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CD10 is frequently expressed in classical seminomas

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Summary. CD10 is a cell surface metalloproteinase widely expressed in various normal tissue and in epithelial, stromal or both components of various malignancies. The aim of our study was to investigate, for the first time, the expression of CD10 in a series of 135 cases of testicular germ cell tumours in order to asses its possible diagnostic and biologic significance.

The expression of CD10 was studied, using immunohistochemistry, in 96 pure forms and 39 mixed forms of germinal cell tumours of the testis.

Immunostaining for CD10 was positive in 68/74 (92%) seminomas and 16/24 (67%) seminomatous component of mixed germ cell tumours. The intratubular germ cell neoplasia of the unclassified type always expressed CD10. Anaplastic seminomas, embryonal carcinomas, teratoma and spermatocytic seminomas were negative for CD10.

Our findings indicate that only seminomas and intratubular germ cell neoplasia, the precursors of germ cell tumours, express CD10, but when they differentiate along embryonal, somatic, trophoblastic, yolk sac lines they lose CD10 expression.

CD10 could be considered a useful marker to differentiate seminoma from other forms of testicular germ cell tumours and for a better estimation of the seminomatous component in mixed germ cell tumours.

Key words: CD10, Seminoma, IGCNU

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Introduction

CD10 is a 90-to 110-kDa membrane metallo-endopeptidase and is a marker of early lymphoid progenitor and normal germinal center cells. It also reacts with some nonhematopoietic cells and in particular against epithelium protein of the renal proximal tubule. Consequently, it is considered a reliable marker of renal cell carcinomas. CD10 also stains other tumours such as endometrial stromal sarcomas, transitional cell carcinomas, prostatic carcinomas, schwannomas, melanomas, rhabdomyosarcomas, leiomyosarcomas, hemangiopericytomas, solitary fibrous tumours, hepatocellular carcinomas, solid-pseudopapillary tumours of the pancreas, tricho-blastomas, atypical fibroxanthomas (Chu and Arber, 2000).

Germ cells tumours (GCTs), which represent more than 90% of all testicular neoplasms, can be divided clinically, epidemiologically and histologically into 3 main categories: infantile/prepuberal (teratoma and yolk sac tumour), postpuberal (classic seminoma and nonseminomatous germ cell tumours) and spermatocytic seminoma.

To the best of our knowledge, only one study (Brox et al., 1986) documented sporadic CD10 expression in a mediastinal mixed germ cell tumour (GCT) composed 90% of the endodermal sinus tumour and 10% seminoma but it did not report whether CD10 immunostained both histological components. A successive study (Chu and Arber, 2000) documented no CD10 epression in a series of 14 GCTs, about which it specified neither histotype nor the site of involvement (gonads or extragonadal). A recent paper (Ota et al., 2013) investigates CD10 expression only in a small series of seminomatous GCTs. These data prompted us to investigate for the first time CD10 expression in a series of seminomatous and nonseminomatous GCTs of

the testis in order to evaluate the possible diagnostic and biologic significance of this finding.

Materials and methods

We included in this study 135 consecutive cases of testicular GCTs (96 pure forms and 39 mixed forms) observed between 2004 and 2012 in adult males in our institutions. The histopathological features of tumours studied are summarized in Table 1. All cases analyzed were orchiectomy specimens. Hematoxylin and eosin stained sections were retrieved to identify the representative paraffin blocks for immunohistochemical analysis. For each case most representative sections including intratubular germ cell neoplasia of the unclassified type (IGCNU) were chosen. For mixed GCTs only the sections showing all the histological elements were considered for immunohistochemical analysis. All GCTs were reevaluated for the following histopathologic features: mitotic figures for 10 high power field (HPF), necrosis and atypia. Atypia was defined, according to Tickoo et al. (2002) as moderate to marked nuclear pleomorphism, nuclear overlapping, lack or sparseness of tumour lymphocytic infiltration and lack or paucity of cytoplasmic clarity.

The immunohistochemistry was performed on 3-4 μm sections using a mouse monoclonal antibody against CD10 (clone 56C6; Leica Microsystem, Newcastle, Novocastra, ready to use). The primary antibody was detected using a biotin-free polymeric-horseradish peroxidase (HRP)-linker antibody conjugate system (Bond Polymer Refine Detection, Vision BioSystems Ltd, Aus) with a heat-induced epitope retrieval, conducted with the Bond Max automated immunostainer (Vision BioSystems Ltd, Aus).

