

# CDX2 immunostaining in primary and metastatic germ cell tumours of the testis

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## Abstract

**Objective:** To evaluate the immunohistochemical staining pattern of caudal type homeobox 2 (CDX2) protein in germ cell tumours (GCTs) of the testis.

**Methods:** This study reassessed archival tissue samples collected from patients diagnosed with primary and metastatic testicular GCTs for CDX2 immunoreactivity using standard immunohistochemical techniques. Positive nuclear immunostaining was evaluated with regard to both the staining intensity and the extent of the staining.

**Results:** Tissue sections from primary and metastatic testicular GCTs ( $n = 104$ ), germ cell neoplasia *in situ* (GCNis) ( $n = 5$ ) and benign testicles ( $n = 15$ ) were analysed. The GCNis and benign testicular tissues showed no immunoreactivity for CDX2. Strong and diffuse staining of CDX2 was demonstrated only in the mature colonic epithelium of teratomas in both primary and metastatic GCTs. CDX2 positivity in other tumours (one pure yolk sac tumour, one yolk sac component of a mixed GCT and one pure seminoma) was infrequent, and was only weak and focal.

**Conclusions:** CDX2 immunostaining should be interpreted based on both the staining intensity and the extent of staining so as not to cause misdiagnosis. Teratomas with colonic-type epithelium should be considered in the differential diagnosis if a metastatic tumour with an unknown primary shows prominent CDX2 immunostaining.

## Keywords

CDX2, germ cell tumour, immunohistochemistry, teratoma, testis

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## Introduction

The caudal type homeobox 2 (*CDX2*) gene is a homeobox gene that encodes a protein that functions as a nuclear transcriptional factor.<sup>1</sup> It plays a role in regulating the

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development and differentiation of the embryonic intestinal epithelium by activating intestine-specific proteins.<sup>1-3</sup> Among normal adult tissues, CDX2 protein is strongly and diffusely produced in all intestinal epithelial cells, including tissue from the duodenum to the distal rectum, and also in the epithelium of pancreatic small ducts and ductules.<sup>4</sup> Moreover, it has been found in intestinal metaplasia occurring in the stomach, oesophagus, gall bladder, and urinary bladder.<sup>5-8</sup> Due to its relative specificity for the gastrointestinal tract, the use of anti-CDX2 antibodies has become part of the immunohistochemistry panel for carcinomas of unknown primary origin; and it is accepted as being highly sensitive for the identification of colorectal adenocarcinomas.<sup>9,10</sup> However, various studies have demonstrated CDX2 positivity in a small percentage of ovarian mucinous tumours,<sup>11</sup> endometrial lesions,<sup>12</sup> carcinoids<sup>13</sup> and some adenocarcinomas of other sites such as uterine cervix,<sup>14</sup> prostate,<sup>15</sup> and lung.<sup>16</sup>

The large majority of primary testicular tumours originate from germ cells.<sup>17,18</sup> These tumours are classified as seminomas, embryonal carcinomas, yolk sac tumours, trophoblastic tumours and teratomas. As they may comprise confusing histological growth patterns, unusual variants, and mimic other tumours, diagnosing germ cell tumours (GCTs) can be challenging.<sup>19</sup> Their correct identification often has important therapeutic and prognostic implications. Particularly when dealing with metastatic tissues, immunohistochemical studies are needed to resolve these dilemmas. The diagnostic utility of CDX2 in GCTs of the testis has not been well studied. This study examined the immunohistochemical staining pattern of CDX2 in GCTs of the testis, germ cell neoplasia *in situ* (GCNis) and normal seminiferous tubules.

## Patients and methods

### Patient population

This retrospective study was conducted on archival tissue samples collected from patients diagnosed with testicular GCTs between January 2005 and December 2014 in the Department of Surgical Pathology, Uludag University, Bursa, Turkey. Normal seminiferous tubules were collected from the testicular tissues of patients who underwent surgery for benign testicular disorders. All of the cases diagnosed as testicular GCTs were included in the study. The study was approved by the Institutional Review Board and Scientific Research Committee of Uludag University (no. 2015-14). There was no requirement for informed consent for the study.

