Immunostain Update: Diagnosis of Metastatic Breast Carcinoma, Emphasizing the Distinction from Gynecologic Cancers

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Up to one third of patients with breast cancer will show evidence of metastatic spread over their course of disease. Common sites of metastasis in breast cancer, in addition to axillary and supraclavicular lymph nodes, include bone, liver, lung, pleura (with associated pleural effusion), brain, skin, gynecologic organs, and GI tract, although any anatomic site can be the potential target. Most metastatic lesions occur after the diagnosis of breast cancer has been established, typically within the first 5-10 years. However, some metastases may take 20 to 30 years to manifest. Furthermore, breast cancer can first present as a metastatic lesion. In addition, breast cancer patients have an increased risk of developing a second primary cancer, this occurring in about 12% of affected women. In particular, women with mutations in BRCA1 or BRCA2 gene have an increased lifetime risk for breast, ovarian, tubal and peritoneal cancer. Therefore, pathologists often face the challenging task to confirm the diagnosis of a clinically suspicious metastatic breast cancer, to evaluate the possibility of breast origin in the workup for a metastatic tumor of unknown primary, or to distinguish a metastatic breast lesion from a new second primary carcinoma. Correct distinction in the latter scenario is critical not only for the different treatment the patient will receive but also for the different prognostic implication the diagnosis will confer. With distant metastasis, breast cancer is essentially incurable.

Although less frequently, non-breast primary tumors can disseminate to the breast. Cutaneous melanoma is the most common non-breast tumor with this propensity. Other common sources of extramammary malignant tumors metastasizing to the breast include carcinomas of the lung, ovary, stomach, or kidney, as well as lymphomas. These metastatic tumors can morphologically simulate breast cancer and lead to misclassification.

Breast cancers may differ in their metastatic patterns according to their morphology and biomarker profile. Compared to ductal carcinoma, invasive lobular carcinoma more likely spreads to the bone, meninges, peritoneum, GYN organs and GI tract. Hormone receptor-positive tumors tend to produce bone metastasis whereas visceral metastasis is correlated with hormone receptor-negative primary tumors.
When evaluating metastatic lesions, clinical history, radiologic findings and review of prior slides are most important and more helpful than any special studies in distinguishing breast from non-breast tumor. However, the clinical history may not be revealing, and the prior slides may not always be available for review. In such situations, selected use of IHC markers may provide an invaluable diagnostic adjunct in elucidating the primary site.

**Cytokeratin profile in breast cancers**

The vast majority (>90%) of breast carcinomas are CK7+ and CK20-, a profile also present in most non-breast carcinomas except for colon cancer (CK7-/CK20+), pancreaticobiliary/other GI cancer (often CK7+/CK20+), and renal clear cell carcinoma and hepatocellular carcinoma (CK7-/CK20-). Therefore, CK7 and CK20 evaluation is often not helpful in the determination of breast primary. A CK20+ or CK7- pattern, however, would make breast origin less likely. Mucinous variants of adenocarcinoma in the lung and ovary are known to express CK20, in contrary to their non-mucinous counterparts. Studies on mucinous carcinoma of the breast have shown conflicting data: one study detected CK20 reactivity in 27% of mucinous carcinoma, while a separate study found no CK20+ mucinous carcinoma.

**Breast specific markers**

**ER and PR:** Approximately 75-80% of the breast cancers are positive for ER and/or PR. In a large study including 5993 breast cancers, the positive rate for ER was noted to correlate with nuclear grade of the tumor: while 100% of tumors with grade 1 nuclei were positive for ER, only 2% of nuclear grade 3 cancers expressed ER. The other major ER/PR expressing tumors are gynecologic and skin adnexal tumors.