Three CD10 staining patterns were observed in neoplastic cells: cytoplasmic, membranous and perinuclear Golgi zone pattern. Only complete cell membrane and/or diffuse cytoplasmic staining were considered. The intensity of immunostaining was graded as no/weak staining, moderate or strong . The tumours were considered positive if at least 10% of neoplastic cells exhibited moderate or strong expression of CD10. To identify IGCNU we used immunohistochemical staining for the stem cell factor receptor OCT3/4 (clone N1NK, Leica Microsystem, Newcastle, Novocastra, ready to use) which shows nuclear reactivity.

Results

Table 2 shows the results of immunohistochemical staining for CD10. Sixty-eight (92%) seminomas had moderate/strong CD10 expression (Fig. 1A,B). CD10 reactivity was frequently cytoplasmic associated with membranous staining in 35 cases, with membranous and perinuclear staining in 22 cases, and with perinuclear staining in 6 cases. The immunostaining was only cytoplasmic in 5 cases and membranous with an associated perinuclear staining in 2 cases. Two (3%) seminomas had low staining for CD10 and 4 (5%) cases were completely negative. The anaplastic seminomas showed no CD10 expression (Fig. 2). All the pure forms of nonseminomatous GCTs were negative for CD10.

In the teratoma, CD10 immunostained the apical surface of mature enteric-type epithelium.

The seminomatous component in the mixed GCTs was positive for CD10 in 16/24 (67%) cases (Fig. 1C). The yolk sac component present in 15 mixed GCTs intensely expressed CD10 in 2 cases (13%). The other histological components in the mixed forms were negative for CD10.

CD10 expression was found in all cases of IGCNU (Fig. 1D) often adjacent to GCTs. This aspect was confirmed by the nuclear expression by the same cells of the stem cell factor receptor OCT3/4

The syncytiotrophoblastic cells, when present, were immunoreactive for CD10. In normal testicular parenchyma, CD10 immunostained the luminal border

Table 1. Histopathological features of cases analyzed.

Tumour	Vascular/lymphatic invasion		Tunical invasion		Necrosis		N
	Present	Absent	Present	Absent	Present	Absent	135
Seminoma	15 (20)	59 (80)	6 (8)	68 (92)	17 (23)	57 (77)	74
Anaplastic seminoma	2 (33)	4 (67)	0	6 (100)	4 (67)	2 (33)	6
Spermatocytic seminoma	0	3 (100)	0	3 (100)	0	3 (100)	3
Embryonal carcinoma	4 (40)	6 (60)	1 (10)	9 (90)	4 (40)	6 (60)	10
Yolk sac tumour	0	2 (100)	0	2 (100)	0	2 (100)	2
Teratoma	0	1 (100)	0	1 (100)	0	1 (100)	1
Mixed germ cell tumours							
Seminoma and embryonal	6 (60)	4 (40)	2 (20)	8 (80)	3 (30)	7 (70)	10
Seminoma, embryonal, and other types	2 (25)	6 (75)	1 (13)	7 (87)	0	8 (100)	8
Embryonal and other types	8 (57)	6 (43)	1 (7)	13 (93)	4 (29)	8 (57)	14
Seminoma and other types	2 (33)	4 (67)	2 (33)	4 (67)	1 (17)	5 (83)	6
Other types	0	1 (100)	0	1 (100)	0	1 (100)	1

Table 2. Results of immunohistochemical staining for CD10.

Tumour	Intensity of CD10 staining			
	No/Weak	Moderate	Strong	N=135
Seminoma	6 (8)	14 (19)	54 (73)	74
Anaplastic seminoma	6 (100)			6
Spermatocytic seminoma	3 (100)			3
Embryonal carcinoma	10 (100)			10
Yolk sac tumour	2 (100)			2
Teratoma	1 (100)			1
Mixed germ cell tumours				39
Seminomatous component	8 (33)	2 (8)	14 (59)	24
Embryonal component	32 (100)			32
Yolk sac component	13 (87)		2 (13)	15
Choriocarcinoma component	3 (100)			3
Polyembrioma	1 (100)			1
Mature-immature teratoma	21 (100)			21
Rabdomiosarcoma	1 (100)			1

of epithelial cells, of epididymis and rete testis.

