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CASE REPORT

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Immunoexpression of CDX2 in metastatic nonintestinal adenocarcinomas: an immunohistochemical pitfall and its pathological implications

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Abstract

Background CDX2, a homeobox gene is the marker of intestinal differentiation. Its expression may lead to misdiagnosis while evaluating metastasis from unknown primary. In the present narrative, we discuss the clinical, morphological, and immunohistochemical (IHC) findings of metastatic adenocarcinoma of the lymph node that displayed nuclear immunoexpression of CDX2. However, the clinical and radiological picture supported the non-intestinal primary; prostate in one and lung in the other.

Case presentation A 68-year-old man presented cervical lymph node enlargement. An epithelial tumor with acinar and cribriform pattern was seen that showed expression of CK, CDX2, and PSA in IHC. The patients complained of nonspecific symptoms related to both the gastrointestinal system and the prostate. Serum PSA was diagnostic (> 500 ng/ml). Similarly, core biopsy from mediastinal lymph node from a 51-year-male was received with possible differential of cancer and tuberculosis. Moderately differentiated adenocarcinoma was observed with the expression of EMA, CK 7, CDX2, and TTF1. The expression of both CDX2 and TTF1 was patchy. When the patient was called and all the details were sought the computed tomography (CT) thorax showed a lower chest wall lesion and multiple metastasis. The case was hence signed off as primary from lung on basis of clinical picture.

Conclusion Such deviants must be reported and recorded. The knowledge of these will make a pathologist cautious and thus avoid misdiagnosis.

Keywords CDX2, Immunohistochemistry, Metastasis, Unknown primary, Adenocarcinoma

Introduction

Carcinoma of unknown primary (CUP) is a term coined for a condition in which there is the presence of metastatic disease without clinical symptom related to the site of origin after proper imaging workup. A situation like this may arise for a reporting pathologist when the patient ignores minor symptoms of the primary and does not cite them. Secondly, when the requisition form is provided with incomplete information. After the tumor is identified at the metastatic site by cytology or histology, a further immunohistochemical (IHC) study is recommended to determine the primary site. (Varadhachary 2007) It must be complimented with clinical and radiological work-up as well. Morphology can be decisive in some tumors such as papillary thyroid carcinoma, renal cell carcinomas, plasmacytoma where IHC confirms the site of origin through specific markers such as

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Thyroglobin, Cluster of differentiation (CD) 10, CD138, etc. (Varadhachary 2007; Bayrak et al. 2012) This might not be the case where just the type of tumor with no identifiable characteristic features of primary (like adenocarcinomas, high-grade tumor, etc.,) is seen.

Adenocarcinomas are known to metastasize to distant lymph nodes. In these cases, to identify the primary site of the malignancy, the role of morphology is limited. IHC plays an important role in this scenario and helps the pathologist narrow the site of origin. One such popular panel is Cytokeratin (CK) 7 and CK20. (Bayrak et al. 2012; Selves et al. 2018) Their expression is divided into four major categories and narrows down the possible site of origin. Further specific antibodies like CK19 for pancreaticobiliary, caudal-related homeobox transcription factor (CDX) 2 for gastrointestinal tract, Mammaglobin and gross cystic dilatory fluid protein (GCDFP) 15 for breast cancer, paired box (PAX) 8 for female genital tract, Thyroid transcription factor (TTF)1 for lung and thyroid may be used to confirm. (Varadhachary 2007).

CDX 2 is a homeobox gene and limited transcription factor in the intestine. CDX2 is an important factor in the development of precancerous lesions such as Barrett's esophagus or intestinal metaplasia in the stomach and as a tumor suppressor in colorectal cancers. It is one of the most sensitive and specific IHC markers which is used for identification for intestinal origin of tumors at metastatic sites. (Werling et al. 2003a) It has been reported to be down-regulated in cases of colorectal carcinomas, indicating a poor prognosis. However, even in tumors of unknown origin, its expression serves as a biomarker for intestinal differentiation. A marker of such specificity when expressed in cancers may lead to a diagnostic dilemma.

