

High expression of SALL4 and fascin, and loss of E-cadherin expression in undifferentiated/dedifferentiated carcinomas of the endometrium

An immunohistochemical and clinicopathologic study

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Abstract

Undifferentiated/dedifferentiated endometrial carcinomas (UCE/DCEs) of the endometrium are rare tumors with poor prognosis. There are few clinicopathologic studies with detailed immunohistochemical analysis regarding UCE/DCEs.

We evaluated the diagnostic value of a selected tumor stem-cell marker and epithelial-mesenchymal transition (EMT) markers, in addition to previously studied markers in identifying UCE/DCEs from other types of high-grade endometrial carcinomas.

Eleven cases of UCE/DCEs with complete clinical follow-up that were diagnosed between 2006 and 2015 were included in the study. For immunohistochemical comparison, 11 clinically matched cases for each type of other high-grade endometrial carcinomas (high-grade endometrioid (F3-EC), serous [SC], and clear cell carcinoma [CCC]) were used as a control group. An immunohistochemical analysis including fascin, SALL4, E-cadherin, and β -catenin, in addition to epithelial and neuroendocrine markers was performed in each case.

The majority of UCE/DCEs displayed diffuse expression of fascin (81.9%) and loss of E-cadherin expression (54.5%). SALL4 expression was detected in 36.3% of the UCE/DCE cases. SALL4 expression was significantly more frequent in UCE/DCEs than all other high-grade carcinomas ($P < 0.001$). Loss of E-cadherin and fascin expression was significantly more frequent in UCE/DCEs than high-grade endometrioid and clear cell adenocarcinomas ($P = 0.012$, 0.014 and $P = 0.01$, 0.003 , respectively).

We suggest that loss of E-cadherin expression together with fascin and SALL4 immunopositivity in addition to morphologic features have an impact in differential diagnosis of UCE/DCEs from other high-grade endometrial carcinomas.

Abbreviations: CCC = clear cell carcinoma, DCE = dedifferentiated endometrial carcinoma, EMT = epithelial-mesenchymal transition, ER = estrogen receptor, F3-EC = FIGO grade 3-endometrioid carcinoma, PR = progesterone receptor, SC = serous carcinoma, UCE = undifferentiated endometrial carcinoma, WHO = World Health Organization.

Keywords: E-cadherin, endometrium, fascin, immunohistochemistry, SALL4, undifferentiated carcinoma

1. Introduction

Undifferentiated carcinoma of the endometrium (undifferentiated endometrial carcinoma [UCE]) is a rare and poorly understood neoplasm with aggressive behavior, defined simply as a “malignant epithelial neoplasm with no differentiation” by the World Health Organization classification.^[1] Few studies have described the clinical and histologic features of UCE.^[2–8] The main character-

istics are the patternless growth with no morphologic evidence of differentiation, such as papillae, trabeculae, nests, squamous metaplasia, or spindled architecture. A related term “dedifferentiated endometrial carcinoma” (DCE) defines a UCE with a differentiated component that is usually low-grade endometrioid carcinoma.^[1,7,9] However, high-grade endometrial carcinomas, such as International Federation of Gynecology and Obstetrics (FIGO) grade 3 endometrioid carcinoma (F3-EC) or mixed carcinomas, have also been reported within DCEs.^[2,8]

Due to misinterpretations of its solid areas, this relatively rare tumor may cause difficulty in the differential diagnosis, which includes other high-grade endometrial carcinomas, both epithelial and neuroendocrine. Several studies have addressed this issue using immunohistochemical markers; both UCE and undifferentiated components of DCE are variably positive for epithelial markers and negative for PAX-8, whereas F3-ECs are usually immunoreactive for these markers.^[8,9] Furthermore, hormone receptor (estrogen [ER] and progesterone receptor [PR]) expressions are more frequently retained in F3-ECs than UCE/DCE (60% vs 12%, respectively).^[2] Focal expression of neuroendocrine markers is seen in almost half of UCE/DCE.^[10]

Relatively new markers have recently been introduced in the subject of endometrial carcinomas, including actin-binding protein fascin, and stem-cell markers such as Sal-like protein 4 (SALL4).^[11–13] Epithelial-mesenchymal transition (EMT)-related proteins, for example, E-cadherin and β -catenin are also subjects