### Histological examination

Using archival tissue samples from the Department of Pathology, Uludag University, haematoxylin and eosin-stained slides of the cases were reassessed and one representative block was selected for immunohistochemical staining. CDX2 staining was performed on 4 µm-thick sections from formalin-fixed, paraffin-embedded tissues, using the Bond Polymer Refine Detection kit according to the manufacturer's instructions (Leica Microsystems UK, Milton Keynes, UK). Immunostaining was carried out using a fully automated immunohistochemistry staining system (Leica Bond-Max System; Leica Microsystems (UK) Ltd) according to the manufacturer's instructions. Ethylenediaminetetra-acetic acid was used for antigen retrieval by the automated system. The sections were incubated with a mouse monoclonal primary antibody against CDX2 (clone AMT28; Leica Microsystems UK). Colonic adenocarcinoma tissue from colectomy specimens from

the Department of Pathology, Uludag University, was used as a positive control and a negative control consisted of no primary antibody. The immunoreactivity was evaluated using a light microscope (model BX51TF, Olympus, Tokyo, Japan). Only nuclear staining was considered as positive immunostaining. The staining intensity was scored as follows: 0 for no staining; 1+ for weak staining; 2+ for moderate staining; 3+ for intense staining. Four groups were categorized according to the percentage of nuclear staining (0 for <1%, 1+ for 1% to 25%, 2+ for >25% to 50%, 3+ for >50%). A total of 10 fields (at least 500 cells) were counted at x400 magnification for each specimen.

**Results**

This retrospective study reassessed tumour samples from 109 patients diagnosed with testicular GCTs, of which 80 were testicular GCTs, 24 were metastatic testicular GCTs, and five were GCNis. Fifteen subjects with benign testicles with normal seminiferous tubules were also enrolled in the study. The 80 GCTs of the testis and 24 metastatic testicular GCTs found in lymph nodes that were included in the study were classified as follows: 37 were pure seminomas, 29 were pure teratomas, two were pure yolk sac tumours, three were pure embryonal carcinomas and 33 were mixed GCTs with various histological components (Table 1).

The most common testicular GCTs were pure seminomas (37 of 104; 35.6%) followed by mixed GCTs (33 of 104; 31.7%). Mixed GCTs had two or more neoplastic components including teratoma, seminoma, yolk sac tumours, embryonal carcinomas and choriocarcinoma (Table 2).

Intense (3+) and diffuse (3+) CDX2 nuclear positivity was identified in all teratomas that had mature colonic-type

**Table 1.** Distribution of the testicular germ cell tumours evaluated in the study (n = 104).

Tumour type	Testicular tumours n = 80	Metastatic tumours n = 24
Seminoma	36	1
Teratoma	9	20
Yolk sac tumour	2	–
Embryonal carcinoma	3	–
Mixed germ cell tumour	30	3

Data presented as n of tumours.

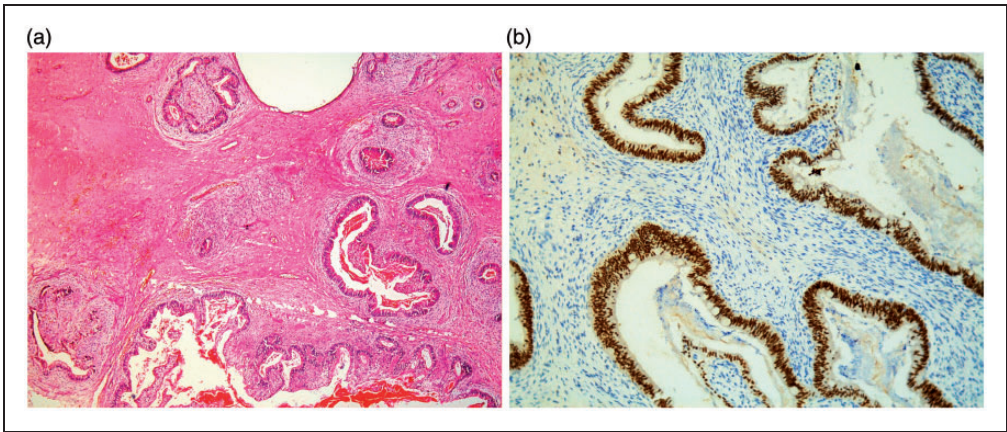
**Table 2.** Histological components of testicular mixed germ cell tumours (n = 33).