Here we present two such cases of lymph node metastasis from unknown primary where in case one, the minor symptom related to primary was ignored and never mentioned unless the metastatic deposit was detected and in the case two, all the relevant clinical details were not provided. Both cases were morphologically adenocarcinoma and displayed nuclear immunoexpression of CDX2. However, further studies after morphology and immunohistochemical diagnosis revealed that the primary site was the prostate in one and the lung in the other. Knowledge of this expression is important to be known to a pathologist who suggests the site of primary and to consider possibility of enteric pulmonary adenocarcinoma as well. The above cases highlight, ancillary techniques are only aids to the diagnosis; not diagnostic. One must look at the entire clinical picture before making final comments, 'it's the patient as a whole that we are diagnosing', not the tissue sample.

Case report

Case 1

A 68-year-old man came to the outpatient department of hematology with left cervical swelling for 6 months that was progressively increasing in size along with backache. The patient was anemic and a provisional diagnosis of probable tuberculosis with differential lymphoma was made.

Investigations

An excision biopsy was performed for histology and confirmation, simultaneously chest radiograph and computed tomography (CT) head and neck was ordered. The head and neck CT revealed a large heterogeneous enhancement nodular lesion in the left supraclavicular region and neck along with enlarged level I, II cervical lymph nodes. On CT the thorax, no significant lymphadenopathy or organomegaly was observed.

A globular enlarged lymph node measuring 2×3x2.5 cm was received; the outer surface was smooth and the cut surface showed grey and white areas. Hematoxylin and eosin-stained sections showed partially capsulated lymph node with effaced architecture. The underlying stroma showed near-complete replacement by malignant tumor cells arranged in a cribriform glandular pattern [Fig. 1a] with areas of necrosis. The individual tumor cells were medium to large in size with vesicular chromatin, prominent nucleoli, and eosinophilic cytoplasm. Provisional diagnosis of metastatic adenocarcinoma was made and as the patient had no specific complaints relating to primary site; it was thought to be either lung or gastrointestinal tract (GIT) so CDX2 and TTF1 were selected, apart from pan CK. As the patient was an elderly male, prostatic specific antigen (PSA) was added. In view of cribriform arrangement in some areas CD117 to rule out adenoid cystic metastasizing from minor salivary glands of paranasal sinuses was included.

The tumor cells were negative for TTF1, CD117 and displayed expression for CK [Fig. 1b], PSA [Fig. 1d] and CDX2 [Fig. 1c]. Both CDX2 and PSA were patchy and moderate. This led to an impasse, and the patient was called with all the investigations and was discussed with the treating physician as well. The patient complained of symptoms related to both systems namely, altered bowel habits, frequent urination, sense of incomplete bladder emptying.

Serum PSA was ordered to rule out prostate. To our surprise the serum PSA levels were > 500 ng/ml.

Diagnosis

Thus, in view of PSA serology and PSA immunoexpression the case was reported as metastatic prostatic