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of interest in the field of endometrial carcinomas; loss of E-cadherin expression in endometrial carcinomas has been correlated with advanced stage and worse prognosis in some studies.^[14–16] None of the aforementioned immunohistochemical markers, namely fascin, E-cadherin, β -catenin, and SALL4, is specific to any type of tumor and used for various reasons. For example, fascin has a role in formation of actin-based cellular protrusions and in cell motility and migration. Therefore, it can be used for predicting the aggressive clinical course of a malignant tumor, as well as differentiating neoplastic cells of classic Hodgkin lymphoma from other types of lymphomas with anaplastic large cells.^[11,12] Similarly, SALL4 can be used to detect tumor stem cells, as well as being a malignant germ cell tumor marker.^[13,17] E-cadherin and β -catenin can be used in the differential diagnosis of subtypes of breast carcinomas and malignant soft tissue tumors, as well as to show tumor aggression.^[14–16] By having roles in dedifferentiation, invasion, and metastasis, these markers appear to be promising tools with regards the prediction of the clinical course of patients with various types of malignant tumors. However, data regarding their roles in the differential diagnosis of UCE/DCEs from other high-grade uterine carcinomas are lacking in the literature.

The aim of this study was to investigate the diagnostic value of a selected tumor stem-cell marker (SALL4), fascin, and EMT markers in identifying UCE/DCE from other types of high-grade endometrial carcinomas (high-grade endometrioid [F3-EC], serous [SC], and clear cell carcinoma [CCC]).

2. Methods

2.1. Participants

Following a digital archive scan of 1420 consecutive cases of endometrial carcinoma diagnosed in the Department of Pathology, Faculty of Medicine, Istanbul University between January 1st, 2006 and December 31st, 2015, 23 cases were found to have a pathologic diagnosis of UCE/DCE, and 87 other types of high-grade endometrial carcinomas. The inclusion criteria were having a pathologic diagnosis of UCE/DCE, availability of adequate tissue for further immunohistochemical analysis, and presence of adequate clinical and follow-up data. Clinical data including patient age, stage, type of surgery, postoperative therapy, and

prognostic findings were retrieved from the patients' files in the Gynecology and Obstetrics Clinic and Institute of Oncology of Istanbul University. Eleven cases of UCE/DCE with adequate clinical and follow-up information, and available archived paraffin blocks were retrieved for the study group. The study was performed in accordance with the Declaration of Helsinki (5th revision, October 2000) of the World Medical Association and approved by the National Medical Ethics Committee of the Republic of Turkey. Institutional review board approval was provided before we started the study. Written consent from patients was not obtained since the study was designed retrospectively and needed no consent.

An appropriate immunohistochemical panel including epithelial (epithelial membrane antigen [EMA], low-molecular-weight cytokeratin [LMWCK], and pancytokeratin [PanCK]), neuroendocrine (chromogranin A, synaptophysin, and CD56), and other markers (Estrogen receptor [ER], progesterone receptor [PR], vimentin, smooth muscle actin [SMA], and desmin) had already been applied to each case of UCE/DCE for routine diagnostic purposes. For comparison, all markers of the additional panel were also examined in the control group of F3-ECs (n = 11), SCs (n = 11), and CCCs (n = 11) of the endometrium.

2.2. Test methods

An additional panel including E-cadherin, β -catenin, fascin, and SALL4 was performed. All immunohistochemical studies were performed using a Ventana Medical System-Benchmark XT/ISH Staining module (Tucson, AZ) in our department. Specifications of the immunohistochemical procedure are shown in Table 1. Nuclear staining for ER, PR, and SALL4 and cytoplasmic or cytoplasmic membranous staining for the rest of the markers were regarded as positive during the immunohistochemical analysis. Any percentage of positive staining for SALL-4 and positive staining of fascin in >50% of the tumoral cells were accepted as positive. Total loss of E-cadherin was accepted as negative, whereas any percentage of positive staining was recorded as positive. All hematoxylin and eosin and immunohistochemical slides were reevaluated by the first and senior authors (SO, EY). All micrographs were taken using an integrated digital camera (Olympus DP71, Japan) on a light microscope (Olympus BX51, Japan).

Table 1
Details of immunohistochemical procedure.

