Int J Clin Exp Pathol 2013;6(3):498-502
www.ijcep.com /ISSN:1936-2625/IJCEP1212024

Original Article

Metastatic seminomas in lymph nodes: CD10 immunoreactivity can be a pitfall of differential diagnosis

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Received December 16, 2012; Accepted January 18, 2013; Epub February 15, 2013; Published March 1, 2013

Abstract: Metastatic seminoma can potentially be confused with lymphoma in a lymph node biopsy. Here, we report a case in which the immunohistochemistry of CD10 was a pitfall in the differential diagnosis of a metastatic seminoma, and further present a brief study of CD10 expression in a seminoma series. A 67-year-old man, who had a history of lobectomy of the lung due to squamous cell carcinoma 2 years prior, showed lymphadenopathy of the neck and the paraaorta on follow-up study by fluorodeoxyglucose-positron emission computer tomography scan. The biopsy of the cervical node demonstrated infiltration of large atypical cells. The results of the screening immunohistochemistry were CD20(-), CD3(-), CD10(+), CD30(-), AE1/AE3(-), and placental alkaline phosphatase(-), providing the impression of CD10-positive lymphoma. However, the following studies revealed germ cell characteristics [OCT3/4(+), SALL4(+), and CLDN6(+)], confirming the diagnosis of seminoma. We further evaluated CD10 expression in a series of seminomas (n=16). Strong positivity was observed in 14 cases; partial and weak positivity, in 2 cases. These findings should be considered in the differential diagnosis of seminoma.

Keywords: Testicular seminoma, CD10, PLAP, OCT3/4

Introduction

Pathological examination of lymph nodes provides a decisive diagnosis in most cases of occult cancer. However, unusual presentation or complicated histories can sometimes blur the diagnostic processes, and the subsequent immunohistochemical studies might confound the decision when unexpected immunostaining results are obtained. In the present paper, we report such a case of metastatic seminoma with positive immunostaining for CD10, a representative marker for germinal-center B cells.

The diagnosis of seminoma that has metastasized to the lymph nodes is difficult by radiological imaging, including computer tomography (CT) and fluorodeoxyglucose-positron emission CT (FDG-PET CT), in patients without a clinical suspicion of primary tumors [1, 2]. Furthermore, in the histological examination of a lymph node biopsy, seminoma may be confused with lymphoma, especially in the cases of massive necrosis or a tiny fragment of tumor tissue [3]. Immunohistochemistry can be used to confirm the diagnosis of seminoma, using specific markers such as placental alkaline phosphatase (PLAP), when a possibility of seminoma is taken into consideration for the differential diagnosis. However, since rare exceptions occur, unexpected results can confound the diagnostic process. In the present paper, we report our experience of an initial confusion on the PLAP-negative and CD10-positive results for metastatic seminoma in a lymph node biopsy.

Case report

A 67-year-old man was admitted to the hospital for a follow up survey of a lung lobectomy, which had been performed for squamous cell carcinoma 2 years previously. FDG-PET CT scan demonstrated positive signals in the neck and para-aortic lymph nodes. The right cervical

