EJGO European Journal of Gynaecological Oncology

CDX2 expression in endometrial endometrioid adenocarcinoma

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Summary

Background: CDX2 is an intestinal-specific gene and activated CDX2, ensures inhibition of cell proliferation, and differentiation of intestinal epithelial cells. Immunohistochemically CDX2 expression is common in neoplastic and non-neoplastic cells in the gastrointestinal system. CDX2 is used especially in differential diagnosis of metastatic tumors in pathology practice. Recently it is reported that CDX2 expression may be found outside of the gastrointestinal system. *Aims:* In the present study, the authors aimed to research the CDX2 expression in endometrioid adenocarcinoma in endometrium and attempt to determine the relationship with grade and stage. *Materials and Methods:* The study included 92 cases with endometrial endometrioid adenocarcinoma. Sections prepared in paraffin blocks were researched immunohistochemically for CDX2 expression. *Results:* Expression was detected 31/92 of the cases. In 13% of cases (12/92), positivity was detected in the glandular area of the tumor tissue while in 20% of cases (19/92) expression was identified in the morular metaplasia area. It was noted that in two cases there was strong expression in the glandular area. *Conclusions:* CDX2 expression may be observed in endometrial endometrioid adenocarcinoma. This situation does not imply intestinal metaplasia. Especially as histopathological evaluation of metastatic tumors may cause misdiagnosis, in endometrioid adenocarcinoma in non-morular tumoral tissue, it should be remembered that, though rare, intense CDX2 expression may be found.

Key words: CDX2; Endometrium; Endometrioid adenocarcinoma.

Introduction

CDX2 protein plays an important role in intestinal epithelial differentiation, and is an intestine-specific homeobox gene transcription factor [1]. Immunohistochemically CDX2 expression in colorectal adenocarcinoma is diffuse and intense. This characteristic is beneficial to distinguish colorectal adenocarcinoma from pulmonary and ovarian adenocarcinoma [2-4]. Additionally CDX2 expression is reported in neuroendocrine tumors, in pancreatic adenocarcinoma, and in transitional cell carcinoma of the bladder [5, 6]. In the gynecological system, CDX2 expression is described in surface epithelial tumors of the ovary, especially in mucinous tumors [2-4, 7]. There are few studies reporting the CDX2 expression of endometrioid adenocarcinoma of the uterus. While CDX2 expression is not observed in normal endometrium, different rates of expression were reported (1.4-27%) in endometrioid adenocarcinoma [5-8].

Uterine endometrioid adenocarcinoma frequently shows various types of squamous differentiation. These are a) fully keratinized cells, b) morules, c) hyaline-like spherules of keratin without nuclei, d) undifferentiated "large cell" non-keratinized cells, e) glassy cells, and mixture of these [9]. The most common types of squamous differentiation are morules and fully keratinized [10].

Materials and Methods

The authors aimed to research the CDX2 expression in endometrioid adenocarcinoma and to evaluate the differences in expression of squamous differentiation areas and neoplastic glandular cells.

The study included 92 endometrial endometrioid adenocarcinoma diagnosed in 2014-2015 at Eskişehir Osmangazi University's Pathology Department. Operation specimens were fixed in formalin. By histopathological evaluation, tumor grade, greatest dimension of the tumor, and depth of invasion were detected. Grades and stages were determined according to the International Federation of Gynecology and Obstetrics (FIGO) criteria. The characteristics of the study population are presented in Table 1. Presence of squamous differentiation was noted. Cells located as islands or in syncytial layers, with rounded-spindle nucleus, broad eosinophilic cytoplasm, and undetermined cytoplasmic boundaries were evaluated as squamous morule. Cell groups with keratinization, clear cytoplasmic boundaries, and intercellular bridges were recorded as typical squamous elements.

The paraffin block best reflecting tumor morphology was studied immunohistochemically for CDX2. Five µm-thick sections were taken from the paraffin blocks. Deparaffinization and immunoperoxidase staining of slides was completed by an automatic staining system in accordance with the manufacturer's instructions. Chromogeneous diaminobenzidine (DAB) was used for marking. Negative staining was applied with Harris hematoxylin. For negative controls, instead of primary antibody, the same concentration of immunoglobulin (IgG1) was used. For positive controls, colonic adenocarcinoma was used. Staining of 1% or less of cells was accepted as negative. Scoring for 1-10% positivity was "+", from 10-50% it was "++", and 50% and above it was

Revised manuscript accepted for publication April 13, 2016

Table 1. — *Age and tumor size in CDX2 positive and negative groups.*

CDX2	(-)	Glandular positivity	Morular positivity	*p*
Age, years (mean)	58.7	58.1	56.6	0.367
Tumor size, cm (mean)	3.3	4.4	3.1	0.472
Total	61	12	19	

Table 2. — *Grade and Stage of CDX2 positive and negative groups.*

CDX2		(-) n	Glandular positivity n	Morular positivity n	*p*
Grade	I	20	3	4	
	II	24	6	13	
	III	17	3	2	0.098
Stage	I	49	9	15	
	II	5	2	2	
	III	6	1	2	
	IV	1	0	0	0.259
Total		61	12	19	

"+++". Only nuclear staining was evaluated.

