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ORIGINAL ARTICLE

Role of epithelial–mesenchymal transition factors in the histogenesis of uterine carcinomas

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Abstract

Several subtypes of high-grade endometrial carcinomas (ECs) contain an undifferentiated component of non-epithelial morphology, including undifferentiated and dedifferentiated carcinomas and carcinosarcomas (CSs). The mechanism by which an EC undergoes dedifferentiation has been the subject of much debate. The epithelial–mesenchymal transition (EMT) is one of the mechanisms implicated in the transdifferentiation of high-grade carcinomas. To improve our understanding of the role of EMT in these tumors, we studied a series of 89 carcinomas including 14 undifferentiated/dedifferentiated endometrial carcinomas (UECs/DECs), 49 CSs (21 endometrial, 29 tubo-ovarian and peritoneal), 17 endometrioid carcinomas (grade 1–3), and 9 high-grade serous carcinomas of the uterus, using a panel of antibodies targeting known epithelial markers (Pan-Keratin AE1/AE3 and E-cadherin), mesenchymal markers (N-cadherin), EMT transcription factors (TFs) (ZEB1, ZEB2, TWIST1), PAX8, estrogen receptors (ER), progesterone receptors (PR), and the p53 protein. At least one of the three EMT markers (more frequently ZEB1) was positive in the sarcomatous component of 98% ($n = 48/49$) of CSs and 98% ($n = 13/14$) of the undifferentiated component of UEC/DEC. In addition, 86% of sarcomatous areas of CSs and 79% of the undifferentiated component of UEC/DEC expressed all three EMT-TFs. The expression of these markers was associated with the loss of or reduction in epithelial markers (Pan-keratin, E-cadherin), PAX8, and hormone receptors. In contrast, none of the endometrioid and serous endometrial carcinomas expressed ZEB1, while 6% and 36% of endometrioid and 11% and 25% of serous carcinomas focally expressed ZEB2 and TWIST1, respectively. Although morphologically different, EMT appears to be implicated in the dedifferentiation in both CSs and UEC/DEC. Indeed, we speculate that the occurrence of EMT in a well differentiated endometrioid carcinoma may consecutively lead to a dedifferentiated and undifferentiated carcinoma, while in a type II carcinoma, it may result in a CS.

Keywords Epithelial mesenchymal transition · ZEB1 · ZEB2 · TWIST · Endometrial carcinoma · Dedifferentiated · Undifferentiated

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Introduction

Poorly differentiated endometrial carcinomas (ECs) with solid pattern comprise various subtypes of endometrial malignancies including grade 3 endometrioid carcinoma, serous carcinoma, undifferentiated/dedifferentiated carcinomas, and carcinosarcomas (CSs), all of which are high-grade tumors. The mechanism leading to the loss of glandular and/or epithelial patterns in these tumors is poorly understood and may differ among histological subtypes.

Undifferentiated endometrial carcinoma (UEC) and dedifferentiated endometrial carcinoma (DEC) are aggressive and high-grade endometrial cancers [1]. The UEC and the undifferentiated component of DEC display a similar morphology with no apparent epithelial differentiation. Histologically, they differ from CS, another subtype of high-grade EC that contain a component of non-epithelial morphology. Indeed, in CSs, the non-epithelial component has a sarcomatous and mesenchymal appearance, often associated with heterologous elements (striated muscle, bone, cartilage, adipose differentiation) admixed with high-grade carcinomatous elements such as serous, clear cell, and endometrioid carcinomas. Conversely, the undifferentiated part of DEC/UEC consists of discohesive, epithelioid cells, with a rhabdoid morphology in some cases, without heterologous tissue, and associated side by side with a low-grade endometrioid carcinoma in DEC. Although morphologically different, both UEC/DEC and CSs display some similarities in their histogenesis, including the implication of epithelial–mesenchymal transition (EMT) [2, 3].

EMT is a process resulting in the transition of a cell from an epithelial to a mesenchymal phenotype. This mechanism is involved in early embryogenesis, including gastrulation and neural crest migration [4]. EMT is believed to be reactivated in carcinoma cells, leading to the loss of certain epithelial characteristics such as cellular adhesion and polarity, in order to acquire a mesenchymal morphology and the ability to migrate [4–6]. EMT not only contributes to cancer onset and progression, but it also bestows resistance to cell death and thus to chemotherapy and immunotherapy [5, 7].

