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Review article

Immunohistochemistry, carcinomas of unknown primary, and incidence rates

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ABSTRACT

Pathologists use immunohistochemistry is their day-to-day practices to assist in distinguishing site of origin of metastatic carcinomas. Here, the work-up is discussed neuroendocrine carcinomas, squamous cell carcinomas and adenocarcinomas with particular attention to tumor incident rates and predictive values of the best-performing immunohistochemical markers.

Introduction

Carcinomas of unknown primary (CUPs), are those carcinomas for which clinical, radiographic and pathologic evidence of a primary site of origin are lacking.[1] Given this definition, it would only seem to apply to those cases that have had a pathologic work-up for which the results of the work-up remain unclear or non-specific. The percentage of carcinomas falling into such a category remains unclear. However, it is the author's experience that it likely represents much lower percentage of malignancy than the percentage reported by some authors.[2]

The reasons for this are multifold. Data regarding number of CUPs may be abstracted from death certificates that notoriously contain incorrect information.[3] Many cases may not have had a complete radiographic and pathologic work-up prior to the patient expiring. Clinicians may even not “believe” pathologic assessment due to their own biases and still may consider cases to be of unknown primary even when relatively specific immunohistochemical results have been obtained.

As autopsies continue to become less frequent, pathologists are predominately exposed to such cases with small biopsies from lymph nodes, the liver, the lungs or bone, each a frequent site of metastatic disease. Here we will discuss the work-up of metastatic carcinoma usually seen on small biopsy for which the primary remains unknown or unclear. We will discuss the results in the face of pre-test probability and positive (and negative) predictive value for some immunohistochemical markers. We will start from the most basic forms of differentiation. Is the tumor predominately squamous, neuroendocrine (well differentiated or high grade), or is it adenocarcinoma (here defined as predominately non-squamous and non-neuroendocrine)? While not discussed here, it is incumbent on the pathologist to exclude germ cell tumors, sarcomas that express cytokeratins (e.g., synovial sarcoma, epithelioid sarcoma and epithelioid angiosarcoma), and mesothelioma before he or she attempts to identify the site of origin for a metastatic carcinoma.

Every case of metastatic carcinoma caries with it some pre-test probability for a primary site of origin. For example, a poorly differentiated carcinoma in a cervical lymph node from a man has no chance of being metastatic uterine carcinoma. A biopsy of small cell carcinoma (SmCC) in the liver is very likely to be from the lung, regardless of the

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clinical impression. Thus sites of involvement and incidence rates carry with them abundant information that should help guide the pathologist in the work-up of a case and his or her final conclusions.

Finally, it has been suggested that putative site of origin need not be determined given the move to treat cancer with targeted therapies based on their molecular abnormalities or interactions with the immune system.[4] The data for such an approach remain less than compelling but clinical trials may still prove the strategy effective for at least a subset of cases.[5,6]

Neuroendocrine carcinoma

Most classification systems now recognize two major groups of neuroendocrine carcinomas.[7] There are well-differentiated neuroendocrine "tumors" (usually further graded based on mitotic activity or ki-67 index) and poorly differentiated neuroendocrine carcinomas (SmCC and large cell neuroendocrine carcinoma). The diagnoses of typical and atypical carcinoid tumors of the lung mostly correspond with well-differentiated neuroendocrine tumor (of the gut and pancreas), grades 1 and 2.

Well-differentiated neuroendocrine tumors are usually readily identified by pathologists based on H and E morphology, however some atypical patterns of growth, artifacts seen on small biopsy (often previously frozen), or strange clinical situations may lead to the pathologist not immediately recognizing them. The tumors should invariably express keratins and should express more specific markers of neuroendrocrine differentiation, such as chromogranin and synaptophysin. Finally, almost all tumors will have ki-67 indices of less than 20% (i.e., they are grade 1 or 2 tumors). Grade 3 tumors are rare and before such a diagnosis is made (as pointed out in the excellent review of this topic by Bellizzi) other tumors should be considered.[8] For example, any tumor believed to be a grade 3 well-differentiated pancreatic neuroendocrine tumor (PNET) must be shown not to be a mixed acinar-endocrine carcinoma (certainly a rare tumor, though nonetheless more common than a grade 3 well-differentiated PNET).[9]

Regarding pre-test probability, studies have shown that when well-differentiated neuroendocrine tumors are biopsied as metastases in the liver, most are likely to be of pancreatic or small intestinal origin.[10,11] In one study, 27 of 69 tumors for which an origin could be determined were thought to have arisen in the pancreas and 30 in the small intestine.[11] Of note, only 2 were believed to have come from the lung and only one from the stomach. This appears to be the case in spite of the relative frequencies of primary well-differentiated endocrine tumors of the lung and stomach. Here it should also be noted that all the primary pancreatic tumors were identified by imaging studies. Indeed, in the same study, when a primary could not definitively be defined by imaging or endoscopy, 13 of 15 cases were found to be of the small bowel during surgical exploration. Thus, it can rightly be said that a metastatic well-differentiated neuroendocrine tumor with occult primary (after detailed radiographic assessment) is usually small bowel in origin (Fig. 1).