The software of SPSS version 21.0 was used for statistical analysis. Statistical analysis were performed with Fisher exact test for any 2×2 tables, Pearson χ^2 test for non-2×2 tables, and χ^2 trend test for ordinal datum. Differences were considered statistically significant when $p < 0.05$.

Results

Aside from the morular differentiation, in 12/92 (13% of the cases), glandular tumor tissue was positive (Figure 1). CDX2 expression was identified in all of the morular differentiation areas (19/92 of the cases) (Figure 2). Mean age in 12 cases with positivity in glandular pattern was 58 years, while the mean age of the 19 cases with positivity in morular differentiation areas was 56 years. The mean tumor size in these cases were 4.4 and 3.1 cm, respectively. Difference between the groups were not statistically significant ($p > 0.05$) (Table 1). Among the three groups that CDX2 was positive in glandular pattern, positive in morular pattern and wholly negative, stages or grade distribution were not statistically significant ($p > 0.05$) (Table 2). In two cases intense staining was identified in glandular tumor tissue (Figure 3). These cases with "++" and "+++" positivity were 42-years-old, Stage 1a, Grade 3, and 79-years-old, Stage 3b and Grade 2, respectively.

Discussion

CDX2 is a homeobox gene. It has been shown to have a role in differentiation of intestinal epithelial cells in development of small and large intestine [11, 12]. Suh *et al.*

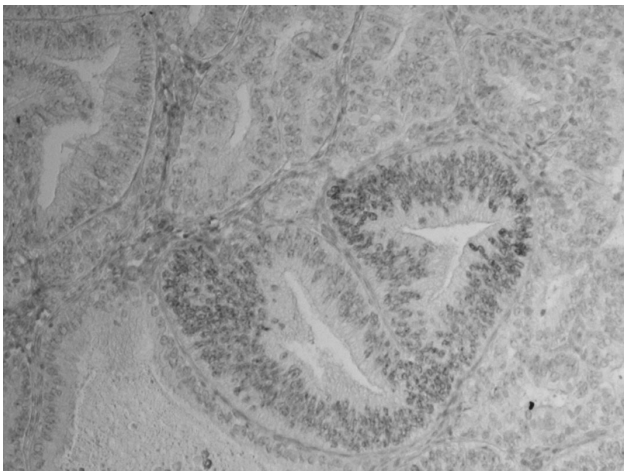

Figure 1. — CDX2 positivity at tumoral glandular structures (magnification: ×200).

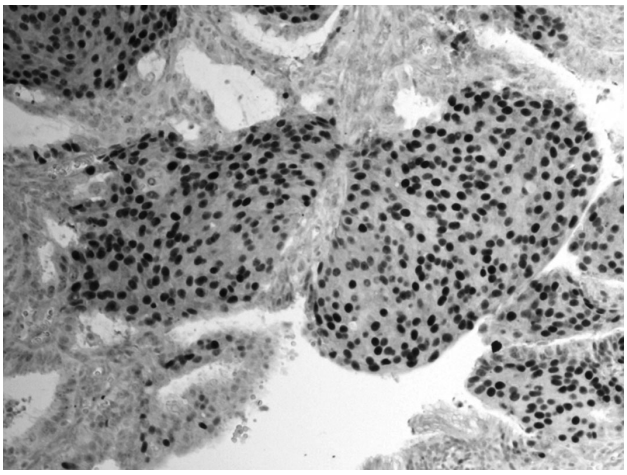

Figure 2. — CDX2 expression in morular differentiation areas (magnification: ×200).