The activation of the transdifferentiation program is orchestrated by a network of EMT-inducing transcription factors (EMT-TFs) that include members of the Zinc finger E-box-binding homebox (ZEB), SNAIL, and basic helix-loop-helix (TWIST) families. Expression of these factors can be aberrantly reactivated in organ fibrosis and oncogenesis, triggering pathological traits of EMT [8]. In human cancers of epithelial origin, morphological features of EMT have mostly been reported at the invasive front of the primary tumor, underlining the role of microenvironmental signals generated by the activated stroma in the completion of the transdifferentiation process [9, 10]. This observation has led to the prevailing concept that the role of EMT in tumor progression is limited to cancer cell invasion and metastatic spread [11, 12]. However, recent observations have highlighted the active role of EMT-TFs in the initiation and the development of primary tumors, including triple-negative breast cancers (TNBCs) and melanomas [6, 13]. Consistent with this notion, EMT inducers act as genuine oncoproteins, fostering malignant transformation and primary tumor growth by alleviating key oncosuppressive mechanisms [14–16] and by providing cells with cancer cell stem features, including self-renewal properties [17, 18]. Moreover, recent data have shown that, by impacting initial steps of malignant transformation, the expression of EMT-TFs is a determinant of the genetic history of breast tumorigenesis [19].

Though EMT has been extensively investigated in ECs [20–22], the combined immunoprofile of all three EMT markers, ZEB1, ZEB2, and TWIST1, was not explored in these tumors.

The aim of this retrospective study was to assess immunohistochemical expression of various EMT-associated proteins in uterine carcinomas, including undifferentiated/dedifferentiated carcinomas and gynecological carcinosarcomas, as well as serous and endometrioid carcinomas in order to improve our understanding of the role of EMT in the histogenesis of various histological types of endometrial carcinomas, in particular those with a non-glandular/epithelial morphology.

Material and methods

Case selection A total of 89 cases were retrieved from the files of the pathology department of the Lyon teaching hospital (Centre Hospitalier Croix Rousse, Lyon, France) from 2005 to 2014. This study was conducted in accordance with the Declaration of Helsinki.

These cases included 49 gynecologic CSs (21 uterine, 20 ovarian, 3 fallopian tubes, and 5 peritoneal tumors) six of which had metastases, occurring in different locations: 2 lymph nodes, 2 peritoneal, 1 ovarian, and 1 inguinal. Furthermore, we studied 7 undifferentiated and 7 dedifferentiated uterine carcinomas, 9 high-grade type II endometrial carcinomas (all of which were high-grade serous carcinomas), and 17 type I endometrial carcinomas of different histological grades (10 cases of grade 1–2 and 7 cases of grade 3 endometrioid carcinomas).

Data retrieval The following clinical information was retrieved from electronic medical records: age, medical history, tumor location, and stage (FIGO 2009 staging system).

Slide review Hematoxylin and eosin saffron-stained slides were reviewed for each case. The percentage and type of each component were assessed. The following information was reviewed and recorded: for CSs, the histological type of the carcinomatous component (endometrioid, serous, clear cell, or undifferentiated) and the homologous or heterologous nature of the sarcomatous component. For the carcinomas, the histological subtypes and grade were noted (endometrioid, serous, clear cell, undifferentiated/dedifferentiated).

Immunohistochemical analysis Slides were stained using a Ventana Medical System Benchmark-XT staining module (Tucson, AZ). The antibodies used for this study included epithelial markers (Pan-Keratin AE1/AE3 and E-cadherin), a mesenchymal marker (N-cadherin), EMT-TFs (ZEB1, ZEB2, and TWIST1), PAX8, estrogen receptors (ER), progesterone receptors (PR), and the p53 protein. Further details on the immunohistochemical procedure are shown in Table 1.

Hormone receptors, P53, PAX8, and EMT-TFs were nuclear staining, E-cadherin and N-cadherin were membranous staining, and AE1/AE3 was cytoplasmic staining. The staining was defined as negative (0) or 1+ (mild), 2+ (moderate), or 3+ (strong) for all markers. The extent of staining was extrapolated from the percentage of marked tumor cells. P53 was considered to be abnormally expressed when none of the tumor cells or > 75% displayed a 3+ staining [23]. Hormonal receptors were considered to be positive when > 10% of cells were stained [24]. Positivity was accepted for other markers when > 5% of tumor cells were stained.