Histological components <sup>a</sup>	Testicular tumours n = 30	Metastatic tumours n = 3
Teratoma	21	2
Embryonal carcinoma	21	2
Yolk sac tumour	17	3
Seminoma	13	–
Choriocarcinoma	8	–

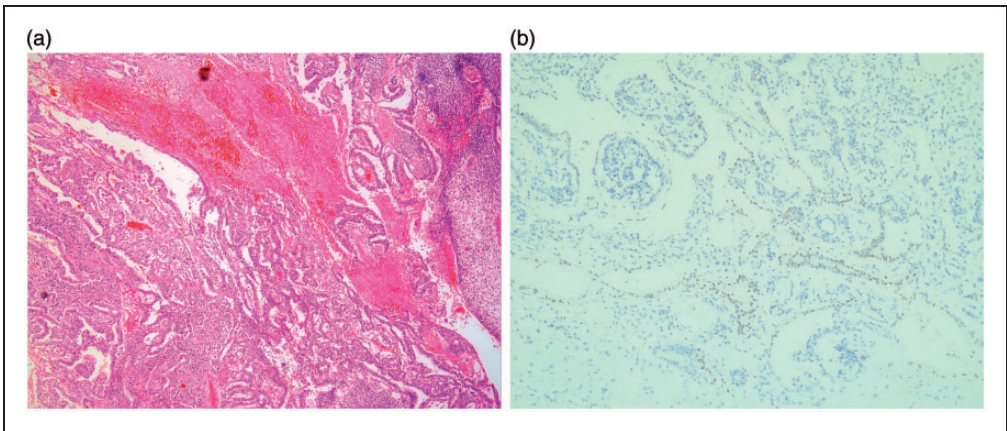
Data presented as n of tumours.

<sup>a</sup>Mixed germ cell tumours can present with two or more neoplastic components.

epithelium, which was 11 of 30 cases of testicular GCTs with teratoma (including mixed GCTs with teratoma) and 16 of 22 cases of metastatic GCTs with teratoma (including mixed GCTs with teratoma) (Figure 1). Except for the teratomas, CDX2 immunoreactivity was detected only in one pure yolk sac tumour (Figure 2), one yolk sac component of a mixed GCT and one pure seminoma, with all three tumours showing weak (1+) and focal (1+) staining. No CDX2 immunostaining was observed in the other components of mixed GCTs including embryonal carcinomas and choriocarcinoma. Similarly, GCNis and



**Figure 1.** Representative photomicrographs showing a section of testicular teratoma including glands with colonic-type epithelium: (a) haematoxylin and eosin staining; (b) showing strong and diffuse immunostaining for caudal type homeobox 2 (CDX2) protein. The colour version of this figure is available at: <http://imr.sagepub.com>. Scale bar 100  $\mu$ m.



**Figure 2.** Representative photomicrographs showing a section of testicular yolk sac tumour: (a) haematoxylin and eosin staining; (b) showing weak and focal immunostaining for caudal type homeobox 2 (CDX2) protein. The colour version of this figure is available at: <http://imr.sagepub.com>. Scale bar 100  $\mu$ m.

benign testicular tissues showed no immunoreactivity for CDX2.

## Discussion

Germ cell tumours are the most common malignancy of the testis.<sup>17,20</sup> These tumours demonstrate a wide range of subtypes with

various histopathological patterns.<sup>17,20–22</sup> The most common and most significant challenges in testicular pathology are differential diagnoses between seminoma and nonseminomatous GCT, as well as between GCTs and testicular tumours of non-germ cell type, particularly the sex cord-stromal tumours.<sup>22</sup> In addition, diverse

morphological (architectural and cytological) features cause diagnostic pitfalls when the tumour is initially diagnosed as a metastatic foci without a known primary tumour.<sup>21</sup> An accurate diagnosis of testicular GCT is required in order for the physician to be able to offer the most appropriate treatment. Therefore, in most cases, supplementary studies are needed to distinguish GCTs from many other morphologically similar tumours.<sup>21–25</sup> In this context, immunohistochemical studies that identify a number of biomarkers, including alpha-fetoprotein, CD30, glypican 3, KIT, octamer-binding transcription factor 3/4, sal-like protein 4, placental alkaline phosphatase, sex determining region Y-box (SOX) 2 and SOX17 have been useful in the diagnosis of GCTs.<sup>17</sup> In addition, the molecular analysis of chromosomes 12 p, X, 7, 8, 21, Y, 1 p, 11, 13, and 18, are being investigated as potential diagnostic tools.<sup>17,26</sup> These molecular analyses could provide an exact diagnosis, particularly in GCTs derived from GCNis.<sup>26</sup> However, many pathology centres are not equipped to use molecular studies and immunohistochemical studies continue to be easy, affordable and reproducible. Therefore, new immunohistochemical markers are still being investigated to differentiate between these testicular tumours.