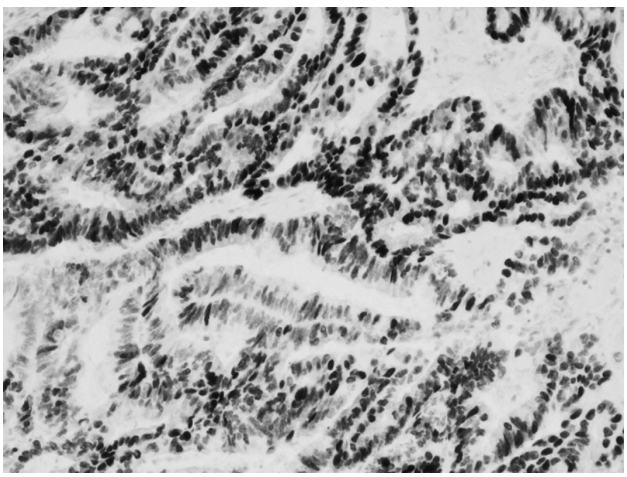

Figure 3. — Diffuse and intense CDX2 expression (magnification: ×200).

demonstrated that CDX2 plays a role as a transcription factor increasing expression of many gene products in mature intestinal epithelium. In cell cultures, CDX2 inhibits the replication and induces the differentiation of intestinal epithelial cells [13].

Moskaluk *et al.* investigated expression of CDX2 in several normal tissue and malignant tumors. Intense and widespread positivity of CDX2 was seen in small intestinal, appendiceal, rectal and colonic epithelium, and in pancreatic centroacinar cells and ducts, while normal endometrial epithelium and stroma were totally negative [7]. In malignant tumors, CDX2 was most commonly seen in colorectal adenocarcinomas (90%). Other adenocarcinomas with extensive nuclear CDX2 staining included gastroesophageal adenocarcinomas (20%), ovarian endometrioid adenocarcinomas (20%) and mucinous adenocarcinomas (20%), uterine endometrioid adenocarcinoma (4.3%), and prostatic adenocarcinoma (1%) [7].

There are few studies in the literature that specifically researched CDX2 expression in endometrial adenocarcinoma [8, 10, 14, 15]. According to these studies, positivity is nearly always found in morular differentiation areas of endometrial adenocarcinoma [10, 14]. Some studies have found expression in neoplastic glandular structures, while some have not separated positivity in morular or neoplastic glandular positivity. In the present study in 12 of 92 cases (13%), expression was found in glandular neoplastic epithelium. As expected, morular differentiation areas of the tumors (19 cases) were consistently positive for CDX2 immunohistochemically. Non-neoplastic endometrial tissue was totally negative. Wani *et al.* proposed that CDX2 positive cell groups identified in tumors without squamous differentiation may be tumor cells that will undergo squamous differentiation [14]; however this theory was not fully accepted in the literature. In the present study, aside from morular metaplasia areas, glandular cells expressing CDX2 were clearly observed. Houghton *et al.* reported that endometrioid adenocarcinoma without squamous elements or morules were negative for CDX2. Only in one case was focal positivity was found [10]. Saegusa *et al.* stated that CDX2 positivity in endometrioid adenocarcinoma may be related to nuclear beta catenin activity. CDX2 immunoreactivity overlaps with nuclear beta catenin and p21 accumulation [15]. They suggested that CDX2 and beta catenin work together for transdifferentiation of morular phenotype in endometrial carcinoma cells. CDX2 expression is under transcriptional control of nuclear beta catenin [8].

In the present study, the authors did not perform p63 immunohistochemistry, hence they could not observe other types of squamous differentiation. In all cases with morular metaplasia, CDX2 was identified in varying intensity.

Aside from the gastrointestinal system, CDX2 positivity may be observed in ovarian and endometrial endometrioid adenocarcinomas [7, 10, 16]. This expression does not signify the intestinal differentiation of the tumor.

In pathology practice, CDX2 is frequently studied immunohistochemically in differential diagnosis of metastatic tumors. The detection of CDX2 expression in endometrioid adenocarcinoma may cause misdiagnosis. Reported CDX2 expression in endometrioid adenocarcinoma is generally weak. In the literature only three cases were reported that had diffuse intense positivity [7, 17]. In the present study, two cases had intense and widespread positivity with CDX2 "++" and "+++", respectively. These cases did not have morular metaplasia. In glandular structures, it is proposed that diffuse expression may be linked to therapeutic modulation [17].

In metastatic tumors, while investigation of the primary tumor, especially in differential diagnosis of colonic adenocarcinoma, it should be kept in mind that although rare, intense CDX2 positivity may be observed in glandular tumoral tissue of the endometrium. Additional immunohistochemical studies may prevent misdiagnosis.

Acknowledgements

The authors thank Dr. Ertuğrul Çolak from Department of Biostatistics and Medical Informatics, Eskişehir Osmangazi University Faculty of Medicine, for his help with statistical analysis.

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