To provide a semi-quantitative measurement of EMT phenotype, we calculated EMT scores, corresponding to the product of staining intensity and staining extent (expressed in percentage), for ZEB1, ZEB2, and TWIST1.

Statistical analysis The chi-square or Fisher tests were applied to correlate the expression of the different markers with the histological nature of the different components (carcinomatous, sarcomatous, or undifferentiated), the histological subtype, FIGO stage, and heterologous component.

Results

Histological findings

Type I uterine carcinomas were all of endometrioid subtype, including 8 grade 1, 2 grade 2, and 7 grade 3, according to the FIGO grading system. Type II uterine carcinomas were all high-grade serous carcinomas. The differentiated components of the seven dedifferentiated carcinomas consisted of grade 1 endometrioid carcinoma (Fig. 1a, b).

CSs consisted of an epithelial component that was either serous (37 cases), grade 3 endometrioid (19 cases), clear cell (5 cases), or undifferentiated (15 cases). Twenty cases contained mixed epithelial components, all of high grade. The sarcomatous component showed heterologous elements in 32 cases (65%).

Immunohistological findings

Endometrioid uterine carcinomas

P53 was abnormally expressed in three endometrioid carcinomas all of which were grade 3 (18%). ER and PR were significantly more expressed than in all of the other carcinomas ($p < 0.05$). N-cadherin was mostly expressed at the invasive front in 13 cases (77%). E-cadherin was expressed in all cases. There was no ZEB1 positivity. ZEB2 was positive in one case (grade 3) (6%). Five cases (grade 1 or 2) (36%) expressed TWIST1, usually at the invasive front.

Uterine serous carcinomas

P53 was abnormally expressed in every uterine serous carcinoma (USC), with a significant discrepancy compared to endometrioid carcinomas ($p < 0.05$). N-cadherin was expressed at the invasive front of seven cases (78%), while E-cadherin was positive in all cases, except in the solid area in one case where the staining was focal. There was no ZEB1 positivity. ZEB2 and TWIST1 were positive in 1 (11%) and 2 (25%) USCs, respectively.

Carcinosarcomas

P53 was overexpressed in 83% of cases, in both components. PAX8 was expressed in 84% of the carcinomatous components and 24% of the sarcomatous components. Pan-Keratin

Table 1 Immunohistochemical procedures

Antibody	Clone	Source	Dilution	Staining localization
Pan-Keratin	AE1/AE3	Dako	1/150	Membranous
E-cadherin	36	Ventana	Prediluted	Membranous
PAX8	MRQ-50	Ventana	Prediluted	Nuclear
P53	DO-1	Dako	1/10	Nuclear
ER	SP1	Ventana	Prediluted	Nuclear
PR	1E2	Ventana	Prediluted	Nuclear
N-cadherin	32	BD science	1/100	Membranous
ZEB1	H102	Santa-Cruz	1/100	Nuclear
ZEB2	Polyclonal	Supplied by E. Tulchinsky	1/1000	Nuclear
TWIST1	Twist2C1a	Abcam	1/50	Nuclear

