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Concise immunohistochemistry in carcinoma of unknown primary origin

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Carcinoma of unknown primary origin is a malignant epithelial neoplasm clinically defined by the presence of metastasis without known primary origin at the time of diagnosis. When it is clinically encountered, further investigations should be considered. Identification of primary origin of such neoplasm is crucial for proper patient management and prognosis. Immunohistochemistry has become an ancillary study for resolving this issue. Initial immunohistochemistry panel including AE1/AE3, S100, CD45 and vimentin is suggested for identification of lineage of tumor cell differentiation. If the tumor cells are diffusely positive for AE1/AE3 confirming the diagnosis of carcinoma, additional immunohistochemistry markers including CK7, CK20 and other tissue-specific markers should be employed in order to determine a primary origin. Interpretation of immunohistochemistry should always be correlated with histopathological findings, clinical context and radiological information. This approach can facilitate determination of the type and origin of carcinoma of unknown primary origin.

Keywords: *Immunohistochemistry, carcinoma of unknown primary origin, cytokeratin, carcinoma.*

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มะเร็งที่มีต้นกำเนิดจากเซลล์เยื่อบุและไม่ทราบอวัยวะต้นกำเนิด หมายถึง มะเร็งที่มีการแพร่กระจายไปจากอวัยวะตั้งต้นเมื่อได้รับการวินิจฉัยครั้งแรก การระบุอวัยวะต้นกำเนิดของมะเร็งกลุ่มนี้ต้องอาศัยการส่งตรวจทางคลินิกหลากหลายวิธีการ เนื่องด้วยส่งผลต่อการให้การรักษาพยาบาลและการพยากรณ์โรค ในปัจจุบันวิธีการตรวจอิมมูโนฮิสโตเคมีทวีบทบาทในการค้นหาอวัยวะต้นกำเนิดของมะเร็งกลุ่มดังกล่าว ชุดการตรวจแรกควรเริ่มจากแอนติบอดีต่อ AE1/AE3, S100, CD45 และ vimentin เพื่อระบุการเจริญพัฒนาของเซลล์มะเร็ง หากเซลล์มะเร็งส่วนใหญ่ให้ผลบวกต่อ AE1/AE3 ซึ่งยืนยันว่าเป็นมะเร็งที่มีต้นกำเนิดจากเซลล์เยื่อบุ ชุดการตรวจถัดไปที่ควรพิจารณา ได้แก่ CK7, CK20 และแอนติบอดีที่มีความจำเพาะต่อเนื้อเยื่ออื่น ๆ เพื่อช่วยระบุอวัยวะต้นกำเนิดของมะเร็งนั้น ๆ การแปลผลการตรวจอิมมูโนฮิสโตเคมีควรพิจารณาให้สอดคล้องกับลักษณะทางจุลพยาธิวิทยา ร่วมกับบริบทของผู้ป่วยทางคลินิกและภาพถ่ายรังสีเสมอ แนวทางการพิจารณาเลือกใช้ตัวบ่งชี้ดังกล่าวสามารถช่วยระบุการเจริญพัฒนาของเซลล์มะเร็งและอวัยวะต้นกำเนิดของมะเร็งที่มีต้นกำเนิดจากเซลล์เยื่อบุได้

คำสำคัญ: *การตรวจอิมมูโนฮิสโตเคมี, มะเร็งที่ไม่ทราบอวัยวะต้นกำเนิด, ไซโตเคอราติน, มะเร็งที่มีต้นกำเนิดจากเซลล์เยื่อบุ.*

"Carcinoma of unknown primary origin (CUP)" or "carcinoma of uncertain origin" is a malignant epithelial neoplasm clinically defined by the presence of metastasis without known primary origin at the time of diagnosis. Although this reflects the advanced stage of the cancer, identification of primary origin of such neoplasm is still crucial for proper patient management and prognosis. The term "cancer of unknown primary origin" is sometimes used interchangeably with the term CUP because carcinoma constitutes the major category of cancer of unknown primary origin.[1, 2] However, not all cancers of unknown primary origin are carcinomas. Searching for primary origin usually requires clinical examination, serological investigation, histopathological finding in biopsy and radiological imaging. Advance of molecular oncology in a few decades leads to continuous discoveries of novel tissue-specific markers/antibodies and effective immunohistochemical panels for elucidating primary origin of CUP. Nowadays, immunohistochemistry (IHC) has become an indispensable ancillary study in the identification and classification of CUP.

It has been traditionally stated that "it may be dangerous to base any distinction in tumor pathology primarily on the basis of the pattern of immunoreactivity of a given marker, no matter how specific it is purported to be".[3] This statement emphasizes the importance of conventional analysis with hematoxylin and eosin (H&E) as the basis of diagnosis in surgical pathology and a gold standard for morphological evaluation by pathologists. Differential diagnoses should be initially constructed based on histopathological findings and available clinical information. IHC is considered as an adjunct study to histopathological examination. This approach will narrow down the possible diagnoses resulting in a cost-effective IHC panel. Additionally, IHC interpretation should always be correlated with histopathological findings in H&E slide and available clinical information. Performing extensive IHC markers without morphological consideration is discouraged in order to avoid inconclusive IHC result.

Histopathological findings sometimes indicate the primary origin if the tumor is well differentiated such as metastatic papillary thyroid carcinoma. However, metastatic carcinoma especially to lymph node at various body locations sometimes shows marked cytological atypia that is not distinctive enough to allow a specific diagnosis of its origin. In this circumstance, diagnosis of poorly differentiated carcinoma in a biopsy specimen and clinical diagnosis of CUP are inevitably rendered. In some cases, the malignant cells are undifferentiated and difficult to be classified based solely on H&E slide whether it is a carcinoma, lymphoma, melanoma or sarcoma. This scenario is frequently encountered in a tiny biopsy specimen where the malignant cells are scant in the sample resulting in limited morphological evaluation. This review mainly focuses on the concept of IHC approach to facilitate the identification of lineage of tumor differentiation and primary origin of CUP.

Step-by-step approach to CUP

There are many approaches to work up a CUP. The following is a concise strategy for performing IHC panel to approach a CUP.

1. Review the slides of metastatic tumor without knowing any clinical information is a crucial step. Diagnosis of malignancy is normally established based on morphological examination, not solely relied

on the IHC result. The most likely broad category of the malignancy (such as carcinoma, melanoma, lymphoma or sarcoma) could be predicted if the morphology is distinctive. A broad differential diagnosis correlated with available clinical and radiological information is then generated.

2. The next step is to determine the first diagnostic IHC panel to perform. There are two likely scenarios: 2.1 Tumor with clear lineage of differentiation and 2.2 Tumor with unclear lineage of differentiation.