Antibody	Producer	Clone	Dilution
PanCK	ThermoFisher Rockford, IL/USA	AE1:AE3	1/100
EMA	ThermoFisher Rockford, IL/USA	GP 1;4	1/1000
LMWK	ThermoFisher Rockford, IL/USA	AE1	1/50
Chromogranin A	Genemed San Francisco, CA/ USA	Rabbit	1/400
Synaptophysin	Novocastra New Castle/ UK	27G12	1/100
CD56	Genemed San Francisco, CA/USA	123C3	1/100
Vimentin	Biocare Concord, CA/USA	V9	1/300
ER	Biocare Concord, CA/USA	SP1	1/50
PR	Spring Pleasanton, CA/USA	SP2	1/400
SMA	ThermoFisher Rockford, IL/USA	1A4, asm1	1/800
Desmin	Genemed San Francisco, CA/USA	D33	1/100
Fascin	ThermoFisher Fremont, CA/USA	FCN01	1/100
SALL4	Biocare Concord, CA/USA	6E3	1/100
E-cadherin	ThermoFisher Rockford, IL/USA	4A2C7	1/200
β -catenin	ThermoFisher Rockford, IL/USA	CAT-5H10	1/100

EMA = epithelial membrane antigen, ER = estrogen receptor, LMWK = low-molecular-weight cytokeratin, PR = progesterone receptor, PanCK = pancytokeratin, SALL4 = Sal-like protein 4, SMA = smooth muscle actin.

2.3. Statistical methods

All statistical analyses were performed using Statistical Package for Social Sciences software for Windows version 21.0 (IBM Corp, Armonk, NY). All comparisons for immunohistochemical results between histologic subtypes were investigated using the Chi-square test, Fisher exact test, or Mann–Whitney *U* test where applicable. *P* values less than 0.05 were considered significant in all comparisons.

Progression-free survival was calculated from the date of diagnosis to the date of the first evidence of clinical progression or death of disease. Overall survival was calculated from the date of diagnosis to the date of death related to any reason.

3. Results

3.1. Participants

UCE/DCE cases ($n=11$) comprised 0.77% of all endometrial carcinomas ($N=1420$) diagnosed between 2006 and 2015. All patients were postmenopausal, and their ages ranged from 54 to 79 years (mean: 62.5 years; median: 59 years). All patients underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy with or without adjuvant therapy. The FIGO tumor stages were as follows: 3 tumors were stage I (27.27%), 2 tumors were stage II (18.18%), 2 tumors were stage III (18.18%), and 4 tumors were stage IV (36.36%).

3.2. Pathologic features

The tumor sizes ranged from 2.5 to 10 cm (average: 6.77 cm). All tumors had deeply (>50%) infiltrated the myometrium and showed extensive necrosis (Fig. 1A, B). The average number of submitted blocks was 14 per tumor (range, 10–18 blocks).

Microscopically, 2 of the 11 cases were pure UCEs, the remainder (9/11) bore an endometrioid component diagnosed as DCE. The endometrioid component, comprising 2% to 3% to 40% of the whole tumor, was low-grade in 5 cases (Fig. 1C), and high-grade in 4 cases. The undifferentiated component was composed of solid sheets of discohesive small to medium-sized neoplastic cells with vesicular nuclear chromatin and conspicuous nucleoli (Fig. 1D). Neither gland formation nor typical adenocarcinomatous growth pattern was observed in the undifferentiated component. Lymphovascular invasion was found in all cases. The lymph node metastasis was composed of undifferentiated component in 3 cases and both differentiated (endometrioid) and undifferentiated component in 2 cases.

3.3. Immunohistochemical features

Immunohistochemical analysis of the undifferentiated component revealed positive staining for pancytokeratin (5/11), epithelial membrane antigen (7/11), low-molecular-weight cytokeratin (6/11), chromogranin A (2/11), synaptophysin (4/11), CD56 (3/11), and vimentin (3/4). In brief, all cases showed intense but focal positivity (<10% of the tumor cells) for at least 1 of the 3 epithelial markers (Fig. 2A, B). With the exception of 2 cases with focal and weak positivity for ER, no cases were positive for hormone receptors. Six cases were focally positive for at least 1 neuroendocrine marker; 3 cases were positive for 2 neuroendocrine markers (Fig. 2C, D). Immunostaining for smooth muscle actin and desmin was performed in 4 cases and none showed positivity.