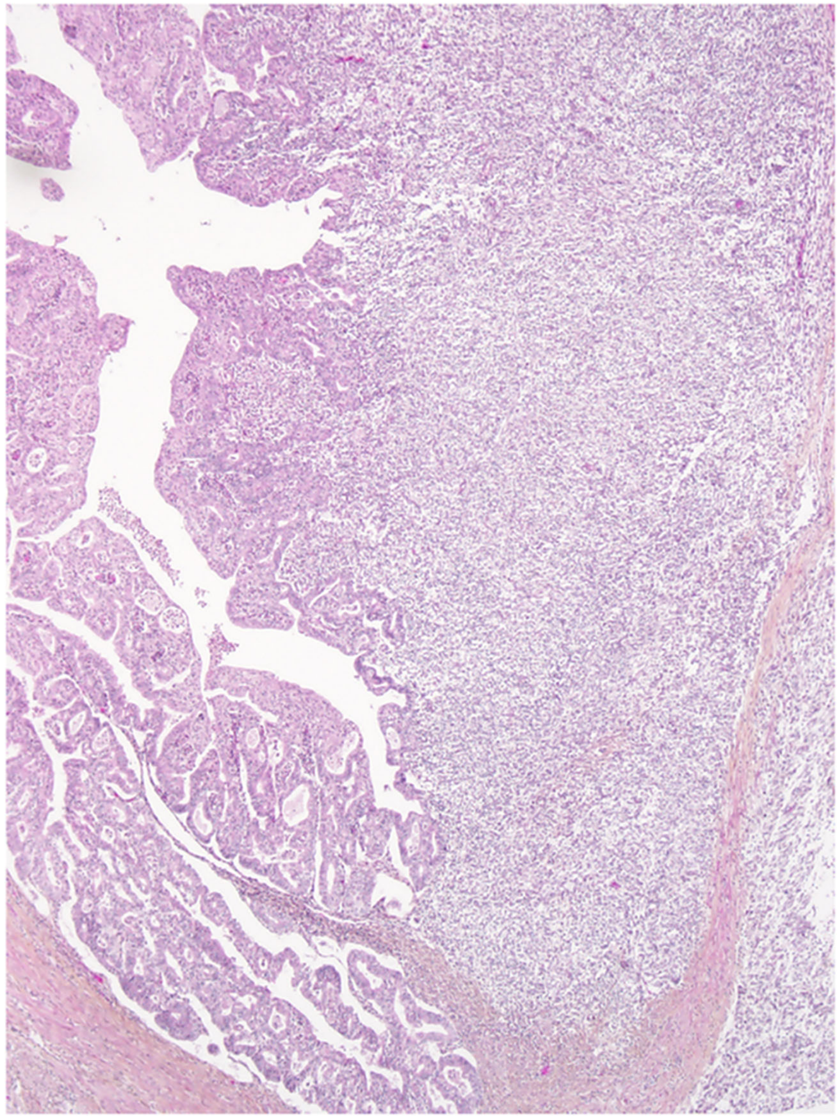

Fig. 1 Dedifferentiated endometrial carcinoma consists of well-differentiated endometrioid carcinoma (left side) and undifferentiated carcinoma (right side) HES, × 250

(AE1/AE3) and E-cadherin were constantly expressed in the carcinomatous components and were focally positive in 44% and 27% of the sarcomatous components, respectively. N-cadherin was expressed in 77% of the carcinomatous components and 84% of the sarcomatous components. In the sarcomatous component, the decrease in epithelial markers was associated with an expression of EMT-related TFs. ZEB1 and ZEB2 were expressed in 98% and 86% of sarcomatous component (EMT scores 1.59 and 0.78) and in 4% and 2% of epithelial component. TWIST1 was expressed in 96% of sarcomatous area (EMT score 1.18) and 21% of carcinomatous zone, usually at the invasive front.

Considering the prognostic value of heterologous components, we compared the different markers between homologous and heterologous components. No significant difference was observed. Furthermore, there was no significant discrepancy in the expression of the different markers according to the FIGO stage.

Metastases versus initial localization for carcinosarcomas ($n = 6$)

In half of the cases, the metastases consisted of the carcinomatous component only. In the other half, both sarcomatous and carcinomatous components had metastasized. No case displayed exclusively sarcomatous metastases. Immunohistochemical staining revealed no significant discrepancy compared to the primary site, specifically regarding EMT-TFs.

UEC and DEC (Table 2) (Fig. 2)

P53 was overexpressed in five UECs and in the dedifferentiated area of one DEC. It was completely lost in the dedifferentiated area of one DEC. In all cases, the undifferentiated areas were negative for ER and PR. In four UECs and three DECs, PAX8 was focally positive, while the differentiated area expressed ER, PR, and PAX8, strongly and diffusely in all cases. AE1/AE3 and

Table 2 Immunohistochemical findings in the 14 cases of undifferentiated endometrial carcinomas and dedifferentiated endometrial carcinomas

Antibody	Expression	Undifferentiated endometrial carcinomas	Dedifferentiated endometrial carcinomas	
			Well differentiated component	Undifferentiated component
		n = 7	*n* = 7	*n* = 7
P53	Abnormal	5 (71%)	0	2 (29%)
ER	Positive	0	7 (100%)	0
PR	Positive	0	6 (86%)	1 (14%)
PAX 8	Positive	4 (57%)	7 (100%)	3 (43%)
AE1/AE3	Positive	5 (71%)	7 (100%)	1 (14%)
E-cadherin	Positive	3 (43%)	7 (100%)	2 (29%)
N-cadherin	Positive	6 (86%)	2 (29%)	7 (100%)
ZEB1	Positive	5 (71%)	0	6 (86%)
ZEB2	Positive	5 (71%)	0	6 (86%)
TWIST1	Positive	5 (71%)	0	7(100%)

