membranous) differentiation markers
- Nuclear transcription factors
Membranous/Cytoplasmic Tumor Specific Markers

- “Luxury proteins” of terminally differentiated cells
- Expression inversely related to degree of differentiation
- Expression may be present in small subset of tumor cell population
Nuclear Transcription Factors

- Proteins with modular structure composed of unique DNA binding and transcriptional/regulation domains
- Bind to short-sequence DNA motifs by distinct molecular mechanisms
- Can be thought of as molecular ‘switches’ that downstream regulate a series of cell-specific proteins
Advantages of Nuclear Transcription Factors as Cell- and Tumor-type Specific Markers

- Usually positive on entire tumor cell population
- Expression not necessarily related to state of tumor differentiation
- Exquisitely sensitive and specific
Examples of Transcription Factor Tumor Markers

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>Tissue Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF-1</td>
<td>Lung, thyroid CA</td>
</tr>
<tr>
<td>CDX-2</td>
<td>Colorectal, GI Adenocarcinoma</td>
</tr>
<tr>
<td>WT-1</td>
<td>Ovarian serous CA</td>
</tr>
<tr>
<td>PAX-2</td>
<td>Renal cell CA</td>
</tr>
</tbody>
</table>
Breast Cancer Markers

GCDFP-15
Mammaglobin
GCDFP-15


- Overall sensitivity 55% (Majouzian, et al., 1989) for breast carcinoma

- Our recent studies show overall sensitivity of 51% (post-HIER era) [Tse C et al., in preparation]

- Sensitivity a function of tumor cell type: highest in ILC and tumors showing apocrine differentiation
**GCDFP-15**

- Expression independent of tumor grade, ER status, mitotic index, etc.
- Also expressed by glands in vulva, eyelid, ear canal, trachea, bronchus
- Adenocarcinomas of lung, colon and ovary almost always negative
- Incidence of expression in TTF-1 positive lung carcinomas: 8/112 (7%) [Hing AW et al., 2004]
Mammaglobin

- 10 kd glycoprotein identified by differential screening techniques
- Function unknown
- Expression highly restricted to breast cancers
- Watson MA et al (Cancer Res 59:3028-31, 1999) showed relatively high levels of expression in >80% of breast cancers
Mammaglobin

*Tse C, et al., in preparation*

- N = 219 breast cancers
- IHC for mammaglobin, GCDFP-15
- Overall sensitivity of mammaglobin 46.6%
- Combined sensitivity of GCDFP-15 and mammaglobin 69%
## Mammaglobin

*Tse C, et al., in preparation*

(N = 219)

<table>
<thead>
<tr>
<th></th>
<th>Mammaglobin Negative</th>
<th>Mammagloblin Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCDFP-15</td>
<td>31%</td>
<td>18%</td>
</tr>
<tr>
<td>GCDFP-15</td>
<td>22%</td>
<td>29%</td>
</tr>
</tbody>
</table>
Mammaglobin

Tse C, et al., in preparation

- Overall sensitivity slightly lower than GCDFP-15 (51.1% vs. 46.6%)
- Identifies an additional 18% of cases not positive for GCDFP-15
- Positivity rate not correlated with estrogen receptor positivity
Mammaglobin
Mammaglobin

Tse C, et al., in preparation

% Mammaglobin positive

N=59

- Gastric: N=16
- Colorectal: N=27
- Pancreatobir: N=27
- Lung: N=10
- Prostate: N=15
- Ovarian: N=15

(revised)
Breast-“Specific” Markers

There is no breast-specific marker that cannot also be expressed by sweat gland tumors.

ER, PR, GCDFP-15, mammaglobin, etc.
Recommendation

If breast carcinoma in the differential diagnosis, consider using antibodies to both GCDFP-15 as well as mammaglobin to maximize sensitivity.
GI Tract Cancer Markers

Villin
CDX-2
Villin

- 95 kd actin-binding protein, found preferentially in microvilli

- Expression in normal tissues largely restricted to epithelial cells of the GI tract

- Highly sensitive marker of colorectal adenocarcinomas (West AB et al., Gastroenterol 94:343-52, 1988)
Villin


- 0% renal cell carcinomas positive
- 5% lung carcinomas positive ("rootlet type" adenocarcinomas)
- 0% head and neck carcinomas (squamous cell, thyroid, salivary gland) positive
- 0% breast carcinomas positive
- 0% ovarian serous carcinomas positive
CDX2

- Intestinal-specific nuclear transcription factor
- Regulates proliferation and differentiation of intestinal epithelial cells
- Expression may be reduced compared with normal in colorectal adenocarcinoma
- Extremely sensitive marker of colorectal adenocarcinoma
CDX2

- Outstanding marker of colorectal adenocarcinoma (e.g., distinguish metastatic colorectal CA from primary lung adenocarcinoma)

- Also marker of non-colorectal GI adenocarcinomas (heterogeneous pattern)

- Exceptions: mucinous ovarian, bladder adenocarcinomas
CDX-2

Expression in Primary Lung Carcinoma?