Positivity of fascin was observed in 81.9% (9/11), 81.9% (9/11), 27.2% (3/11), and 18.1% (2/11) of UCE/DCE SC, F3-EC, and CCC cases, respectively (Fig. 3A, B). Regarding fascin expression, there was a significant difference between UCE/DCE

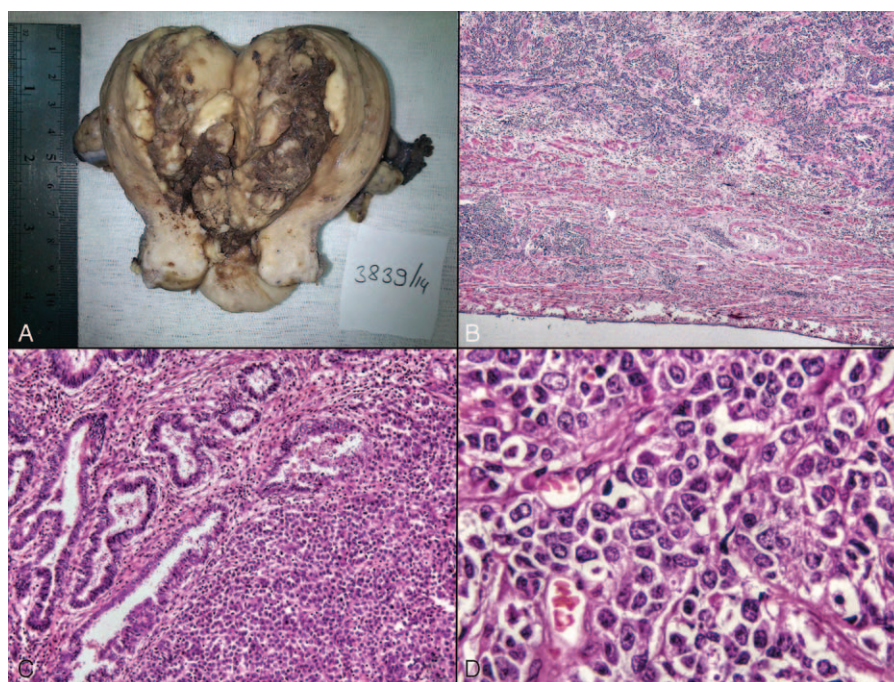


Figure 1. (A) Large exophytic and polypoid tumoral lesion filling the entire endometrial cavity and extending to the endocervical region. (B) Microscopically, tumoral cells infiltrating deep myometrium (H&E, $\times 10$, original magnification). (C) The dedifferentiated endometrial carcinoma consisting of well-differentiated endometrioid carcinoma (left side) and undifferentiated carcinoma (right side) (H&E, $\times 20$, original magnification). (D) The neoplastic cells have medium-sized nuclei with vesicular chromatin (H&E, $\times 100$, original magnification).

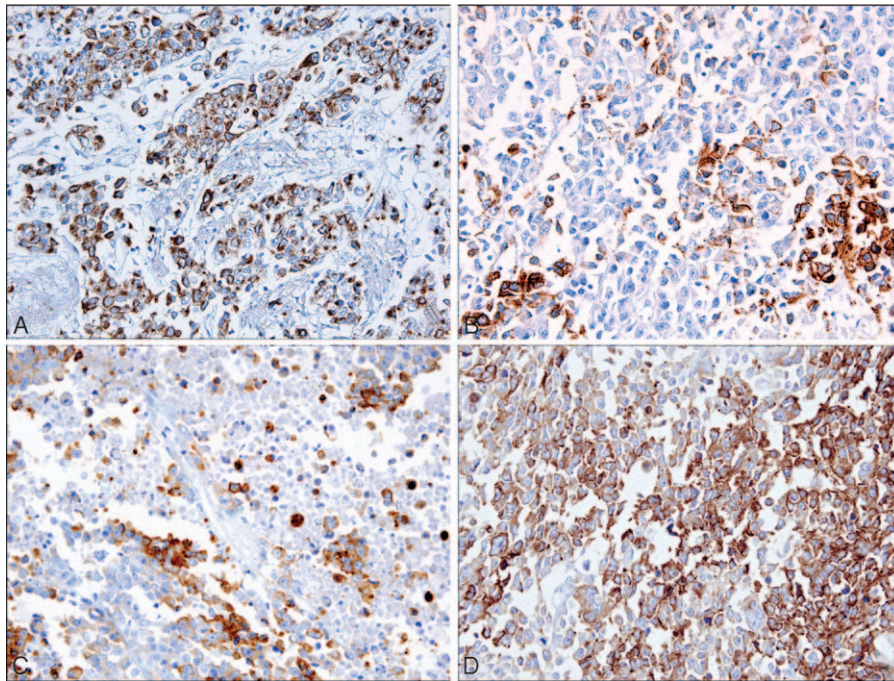


Figure 2. (A) EMA is also focal positive in neoplastic cells of a case of undifferentiated carcinoma (EMA, $\times 20$, original magnification). (B) The neoplastic cells are focally positive for PanCK ($\times 20$, original magnification). (C) The neoplastic cells display patchy staining with Chr A in a case of undifferentiated carcinoma (Chr A, $\times 20$, original magnification). (D) Syn is also focal positive in neoplastic cells in a case of undifferentiated carcinoma (Syn, $\times 40$, original magnification). Chr A=chromogranin A, EMA=epithelial membrane antigen, PanCK=pancytokeratin, Syn=synaptophysin.

and F3-EC and CCC cases ($P=0.01$ and $P=0.003$, respectively), but there was no difference between UCE/DCE and SC.