Discussion

The etiology of testicular cancer is still very poorly understood. It has been demonstrated that all adult GCTs are derived from a common precursor recognized as "intratubular germ cell neoplasia of the unclassified type" (IGCNU) and there is a close histogenetic link between seminoma and nonseminamatous germ cell tumours (Srigley et al., 1988). The underlying molecular mechanism of germ cell transformation to IGCNU or fully developed seminoma is poorly understood. Recently, studies have been performed to identify new diagnostic markers and to investigate the origin, as well as genes, characterizing progression of seminoma. In fact, OCT3/4, NANOG, SALL4, Glypican 3, SOX2, SOX17 and MAGE-4 are proposed as new immuno-

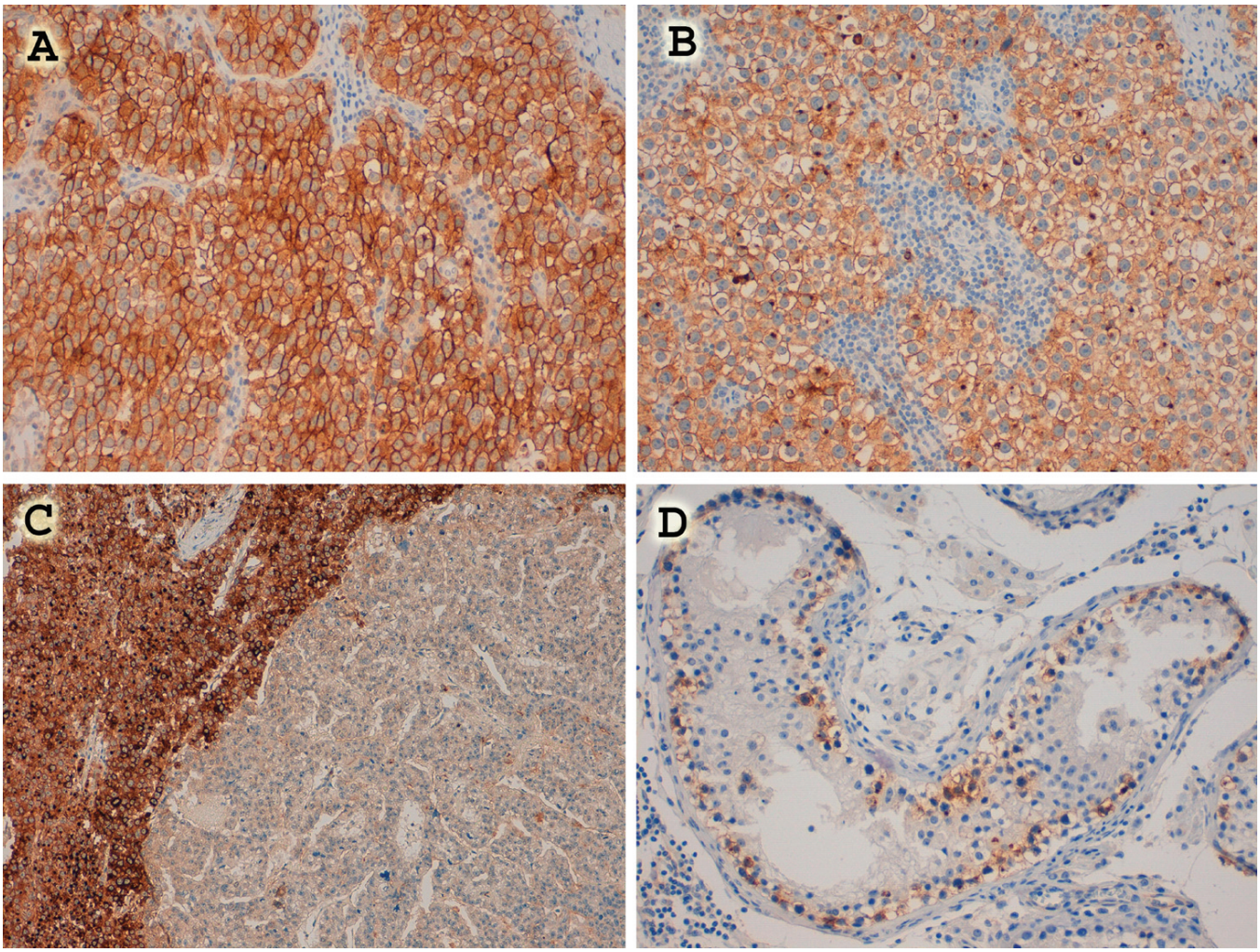

Fig. 1. A, B. CD10 staining pattern in seminomatous cells: in **(A)** cytoplasmic associated to membranous staining in **(B)** cytoplasmic associated to perinuclear staining. **C.** Mixed seminoma and embryonal carcinoma. CD10 immunoreactivity in seminoma (left). **D.** Cytoplasmic and membranous immunoreactivy for CD10 in intratubular germ cell neoplasia. A-C, x 200; D, x 100