The *CDX2* gene is a member of the caudal-related homeobox gene family that is expressed during the normal development of the intestinal tract and is important for hindgut development in humans.<sup>1–3</sup> As expected, it has been shown to be expressed strongly and extensively in colonic epithelial tumours, and it is considered as an effective marker for the identification of colorectal adenocarcinomas.<sup>4,27</sup> Although anti-CDX2 antibodies are included in a panel of antibodies to identify metastatic colonic adenocarcinomas,<sup>4,27</sup> subsequent studies have also demonstrated CDX2 immunoreactivity in adenocarcinomas of the oesophagus, stomach, small intestine, ampulla of Vater,

mucinous-type ovary, intestinal-type sino-nasal cavities, urinary bladder, urachus, prostate and gastrointestinal neuroendocrine neoplasms.<sup>4,11,23,28–30</sup> Staining in these tumours was less frequent and usually patchy/focal with less intense immunoreactivity than that observed in colonic adenocarcinomas.<sup>4</sup> However, some researchers concluded that the anti-CDX2 antibody is not suitable for distinguishing primary tumours from metastatic colorectal adenocarcinoma.<sup>11,29,30</sup>

There are very limited data on CDX2 immunoreactivity in GCTs, and the studies published so far had small numbers of cases. For example, one study examined 52 malignant GCTs for CDX2 expression.<sup>20</sup> CDX2 positivity was detected in four (100%) out of four cases of teratoma, three (38%) out of eight cases of yolk sac tumour, three (38%) out of eight cases of embryonal carcinomas, two (100%) out of two cases of choriocarcinoma and only one (3%) out of 29 cases of seminoma.<sup>20</sup> As the percentage of positive cases was quite high, the authors concluded that CDX2 expression in a metastatic tumour with an unknown primary should not only point to a colorectal origin, but also testicular GCT.<sup>20</sup> However, in the present study CDX2 positivity was determined in 27 (52%) of 52 tumours with a teratoma component, two (9%) of 22 tumours with a yolk sac tumour component, and one (2%) of 50 tumours with a seminoma component. This present study not only evaluated the tumours located in the testes, but also those at metastatic sites. At both sites, the only strong and diffuse CDX2 positivity was in the mature colonic epithelium of teratomas. The previous study scored positive cases as 2+ and 3+, but the scoring system was not described clearly in the article.<sup>20</sup> This causes confusion when interpreting the extent of the CDX2 positivity. In the present study, because the other positively-stained tumours (two yolk sac tumours and one seminoma) only demonstrated weak and

focal staining, only teratomas with colonic epithelium were considered to have positive immunoreactivity for CDX2. In contrast to the previous study,<sup>20</sup> the findings of the present study suggest that CDX2 positive immunoreactivity is unique for colorectal epithelium. The inconsistency between these present findings and those of the previous study might result from the different interpretations of the immunohistochemical staining patterns.<sup>20</sup>

In a series of 40 cases of testicular GCT and eight cases of metastatic GCT that were evaluated for CDX2 immunoreactivity, none of the tumours showed positivity except the yolk sac tumours (12 of 26) and the teratoma components with mature colonic epithelium (seven of 14).<sup>31</sup> Only the extent of the staining was evaluated in that study.<sup>31</sup> While positive yolk sac tumours were mostly interpreted as 1+ (1% to 25%), the report did not state what the score was for the CDX2-positive teratomas. In the present study, there were also yolk sac tumours (two of 22 tumours) showing positive immunostaining, and similar to the previous study,<sup>31</sup> the staining was focal rather than diffuse. In addition, the present study demonstrated that the staining intensity was weak in both of the yolk sac tumours.

In another study, CDX2 positivity of 26 ovarian and testicular yolk sac tumours was evaluated in association with the pattern of tumour growth.<sup>23</sup> All cases with classical patterns showed positivity only in isolated epithelial cells, and staining was patchy in those displaying a somatic glandular pattern.<sup>23</sup> The authors demonstrated diffuse positivity in only two yolk sac tumours with a somatic glandular pattern.<sup>23</sup> Unfortunately, the intensity of staining was not defined in the study.<sup>23</sup>

In a study of 124 cases, CDX2 positivity was more frequent in glandular yolk sac tumours (83%) than in adenocarcinomas (63%) arising from GCTs.<sup>32</sup> Despite the fact

that the majority of the adenocarcinoma cases (64%) showed no colonic/enteric differentiation morphologically, the rate of CDX2 positivity was quite high, as for the glandular yolk sac tumours.<sup>32</sup> These high rates of CDX2 positivity were striking and may be due to the methodology used to evaluate the staining pattern, as tumours were defined as positive when > 10% of the tumour cells were stained.