E-cadherin were negative in six of seven and five of seven undifferentiated areas of DEC (86% and 71%, respectively) or were only focally positive in the remaining cases. In UEC, AE1/AE3 was expressed in most cases (five of seven, 71%), although focally, while E-cadherin was negative (four of seven cases 57%) or only focally positive (three of seven cases 43%). The majority of UECs (five of seven cases) expressed, strongly and diffusely, all three EMT markers and one case was positive for two markers. Undifferentiated areas of all DEC expressed at least one EMT marker, and six cases (86%) showed strong and diffuse positivity of all three EMT markers, while the well-differentiated areas were totally negative**.** EMT scores as calculated above were quite similar between UEC (ZEB1 1.54, ZEB2 0.37, and TWIST1 0.91) and DEC (ZEB1 1.92, ZEB2 0.3, and TWIST1 0.7).

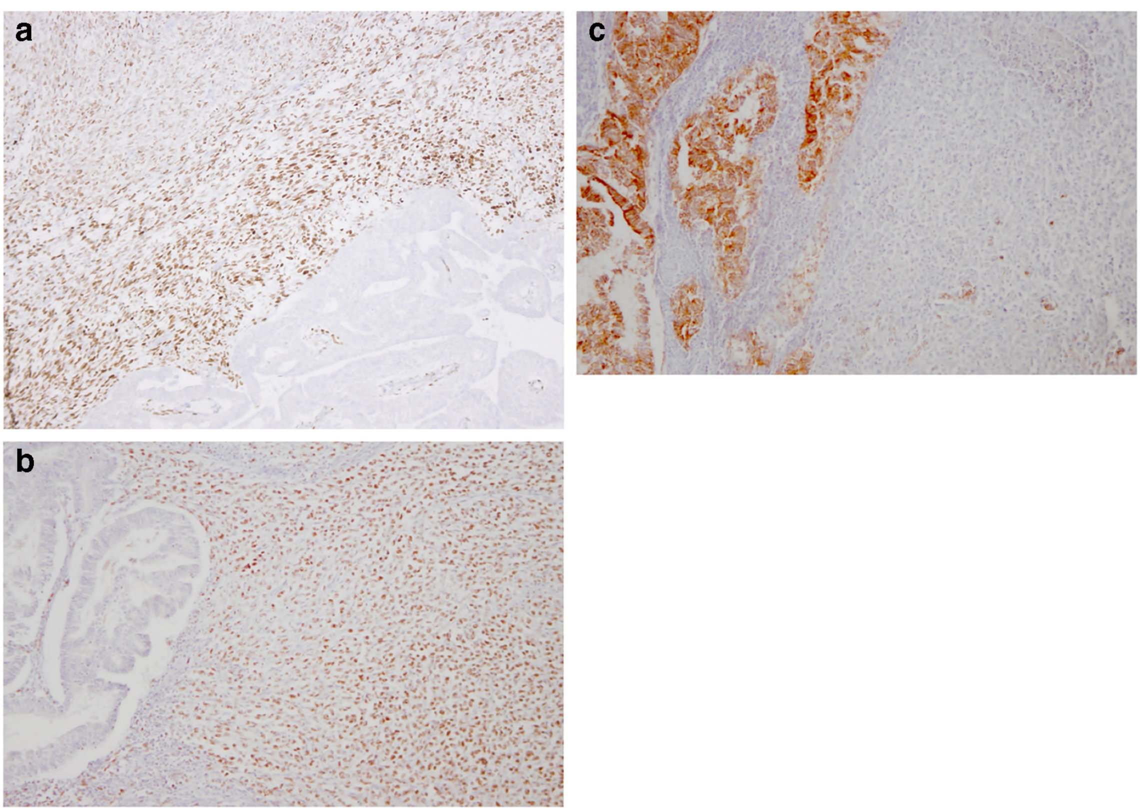

Fig. 2 Immunohistochemical findings in dedifferentiated endometrial carcinomas. TWIST1 (**a** Immunoperoxidase × 100) and ZEB1 (**b** Immunoperoxidase × 100) are positive only in the undifferentiated component, while E-cadherin expression is lost in the undifferentiated component (**c** Immunoperoxidase × 100)

Comparison of the expression of EMT markers in various types of ECs

There was no significant difference in EMT-related markers between the carcinomatous component of CSs and the other types of carcinomas ($p > 0.05$). However, EMT markers were generally more frequently positive in CSs (both components) than in endometrioid and USC.