2.1 Tumor with clear lineage of differentiation on morphological examination. If a diagnosis of metastatic adenocarcinoma is morphologically established, the next step is to determine the likely primary origin of the tumor. The first IHC panel including cytokeratin 7 (CK7), CK20 and other tissue-specific markers should be considered. If a lymphoma is diagnosed, the next step is to classify whether it is B-cell or T-cell lymphoma, and to define the specific type of a lymphoma. The initial IHC panel including cluster of differentiation 3 (CD3), CD20 and other lymphoid markers should be exercised in this setting, but the approach of lymphoma is beyond the scope of this review.

2.2 Tumor with unclear lineage of differentiation on morphological examination. This scenario is frequently encountered in a very tiny biopsy specimen causing limited morphological feature of malignant cells. Elucidating the lineage of tumor cell differentiation by IHC markers covering a broad category of neoplasms is an initial aim in this setting. The first IHC panel would include AE1/AE3, S100, CD45 and vimentin. This approach will be discussed further in the following sections.

IHC markers of carcinoma

Carcinoma is referred to a malignant neoplasm derived from epithelial tissue regardless of the organ of origin.[4] The epithelia of the body are derived from all three germ layers such as epidermis and skin adnexa (ectoderm), gastrointestinal epithelium (endoderm), respiratory epithelium (endoderm), urothelium (endoderm), renal tubular epithelium (mesoderm), etc. Thus, malignant neoplasms arising in such epithelia are all termed carcinomas. Adenocarcinoma constitutes a major category (~60%) of CUP[5], but precise percentage varied among studies. Poorly differentiated carcinoma including poorly differentiated squamous cell carcinoma or neuroendocrine carcinoma comprises the remaining cases of CUP.

Because epithelial cells normally contain different types of intracytoplasmic (CK) [6], these filaments are also present in the cytoplasm of carcinoma cells. Antibody to CK is an excellent marker of epithelial differentiation, and shows strong and diffuse staining in carcinoma.[7] Antibody to different types of CK is currently available for determination of epithelial differentiation of the tumor. Because AE1/AE3 is composed of antibodies targeting both acidic CK (except CK9, CK12, CK17 and CK18) and basic CK [8], it is therefore used as a pan-epithelial marker. The pitfall is that there are few AE1/AE3-negative carcinomas, notably hepatocellular carcinoma, because the tumor cells contain CK18 that is not recognized by AE1/AE3. In this regard, Cam5.2 containing antibodies to CK8 and CK18 should be utilized instead of AE1/AE3 in a case suspicious for hepatocellular carcinoma.

Positive keratin in non-carcinoma showing true epithelial differentiation has been described.[8] Examples of this category include synovial sarcoma, epithelioid sarcoma and epithelioid angiosarcoma. In spite of this concern, the most likely diagnosis of carcinoma should be considered when epithelioid tumor cells are diffusely positive for AE1/AE3 (Figure1).

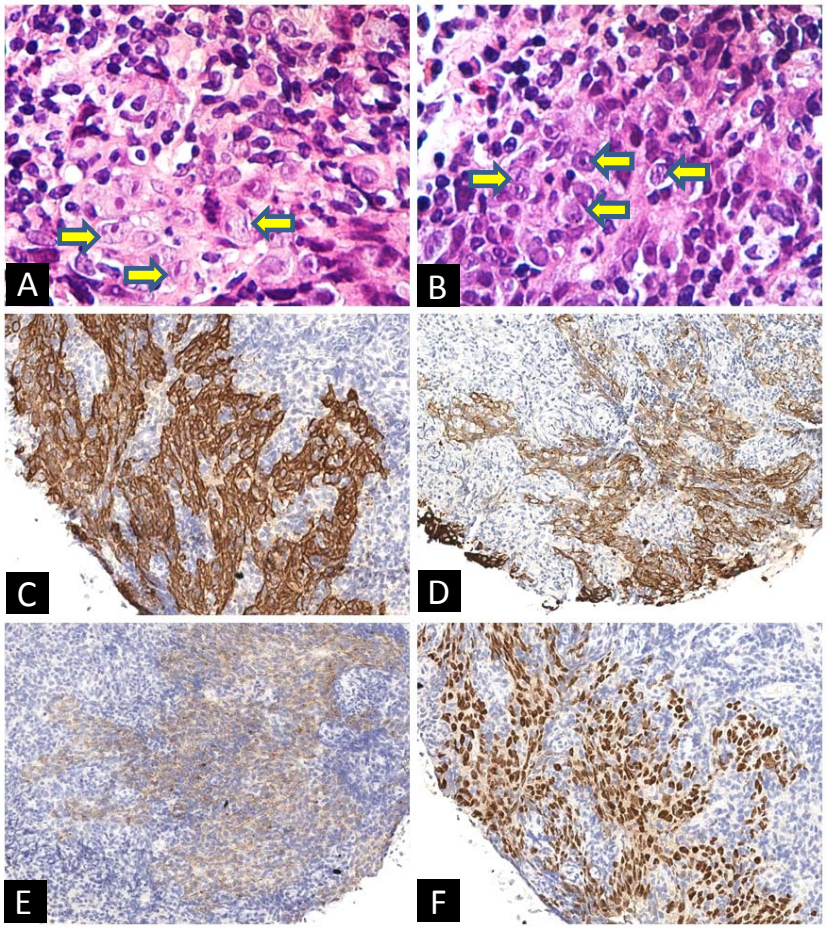

Figure 1. Histopathological findings of non-keratinizing nasopharyngeal carcinoma in a tiny biopsy specimen from nasopharynx

A. Cluster of tumor cells (arrows) showing enlarged vesicular nuclei with nucleoli (H&E, 400x)

B. Isolated tumor cells (arrows) infiltrating between lymphoid cells (H&E, 400x)

C. Cytoplasmic staining for AE1/AE3 in the tumor cells (AE1/AE3, 200x)

D. Cytoplasmic staining for CK5/6 in the tumor cells (CK5/6, 200x)

E. Weakly cytoplasmic staining for EBV-LMP1 in the tumor cells (EBV-LMP1, 200x)

F. Nuclear staining for p63 in the tumor cell nuclei (p63, 200x)

IHC markers of melanoma, lymphoma and sarcoma

In some cases, the malignant cells are undifferentiated and difficult to be classified whether they are carcinoma, melanoma, lymphoma or sarcoma, utilization of initial IHC panel including AE1/AE3, S100, CD45 and vimentin is helpful for distinguishing these entities.[9, 10] Initial IHC panel for evaluating cancer of unknown primary origin or poorly differentiated malignancy is summarized in Table 1.