**GCDFP-15:** GCDFP-15 is a highly specific marker for breast cancer. Salivary gland and skin adnexal tumors are the only two major sources with significant immunoreactivity for GCDFP-15. The reported sensitivity of GCDFP-15 for breast cancer in the literature is highly variable, ranging from as low as 14% up to 75%. The sensitivity in our experiences is highly dependent on the antibody clone used as well as the hormone receptor and HER2 status of the tumors. ER+ and HER2+ breast cancers are significantly more likely to express GCDFP-15 than triple negative (ER, PR and HER2 negative) tumors.

**Mammaglobin:** Mammaglobin is a recently described marker of breast differentiation. It is a member of the secretoglobin family and the gene product is a secretory protein found in normal breast epithelium and to a variable degree, in breast carcinomas. Compared to GCDFP-15, mammaglobin is more sensitive in detecting breast cancers. Mammaglobin expression is not altered at the metastatic site. The specificity is yet to be fully elucidated. However, emerging data have shown overall good specificity, with extra-mammary expression mainly seen in endometrioid adenocarcinoma and salivary gland tumor. Of note,
mammaglobin and GCDFP-15 often show patchy staining in breast cancer and may be differentially expressed in breast cancers with various morphologic features. Thus they are complementary to each other in supporting mammary origin for a carcinoma.

Triple negative breast carcinomas are aggressive tumors associated with a high incidence of metastasis. Due to their lack of staining with hormone receptors and HER2, confirmation that a metastatic lesion originates from a primary triple negative breast carcinoma can be particularly problematic. We have recently shown that mammaglobin, GCDFP-15 and androgen receptor (AR) are expressed by TN breast cancers, with at least one expressed in 44% of tumors. Therefore, positive staining with these markers in a metastatic tumor from a patient with history of breast cancer is supportive of a breast primary even when ER, PR, HER2 are absent.

**Immunophenotype between primary and metastatic breast carcinomas**

Most metastatic breast cancers retain the CK7+ and CK20- immunoprofile of their primary tumors. Occasionally, the metastatic lesions can show loss of CK7 and/or gain of CK 20. Likewise, mammaglobin and GCDFP-15 expression also shows excellent concordance between primary and metastatic tumors. In one study, all the positive primary cases remain positive for mammaglobin and GCDFP-15 in their metastatic lesions. On the contrary, change in ER, PR and HER2 status has been described in a significant number of patients over the course of disease progression in breast cancer. For instance, the discordance rates between primary tumor and recurrent/metastatic lesion for ER, PR and HER2 have been reported around 20%, 40% and 15% respectively. This discrepancy is likely to be increased with target therapy. In a recent study, up to one third of patients who had HER2 amplified tumors and received neoadjuvant trastuzumab demonstrated loss of HER2 gene amplification in the residual treated tumor. Therefore, discrepancy of immunophenotype compared to primary tumor is not uncommon and does not entirely exclude the possibility of a metastatic breast primary.

The basic antibody panel of metastatic workup for positive identification of breast cancer usually includes ER (with/without) PR, mammaglobin and GCDFP-15. Depending on the clinical scenario, morphologic features, and anatomic site involved by the tumor, additional markers are usually required to distinguish metastatic breast cancer from other primary.

**Distinguishing breast cancer from gynecological tumors**

Breast and gynecologic cancers are common in the same patient population, especially in women with BRCA mutations. Pathologists may face the challenge to distinguish gynecologic from breast primary in the lymph nodes and effusions, or to identify a metastatic breast cancer in a gynecologic site.
Gynecologic tumors are frequently positive for ER and PR, thus reactivity for ER and PR does not discriminate between breast and gynecologic metastases. However, immunohistochemical studies with GCDFP-15, mammaglobin, WT1, and PAX8 may assist in making this distinction.