The comparison of EMT scores between the sarcomatous components of CSs and of UEC/DEC revealed no significant discrepancy.

Discussion

EMT is a central mechanism to both physiological and pathological processes, occurring during embryonic development, tissue regeneration, wound healing, and cancer invasion [25, 26]. It is a significant driver of carcinogenesis and also a major mechanism for generating cancer stem cells [17, 27]. During EMT, the epithelial phenotype marker E-cadherin is replaced by mesenchymal cadherins such as N-cadherin or P-cadherin ("cadherin switch"), leading to a loss of intercellular adhesion [2], promoting not only stromal invasion but also extravasation, circulatory survival, and initial implantation at distant metastatic sites [28]. Several TFs coordinate this process, including the ZEB family (ZEB1/ZEB2), Snail family (SNAI1/Snail, SNAI2/Slug), or TWIST family (TWIST1, TWIST2) [7, 29]. These TFs are regulated by micro-RNAs (miRs), especially the miR-200 family, which inhibits ZEB1 and ZEB2 expressions (transcriptional repressors of E-cadherin), thus maintaining an epithelial phenotype [30, 31]. In turn, ZEB1 and ZEB2 target and downregulate miR200 [32, 33], thus resulting in a double negative feedback loop between ZEB1/ZEB2 and miR-200 in EMT regulation. Loss of E-cadherin expression is characteristic of epithelial cells undergoing EMT. Our results showing a positivity of EMT markers (ZEB1, ZEB2, TWIST1) in all but one case of UECs (86%), in the dedifferentiated component of all DECs (100%), in the sarcomatous component of carcinosarcomas (98%, 86% and 96% of cases), along with a gain in N-cadherin expression and loss of or decrease in E-cadherin positivity in all cases, are consistent with the reactivation of EMT in these subtypes of high-grade endometrial carcinomas. In contrast, endometrial serous and endometrioid carcinomas (grade 1, 2 or 3) displayed EMT-TFs in a lower number of cases. Indeed, ZEB1 was never positive, while ZEB2 was focally expressed in 11% and 6% of cases and TWIST1 in 25% and 36% of cases, respectively. Moreover, E-cadherin expression was maintained in all cases, including high-grade endometrioid and serous carcinomas.

Roméro-Pérez et al. [3] studied 21 cases of UEC and reported frequent overexpression of ZEB1 (62%) associated with a reduced or lost expression of E-cadherin (100%) and a positivity of N-cadherin (50%). MicroRNA of the miR-200 family (targets of ZEB TFs) was significantly decreased, undoubtedly linked to the upregulation of ZEB1. In 2015, Stewart et al. [34] studied the expression of fascin in 5 UECs and 17 DECs. The undifferentiated components showed diffuse fascin expression in 4 out of 5 UECs and 16 out of 17 DECs (91%). The actin-binding protein fascin-1 enhances cellular motility and migration and is normally expressed in mesenchymal tissue only. Fascin expression is upregulated in EMT [35–37] and associated with poorer prognosis [38]. In 2015, Ramalingam et al. [39] studied 32 UECs/DECs and reported altered expression of E-cadherin in 75% of cases (50% of total loss and 25% of focal or patchy positivity). Most recently, Onder et al. [40] studied 11 UECs/DECs and reported total loss of E-cadherin expression in 6 (54.5%) cases and fascin positivity in 9 (81.9%) cases. We also found a decreased expression of this epithelial marker in all cases (totally negative in 64% and focal positivity in 36% of cases). Herein, we demonstrated that in addition to ZEB1 positivity as shown in these studies, UEC/DEC also express ZEB2 and TWIST1. At least one of these three markers was positive in all DEC and six of seven UECs. These results, along with the loss of or decrease in E-cadherin and acquisition of N-cadherin expression (100%), reinforce the notion that EMT may lead to the dedifferentiated morphology in UECs/DECs.