Although it was incompatible with the results of the present study, a recent study of 93 testicular GCTs, two primitive neuroectodermal tumours, eight Leydig cell tumours and six Sertoli cell tumours, suggested that CDX2 was a sensitive and relatively specific marker for yolk sac tumours. CDX2 immunostaining was uniformly and strongly positive in the epithelioid cells of all yolk sac tumours ( $n=25$ ). Similar to the findings of the present study, glands with intestinal differentiation in teratomas were also CDX2 positive in 11 of 14 cases.<sup>33</sup>

The findings of most of these studies give rise to the idea that CDX2 positivity in testicular GCTs could be a potential pitfall when examining metastatic tumours of unknown primary origin.<sup>20,23,31,32</sup> Nevertheless, the present study had a higher number of GCT cases and the staining pattern was described in more detail compared with previous studies.<sup>20,23,31,32</sup> Hence, these current findings suggest that tumours should only be considered to have positive CDX2 immunoreactivity if the extent of the staining is diffuse and the intensity is strong.

In conclusion, this present study demonstrated strong and diffuse immunostaining for CDX2, as is observed in colorectal adenocarcinomas, only in the mature colonic epithelium found in teratomas in testicular GCTs. CDX2 positivity in other tumour types was infrequent, and presented as weak and focal staining. These current findings suggest that CDX2 immunoreactivity should be interpreted by considering both the staining intensity and the extent of

staining so as not to cause misdiagnosis. If a metastatic tumour with an unknown primary tumour shows prominent CDX2 immunoreactivity, aside from colorectal adenocarcinoma, a teratoma with colonic-type epithelium should be considered in the differential diagnosis instead of other GCTs.

### Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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### References

1. Drummond F, Putt W, Fox M, et al. Cloning and chromosome assignment of the human CDX2 gene. *Ann Hum Genet* 1997; 61(Pt 5): 393–400.
2. Guo RJ, Suh ER and Lynch JP. The role of Cdx proteins in intestinal development and cancer. *Cancer Biol Ther* 2004; 3: 593–601.
3. Stainier DY. No organ left behind: tales of gut development and evolution. *Science* 2005; 307: 1902–1904.
4. Moskaluk CA, Zhang H, Powell SM, et al. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. *Mod Pathol* 2003; 16: 913–919.
5. Barros R, da Costa LT, Pinto-de-Sousa J, et al. CDX2 autoregulation in human intestinal metaplasia of the stomach: impact on the stability of the phenotype. *Gut* 2011; 60: 290–298.
6. Phillips RW, Frierson HF Jr and Moskaluk CA. Cdx2 as a marker of epithelial intestinal differentiation in the esophagus. *Am J Surg Pathol* 2003; 27: 1442–1447.
7. Sung MT, Lopez-Beltran A, Eble JN, et al. Divergent pathway of intestinal metaplasia and cystitis glandularis of the urinary bladder. *Mod Pathol* 2006; 19: 1395–1401.
8. Pinto R, Barros R, Pereira, Castro I, et al. CDX2 homeoprotein is involved in the regulation of ST6GalNAc-I gene in intestinal metaplasia. *Lab Invest* 2015; 95: 718–727.
9. Bakaris S, Cetinkaya A, Ezberci F, et al. Expression of homeodomain protein CDX2 in colorectal adenoma and adenocarcinoma. *Histol Histopathol* 2008; 23: 1043–1047.
10. Li MK and Folpe AL. CDX-2, a new marker for adenocarcinoma of gastrointestinal origin. *Adv Anat Pathol* 2004; 11: 101–105.
11. Frassetto F, Pelosi G, Cafici A, et al. CDX2 immunoreactivity in primary and metastatic ovarian mucinous tumours. *Virchows Arch* 2003; 443: 782–786.
12. Wani Y, Notohara K, Saegusa M, et al. Aberrant Cdx2 expression in endometrial lesions with squamous differentiation: important role of Cdx2 in squamous morula formation. *Hum Pathol* 2008; 39: 1072–1079.
13. Jaffee IM, Rahmani M, Singhal MG, et al. Expression of the intestinal transcription factor CDX2 in carcinoid tumors is a marker of midgut origin. *Arch Pathol Lab Med* 2006; 130: 1522–1526.
14. Sullivan LM, Smolkin ME, Frierson HF Jr, et al. Comprehensive evaluation of CDX2 in invasive cervical adenocarcinomas: immunopositivity in the absence of overt colorectal morphology. *Am J Surg Pathol* 2008; 32: 1608–1612.
15. Herawi M, De Marzo AM, Kristiansen G, et al. Expression of CDX2 in benign tissue and adenocarcinoma of the prostate. *Hum Pathol* 2007; 38: 72–78.
16. Cowan ML, Li QK and Illei PB. Cdx-2 expression in primary lung adenocarcinoma. *Appl Immunohistochem Mol Morphol* 2016; 24: 16–19.
17. Ulbright TM, Amin MB, Balzer B, et al. Germ cell tumors. In: WHO classification of tumours of the urinary system and male genital organs. In: Moch H, Humphrey PA, Ulbright TM, Reuter VE (eds). IARC Press, Lyon 2016; 189–226.
18. Jones TD, Wang M, Sung MT, et al. Clonal origin of metastatic testicular teratomas. *Clin Cancer Res* 2006; 12: 5377–5383.
19. Nogales FF, Preda O and Nicolae A. Yolk sac tumours revisited. A review of their many