A diffuse strong staining of S100 in an AE1/AE3-negative malignant tumor is a good evidence that such malignancy may be a melanoma or other S100-positive malignancies. S100, although not specific, is a sensitive marker for melanocytic differentiation. SRY-related HMG-box 10 (SOX10) protein is a neural crest transcription factor crucial for maturation of melanocyte and Schwann cell. SOX10 is a more sensitive marker for melanocytic and schwannian differentiation than S100. SOX10 is also proposed as a useful marker for identifying metastatic melanoma in the appropriate clinical context.[11, 12] However, SOX10 and S100 cannot be used to differentiate between benign and malignant pigmented skin lesions. More specific melanocytic markers including HMB-45, melan-A and tyrosinase should be further utilized for diagnostic confirmation of melanoma (Figure 2).

Table 1. Initial and additional IHC panels for evaluation of cancer of unknown primary origin or poorly differentiated malignancy

Cancers / Markers	Carcinoma	Melanoma	Lymphoma	Sarcoma
AE1/AE3	+	-	-	-/+
S100	-	+	-	-
CD45	-	-	+	-
Vimentin	-/+	+	+	+
Additional markers	CK5/6, p63, CK7, CK20, other tissue-specific markers	HMB-45, melan-A, tyrosinase	CD3, CD20, Ki-67, other lymphoid markers	Other mesenchymal markers

Abbreviation: +, positive reactivity; -, negative reactivity; -/+, commonly negative reactivity.

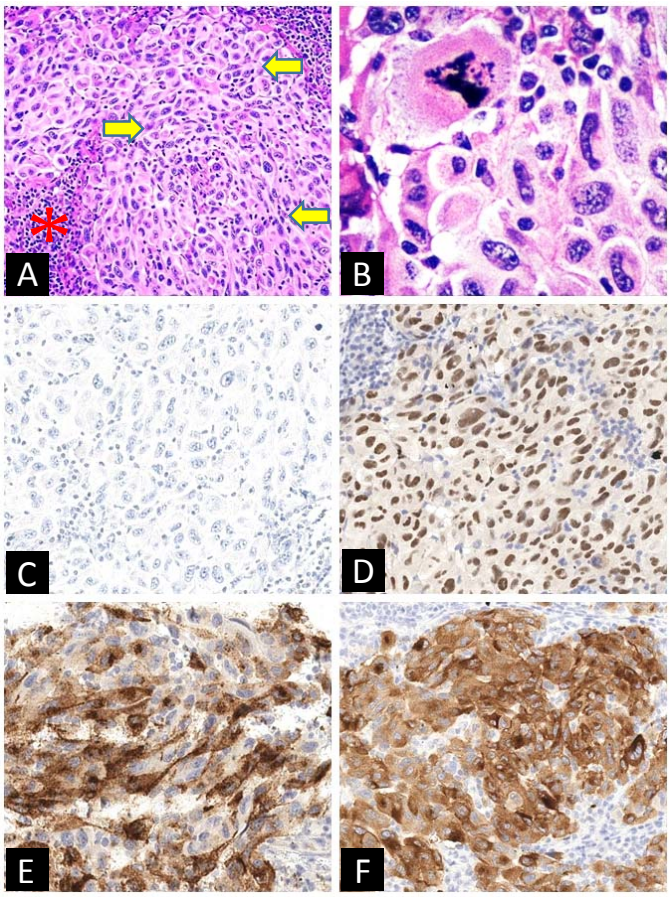

Figure 2. Histopathological findings of metastatic melanoma to the right axillary lymph node
A. Cluster of tumor cells (arrows) infiltrating between lymphoid cells (asterisk) (H&E, 200x)
B. Tumor cells showing marked cytologic atypia without melanin pigments seen (H&E, 400x)
C. No cytoplasmic staining for AE1/AE3 in the tumor cells (AE1/AE3, 200x)
D. Nuclear staining for SOX10 in the tumor cell nuclei (SOX10, 200x)
E. Cytoplasmic staining for HMB-45 in the tumor cells (HMB-45, 200x)
F. Cytoplasmic staining for melan-A in the tumor cells (melan-A, 200x)

CD45 or leukocyte common antigen (LCA) is regarded as a pan leukocyte marker. More than 99% of Non Hodgkin B-cell lymphoma and more than 90% of T-cell lymphoma are positive for CD45.[13] If the malignant tumor cells are positive for CD45 and negative for CK and S100, further IHC panel toward classifying the lymphoma subtype by using CD3, CD20 and other lymphoid markers should be exercised. Vimentin is usually positive in lymphoma. There is a caveat that few lymphomas are not recognized by CD45. Plasmablastic lymphoma is a rare aggressive lymphoid neoplasm resembling B immunoblast or plasmablast and expressing CD38 and CD138. CD45, CD20 and paired box gene 5 (PAX5) are typically negative or sometimes weakly positive in a minority of cells of plasmablastic lymphoma.[14] Anaplastic large cell lymphoma is also variably positive for CD45.[14] CD30 and anaplastic lymphoma kinase (ALK) should be investigated if anaplastic large cell lymphoma is included in the

differential diagnosis. Reed-Sternberg cell in classical Hodgkin lymphoma is typically negative for CD45 and CD20, but positive for PAX5, confirming B cell origin. Fortunately, morphology of classical Hodgkin lymphoma is distinctive for histopathological recognition, and not usually included in the differential diagnosis of CUP. Utilization of CD3 and CD20 instead of CD45 in the first IHC panel is an alternative option especially in a case suspicious for lymphoma morphologically (Figure 3). Adding Ki-67 into the first panel is very helpful in this setting. If poorly differentiated malignant cells predominantly express either CD3 or CD20 together with high Ki-67 index, such neoplasm is very likely to be T-cell or B-cell lymphoma, respectively.