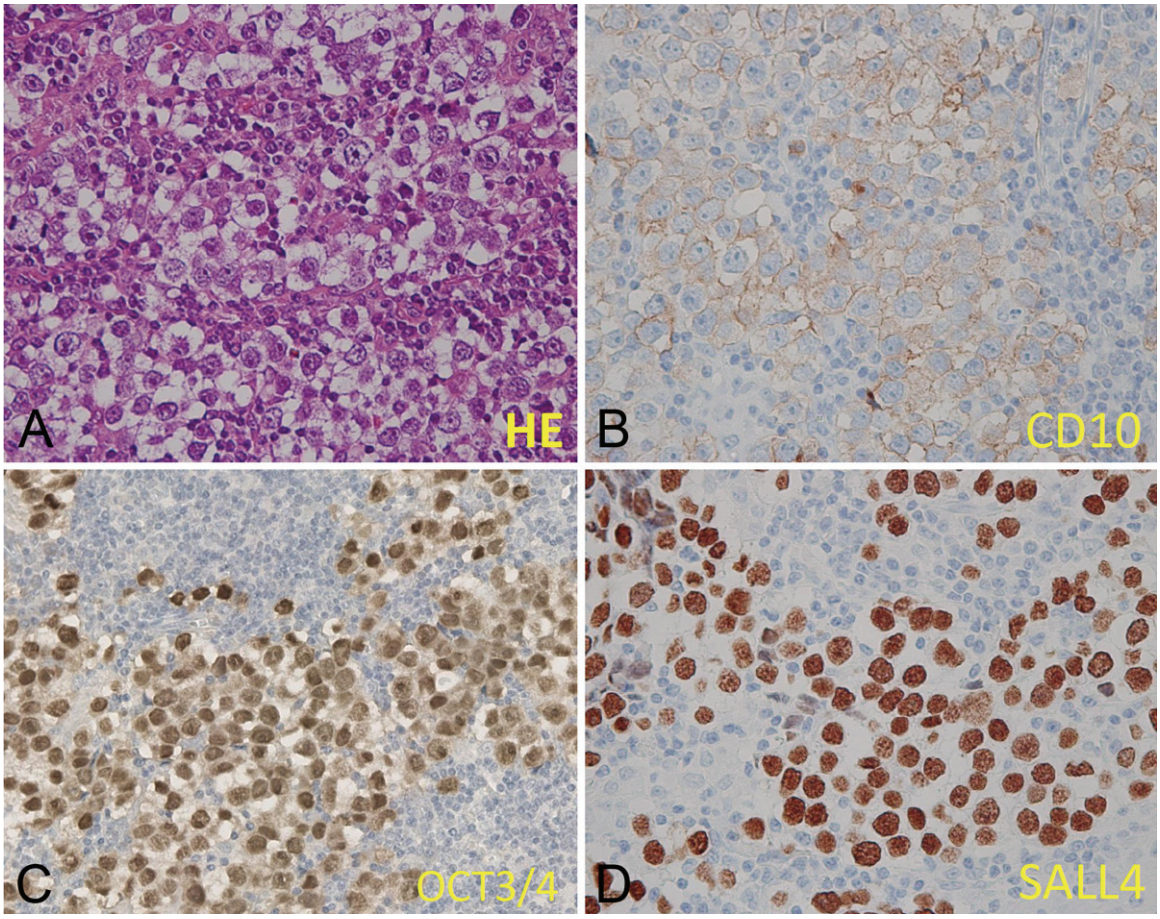

Figure 1. Histopathological and immunohistochemical features of infiltrating neoplastic cells in the lymph node. A: Round cells containing clear or lightly eosinophilic and granular cytoplasms were admixed with small lymphocytes. B: Neoplastic cells with a puzzling immunophenotype [AE1/AE3(-), PLAP(-), and LCA(-), CD3(-), CD20 (-)] showed a membranous staining pattern for CD10. These cells were also positive for (C) OCT3/4 and (D) SALL4.

lymph node, which was swollen to about 1.5cm in diameter, was biopsied. Normal lymph node architecture was histologically preserved in most parts of the lymph node, but large tumor cells were found to be proliferating in a sheet. The tumor cells were round with clear or lightly eosinophilic and granular cytoplasms (**Figure 1A**). Malignant lymphoma was suspected at a glance, and the immunohistochemical staining showed the puzzling results of AE1/AE3(-), PLAP(-), and LCA(-), CD3(-), CD20(-), with membranous staining of CD10 (**Figure 1B**). Details of the antibodies used are presented in **Table 1**.

After a discussion about the morphology of the tumor cell nuclei, metastatic seminoma was still suspected, and immunohistochemical staining was then performed; OCT3/4 and SALL4 was strongly positive (**Figure 1C** and **1D**), and CLDN6 was weakly positive. We diagnosed this case as seminoma metastasis to the lymph node and recommended that the clinician investigate the testis. After a tumor was demonstrated by ultrasonography, the patient received chemotherapy at another hospital. High orchiectomy was then performed, demonstrating a scar lesion with granulomatous reaction.