Total loss of E-cadherin expression was observed in 54.5% (6/11) of UCEs, whereas only 2 SCs of the control group (6.06%) showed a similar staining pattern (Fig. 3C). With regard to total

loss of E-cadherin expression, the difference between UCE and F3-EC and CCC was statistically significant ($P=0.012$, $P=0.014$), but there was no significant difference between UCE/DCE and SC, of the 6 UCEs with loss of E-cadherin expression, 4 cases had punctate/Golgi staining of β -catenin (Fig. 3D). Besides these

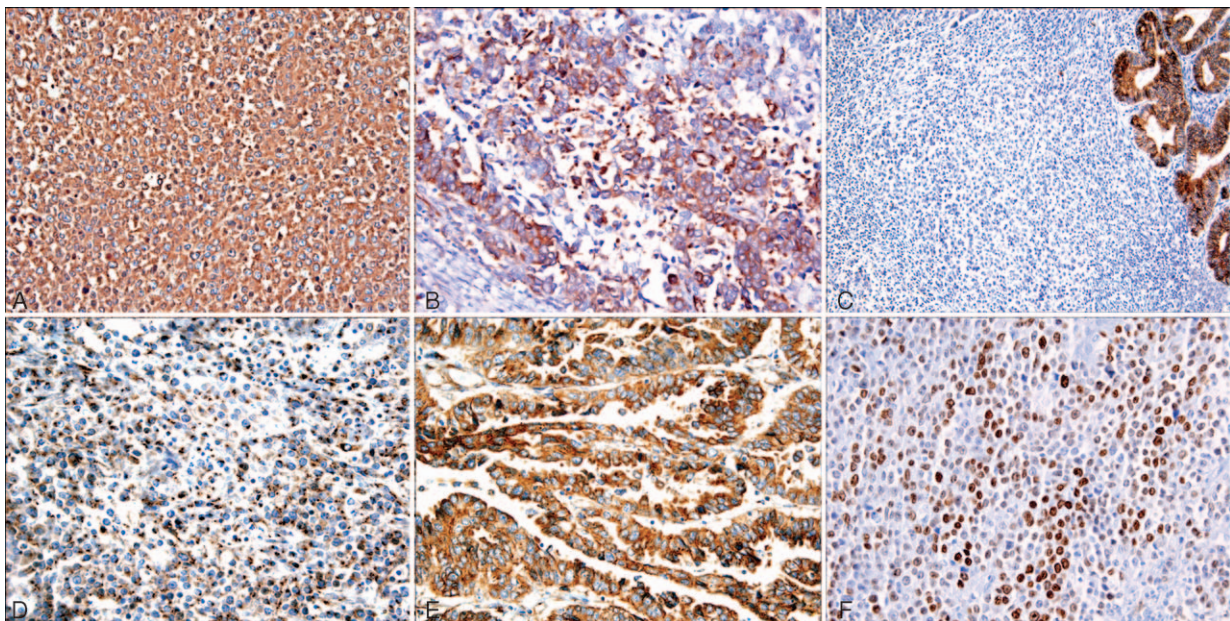


Figure 3. (A, B) Positive immunostaining for fascin in an undifferentiated (A) and serous adenocarcinoma (B) (Fascin, $\times 40$, original magnification). (C) Loss of immunostaining of E-cadherin in undifferentiated component and diffuse immunostaining in differentiated component in a dedifferentiated carcinoma (E-cadherin, $\times 20$, original magnification). (D, E) Punctate staining in an undifferentiated carcinoma (D) and cytoplasmic staining in endometrioid adenocarcinoma (E) (β -catenin, $\times 40$, original magnification). (F) Positive immunostaining in an undifferentiated carcinoma (SALL4, $\times 40$, original magnification).

Table 2
Immunohistochemical findings of undifferentiated carcinomas.