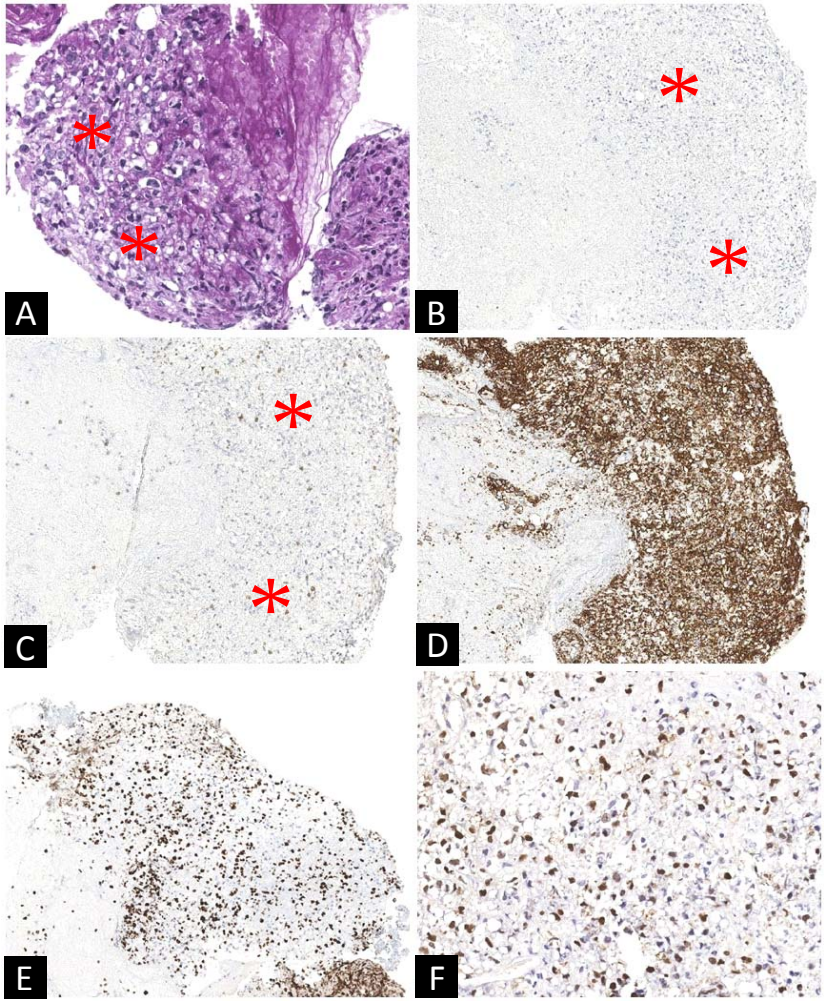

Figure 3. Histopathological findings of diffuse large B-cell lymphoma at the right thalamus of HIV-positive man

A. Round tumor cells (asterisks) without definite differentiation (H&E, 200x)
B. No cytoplasmic staining for AE1/AE3 in the tumor cells (asterisks) (AE1/AE3, 100x)
C. No membrane staining for CD3 in the tumor cells (asterisks) (CD3, 100x)
D. Diffusely membrane staining for CD20 in the tumor cells without CD20 staining in the brain tissue on the left (CD20, 100x)
E. Nuclear staining for Ki-67 in 40% of the tumor cell nuclei (Ki-67, 100x)
F. Nuclear staining for EBER in the tumor cell nuclei (In situ hybridization for EBER, 200x)

Vimentin is one of an intermediate filament class 3 normally found in the cytoplasm of mesenchyme-derived cells including lymphoid cell. Strongly positive vimentin expression in a non-melanocytic, non-lymphoid malignancy is generally an indicator of sarcoma. Additional markers of specific mesenchymal differentiation are sequentially required in order to reach a definite diagnosis, but this is beyond the scope of this review. Although vimentin is traditionally considered as a mesenchymal marker, it is practically non-specific due to an expression of vimentin in some carcinomas, lymphoma and melanoma. Among the above markers, vimentin is generally the least helpful and should be carefully interpreted with caution. However, a vimentin-negative tumor is unlikely to be a sarcoma (with the exception of alveolar soft part sarcoma), lymphoma, or melanoma.[9, 10]

IHC markers of squamous cell carcinoma

CK5 and CK6 are basic CK. Almost all squamous cell carcinomas and half of urothelial carcinomas are positive for CK5/6 and p63.[15, 16] Coexpression of CK5/6 and p63 is highly predictive of squamous differentiation, even in poorly differentiated squamous cell carcinoma.[15, 16] p40, a more specific marker of squamous differentiation than p63 or CK5/6, has been recently addressed in pulmonary squamous cell carcinoma.[17] However, further studies are required whether p40 should be included in an IHC panel to evaluate squamous differentiation of the tumor in other sites. Primary origin of squamous cell carcinoma is established clinically. No IHC marker is used to precisely facilitate the primary origin of squamous cell carcinoma till date.

Interestingly, squamous cell carcinoma arising in some organs has a unique feature. A worldwide study of over 10,000 cervical cancers reported that virtually all squamous cell carcinomas of the cervix are associated with high-risk human papilloma virus (HPV).[18] A major subset of cervical squamous cell carcinoma is, therefore, p16 positive by IHC.[19] Nasopharyngeal carcinoma is a cancer showing squamous differentiation and arising at the nasopharynx. Strong expression of AE1/AE3, CK5/6 and p63 is observed in this neoplasm (Figure 1). Moreover, non-keratinizing nasopharyngeal carcinoma is associated with Epstein-Barr virus (EBV) in almost all cases.[20] In situ hybridization (ISH) for EBER is currently the most reliable method to demonstrate EBV infection in the tumor. IHC for EBV latent membrane protein 1 (EBV-LMP1) is not as sensitive as EBER.

IHC markers of adenocarcinoma

CK7 and CK20 are low molecular weight CK showing distinctive pattern of expression in the epithelium of various organs. The restricted distribution of CK7 is useful in evaluating the origin of adenocarcinoma because CK7 is present in most, but not all, breast, lung, thyroid, salivary gland, ovarian uterine, pancreatobiliary and urothelial carcinomas.[10] CK20 is limitedly expressed to gastrointestinal epithelium, pancreatobiliary tumor, mucinous ovarian tumor and Merkel cell carcinoma. Expression pattern of CK7 and CK20 in adenocarcinoma arising in such visceral organs can be separated into 4 main diagnostic groups[21]: (1) CK7+/CK20+, (2) CK7+/CK20-, (3) CK7-/CK20+, and (4) CK7-/CK20-, as summarized in Table 2. These expression patterns of CK7 and CK20 are common immunophenotype

of adenocarcinoma in such organs. Although, typical expression of CK7 and CK20 can somehow narrow down the possible origin of adenocarcinoma, there are still some exceptions of this approach.

Because cancer is a disease having molecular and genetic heterogeneity, some subset of carcinoma in one organ may express more than one pattern of CK7 and CK20. For example, all four patterns of CK7 and CK20 had been described in gastric adenocarcinoma, but CK7+/CK20+ gastric adenocarcinoma is the most common one among others.[22] CK7-/CK20+ colorectal adenocarcinoma is a well-recognized pattern, but CK7+/CK20+ and CK7-/CK20- colorectal adenocarcinomas were reported in 32% of the cases in one study.[23] Therefore, reactivity of CK7 and CK20 should be interpreted with caution, and should not be used as the sole basis for defining a primary origin of CUP. Combination of CK7, CK20 and other tissue-specific markers is inevitably crucial to reaching a correct diagnosis.