It is good that such is the case, as the use of IHC for finding the site of origin in such cases is of questionable use. TTF1 expression has been noted to variable degrees in pulmonary carcinoid and atypical carcinoid tumors with an overall expression rate of compiled cases from multiple studies being 32% whereas less than 1% of other metastatic well-differentiated neuroendocrine tumors have shown staining.[8,12–18] Pulmonary typical and atypical carcinoid tumors express OTP consistently (up to 80% of cases) and the protein is not expressed by well-differentiated neuroendocrine tumor from other sites.[19] Given the rarity of well-differentiated thoracic neuroendocrine tumors presenting a metastases of unknown primary site, this antibody may nonetheless remain of limited use with neuroendocrine tumors.

Well-differentiated PNETs frequently express PAX6, Islet 1, and PR.[8,20–23] PR expression and its use for distinguishing PNETs from other WDNETs has long been known.[23] While it is expressed in almost 60–70% of cases, it is less commonly expressed in higher grade and higher stage tumors (i.e., metastases).[24] PAX6 is expressed in the majority of PNETs and is also expressed in most duodenal and rectal WDNETs.[20,25] It is not expressed in ileal-jejeunal WDNETs, however. Islet 1 is expressed in a relatively similar fashion although it appears to be slightly more robust in function than PAX6.[21,22,26] Of note, there is an abundance of literature regarding PAX8 expression with PNETs. This appears secondary to cross-reactivity of the polyclonal antibody and monoclonal PAX8 antibodies are universally non-reactive with PNETs.[25]

CDX2-expression is somewhat more ubiquitous although diffuse positivity is generally seen jejeunal and ileal WDNETs.[8,21,27–30] PNETs and duodenal, gastric and rectal WDNETs stain less frequently and will typically not show strong and diffuse staining. Given the numbers of tumors from these sites that present with occult primaries, diffuse CDX2 immunoreactvity is almost always indicative of a jejeunal or ileal WDNET. SATB2 is most strongly and consistently expressed in rectal WDNETs, however significant expression is seen with other WDNETs.[31] Given the relative frequency of the various WDNETs to present with metastases, its use is unclear.

Finally, a note should be made about appendiceal goblet cell carcinoid. While the tumors do not typically present with widespread disease, they do sometimes present as ovarian metastases.[32] Here the differential may include metastatic gastric and breast cancer and neuroendocrine markers may be overlooked when the pathologist attempts to identify the site of origin. Aside from often expressing chromogranin and synaptophysin, appendiceal carcinoids are frequently immunoreactive with antibodies to SATB2 and CDX2.[33]

Poorly differentiated neuroendocrine carcinomas (PDNECs), especially SmCCs, are aggressive malignancies that often metastasize early (thus the majority (50–60%)) of pulmonary SmCCs are "extensive stage" at the time of their original diagnosis. Not surprisingly then, a proportion present as metastatic malignancies without a clinically or radiographically obvious primary tumor. For statistics purposes these are generally considered to be extra-pulmonary SmCC.

There are approximately 1000 cases of extrapulmonary SmCCs in the US every year (an estimate that is assumed not to include Merkel cell carcinoma of which there are approximately 1600 diagnosed per year in the US).[34,35] This is pretty infrequent compared to the 25–30 thousand cases of pulmonary SmCC.[36] The majority of extrapulmonary SmCCs have an easily identified primary site, however up to 30% may not have an obvious primary site. This translates to up to 300 cases in the US per year. Given the relative frequency of the primary tumor sites, one would expect that more than 90% of cases with no known primary are pulmonary in origin.

Unfortunately, determining site of origin with IHC is not straightforward with these lesions as site-specific markers tend to be expressed less sensitively and specifically than they are with other tumors. For example, TTF1 is expressed in about 85% of pulmonary SmCCs and in about 35% of non-cutaneous extrapulmonary SmCCs.[8] This holds for other site specific markers (e.g., CDX2) as well.[37] Given the skewed incidence rates, the predictive value of such markers is extremely limited.

There are two notable exceptions. MCCs do not express TTF1 (< 1%) and do express CK20 (approximately 90% of cases).[8,38–42] CK20 expression is uncommon in pulmonary SmCCs (approximately 5% of cases express it). Also, MCC is now well known to be associated with Merkel Cell Polyoma Virus. Antibodies to the Merkel Cell Polyomavirus Large T Antigen interact with 60–75% of MCCs and are extremely specific.[8,43–45] Other tumors that can be distinguished from pulmonary SmCC are extrapulmonary SmCCs secondary to high-risk human papillomavirus (HR-HPV) infection. Although the number of cases is somewhat obscure, SmCCs of the cervix, anus, oropharynx and sinonasal tract are often secondary to HR-HPV infection.[46–51] Here, in situ hybridization for the virus is necessary as p16 IHC highlights most SmCCs regardless of site of origin due to the mutations of