Pt	EMA	LMWK	PanCK	Chr A	Syn	CD56	ER-PR	SMA-desmin	Vimentin
1	10%–20%	10%–20%	10%–20%	(–)	(–)	(–)	Focal+	(–)	(–)
2	(–)	<10%	(–)	%20 W	(–)	(–)	N/A	N/A	N/A
3	<10%	10%–20%	<10%	(–)	(–)	(–)	N/A	N/A	N/A
4	10%–20%	(–)	(–)	(–)	%10 W	%10 W	N/A	N/A	N/A
5	<10%	(–)	(–)	(–)	(–)	(–)	(–)	N/A	N/A
6	(–)	<10%	<10%	(–)	(–)	(–)	N/A	N/A	N/A
7	(–)	10%–20%	10%–20%	(–)	(–)	<10 W	N/A	(–)	(–)
8	10%–20%	(–)	(–)	(–)	(–)	(–)	N/A	N/A	Focal+
9	<10%	(–)	(–)	(–)	%5 S	<10% W	Focal+	N/A	(–)
10	<10%	(–)	(–)	%20 S	%10 S	(–)	(–)	(–)	N/A
11	(–)	<10%	<10%	(–)	%60 W	(–)	N/A	(–)	N/A

Chr A = chromogranin A, EMA = epithelial membrane antigen, ER = estrogen receptor, LMWK = low-molecular-weight cytokeratin, N/A = not available, PanCK = pancytokeratin, PR = progesterone receptor, Pt = patient, S = strong, SMA = smooth muscle actin, Syn = synaptophysin, W = weak.

4 cases, this distinct pattern of staining for β-catenin was observed in 1 UCE and 2 CCC cases without any loss of E-cadherin. Cytoplasmic and/or membranous staining of β-catenin was seen in all cases, both in the study and control groups (Fig. 3E). There was no difference between the control and study groups in terms of β-catenin staining.

SALL4 positivity was observed in 36.3% (4/11) of all UCE/DCEs; no positivity was observed in the rest of the cases (Fig. 3F). The difference between UCE/DCEs and each control group was statistically significant ($P < 0.001$). Detailed immunohistochemical results are shown in Tables 2 and 3.

3.4. Clinical features, follow-up, and prognostic data

The follow-up period ranged from 3 to 78 months (mean: 23 months, median: 11 months) in UCE/DCEs. The median progression-free survival was 7 months (range, 3–78 months) and median overall survival was 10 months (range, 4–78 months) in UCE/DCEs (Table 4).

Among 10 patients with pelvic and paraaortic lymph node dissection, 5 showed lymph node metastases, the most common metastatic sites were the obturator and pelvic lymph nodes. At the time of the initial diagnosis, 4 patients had distant metastases in the liver, brain, and supradiaphragmatic/infradiaphragmatic lymph nodes. Additionally, 4 of the 11 patients also developed distant metastases during the follow-up period. Local recurrence was detected in 2 patients (Table 4).

Adjuvant treatment strategies for patients with UCE were chemotherapy and/or radiotherapy, with regimens similar to those for F3-EC (Table 4). During the follow-up, only 2 patients were alive with no evidence of disease, 1 with stage I (patient 2) and 1 with stage II (patient 5) disease at 73 and 78 months, respectively. Eight patients died of their disease, and 1 (patient 1) of disseminated rectal carcinoma.

4. Discussion

UCEs with a differentiated component are defined as DCE.^[1,7] DCEs comprise 38% to 86% of UCEs,^[2,3,7,8] and in accordance with previous studies, 81.8% of our cases were DCEs. In addition to epithelial and neuroendocrine immune markers studied by other investigators,^[1,4,7,9,10] we analyzed expressions of fascin, SALL4, and EMT markers in UCE/DCE cases in the current study.

Immunohistochemically, UCE shows variable expression of epithelial markers,^[1–4,7–9] is usually positive for vimentin,^[1,3]

Table 3
Immunohistochemical findings of the cases.