Materials and methods

We selected testicular seminoma from the histological files of Toranomon Hospital from 2007 to 2011. Sixteen cases of pure seminoma were retrieved. The age of the patients was 19 to 60 years median, 43 years), and the tumor size was 20 to 107 mm in diameter (median, 62.5mm). (**Table 2**) The tissues from each of the cases were fixed in 10% neutral buffered

Table 1. Details and dilutions of the antibodies inin the present study

Antibody	Clone	Source	Dilution
CD3	PS1	Nichirei biosciences, Tokyo, Japan	1:100
CD10	56C6	DAKO, Glostrup, Denmark	1:50
CD20	L26	DAKO, Glostrup, Denmark	1:100
LCA	PD7/26	DAKO, Glostrup, Denmark	1:100
AE1/AE3	AE1/AE3	DAKO, Glostrup, Denmark	1:100
PLAP	8A9	DAKO, Glostrup, Denmark	1:100
SALL4	6E3	Abnova Corporation, Taiwan, Republic of China	1:100
OCT3/4	C-20	Santa Cruz Biotechnology, Santa Cruz, California, USA	1:500
CLDN6		Immuno-Biological Laboratories, Fujioka, Japan	1:100

Table 2. Clinical and pathological summary of tested testicular seminomas for 16 individual cases and the present case

Case	Age	Diameter (mm)	PLAP*	CD10*	CD10 staining pattern
1	51	36	3+	3+	Membranous
2	34	46	3+	2+	Cytoplasmic
3	35	60	3+	3+	Membranous+Golgi
4	60	60	2+	3+	Membranous+Golgi
5	43	70	3+	3+	Membranous+Golgi
6	40	20	1+	3+	Cytoplasmic
7	50	44	3+	3+	Cytoplasmic+Golgi
8	60	75	3+	3+	Membranous
9	43	70	3+	3+	Membranous
10	23	34	2+	3+	Membranous+Golgi
11	35	83	3+	3+	Cytoplasmic+Golgi
12	47	65	3+	3+	Cytoplasmic+Golgi
13	36	76	3+	3+	Membranous+Golgi
14	19	65	3+	3+	Membranous+Golgi
15	60	107	3+	2+	Membranous+Golgi
16	47	45	3+	3+	Membranous
Present case	67	20#	0	3+	Membranous

*: The staining results were scored as follows: 0 (no detectable staining), 1+ (1–30% positive cells), 2+ (30–70%), and 3+ (71–100%). #: The size of the primary tumor was measured by ultrasonography before chemotherapy.

formalin and embedded in paraffin. Four-micrometer sections were obtained from each sample for further analysis.

Immunohistochemistry

CD10 immunophenotypic studies were performed with immunohistochemistry for PLAP. Immunohistochemical staining was performed on an automated immunostainer Ventana Benchmark GX (Roche Diagnostics, Switzerland). The staining results were scored as follows: 0 (no detectable staining); 1+ (1–30% positive cells); 2+(30–70%); and 3+ (71–100%).

Results

CD10 immunoreactivity was observed in the epithelial cells of the epididymis and the rete testis in the non-neoplastic testis. For the seminoma series, 14 out of 16 cases were strongly positive for CD10, and 2 cases were weakly positive (**Table 2**). CD10 staining was present in the membrane (n=11), Golgi apparatus (n=10), or cytoplasm (n=5) of the neoplastic cells in various combinations (**Figure 2A** and **2B**). Membranous staining without other staining patterns was observed in 4 cases as well as in the patient in this study. Of interest, the seminomas with cytoplasmic staining (20 and 46 mm) were relatively small in size compared to others.

Discussion

The diagnosis of metastatic germ cell tumor in an adult, with or without a history of testicular germ cell tumors, can be challenging. Distinguishing metastatic germ cell tumors