- faces and names. *Histopathology* 2012; 60: 1023–1033.
20. Lee MJ, Vogt AP, Hsiao W, et al. CDX-2 expression in malignant germ cell tumors of the testes, intratubular germ cell neoplasia, and normal seminiferous tubules. *Tumour Biol* 2012; 33: 2185–2188.
  21. Ulbright TM. Germ cell tumors of the gonads: a selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. *Mod Pathol* 2005; 18(Suppl 2): S61–S79.
  22. Ye H and Ulbright TM. Difficult differential diagnoses in testicular pathology. *Arch Pathol Lab Med* 2012; 136: 435–446.
  23. Nogales FF, Quiñonez E, López-Marín L, et al. A diagnostic immunohistochemical panel for yolk sac (primitive endodermal) tumours based on an immunohistochemical comparison with the human yolk sac. *Histopathology* 2014; 65: 51–59.
  24. Young RH. Testicular tumors—some new and a few perennial problems. *Arch Pathol Lab Med* 2008; 132: 548–564.
  25. Ulbright TM. Germ cell neoplasms of the testis. *Am J Surg Pathol* 1993; 17: 1075–1091.
  26. Looijenga LH, Zafarana G, Grygalewicz B, et al. Role of gain of 12p in germ cell tumour development. *APMIS* 2003; 111: 161–171; discussion 172–173.
  27. Werling RW, Yaziji H, Bacchi CE, et al. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol* 2003; 27: 303–310.
  28. Raspollini MR, Amunni G, Villanucci A, et al. Utility of CDX-2 in distinguishing between primary and secondary (intestinal) mucinous ovarian carcinoma: an immunohistochemical comparison of 43 cases. *Appl Immunohistochem Mol Morphol* 2004; 12: 127–131.
  29. Logani S, Oliva E, Arnell PM, et al. Use of novel immunohistochemical markers expressed in colonic adenocarcinoma to distinguish primary ovarian tumors from metastatic colorectal carcinoma. *Mod Pathol* 2005; 18: 19–25.
  30. Tornillo L, Moch H, Diener PA, et al. CDX-2 immunostaining in primary and secondary ovarian carcinomas. *J Clin Pathol* 2004; 57: 641–643.
  31. Bing Z, Pasha T, Tomaszewski JE, et al. CDX2 expression in yolk sac component of testicular germ cell tumors. *Int J Surg Pathol* 2009; 17: 373–377.
  32. Magers MJ, Kao CS, Cole CD, et al. “Somatic-type” malignancies arising from testicular germ cell tumors: a clinicopathologic study of 124 cases with emphasis on glandular tumors supporting frequent yolk sac tumor origin. *Am J Surg Pathol* 2014; 38: 1396–1409.
  33. Osman H, Cheng L, Ulbright TM, et al. The utility of CDX2, GATA3, and DOG1 in the diagnosis of testicular neoplasms: an immunohistochemical study of 109 cases. *Hum Pathol* 2016; 48: 18–24.