histochemical markers for invasive and in situ cell neoplasm (Emerson and Ulbright, 2010). Moreover, MCFD2, BOB1 and PROM1 are new germ cell marker genes for seminoma that provide evidence for the origin of seminoma cells from early gonocytes or from spermatogonia (Gashaw et al., 2007).

CD10 is a cell surface zinc metalloendopeptidase expressed on several tumours and associated with their progression. In fact, the cell surface peptidases are involved in the various stages of neoplasia such as tumour initiation, progression and the development of metastatic disease (Nanus, 2003). However, the exact biological significance of CD10 is still largely unknown. Interestingly, CD10 function in cancer varies by tissue type. In fact, CD10 expression in stromal cells is considered to play an important role in tumour invasion, metastasis and recurrence in carcinomas of colorectum, stomach, breast, skin and lung (Gürel et al., 2012). CD10 expression is a favorable prognostic factor in hepatocellular carcinoma (Mondada et al., 2006), bladder cancer (Seiler et al., 2012) and diffuse large B-cell lymphoma (Ohshima et al., 2001), whereas it predicts a poor outcome in cancers of the prostate (Fleischmann et al., 2011), kidney (Langner et al., 2004), breast (Makrestov et al., 2007), endocrine pancreas (Deschamps et al., 2006) and melanoma (Oba et al., 2011).

It has been shown that many neoplasms expressing CD10 antigen arise from tissues that normally express this peptidase. In normal tissue of the male genital tract CD10 positivity has been shown in the apical surface of the prostatic glandular epithelium, in epididymis ducts and in rete testis, but not in seminiferous tubuli (Mclntosh et al., 1999) . Therefore, the cells of the precursor lesion of germ cells tumours express CD10 during malignant transformation. To the best of our knowledge there is no study investigating CD10 expression in GCTs of the testis. Only one study documents CD10 expression in a mediastinal mixed GCT using both immunofluorescence and immuno-peroxidase techniques (Brox et al., 1986), but a successive study in a series of 14 GCTs about which the histotype and site of involvement is unknown, did not document any CD10 expression (Chu and Arber, 2000). In a recent paper Ota et al. (2013) show CD10 expression in 16 seminomas and in one case of seminoma metastasis to the lymph node.