Tissue-specific markers of adenocarcinoma

Because most antigens and proteins are typically present in more than one tissue, there is currently no single IHC marker having 100% specific to any given organ or tissue. Using IHC markers in combination as IHC panel is critically important for clinical application in oncologic pathology. Many metastatic carcinomas still retain some, but not all, antigens and proteins similarly to the organ of primary origin. Understanding the immunoprofile of epithelium in each organ is beneficial for determining the primary origin of the metastatic carcinoma. This review introduces IHC markers of carcinoma originating from the breast, lung, colon, rectum, prostate gland, urinary bladder and liver. A concise immunophenotype of such neoplasms is summarized in Table 3.

Table 2. Profile of CK7 and CK20 expression of adenocarcinoma in visceral organ.

CK7 and CK20 expression	Visceral organ
CK7+, CK20+	Urinary bladder, stomach, pancreatobiliary organ, mucinous ovarian tumor
CK7+, CK20-	Breast, lung, thyroid, salivary gland, uterus, non-mucinous ovarian tumor
CK7-, CK20+	Colon, rectum, small intestine, appendix, Merkel cell carcinoma
CK7-, CK20-	Liver, kidney, prostate gland, adrenal gland

Table 3. Summary of tissue-specific markers of adenocarcinoma.

Carcinomas and primary origins	Markers
Breast carcinoma	GCDFP-15, GATA-3, ER, mammaglobin
Pulmonary adenocarcinoma	TTF-1, napsin A
Colorectal adenocarcinoma	CDX2, SATB2
Prostatic adenocarcinoma	PSA, PAP, prostein, NKX3.1
Urothelial carcinoma	S100P, uroplakin II, uroplakin III, GATA3, CK5/6, p63, 34 βE12
Hepatocellular carcinoma	arginase-1, HepPar1, glypican-3

1. **Breast.** Invasive ductal carcinoma, not otherwise specified (NOS) is the most common type of breast cancer. It is a group of breast cancer showing no specific differentiation on histopathological examination. Breast carcinoma is typically positive for CK7 and negative for CK20. Gross cystic disease fluid protein 15 (GCDFP-15) has been used as the most specific marker of breast carcinoma, although it is not a sensitive marker for breast origin.[24] Breast carcinoma variably expresses estrogen receptor (ER), GATA binding protein 3 (GATA-3) and mammaglobin. Using these markers as a panel is beneficial for defining the metastatic breast carcinoma (Figure 4). Breast cancer is classified into 4 major molecular subtypes based on gene expression profiling: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2) enriched and basal-like subtypes. The basal-like carcinoma shows strong expression of basal CK (CK5/6 and CK14) without expression of ER, progesterone receptor (PR) and HER2, thus also called triple-negative phenotype. In a case with a past history of triple-negative breast carcinoma, CK7+/CK20-/ER-/PR- profile in a metastatic lesion cannot totally exclude a primary breast origin. Performing GATA-3 may be helpful in this context because GATA-3 expression was seen in up to 73% of triple-negative breast cancer.[25] However, GATA-3 expression had been reported in salivary neoplasms[26] and urothelial carcinoma[27]. Clinical correlation is also essential.

2. **Lung**. Pulmonary adenocarcinoma is defined by World Health Organization (WHO) as a malignant epithelial neoplasm showing glandular differentiation, mucin production, or pneumocyte marker expression. Currently, the most commonly used pneumocyte markers are thyroid transcription factor-1 (TTF-1) and napsin A.[17] TTF-1 is positive in vast majority of pulmonary carcinoma including adenocarcinoma (70-75%), large cell neuroendocrine carcinoma and small cell carcinoma. It is worth noting that small cell carcinoma in other sites (gastrointestinal tract, urinary bladder, cervix and prostate) and thyroid carcinoma also express TTF-1.[10] Napsin A is sometimes expressed in other tumors such as renal cell carcinoma. IHC panel of CK7, CK20, TTF-1 and napsin A is therefore suggested in a case suspicious for metastatic pulmonary adenocarcinoma (Figure 5). TTF-1 and napsin A show nuclear and granular cytoplasmic reactivity in the tumor cells, respectively.

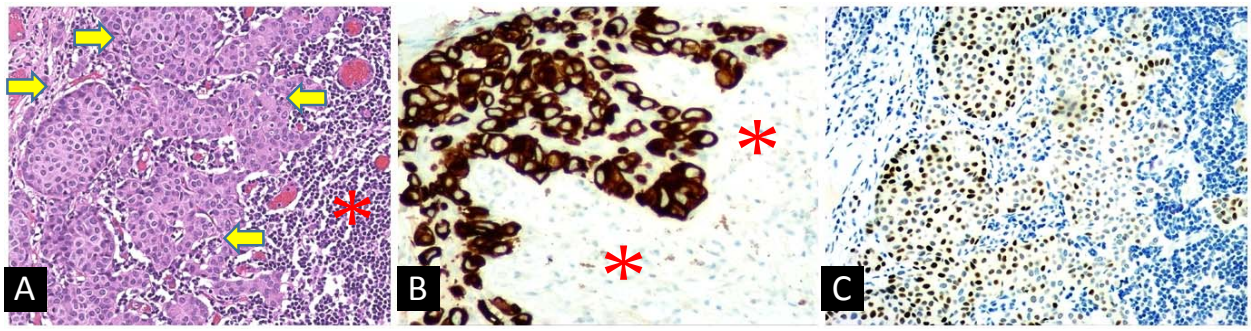

Figure 4. Histopathological findings of metastatic breast carcinoma to the left axillary lymph node. Positive for estrogen receptor and absent CK20 were observed in the tumor (not shown).