Pt	Diagnosis	E-Cadherin	β-Catenin	Fascin	SALL4
1	UCE/DCE	+	+	+	+
2	UCE/DCE	–	+	–	+
3	UCE/DCE	–	+	+	+
4	UCE/DCE	+	+	+	–
5	UCE/DCE	+	+	+	–
6	UCE/DCE	–	+	+	–
7	UCE/DCE	+	+	–	–
8	UCE/DCE	–	+	+	–
9	UCE/DCE	–	+	+	–
10	UCE/DCE	–	+	+	+
11	UCE/DCE	+	+	+	–
12	SC	–	+	+	–
13	SC	+	+	+	–
14	SC	+	+	+	–
15	SC	+	+	+	–
16	SC	+	+	+	–
17	SC	+	+	+	–
18	SC	–	+	–	–
19	SC	+	+	+	–
20	SC	+	+	–	–
21	SC	+	+	+	–
22	SC	+	+	+	–
23	F3-EC	+	+	–	–
24	F3-EC	+	+	–	–
25	F3-EC	+	+	–	–
26	F3-EC	+	+	+	–
27	F3-EC	+	+	+	–
28	F3-EC	+	+	–	–
29	F3-EC	+	+	+	–
30	F3-EC	+	+	–	–
31	F3-EC	N/A	+	–	–
32	F3-EC	N/A	+	–	–
33	F3-EC	+	+	–	–
34	CCC	+	+	+	–
35	CCC	+	+	+	–
36	CCC	+	+	–	–
37	CCC	+	+	–	–
38	CCC	+	+	–	–
39	CCC	+	+	–	–
40	CCC	+	+	–	–
41	CCC	+	+	–	–
42	CCC	+	+	–	–
43	CCC	+	+	–	–
44	CCC	+	+	–	–

CCC = clear cell carcinoma, F3-EC = FIGO grade 3 endometrioid carcinoma, N/A = not available, Pt = patient, SC = serous carcinoma, UCE/DCE = Undifferentiated/dedifferentiated carcinoma.

Table 4**Clinicopathologic features of the patients with undifferentiated carcinoma.**

Patient no	Age	Tumor size, cm	LVI	LN status (metastasis vs total)	Differentiated component	Stage (FIGO)	Adjuvant/primary CT	Adjuvant RT	Site of progression	PFS months	OS months	Survival
1	65	10	+	0/10	2%–3%, grade III E	II	Not received	EBRT + BR	Local	4	32	DOAD
2	70	7.5	+	0/3	5%, grade III E	IB	4 cycles PAC*–CAR*	BR	No progression	73	73	NED
3	58	8	+	4/20	25%, grade I E	IIIC2	6 cycles PAC*–CAR*	BR	Lung, abdomen	6	10	DOD
4	56	2.5	+	0/16	10%, grade I E	IA	Not received	BR	Lung, abdomen	4	5	DOD
5	58	6	+	0/13	30%, grade I–II E	II	6 cycles PAC*–CAR*	BR	No progression	78	78	NED
6	54	4.5	+	9/9	40%, grade III E	IVB	4 cycles PAC*–CAR*	Not received	Local + multiple ID/SD LN	3	4	DOD
7	79	9	+	0/24	5%, grade II E	IB	Not received	BR	Lung	14	27	DOD
8	61	8	+	5/7	5%, grade I–II E	IVB	9 cycles PAC*–CAR*	Not received	Brain	9	11	DOD
9	59	6	+	2/8	2%–3%, grade III E	IIIC1	6 cycles PAC*–CAR*	EBRT + BR	Lung	7	8	DOD
10	58	7	+	3/27	Absent	IVB	15 cycles PAC*–CAR**	Not received	Multiple ID LN	8	9	DOD
11	70	6	+	NA	Absent	IVB	9 cycles PAC*–CAR**	Not received	Liver, multiple ID LN	3	5	DOD

PAC*–CAR*: triweekly paclitaxel 175 mg/m² – carboplatin AUC 5. PAC**–CAR**: weekly paclitaxel 80 mg/m² – carboplatin AUC 2. BR = brachytherapy, C = carboplatin, CT = chemotherapy, DOAD = died of another disease, DOD = died of disease, E = endometrioid carcinoma, EBRT = external beam radiotherapy, ID = infradiaphragmatic, LN = lymph node, NED = no evidence of disease, OS = overall survival, PFS = progression free survival, RT = radiotherapy, SD = supradiaphragmatic.

and negative for hormone receptors.^[1,8] In accordance with the literature, our UCE cases showed frequent expression of vimentin, focal expression of at least 1 of 3 epithelial markers, and were usually negative for ER and PR.

Contrary to small cell carcinomas of the endometrium, positive immunostaining in more than 20% of neoplastic cells with at least 2 neuroendocrine markers is not an expected feature of UCEs/DCEs.^[10] Rather, focal expressions of one or more neuroendocrine markers can be observed in UCE/DCEs, similar to our cases.