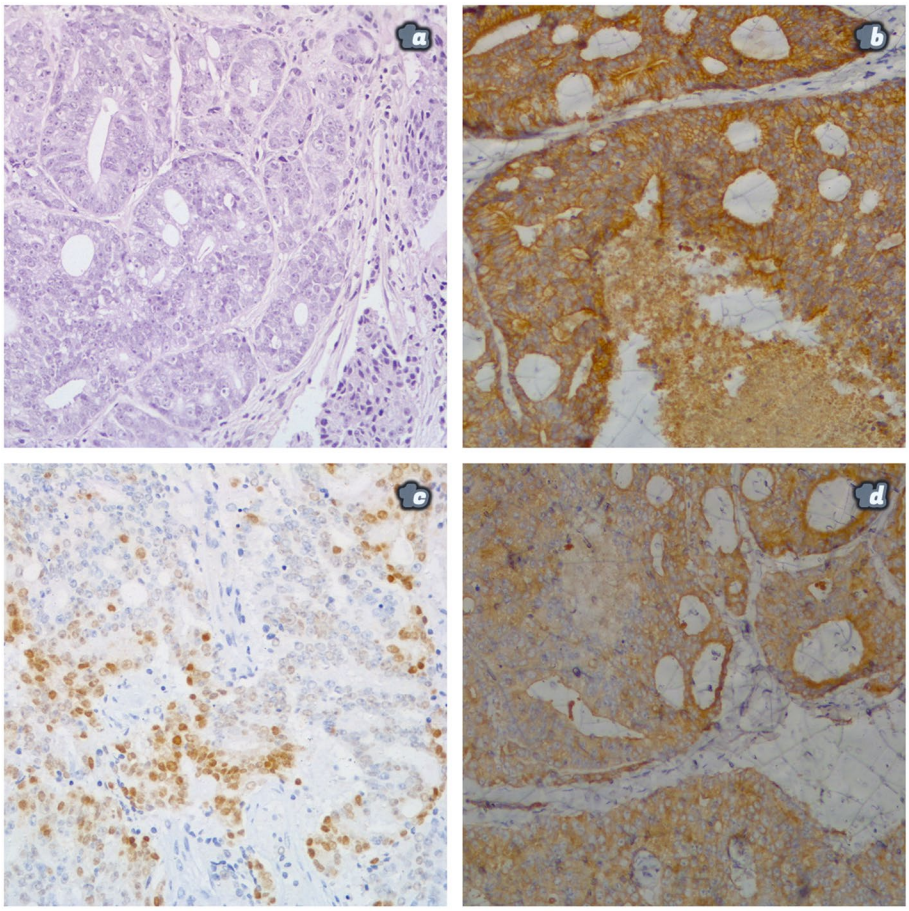

Fig. 1 Histomorphology image of Case 1 shows tumor cells disposed in a cribriform pattern with prominent nucleoli and scant cytoplasm in 1a (H&E × 200). Immunohistochemistry showed immunoexpression for CK in 1b, CDX2 in 1c and PSA in 1d (IHC × 200)

adenocarcinoma to cervical lymph node with aberrant CDX2 expression.

Treatment and follow- up

The patient was referred to the Urology Department for further study, where the prostate biopsy established adenocarcinoma with score 4 + 4. The patient opted for androgen deprivation therapy and is currently apparently asymptomatic with reduction in nodal size.

Case 2

A 51-year-old male presented with complains of mild pain in chest and few episodes of fever. There was history of tobacco chewing for 10 years.

Investigations

Core biopsy from mediastinal lymph node was performed and submitted to the pathology department with two differentials mentioned on the requisition form (? Tuberculosis/ carcinoma).

The biopsy revealed tumor tissue disposed in sheets, nests, and poorly formed glands [Fig. 2a]. Individual tumor cells were highly pleomorphic with vesicular chromatin, prominent nucleoli, and micro vacuolated cytoplasm. Brisk mitosis and necrosis were noted. To confirm the diagnosis and origin of the primary, IHC panel was ordered. Pan CK, CK7, CK 20, CDX2, TTF1, and p63. Tumor cells displayed pan cytokeratin expression [Fig. 2b] with CK7. Both CDX2 [Fig. 2c] and TTF1 [Fig. 2d] was patchy. P63, CK20 were negative.

When the results of immunohistochemistry overlapped, the patient was called with all the clinical and radiological details. To our surprise, he carried a CT thorax and abdomen which showed a large, relatively well defined homogeneous soft tissue enhancing lesion involving the left lingual segment with bilateral and pulmonary adrenal metastases, deposits in the abdominal wall, and mediastinal lymphadenopathy. The attendant also gave a history of swelling on the right lower chest wall for 1 month, which gradually increased in size.