A. Irregular sheet of tumor cells (arrows) infiltrating between lymphoid cells (asterisk) (H&E, 100x)

B. Cytoplasmic staining for CK7 in the tumor cells without CK7 staining in the lymphoid cells (asterisk) (CK7, 200x)

C. Nuclear staining for GATA-3 in the tumor cell nuclei (GATA-3, 100x)

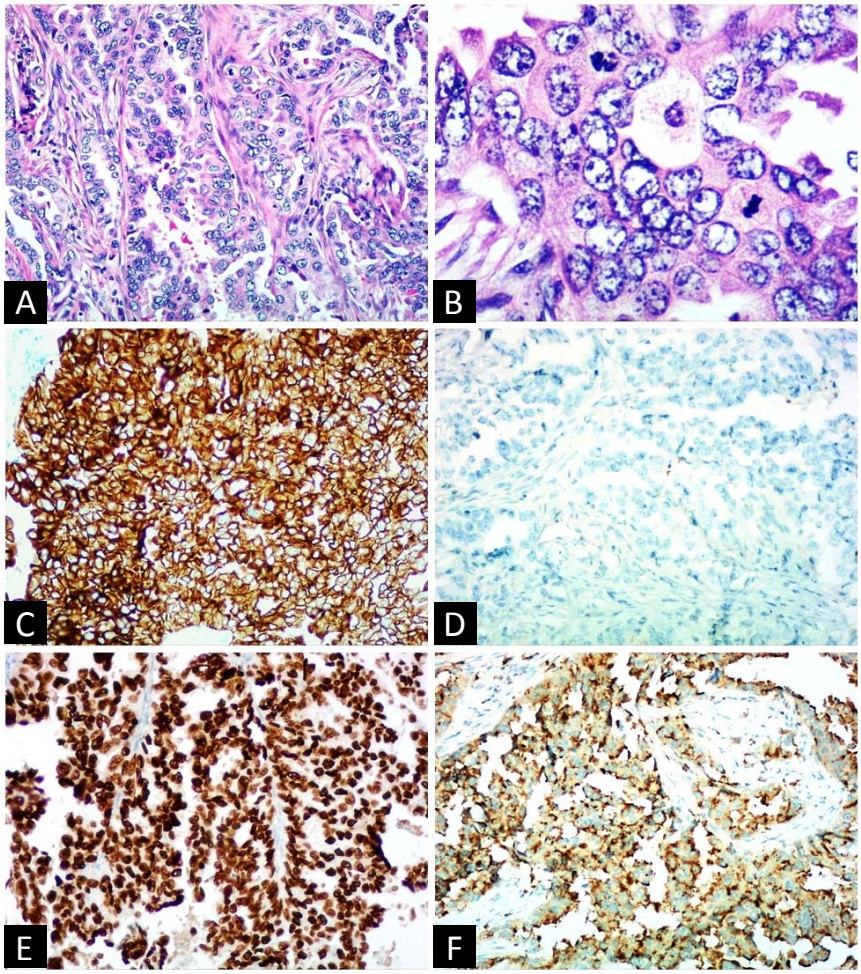

Figure 5. Histopathological findings of metastatic pulmonary adenocarcinoma to the right supraclavicular lymph node

A. Tumor cells arranging in glandular structure (H&E, 200x)

B. Tumor cells showing vesicular nuclei and increased mitoses (H&E, 400x)

C. Cytoplasmic staining for CK7 in the tumor cells (CK7, 200x)

D. No cytoplasmic staining for CK20 in the tumor cells (CK20, 200x)

E. Nuclear staining for TTF-1 in the tumor cell nuclei (TTF-1, 200x)

F. Granular cytoplasmic staining for napsin A in the tumor cells (napsin A, 200x)

3. **Colon and rectum**. More than 90% of colorectal carcinoma are adenocarcinoma.[28] Caudal type homeobox transcription factor 2 (*CDX2*) is a homebox gene responsible for intestinal cell proliferation and differentiation, and is expressed in the nuclei of intestinal epithelial cells. CDX2 protein is expressed in primary and metastatic colorectal adenocarcinomas and other lesions/neoplasms showing intestinal differentiation. The latter category includes intestinal metaplasia of the esophagus and stomach, intestinal-type gastric adenocarcinoma, mucinous neoplasm of the lung, ovary and urinary bladder. The classic immunoprofile suggestive of colorectal adenocarcinoma is positive for CK20 and

CDX2, and negative for CK7. However, unusual CK immunophenotypes had been reported as mentioned previously.[23] Special AT-rich sequence-binding protein 2 (SATB2) has been proposed as a new marker for colorectal differentiation.[29, 30] IHC for SATB2 in combination with CK20 could identify more than 90% of colorectal carcinoma in one study[29], therefore SATB2 is proved to be a complementary marker for metastatic tumor with colorectal differentiation. Recently, SATB2 had been reported as a marker of osteoblastic differentiation in benign and malignant mesenchymal tumors, especially for confirmation of osteosarcoma.[31] This finding indirectly supports that using IHC as a panel is practically favored.

4. **Prostate gland**. Acinar adenocarcinoma or prostatic adenocarcinoma consists of neoplastic prostatic epithelial cells with secretory differentiation in a variety of histomorphological patterns in the absence of basal cells. This malignancy is highly associated with a rising of serum prostate-specific antigen (PSA) level. IHC for PSA, prostatic acid phosphatase (PAP), prostein (P501S) and NKX3.1 are all highly sensitive markers in diagnosis of metastatic prostatic adenocarcinoma.[32] PSA immunopositivity had been observed in salivary gland neoplasm, while PAP is positive in salivary gland neoplasm and neuroendocrine tumor. NKX3.1 seems to be a highly sensitive and specific marker in this setting.[33] Nuclear expression of NKX3.1 along with other positive prostate-restricted markers may prove to be a valuable adjunct in order to determine prostatic origin of CUP.

Alpha-methylacyl-CoA racemase (AMACR, racemase or P504S) is another diffusely positive marker for prostatic adenocarcinoma.[32] Other prostatic lesions expressing AMACR include high-grade prostatic intraepithelial neoplasia, atrophy, adenosis and nephrogenic adenoma. Strong expression of AMACR had been found in papillary renal cell carcinoma, mucinous tubular and spindle cell carcinoma, and acquired cystic disease-associated renal cell carcinoma.[34] Using AMACR alone in a metastatic lesion is discouraged because of its non-specificity. On the other hand, performing AMACR in conjunction with basal cell markers, including 34 βE12 and p63, is very helpful to facilitate the distinction between benign and malignant prostate lesions in a small prostatic core needle biopsy.[35] Overexpression of granular cytoplasmic staining of AMACR in the absence of basal cell markers in small foci in core needle biopsy has proved to be the greatest utility in establishing the diagnosis of prostatic adenocarcinoma (Figure 6).

5. **Urinary bladder**. Infiltrating urothelial carcinoma is the most common malignancy of the urinary tract, and is characterized by a propensity for divergent differentiation. More than 90% of urothelial carcinoma arises in the urinary bladder.[32] Positivity for uroplakin II, uroplakin III, GATA3, CK5/6, p63 and 34 βE12 is of value in proving urothelial differentiation in the appropriate morphologic and clinical context.[32] Coexpression of CK7 and CK20 is commonly observed in 50-60% of the cases. Negative CK7 is highly unusual for urothelial carcinoma.[8] Although uroplakin III is considered the most specific marker for urothelial differentiation, this marker has low sensitivity. Placental S100 (S100P) is an emerging marker for urothelial differentiation.[36] Please note that both squamous cell carcinoma and urothelial carcinoma coexpress CK5/6 and p63 which cannot be used solely for distinguishing these two entities immunohistochemically.