In 2014, Kuhn et al. [41] performed a molecular characterization of UEC/DEC. They provided molecular evidence that these tumors are clonally related to ECs from which they had arisen. Indeed, all somatic mutations detected in the endometrioid components were also found in the undifferentiated components. They also detected additional mutations in the undifferentiated component, supporting the hypothesis of ECs as precursors. They suggested that mutations in *CTNNB1*, *PPP2R1A*, and *TP53* genes may contribute to tumor progression from EC to UEC. However, it has been shown that UEC/DEC are molecularly heterogeneous carcinomas and that different pathways may be implicated in their dedifferentiation [42]. Most (45%) were classified in the hypermutated group with microsatellite instability, and loss of *ARID1* seemed to play a role in the dedifferentiation of endometrioid carcinoma in such cases. However, the copy number low group (28%) with molecular alterations typical of endometrioid carcinomas displayed *PTEN* mutations as main drivers and might acquire an undifferentiated phenotype through mutations in *TP53*. Furthermore, 11% were classified in the serous-like group, lacking the characteristic molecular alterations of endometrioid carcinomas, suggesting that at least some undifferentiated carcinomas might develop through the "serous-like" pathway [42].

In the present study, we show that the loss of epithelial markers reported in the undifferentiated areas of DECs might be related to EMT. UECs/DECs frequently display loss of claudin-4 expression in the undifferentiated areas of the tumor [43], while it has been demonstrated that loss of claudin-4 promotes EMT [44]. Then again, it has been shown that ZEB1 induces EMT by recruiting the SWI/SNF chromatin remodeling protein BRG1 [45] and that inactivating mutations in members of the SWI/SNF complex, such as SMARCA4 (BRG1), are associated with dedifferentiation in these ECs [46]. Taken together, these results are consistent with a role of EMT in the histogenesis of these undifferentiated tumors, leading to dedifferentiation of the endometrioid component. This may be a key issue to understanding the poorer prognosis of dedifferentiated carcinomas compared to grade 3 endometrioid carcinomas, through higher metastatic rates, and resistance to chemotherapy. At least some cases of UEC appear to represent the late stage of DECs, in which the overgrowth of the dedifferentiated area has completely obscured the well-differentiated part of the lesion, likely through EMT. A minority of grade 1 or 2 endometrioid carcinomas expressed TWIST1 (36%) in our series, with only one case of grade 3 showing positivity for one EMT marker (ZEB2). These cases with focal EMT-TF positivity may represent a subset of low-grade endometrial carcinomas that would have progressed to UECs/DECs if left untreated.

The role of EMT in the histogenesis of carcinosarcomas has been comprehensively shown [47–50]. The cases we studied by immunohistochemistry displayed also a significant decrease in epithelial markers (Pan-Keratin and E-cadherin) associated with an expression of EMT-TFs. Indeed, ZEB1, ZEB2, and TWIST1 were expressed in 98%, 86%, and 96% of sarcomatous components and only in 2%, 4%, and 21% of carcinomatous part of CSs, respectively. We found no significant link between the expression of EMT-related markers and FIGO stage at diagnosis or between the carcinomatous component of CSs and the other carcinomas, regarding the expression of these markers. We chose to include CSs of different origins (uterine, ovarian, peritoneal, fallopian tube) in order to compare the expression profile of EMT markers according to the site of origin and found no significant difference. These results confirm the well-established preponderant role of EMT in the histogenesis of gynecological CSs, irrespective of their origin, also supported by numerous other studies. Chiyoda et al. [49] studied the expression of 39 EMT-related genes in 14 CSs compared to 24 endometrioid carcinomas and 8 uterine sarcomas and found that acquired markers of EMT were upregulated in CSs. Castilla et al. [48] compared the epithelial and mesenchymal components of CSs and found a loss of epithelial characteristics, along with acquisition of a mesenchymal phenotype. They studied the microRNA (miRNA) signatures and found a typical EMT expression profile. They also noted a downregulation in miR-203 in endometrial CSs, which is involved in the inhibition of cell stemness. The authors therefore hypothesized a link between stemness phenotypes and EMT in endometrial CSs. As such, many studies suggest a connection between the EMT phenotype and stemness. EMT generates cells with stem-like properties such as self-renewal potential [17, 18, 27].

Regarding the role of miRNAs in EMT regulation, Diaz-Martin et al. [51] identified a core subset of deregulated miRNAs, defining an EMT core miRNA signature, and evaluated the DNA methylation status of selected miRNA loci. Both were evaluated in vitro as well as in vivo, in endometrial CSs, and in other types of endometrial carcinomas. The miRNAs most frequently repressed belonged to the miR200 family. The EMT core signature was validated in endometrial CSs, with a negative correlation between downregulated miRNAs (in particular the miR-200 family) and the level of expression of EMT inducers. They also demonstrated that the downregulation of the miR-200 and miR-205 family occurred through DNA methylation, both in the in vitro model and in endometrial CSs, and showed that the DNA methylation levels on some loci could discriminate CSs from endometrioid endometrial carcinomas. Overall, these results underscore the crucial role of miRNAs in EMT regulation.