**WT1**: In normal adult tissue, WT1 expression is identified in mesothelium, ovarian surface epithelium, and a subset of mesenchymal cells. In neoplasms, mesotheliomas, carcinomas of ovarian surface epithelium, Wilms tumors, and desmoplastic small round cell tumors are the major tumor types that are consistently positive for WT-1. Nearly all ovarian serous carcinomas (>90%), but not those arising from endometrium, are positive for WT-1. A small fraction (<10%) of breast carcinomas may be immunoreactive for WT-1, but the reaction is usually weak and patchy, in contrast to the diffuse and strong expression typically observed in ovarian serous carcinomas. Although WT-1 is a highly sensitive marker for ovarian serous carcinoma, other epithelial carcinomas from ovary and carcinomas arising from the endometrium and endocervix are negative or only weakly positive for WT-1.

**PAX8**: PAX8, a nuclear transcription factor, is a new sensitive and useful marker for tumors derived from Müllerian system, thyroid glands and metanephros. It is positive in most ovarian non-mucinous surface epithelial tumors (serous, endometrioid and clear cell types), renal epithelial tumors (all types of RCC, renal medullary ca and oncocytoma) and thyroid tumors. The sensitivity of PAX8 for ovarian tumors is significantly higher than that of WT1 and PAX2, especially for endometrioid and clear cell ca where WT1 expression is generally negative or only focally positive. As is with any new antibody, the specificity of PAX8 is not fully elucidated. Breast cancers are reported to be consistently negative for PAX8. A majority of malignant mesotheliomas (>90%) are negative for PAX8; and in the few that are reactive show only focal and/or weak staining. Therefore, PAX8 is a useful marker in the differential diagnosis of ovarian and breast carcinomas and in the work-up for malignant effusion.

**Invasive micropapillary carcinoma**: Invasive micropapillary carcinoma (IMC) is an aggressive morphologic variant of carcinoma that has been described in multiple anatomic sites, including breast, ovary, urinary bladder, lung, salivary gland and GI tract. These tumors share similar morphologic features and demonstrate a propensity for lymphatic invasion and metastasis. Therefore, immunostaining is often necessary to help accurately identify the primary site in the metastatic setting. A panel of useful markers will include ER, mammaglobin, PAX8, WT-1, TTF-1, CD20 and uroplakin. The typical immunoprofiles for various IMC are as follows: ovarian IMC ER+, WT-1+, PAX8+/negative for other markers; mammary IMC ER+, mammaglobin +/negative for other markers; bladder IMC uroplakin +, CK20+/negative for other markers; and pulmonary IMC TTF-1 + (some ER+, CK20+)/negative for other markers.
**Mammaglobin expression in endometrioid adenocarcinoma:** Among the gynecologic tumors, approximately 40-70% of endometrioid adenocarcinomas arising from both uterus and ovary are noted to show significant staining for mammaglobin. Other subtypes of adenocarcinomas appear to be negative (such as serous carcinoma) or infrequently positive (such as endocervical adenocarcinoma). However, more studies are needed to address the frequency and pattern of mammaglobin expression in various tumors of the female genital tract, and to understand the limitation of using mammaglobin in discriminating between breast and gynecologic tumors.

**Metastatic breast cancer and its ovarian mimics:** Breast cancer patients with BRCA mutations or strong risk factors for hereditary breast/ovarian carcinoma, often undergo risk-reducing salpingo-oophorectomy (RRSO) either at the time of breast surgery or at a later time, and not uncommonly following systemic chemotherapy. Chemotherapy can induce histologic alterations in an occult metastatic breast cancer that may mimic foamy histiocytes and a spectrum of ovarian lesions (including stromal hyperthecosis, hilus cell nodules and an occult primary ovarian ca). Correct diagnosis requires awareness of the masquerading effects of chemotherapy, awareness of the spectrum of ovarian lesions that may mimic occult metastatic breast cancer, and confirmation with a panel of IHC markers.