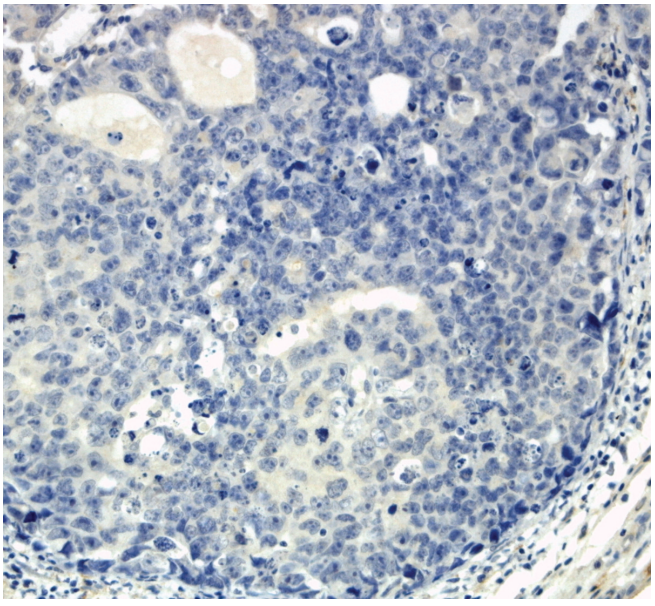

Fig. 2. Negative staining for CD10 in anaplastic seminoma. x 200

In this study we tested for the first time CD10 expression in 135 GCTs of the testis and demonstrate that a very high percentage of seminomas strongly express CD10. In fact we found that 68/74 (92%) seminomas and 16/24 (67%) seminomatous component in mixed GCTs are positive for CD10. On the contrary, CD10 was negative in embryonal carcinoma, teratoma and in almost all yolk sac tumours. These findings suggest that CD10 could be included in panels of immunostains to differentiate seminoma from nonseminomatous GCT. In fact, there are several potential mimickers of seminomas (Ulbright, 2008; Ye and Ulbright, 2012) such as solid embryonal carcinoma, solid pattern yolk sac tumour, Sertoli cell tumour and anaplastic spermatocytic seminoma. Furthermore, in mixed GCTs CD10 could help to identify the seminomatous component and in the estimation of its relative amount. Moreover, despite the recent markers proposed in differential diagnosis of GCTs, such as OCT3/4, SOX2, SOX17 etc, CD10 is an immunomarker easily available in all laboratories considering its large use also in lymphoid neoplasms. The knowledge of CD10 expression in seminomas is very important in routine differential diagnosis particularly on lymph node biopsy of seminoma metastases (Ota et al., 2013).

Another interesting aspect that we observed was the constant expression of CD10 in the cells of IGCNU. The malignant type of these cells was confirmed by their reactivity to OCT3/4. The expression of CD10 in IGCNU confirmed that they share morphologic and molecular characteristics with tumour cells in seminoma and that there is a close histogenetic link between these cells and seminoma. Unlike classical seminomas, all anaplastic seminomas in our study were negative for CD10 immunostaining.

Anaplastic seminoma, also called seminoma with high mitotic index or atypical seminoma (Tickoo et al., 2002), is a new entity described for the first time by Mostofi (Mostofi, 1973) and characterized by cellular pleomorphism, high mitotic activity (an average of 3 or more mitotic figures per HPF), necrosis and paucity of lymphocytic infiltrate. The anaplastic seminoma is a controversial entity for both biological behaviour and histogenesis. In fact, according to some authors (Tickoo et al., 2002) it has a poorer prognosis, a higher incidence

of metastasis, is at a higher stage at clinical presentation and represents (von Hochstetter, 1981) an intermediate tumour between seminoma and embryonal carcinoma. On the contrary, according to Srigley (Srigley et al., 1988), anaplastic seminoma shows no different ultrastructural morphology from classic seminoma and there are no clinicopathological or immunohistochemical parameters to distinguish it from typical seminoma (Suzuki et al., 1993). In our study, the absence of CD10 expression in the cells of anaplastic seminoma supports the hypothesis that it is an intermediate step between seminoma and embryonal carcinoma. Therefore, the absence of CD10 immunoreactivity, together with the presence of the histopathological features mentioned above could help to identify anaplastic seminoma, even if more studies are necessary to validate this observation. Equally, spermatocytic seminoma is negative for CD10. The spermatocytic seminoma according to the new model of GTC histogenesis, based on the tetrahedron model of Srigley et al (Srigley et al., 1988), has a different pathogenesis from seminoma. A series of studies revealed that this tumour does not arise from embryonic/foetal germ cells because it does not express embryonic markers. Rather, it seems to originate from more differentiated, postnatal germ cells, that is, spermatogonia or primary spermatocyte (Looijenga et al., 2006). This different histogenesis could justify the absence of CD10 expression in this neoplasm. The syncytiotrophoblastic cells, in seminoma and in choriocarcinoma component of mixed GCTs, express CD10, as already shown in placenta (Mclntosh et al., 1999).

To summarise, we found that only IGCNU and seminomas express CD10, but nonseminomatous GCTs do not express CD10. Interestingly, it seems that the seminoma and IGCNU, the precursors of GCTs, express CD10 but, according to the histogenetic pathway, when they differentiate along embryonal, somatic , trophoblastic, yolk sac lines they lose CD10 expression. Also, the anaplastic seminoma represents a possible step in the transformation of seminoma towards embryonal carcinoma which does not express CD10.

In conclusion, our findings suggest that CD10 could be routinely used in the panel of immunostaining to differentiate seminoma from other forms of testicular GCTs and for a better estimation of the seminomatous component in mixed tumours. A further possible utility could be to differentiate IGCNU from atypical germ cells found in cryptorchidism, hypofertility or infertility.

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