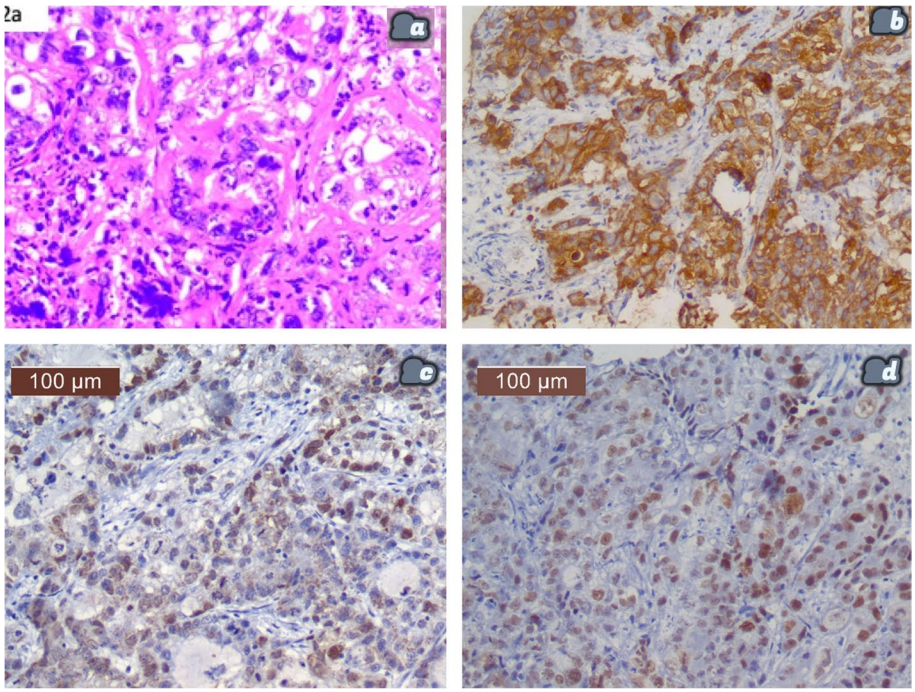

Fig. 2 The histomorphology image of Case 2 shows an irregular acinar pattern of tumor cells with round pleomorphic nuclei, prominent nucleoli, and a scant pale cytoplasm in 2a (H&E × 200). Immunohistochemistry showed immunoexpression for panCK in 2b (IHC × 100), CDX2 in 2c and TTF1 in 2d (IHC × 200)

Clinically, the patient had been completely worked up and the primary tumor was identified as in the lung.

Diagnosis

Given the radiological lung mass arising from the lingual lobe and no evidence of any GIT complaints or lesion, diffuse expression of CK7 and TTF1 in some areas. The diagnosis was signed as metastatic adenocarcinoma of the primary lung with a expression of CDX2.

Treatment and follow-up

The patient was referred to medical oncology where chemotherapy was administered and additional lung epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), and ROS were requested. However, the patient left against medical advice and did not respond to follow- up telephonic calls.

Discussion

The above cases are examples of expression of CDX2 in tumors arising from two different sites, namely lung and prostate. Complete clinical workup and other markers helped to achieve the correct diagnosis. Serum PSA was valuable in the first case. In the second case, the expression of CK7, patchy TTF1, and complete clinical picture were the decisive elements.

Through search of published English literature, we found mention of both prostate and lung cancers displaying CDX2 expression, along with urothelial, endometrial, and hepatocellular cancers as well. (Ortiz-Rey et al. 2004; D'Antonio 2011; Riedt et al. 2009; Shah et al. 2017; Yatabe et al. 2004) Surprisingly, Riedt T et al. in 2009 have also reported CDX2 expression in abnormal lymphoid cells of acute lymphoblastic leukemia. (Riedt et al. 2009) It has been reported that in cancers with neuroendocrine differentiation from the gastrointestinal or other sites (like urinary bladder, uterus, breast, prostate lung, salivary gland) this marker can also show low expression. (Barbareschi et al. 2004) Urinary bladder adenocarcinomas also show to some extent the expression of CDX2 due to their derivation from intestinal urachus. To differentiate these tumors from colonic adenocarcinomas beta catenin, CK7, and thrombomodulin help. Colon cancers likely display beta -catenin expression with negativity for CK7 and thrombomodulin. (Wang et al. 2001).