**Distinguishing breast cancer from pulmonary carcinoma**

Approximately 3% of women with breast cancer demonstrate solitary pulmonary nodules, of which more than 40% are pulmonary metastases. Expression of ER may be detected in up to 18% of pulmonary adenocarcinoma, usually in a focal and weak pattern. Therefore, reactivity for ER does not exclude a lung primary and additional information including staining for TTF-1 is essential in arriving at the correct diagnosis. Diffuse and strong ER expression, however, would support breast primary and argue against lung cancer. Thyroid transcription factor-1 (TTF-1) is a very useful reagent in distinguishing pulmonary adenocarcinomas from other primary carcinomas. TTF-1 is normally identified in thyroid epithelial cells (both follicular and parafollicular C cells), type II pneumocytes, nonciliated bronchiolar epithelial cells, and areas of the developing brain. Neoplasms of lung and thyroid origins retain expression of TTF-1, while non-small cell carcinomas of other sources rarely express this marker. Until recently, no breast carcinomas have been reported to be positive for TTF-1 except rare primary small cell carcinomas of the breast. In an abstract form, Robens et al found TTF-1 expression in 12 of 466 (2.6%) of primary breast carcinomas. In a majority of cases, the expression is focal and/or weak. Therefore, the presence of TTF-1 reactivity cannot by itself be used to rule out a breast origin in a carcinoma of unknown primary site. For breast-specific markers, no immunoreactivity for mammaglobin has been observed in primary lung adenocarcinomas in the literature, whereas GCDFP-15 expression has been reported to range from 0% to 15%.
Diagnosis of metastatic breast carcinoma in malignant effusion

In malignant effusion, the main differential diagnoses include mesotheliomas and metastatic carcinomas from the breast, lung and ovary. Calretinin, WT1 and CK5/6 are considered relatively specific markers for mesothelioma. However, a recent report found calretinin immunoreactivity in 21% of breast cancer. Furthermore, CK5/6 is expressed in 10-15% of breast cancer, predominantly in basal-like carcinoma, metaplastic carcinoma and adenoid cystic carcinoma. Recent gene expression studies on breast cancer have identified basal-like carcinoma as a distinct subtype of invasive ductal carcinoma which carries an aggressive clinical behavior. Basal-like carcinoma is positive for basal type CKs (CK5/6, CK14) and epidermal growth factor receptor (EGFR), and typically negative for ER, PR and HER2. Distinction of metastatic basal-like breast carcinoma from mesothelioma in pleural effusion may be challenging and requires correlation with clinical history and radiologic findings. Based on limited data reported in the literature, mesotheliomas are immunonegative for both mammaglobin and GCDFP-15.

Distinguishing cutaneous metastasis of breast cancer from primary cutaneous adnexal tumors

Skin adnexal carcinomas metastasize infrequently and thus are rarely confused with breast cancer in the work-up of the origin of a carcinoma of unknown primary. On the other hand, breast cancer is the most common source for cutaneous metastasis from internal malignancies, and it is not uncommon that cutaneous metastasis of breast cancer needs to be distinguished from primary cutaneous adnexal tumors in a given patient. This distinction can be difficult as there are overlapping morphologic features and expression patterns for ER, PR, and GCDFP-15 in these tumors. However, our recent data have shown that primary cutaneous adnexal tumors typically do not express mammaglobin and in those that do (2 of 63 cases, 3%), the pattern is patchy and weak. Another recent report found significant differential expression of p63, CK15 and D2-40 between primary adnexal carcinomas and cutaneous metastasis from breast cancers (91%, 40% and 44% vs 0%, 6% and 0%). Therefore, positive mammaglobin expression along with negative p63, CK15 and D2-40 by an adenocarcinoma in the skin argues in favor of a metastatic breast cancer.