The differential diagnosis of UCE/DCE includes F3-EC, SC, carcinosarcoma, and lymphomas of the endometrium.^[2–4] Glandular or trabecular growth patterns in addition to solid areas that lack cellular discohesion favor F3-EC, whereas a glandular component with papillary features, slit-like lumens, and variations in cytologic and architectural features encourage the diagnosis of SC. If a tumor contains an obvious sarcomatous component, the diagnosis of carcinosarcoma is relatively easy.^[3,4] Immunohistochemistry can be used if the differentiation of these entities is problematic. Focal, but not diffuse positivity of epithelial markers,^[3,4] and negativity of PAX-8^[8,9] and lymphoid markers^[2,3] favor the diagnosis of UCE/DCE.

One of the main objectives of the current study was to enlighten the immunohistochemical profile of UCE/DCE from the point of view of the diagnostician. With this perspective, we investigated the expression of various immunohistochemical markers including actin-binding protein fascin, SALL4 as a stem cell marker, and EMT-related proteins including E-cadherin and β -catenin in these tumors.

A recent publication of Stewart and Crook^[11] suggested that fascin expression may contribute to the aggressive behavior of UCE/DCEs. In our study, fascin immunoreactivity was higher in UCE/DCEs than other high-grade endometrial carcinomas except for SC, which supported its role in the poorer clinical outcomes of UCE/DCE and SC.^[1,2,18]

We observed total loss of E-cadherin expression in more than half of our UCE/DCE cases, similar to the study of Ramalingam et al.^[8] Total loss of E-cadherin expression was significantly higher in UCE/DCEs than other high-grade endometrial carcinomas, except for SC. These results were not unexpected, given the discohesive nature of the tumor cells in UCE/DCEs. An interesting finding was the unusual punctate/Golgi staining of β -catenin in two thirds of the UCE/DCE cases with total loss of E-

cadherin expression. Jamieson et al^[19] also noted the same staining pattern, and linked this finding to a defective intracellular transportation of β -catenin.

Tumor stem-cell markers are relatively new in the field of endometrial carcinomas. Although high expression of SALL4 was found associated with poorer prognosis in endometrial carcinomas,^[17] it has not yet been used for diagnostic purposes. SALL4 showed focal-to-diffuse positivity in 36.3% of our UCE/DCEs. All other high-grade endometrial carcinoma subtypes were negative for SALL4. However, contrary to our results, 56 of 80 endometrial carcinomas with SALL4 expression were detected in Liu et al study, in which subtypes of endometrial carcinomas were not specified.^[19] We think that the inconsistency between the 2 studies regarding SALL4 expression in endometrial carcinomas may be related to methods used for interpretation (cytoplasmic vs nuclear immunostaining). Nevertheless, we think that SALL4 expression in all subtypes of endometrial carcinomas should be investigated in larger series.

The limited number of cases is the main weakness of this study. Nevertheless, UCE/DCEs are extremely rare subtypes of endometrial carcinomas and our results prompt us to suggest that frequent expression of SALL4 is a reflection of the undifferentiated morphology of these tumors and contributes to the aggressive clinical course of UCE/DCEs. Furthermore, we may suggest that the loss of E-cadherin expression together with fascin and SALL4 immunopositivity, in addition to morphologic features, have an impact in the differential diagnosis of UCE/DCEs from other high-grade endometrial carcinomas. However, SC is partly similar to UCE/DCE in terms of E-cadherin and fascin expression. Nevertheless, we think that our results need to be supported by others from different populations.

When compared with other high-grade uterine carcinomas, UCE/DCEs more frequently present at advanced stage as large tumors with deep myometrial invasion, and displaying abdominal lymph node metastases more frequently,^[3,4,9] as detected in our series. UCE/DCE has worse clinical outcomes than other high-grade endometrial carcinomas with death or recurrence in 55% to 95% of patients.^[1–4,9] Although the median survival of our patients with UCE/DCE was found a little longer than that of previously reported studies (10 vs 6 months, respectively),^[2,4] poor clinical outcomes were evident in our study.

Treatment modalities used in patients with UCE/DCE are very similar to F3-EC in the literature.^[4] The major cause of

progression and death was distant metastasis in the majority of our patients, and local recurrence was experienced in only 2 of the 11 patients. Thus, we advocate the use of chemotherapy, even for patients with early-stage disease.

In conclusion, this is the first study to compare the expressions of stem cells, EMT markers, and fascin between UCE/DCEs and other high-grade endometrial carcinomas. Although nonspecific when used alone, immunohistochemical analysis using a panel including E-cadherin, SALL4, and fascin has shown to have a diagnostic value in patients with UCE/DCE in our study.

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