Primary lung adenocarcinomas with 'enteric' differentiation

High grade neuroendocrine carcinomas
CDX-2


N = 476

Colorectal: 99%
Gastric: 70%
Pancreatic: 32%
Cholangio: 25%
Ovarian Mucinous: 64%
Bladder Adeno: 100%
Villin


N = 476

Colorectal: 82%
Gastric: 42%
Pancreatic: 40%
Cholangio: 60%
Ovarian Mucinous: 64%
Bladder Adeno: 100%
Recommendation

If GI tract adenocarcinoma in the differential diagnosis, consider using antibodies to both villin as well as CDX-2 to maximize sensitivity
Lung Cancer Marker

TTF-1
TTF-1

- 38 kd member of the NKx-2 family of nuclear transcription factors
- Selectively expressed in early embryogenesis in thyroid, respiratory epithelium and diencephalon
- In lung, TTF-1 binds to and activates promoters for Clara cell secretory protein and surfactant proteins A, B and C
TTF-1

- Expressed in both neuroendocrine and non-neuroendocrine carcinomas of the lung
- Sensitivity varies greatly among different histologic subtypes (highest in small cell and bronchioloalveolar; lowest in squamous and mucinous)
Lung Adenocarcinoma
### Frequencies of TTF-1 Expression in Histologic Subtypes of Lung Cancer

**Non-neuroendocrine carcinomas**

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>0-5%</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>70-80%</td>
</tr>
<tr>
<td>Bronchioloalveolar, nonmucinous</td>
<td>90%</td>
</tr>
<tr>
<td>Bronchioloalveolar, mucinous</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>
Frequencies of TTF-1 Expression in Histologic Subtypes of Lung Cancer

*Neuroendocrine carcinomas*

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Frequency Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoid tumor</td>
<td>0-35%</td>
</tr>
<tr>
<td>Atypical carcinoid</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Small cell undifferentiated</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Large cell undifferentiated</td>
<td>50-70%</td>
</tr>
</tbody>
</table>
TTF-1

- Extremely high specificity for carcinomas of the lung (>95%)
- Near-zero expression in carcinomas of the breast, liver, pancreas, stomach
- Can see expression in small subset of rectal adenoCAs, ovarian serous CAs
- Monoclonal antibody SPT24 significantly more sensitive than 8G7G3/1 clone
- Even greater sensitivity for thyroid than lung carcinomas
TTF-1: CAVEATS

- TTF-1 expression is not lung-restricted in the context of neuroendocrine carcinomas

- When positive, generally ~100% of cells positive; beware of lower levels of positivity (e.g., 1-25%)

- Antigen is poorly preserved in alcohol-fixed cells (e.g., in smears, fluid cell blocks)
Monoclonal Antibodies to TTF-1
A Tale of Two Clones

8G7G3/1 v. SPT24

SHORT REPORT
Positive immunostaining for thyroid transcription factor-1 in primary and metastatic colonic adenocarcinoma: a note of caution
D Penman, I Downie, F Roberts

Serous Ovarian CA Marker

WT-1
WT-1 Gene Product

- Wilms tumor gene located on Chromosome 11p13
- DNA-binding protein that plays a critical role in the development of the genitourinary tract
- Adult tissues: mesangial cells, Sertoli cells, ovarian stromal and surface epithelium, mesothelium
WT1 As Marker of Ovarian (Serous) Carcinoma

- Ordoñez (2000): WT-1 in 83% ovarian carcinomas; 0% breast, lung, colon, kidney, thyroid, prostate

- Goldstein et al (2001): WT-1 in 93% of carcinomas of OSE origin (0% ovarian mucinous, 0% pancreatobiliary tract CAs)

- Lee et al (2002): 97% specificity in effusion cell blocks
WT-1
Hwang, H et al., Appl Immunohistochem Molle Morphol 12:122-6, 2004

91% (N=57)