Distinguishing hepatic metastasis of breast cancer from primary hepatic carcinomas

Breast cancers metastatic to the liver may morphologically mimic hepatocellular carcinoma (HCC) if the tumor cells assume a trabecular growth pattern or they may be confused with cholangiocarcinoma if the tumor shows a glandular growth. Antibody to hepatocyte paraffin 1 (HepPar1) has been widely used as a marker for HCC. HepPar1 is expressed in most (82-100%) HCC, and is occasionally identified in gastric (especially signet ring gastric carcinoma),
pancreatic and gallbladder cancers. However, rare breast cancers have also been reported to express HepPar1. Addition of MOC31 may be of benefit in the differential diagnosis. MOC31 is a relatively sensitive and specific indicator of adenocarcinoma when compared with HCC. Immunoreactivity for MOC31 is reported in almost all cholangiocarcinoma and metastatic adenocarcinoma (including breast) but rarely in HCC.

Signet-ring cell carcinomas
Signet-ring cell carcinomas can arise from virtually all organs, however, most (>90%) cases originate from the stomach, breast and colon. Breast cancers with signet-ring cell morphology show a propensity of metastasis to the GI tract, serosal surface and gynecologic organs. Metastatic breast signet-ring cell carcinomas may mimic primary GI carcinomas radiologically, endoscopically and histologically. Immunohistochemical study is a valuable tool in identification of the primary site for signet-ring cell carcinomas. The staining pattern and sensitivity of IHC markers for signet-ring cell carcinomas from breast and GI tract are summarized in table 1. ER, PR, and GCDFP-15, when positive, are most helpful in confirming the diagnosis of breast primary. Expression of mammaglobin in signet-ring cell carcinomas has not been explored. Of note, mammaglobin reactivity is detected in one colonic adenocarcinoma. CK7 and CK20 are less useful and relatively lack sensitivity, however, immunoreactivity for CK20 would strongly favor GI tract over breast origin. CDX2 and HepPar1 may enable the differential diagnosis in difficult cases with negative ER, PR and GCDFP-15. CDX2 expression in neoplastic tissue is mainly limited to adenocarcinomas of the GI tract. Expression in breast cancers has not been reported to date.

Table 1. Immunostains for signet-ring cell carcinomas from breast and GI tract*

<table>
<thead>
<tr>
<th></th>
<th>Breast</th>
<th>Stomach</th>
<th>Colon</th>
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<tbody>
<tr>
<td>ER</td>
<td>70-80%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>GCDFP-15</td>
<td>78%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CK7</td>
<td>95%</td>
<td>64-67%</td>
<td>44%</td>
</tr>
<tr>
<td>CK20</td>
<td>0-5%</td>
<td>50-100%</td>
<td>78-100%</td>
</tr>
<tr>
<td>CDX2</td>
<td>0%</td>
<td>63-90%, patchy</td>
<td>90%, diffuse</td>
</tr>
<tr>
<td>HepPar1</td>
<td>0%*</td>
<td>83%</td>
<td>22%</td>
</tr>
<tr>
<td>Mucin markers</td>
<td>MUC1</td>
<td>Variable</td>
<td>MUC2, MUC5AC</td>
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*Based on the studies from Tot, O’Connell et al, and Chu et al.
*HepPar1 expression has been reported in rare breast cancer, although not in signet-ring type.

Metastatic melanoma to the breast
Metastatic melanoma can imitate primary breast carcinoma, especially high-grade invasive ductal carcinoma with an expansile nodular growth pattern. We have also observed patterns indistinguishable from lobular carcinoma (with discohesive cells arranged in trabeculae and small nests) and metastatic...
carcinoma. Helpful clues to the possibility of melanoma include the presence of melanin pigment (although the lesions may be amelanotic) and the lack of an in situ breast component. Immunoreactivity with melanoma markers (HMB-45, tyrosinase, and melan A) and negative reaction with CK confirm the diagnosis of metastatic melanoma. It should be noted that focal reactivity (ranging from 2-40% of tumor cells) for CK has been reported in melanoma and S100 can be positive in breast carcinoma. Therefore, S100 should not be used as the only marker for diagnosis of metastatic melanoma in the breast.

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