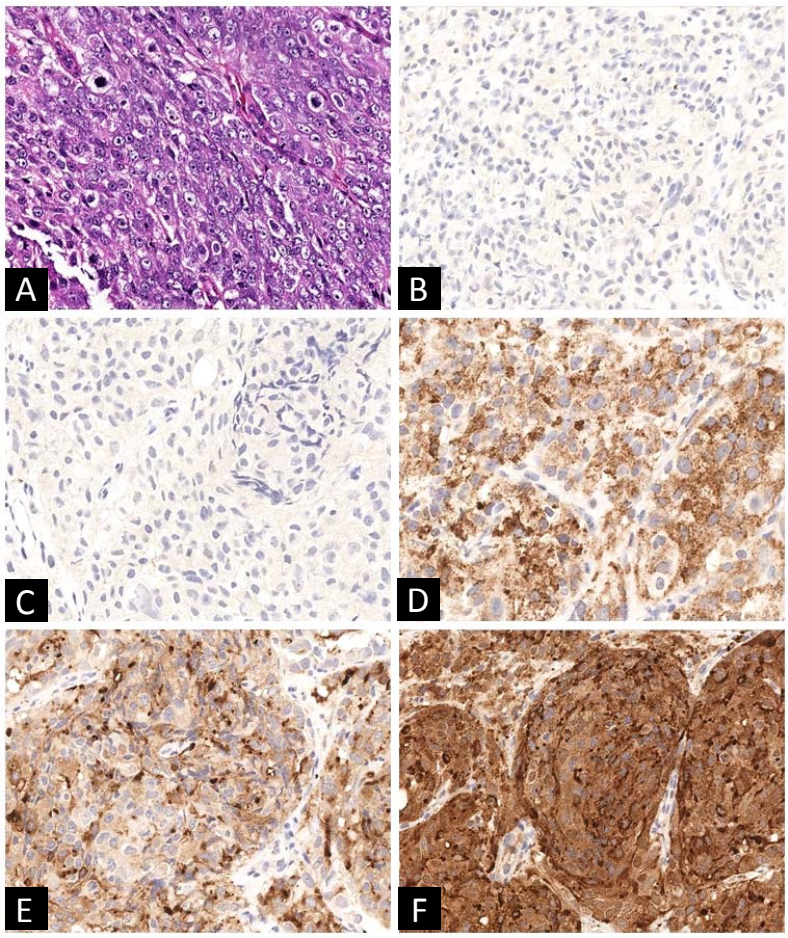

Figure 6. Histopathological findings of poorly differentiated prostatic adenocarcinoma, Gleason score 5 + 5 =10, in the prostate core needle biopsy

A. Solid sheet of tumor cells without glandular structure (H&E, 200x)

B. No basal cell in the tumor because of no cytoplasmic staining for 34βE12 (34βE12, 200x)

C. No basal cell in the tumor because of no nuclear staining for p63 (p63, 200x)

D. Cytoplasmic staining for alpha-methylacyl-CoA racemase (AMACR) in the tumor cells (AMACR, 200x)

E. Cytoplasmic staining for prostate-specific antigen (PSA) in the tumor cells (PSA, 200x)

F. Cytoplasmic staining for prostatic acid phosphatase (PAP) in the tumor cells (PAP, 200x)

6. **Liver**. Hepatocellular carcinoma is a malignant neoplasm with hepatocellular differentiation. Chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) or coinfection approximately accounts for 85% of cases.[28] Alcohol-induced liver injury constitutes the most important non-viral risk factor. Hepatocellular carcinoma is traditionally characterized by cytoplasmic positivity with hepatocyte paraffin 1 (HepPar1) antibody. About 90% of hepatocellular carcinoma are positive for HepPar1, but positivity is observed in less than 50% of poorly differentiated hepatocellular carcinoma. Arginase-1 is the most sensitive and specific marker for hepatocyte compared to HepPar1 and glypican-3.[37, 38] Arginase-1 demonstrates diffuse cytoplasmic expression in both normal hepatocyte and in hepatocellular neoplasms.

Glypican-3, although not specific for hepatocellular differentiation, is frequently positive in poorly differentiated hepatocellular carcinoma. IHC panel for arginase-1, HepPar1 and glypican-3 is the most effective panel in distinguishing hepatocellular carcinoma from other metastatic tumors.[37] Absent CK7 and CK20 is typically observed in most hepatocellular carcinoma.[21]

Cholangiocarcinoma is an adenocarcinoma showing biliary epithelial differentiation. Intrahepatic and extrahepatic types are defined according to the primary involvement. It is the second most common primary hepatic cancer after hepatocellular carcinoma.[28] No specific marker for biliary differentiation is discovered till date. Cholangiocarcinoma commonly expresses CK7, CK19, carcinoembryonic antigen (CEA) and epithelial membrane antigen (EMA).[28] None of them is specific to biliary differentiation. Histopathological findings of cholangiocarcinoma usually show well to moderately differentiated adenocarcinoma without unique feature. In the surgical pathology point of view, it is the diagnosis of exclusion of other differential diagnosis. Definite diagnosis of cholangiocarcinoma usually requires correlation with clinical, radiological and histopathological findings.

Conclusion

Conventional analysis with H&E still remains the gold standard for morphological evaluation and determination whether a tumor is malignancy. To generate a proper differential diagnosis of CUP based primarily on histopathological finding, clinical information is also essential to reach such goal. Application of IHC has become a valuable ancillary study in the diagnosis and classification of CUP. The diagnostic accuracy in recognizing the primary origin of CUP has been continuously improved due to emerging discovery of additional tissue-specific markers. Utilization of IHC as a panel is encouraged in order to avoid confounding reactivity of single IHC marker, and increase specificity of the IHC result. Interpretation of IHC should always be correlated with clinical and radiological context. Explored here are a few examples of diagnostic application of IHC. Nowadays, there are increasing applications of IHC for its predictive and prognostic utility in many cancers.

References

1. Blaszyk H, Hartmann A, Bjornsson J. Cancer of unknown primary: clinicopathologic correlations. APMIS 2003;111:1089-94.
2. Yakushiji S, Ando M, Yonemori K, Kohno T, Shimizu C, Katsumata N, et al. Cancer of unknown primary site: review of consecutive cases at the National Cancer Center Hospital of Japan. Int J Clin Oncol 2006;11:421-5.
3. Chetty R. Cytokeratin expression in cauda equina paragangliomas. Author's response to letter. Am J Surg Pathol 1999;23:491.
4. Kumar V, Abbas AK, Aster JC. Neoplasia. In: Kumar V, Abbas AK, Aster JC. Robbins basic pathology. 10th ed. Philadelphia: Elsevier; 2018. p.189-242.
5. Hammar SP. Metastatic adenocarcinoma of unknown primary origin. Hum Pathol 1998;29: 1393-402.
6. Ross MH, Pawlina W. Cell cytoplasm. In: Ross MH, Pawlina W. Histology: a text and atlas with correlated cell and molecular biology. 7th ed.