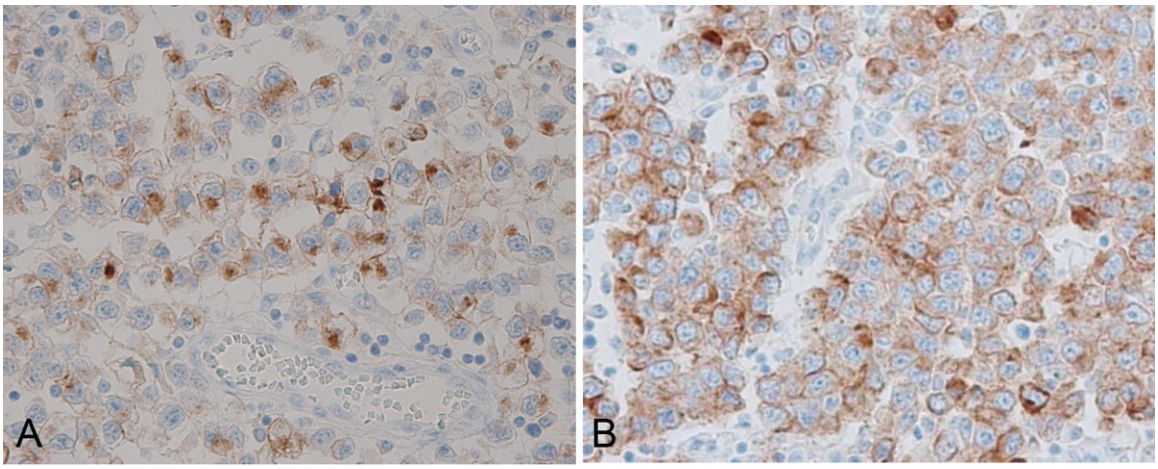

Figure 2. CD10 expression in seminoma. A: Membranous/Golgi positivity for Case 3 in Table 2. B: Cytoplasmic/Golgi positivity for Case 16 in Table 2.

from non-germ cell tumors, especially lymphomas, can be difficult by morphology alone. In the present report, an initial screening of immunohistochemistry included AE1/AE3, PLAP, LCA, CD3, CD20, CD30, and CD10. The unexpected findings were that a metastatic seminoma was PLAP-negative and CD10-positive.

In terms of the diagnosis of seminoma, PLAP is a reliable marker, but several studies have demonstrated that PLAP is neither a specific nor sensitive marker for seminoma [4, 5]. PLAP is also well known to be expressed in lung cancer [4]. The expression of PLAP may decrease in extragonadal or metastatic seminoma compared to primary seminoma [5]. In the present case of the PLAP-negative tumor, we should also consider the possibility of metastatic spermatocytic seminoma [6]. In such a specific situation, more specific markers are useful for the differential diagnosis of germ cell tumors, including OCT4 [7, 8], SALL4 [9], and Claudin 6 (CLDN6) [10]. OCT4 is a marker for seminoma and embryonal carcinoma. SALL4 and CLDN6 are expressed in seminoma, yolk sac tumors, and embryonal carcinoma. Spermatocytic seminoma shows a characteristic expression pattern, that is, OCT4(-), SALL4(+), and CLDN6(-). Since the present tumor was OCT4(+), SALL4(+), and CLDN6(+), we were able to diagnose the tumor as a germ-cell tumor, and more specifically seminoma. Since the testicular tumor had completely burned out and had become a fibrotic scar, we were able to finally diagnose the tumor as a PLAP-negative testicular seminoma metastasis to the lymph node.

CD10, also known as common acute lymphoblastic leukemia antigen (CALLA) or neprilysin, is a zinc-dependent metalloprotease enzyme expressed in a variety of tissues and malignancies. In tumor tissues, CD10 expression has been found in lymphoblastic leukemia/lymphoma and mature lymphoma from a germinal center origin, such as follicular lymphoma and Burkitt lymphoma. In non-hematopoietic tumors, CD10 has been found to be expressed in renal cell carcinoma, glioma, melanoma, colorectal carcinoma [11], and germ cell tumors of the mediastinum [12]. Moreover, CD10 expression has also been reported in endometrial stromal sarcoma. However, only a few studies have reported on its expression in testicular seminoma [13, 14]. Using flow cytometry, CD10 was weakly positive in 2 out of 2 seminoma cases [13]. Moreover, Peiguo Chu et al. reported that CD10 expression was not detected in 14 cases of germ cell tumors; however, the histological details of the germ cell tumors were not described in their study [14]. Thus, we performed a brief study of CD10 expression in testicular seminoma, demonstrating its expression in most of the seminomas. The biological significance of CD10 expression in testicular seminoma is unclear, but CD10 expression is more common in metastatic melanomas than primary melanomas [15]. CD10 is an independent marker that predicts liver metastasis of colon cancers [16]. As such, its expression in the cell membranes of seminoma might be related to the capacity for tumor progression, as illustrated in the present case.

In conclusion, testicular seminoma may metastasize to the lymph nodes and gastrointestinal tract [17]; therefore, knowledge of CD10 expression in tumors is highly important for the practice of diagnostic pathology.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

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