Subsets of ovarian carcinomas and lung carcinomas with intestinal differentiation or with mucinous features may also express CDX2. (Rossi et al. 2004; Werling et al. 2003b) The former may be confirmed due to its expression of CK7 and later by TTF1 expression. However, in our case the biopsy did not display any features suggestive of mucinous differentiation, but as

it was a small core needle biopsy the complete nature of the tumor cannot be commented on. Therefore, the present case highlights that one should be aware that lung cancers may also express CDX2.

Variable percentages of cervical adenocarcinomas may also express CDX2 the morphology being consistent with either mucinous differentiation or even endometroid one. (Sullivan et al. 2008).

Focal expression of CDX2 has also been reported in the prostate and thyroid. Immunoexpression of villin may be of help in this scenario apart from their specific antibodies like PSA and thyroglobin. (Sullivan et al. 2008) However, CDX2 is consistently seen to not immunostain liver, hepatocellular carcinoma, or carcinomas of the kidney, breast, lung, or salivary gland. (Hornick et al. 2022) The specificity of CDX2 in the determination of primary must be enhanced by using CK7 and 20 and should be interpreted with caution. (Tot 2004).

Herawi M et al., in 2007 after finding incidental expression of CDX2 in two cases of prostatic biopsy, carried out a large microarray-based study where they included 195 samples of benign prostatic tissue and 185 samples of prostate cancer. They observed 5.7% and 11.7% of both malignant and benign prostate tissue displaying CDX2 expression. They clearly mentioned that the solid cribriform ductal architecture of prostate cancer with CDX2 expression can be easily mistaken for colonic metastasis in the case of prostatic biopsy. Hence, they recommended serum PSA and PSA immunohistochemistry in these cases well. (Herawi et al. 2007) Our case highlights the same confusion may occur in metastatic sites as well, hence inclusion of serum PSA and PSA IHC in metastatic work-up of elderly male must be performed irrespective of presence or absence of prostate related symptoms.

Another interesting finding in the case one was that there was metastasis from prostatic adenocarcinoma to the cervical lymph nodes. Prostate cancer is the most common cancer in men, often presenting with regional lymph node or bone metastasis and rarely supradiaphragmatic or distant lymph node metastasis (Sepúlveda et al. 2015). Despite the high incidence and prevalence of prostate cancer, there are less than 50 case reports of metastatic involvement of the cervical lymph nodes and only one case presenting in men younger than 45 years. The reported incidence varies between 0.28 and 0.4%. (Sepúlveda et al. 2015; Copeland et al. 2001) Batson postulated that head and neck metastases of prostate cancer occur due to hematogenous spread through the vertebral venous system (or Batson's plexus).

Learning point

The present cases not only highlight expression of CDX2 in metastatic adenocarcinoma from two different sites but also emphasize the fact that a complete clinical picture must be sought and one must not totally rely on ancillary tests in decision making. Secondly knowledge of expression of the known markers makes the diagnostic pathologist cognizant and avoids confusions.

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Conceptualization Preeti Agarwal. Methodology Krachi Agarwal, Shipra Singh and Preeti Agarwal. Software Krachi Agarwal, Shipra Singh and Preeti Agarwal. ValidationKrachi Agarwal, Shipra Singh and Preeti Agarwal. Formal analysis Krachi Agarwal, Shipra Singh and Preeti Agarwal. Investigation Mili Jain and Sumiara Qayoom. Resources Mili Jain and Sumiara Qayoom. Data Curation Mili Jain and Sumiara Qayoom. Writing—Original DraftKrachi Agarwal and Preeti Agarwal. Writing—Review & Editing Preeti Agarwal. Visualization Preeti Agarwal. Supervision Preeti Agarwal. Project administrationPreeti Agarwal. Funding acquisition None. The author(s) read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
Informed consent of the both patients were taken.

Consent for publication
Consent for publication was taken from both patients.

Competing interests
Dr. Preeti Agarwal and Dr. Krachi Agarwal have equal contribution to the above work.

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