Philadelphia: Lippincott Williams & Wilkins; 2016. p.23-73.

7. Spagnolo DV, Michie SA, Crabtree GS, Warnke RA, Rouse RV. Monoclonal anti-keratin (AE1) reactivity in routinely processed tissue from 166 human neoplasms. Am J Clin Pathol 1985; 84:697-704.
8. Rekhtman N, Bishop JA. Quick reference handbook for surgical pathologists. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011.
9. Lin F, Liu H. Immunohistochemistry in undifferentiated neoplasm/tumor of uncertain origin. Arch Pathol Lab Med 2014;138:1583-610.
10. Bhargava R, Dabbs DJ. Immunohistology of metastatic carcinoma of unknown primary site. In: Dabbs DJ, editor. Diagnostic immunohistochemistry: theranostic and genomic applications. 4th ed. Philadelphia: Elsevier/Saunders; 2014. p.204-44.
11. Mohamed A, Gonzalez RS, Lawson D, Wang J, Cohen C. SOX10 expression in malignant melanoma, carcinoma, and normal tissues. Appl Immunohistochem Mol Morphol 2013; 21:506-10.
12. Willis BC, Johnson G, Wang J, Cohen C. SOX10: a useful marker for identifying metastatic melanoma in sentinel lymph nodes. Appl Immunohistochem Mol Morphol 2015;23: 109-12.
13. O'Malley DP, Grimm KE, Banks PM. Immunohistology of non-Hodgkin lymphoma. In: Dabbs DJ, editor. Diagnostic immunohistochemistry: theranostic and genomic applications. 4th ed. Philadelphia: Elsevier/ Saunders; 2014. p.148-88.
14. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Revised 4th ed. Lyon: IARC; 2017.
15. Chu PG, Weiss LM. Expression of cytokeratin 5/6 in epithelial neoplasms: an immunohistochemical study of 509 cases. Mod Pathol. Mod Pathol 2002;15:6-10.
16. Kaufmann O, Fietze E, Mengs J, Dietel M. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. Am J Clin Pathol 2001;116:823-30.
17. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG, editors. WHO classification of tumours of the lung, pleura, thymus and heart. 4th ed. Lyon: IARC; 2015.
18. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048-56.
19. Kurman RJ, Carcangiu ML, Herrington CS, Young RH, editors. WHO classification of tumours of female reproductive organs. 4th ed. Lyon: IARC; 2014.
20. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, editors. WHO classification of head and neck tumours. 4th ed. Lyon: IARC; 2017.
21. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial

neoplasms: a survey of 435 cases. Mod Pathol 2000;13:962-72.

22. Krasinskas AM, Goldsmith JD. Immunohistology of the gastrointestinal tract. In: Dabbs DJ, editor. Diagnostic immunohistochemistry: theranostic and genomic applications. 4th ed. Philadelphia: Elsevier/Saunders; 2014. p.508-39.
23. Bayrak R, Yenid□nya S, Haltas H. Cytokeratin 7 and cytokeratin 20 expression in colorectal adenocarcinomas. Pathol Res Pract 2011 15; 207:156-60.
24. Bhargava R, Dabbs DJ. Immunohistology of the breast. In: Dabbs DJ, editor. Diagnostic immunohistochemistry: theranostic and genomic applications. 4th ed. Philadelphia: Elsevier/Saunders; 2014. p.738-9.
25. Cimino-Mathews A, Subhawong AP, Illei PB, Sharma R, Halushka MK, Vang R, et al. GATA3 expression in breast carcinoma: utility in triple-negative, sarcomatoid, and metastatic carcinomas. Hum Pathol 2013;44: 1341-9.
26. Schwartz LE, Begum S, Westra WH, Bishop JA. GATA3 immunohistochemical expression in salivary gland neoplasms. Head Neck Pathol 2013;7:311-5.
27. Liu H, Shi J, Wilkerson ML, Lin F. Immuno-histochemical evaluation of GATA3 expression in tumors and normal tissues: a useful immunomarker for breast and urothelial carcinomas. Am J Clin Pathol 2012;138: 57-64.
28. Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system. 4th ed. Lyon: IARC; 2010.
29. Magnusson K, de Wit M, Brennan DJ, Johnson LB, McGee SF, Lundberg E, et al. SATB2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. Am J Surg Pathol 2011;35:937-48.
30. Dragomir A, de Wit M, Johansson C, Uhlen M, Pont□n F. The role of SATB2 as a diagnostic marker for tumors of colorectal origin: Results of a pathology-based clinical prospective study. Am J Clin Pathol 2014;141:630-8.
31. Conner JR, Hornick JL. SATB2 is a novel marker of osteoblastic differentiation in bone and soft tissue tumours. Histopathology 2013;63: 36-49.
32. Moch H, Humphrey PA, Ulbright TM, Reuter VE, editors. WHO classification of tumours of the urinary system and male genital organs. 4th ed. Lyon: IARC; 2016.
33. Gurel B, Ali TZ, Montgomery EA, Begum S, Hicks J, Goggins M, et al. NKX3.1 as a marker of prostatic origin in metastatic tumors. Am J Surg Pathol 2010;34:1097-105.
34. Molinié V, Balaton A, Rotman S, Mansouri D, De Pinieux I, Homsi T, et al. Alpha-methyl CoA racemase expression in renal cell carcinomas. Hum Pathol 2006;37:698-703.
35. Evans AJ. α-Methylacyl CoA racemase (P504S): overview and potential uses in diagnostic pathology as applied to prostate needle biopsies. J Clin Pathol 2003;56:892-7.
36. Higgins JP, Kaygusuz G, Wang L, Montgomery K, Mason V, Zhu SX, et al. Placental S100 (S100P) and GATA3: markers for transitional

epithelium and urothelial carcinoma discovered by complementary DNA microarray. Am J Surg Pathol 2007;31: 673-80.

37. Timek DT, Shi J, Liu H, Lin F. Arginase-1, HepPar-1, and Glypican-3 are the most effective panel of markers in distinguishing hepatocellular carcinoma from metastatic tumor on fine-needle aspiration specimens. Am J Clin Pathol 2012;138:203-10.
38. Radwan NA, Ahmed NS. The diagnostic value of arginase-1 immunostaining in differentiating hepatocellular carcinoma from metastatic carcinoma and cholangiocarcinoma as compared to HepPar-1. Diagn Pathol 2012;7: 149.