Sensitivity

- 0% (N=31) - Serous Papillary
- 0% (N=15) - Mucinous
- 0% (N=13) - Endometrioid
- 0% (N=13) - Clear Cell
WT1

Hwang, H et al., Appl Immunohistochem Molec Morphol 12:122-6, 2004

91%
(N=57)

6.8%
(N=118)

0%
(N=46)

0%
(N=45)

Ovarian Serous Papillary

Breast

Colorectal

Lung NS Cell
Hepatocellular CA Markers

HepPar1
CEA
CD34
HepPar1

Liver-specific marker defined by antibody HepPar1

Minervi et al (1997) sensitivity 82%, specificity 90%

Helpful in distinguishing metastatic carcinomas to liver from primary hepatocellular carcinoma
HepPar1

- Uniform expression most helpful
- ‘Hepatoid’ carcinomas of stomach, ovary, and lung may also show significant positivity
- Beware of endogenous biotin mimicking appearance of HepPar1 immunostaining!
Bile Canalicular Structures

- Use polyclonal antibodies to CEA (identifying NCA, not true CEA)
- Antibodies to CD10 can yield similar results
- Extremely high specificity (>95%) but relatively low sensitivity (<50%)
CD34-Positive Sinusoidal Lining Cells

- Not present in normal liver but can be seen in dysplastic hepatic nodules and hepatocellular carcinoma

- Indicates presence of neovascularization or “capillarization” of tumor

- Extremely high specificity (>95%) but relatively low sensitivity (<50%)
CAVEAT

Whatever markers identify hepatocellular carcinoma will also identify hepatoid carcinomas arising outside of the liver


Renal Cell Cancer Marker

PAX-2
Renal Cell Carcinoma
Markers of Christmases Past

- CK7-negative, CK20-negative
- Vimentin
- CD10
- ‘RCC’ (gp100)
PAX-2 most sensitive renal cell carcinoma marker tested

More sensitive than CD10 or aquaporin
PAX-2


- 88% of clear cell CAs positive (most strongly)
- 18% of papillary renal cell CAs positive
- Small percentage of chromophobe, oncocytomas positive
PAX-2 Specificity is Very High

- Breast
- Lung
- Colorectal
- Bladder
- Hepatocellular
- Ovarian

Studies in progress, 2006
PAX2
Scenario 1
Mediastinal Lymph Node Patient with Hx of Breast Cancer
## Lung vs. Breast Carcinoma

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lung</th>
<th>Breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 7</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Cytokeratin 20</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>TTF-1</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Surfactant apoA</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>ER, PR</td>
<td>○</td>
<td>4</td>
</tr>
<tr>
<td>GCDFP-15</td>
<td>○</td>
<td>4</td>
</tr>
<tr>
<td>Mammaglobin</td>
<td>○</td>
<td>4</td>
</tr>
</tbody>
</table>
GCDFP-15
Metastatic breast carcinoma
Scenario 2
Tumor in the colon in a 52-year-old female
CK7
Metastatic ovarian serous papillary CA
## Ovarian vs. Colorectal AdenoCA (Serous)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ovarian Serous</th>
<th>Colorectal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 7</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Cytokeratin 20</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Villin</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>CDX-2</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>ER, PR</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>WT-1</td>
<td>●</td>
<td>○</td>
</tr>
</tbody>
</table>
### Ovarian vs. Colorectal AdenoCA (Mucinous)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ovarian Mucinous</th>
<th>Colorectal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 7</td>
<td>● ●</td>
<td>○ ○</td>
</tr>
<tr>
<td>Cytokeratin 20</td>
<td>● ●</td>
<td>● ●</td>
</tr>
<tr>
<td>Villin</td>
<td>● ●</td>
<td>● ●</td>
</tr>
<tr>
<td>CDX-2</td>
<td>● ●</td>
<td>● ●</td>
</tr>
<tr>
<td>ER, PR</td>
<td>○ ○</td>
<td>○ ○</td>
</tr>
<tr>
<td>WT-1</td>
<td>○ ○</td>
<td>○ ○</td>
</tr>
</tbody>
</table>
Scenario 3

Lung Mass in Elderly Male with Smoking History
Metastatic colorectal adenocarcinoma to lung
# Lung vs. Metastatic Colorectal Adenocarcinoma

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lung</th>
<th>Colorectal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 7</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Cytokeratin 20</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>TTF-1</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Surfactant apoA</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>CDX2</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Villin</td>
<td>④</td>
<td>●</td>
</tr>
</tbody>
</table>
Thank you for your attention.

Questions?

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