Genomic alterations in CSs have also been widely studied, confirming their relatedness to ECs [52–54]. Recently, Cherniack et al. performed a comprehensive genomic analysis of uterine CSs. They found *TP53* mutations in over 90% of cases, mutations in PI3-kinase pathways in half of the cases, as well as other mutations shared with endometrioid or serous endometrial tumors. They calculated EMT scores based on gene signature, providing a quantitative measurement of the phenotype, low scores corresponding to an epithelial phenotype. and high scores to a mesenchymal phenotype. EMT scores in uterine CSs ranked “intermediate” between other gynecologic tumors and sarcomas [53].

Another series revealed an increased frequency of somatic mutations in histone genes H2A and H2B in uterine CSs, possibly indicating a role of these mutations in EMT, therefore possibly instrumental in the evolution of carcinoma to sarcoma [54].

In our study, there was no difference in the expression of EMT markers between primary CSs and the associated metastatic lesions in six cases (100%). As mentioned above, EMT is implicated in promoting invasion and metastatic dissemination. It allows extravasation, circulatory survival, and initial implantation at distant metastatic sites. These processes can be transient, and once the metastatic site has been reached, cells redifferentiate through mesenchymal–epithelial transition (MET) [10]. This may explain the similar immunohistochemical phenotypes we observed between the primary tumors and the metastases. Other studies found similar results regarding the expression of p53, HR, E-cadherin, Snail, and Slug [55, 56].

We found a positivity for ZEB2 and TWIST1 in 6% and 36% of endometrioid carcinomas and in 11% and 25% of serous carcinomas, respectively, with no correlation to FIGO stage. There was no expression of ZEB1.

There was no significant discrepancy in EMT markers between type II *TP53*-mutated carcinomas and type I *TP53*-wild-type carcinomas, despite the role of the p53 protein in EMT. Several studies have shown that the ZEB and TWIST proteins can inhibit p53 and Retinoblastoma protein-dependent pathways, thus preventing cells from undergoing oncogene-induced senescence and apoptosis [14–16]. Wild-type p53 plays a critical function in preventing EMT, by downregulating the expression of ZEB1 and ZEB2, through members of the miR-200 and miR-192 family [57, 58]. In 2013, Dong et al. [59] studied the role of mutant-p53 in endometrial cancer. They found that mutant p53 can bind to, and repress, the promoter of miR-130b, a specific inhibitor of ZEB1, leading to an overexpression of ZEB1. We found no expression of ZEB1 in our nine cases of type II p53-mutated uterine carcinomas, suggesting that *TP53* mutation might not be in itself sufficient to trigger ZEB1-related EMT pathways. Several studies have suggested that a loss of function of p53 is not sufficient to trigger complete transdifferentiation [60]. According to our results, EMT does not seem to be involved in the development of endometrioid or high-grade serous carcinomas.

We found no significant difference in EMT-related markers expression among the epithelial component of CSs and the other histological types of carcinomas. However, Castilla et al. [48] report a significantly more frequent expression of EMT markers as well as "cadherin switch" in the carcinomatous component of CS compared to endometrial endometrioid carcinomas. They suggest that EMT might happen when a specific epithelial context is present, such as in CSs.

Romero-Pérez et al. [47] suggested that ZEB1 overexpression, characteristic of UEC, could help to tell them apart from endometrioid grade 3 carcinomas. Our study confirms that the expression of EMT markers may help pathologists in the differential diagnosis of endometrial carcinomas. Indeed, endometrioid grade 3 carcinomas differ from dedifferentiated carcinomas by the absence of EMT markers (ZEB1 (0%), ZEB2 (6%), TWIST1 (0%)), as assessed by immunohistochemical study, while these markers are positive in all DECs.

Conclusion

We have reported the role of EMT in the histogenesis of undifferentiated/dedifferentiated endometrial carcinomas and confirmed its implication in the histogenesis of gynecological carcinosarcomas, with no difference according to location (uterine, ovarian, peritoneal, or fallopian) or primary versus metastatic site. From this study, we can hypothesize that when EMT occurs in a well-differentiated endometrioid carcinoma, it may consecutively lead to a dedifferentiated and undifferentiated carcinoma, while it may result in a carcinosarcoma when this process occurs in a type II carcinoma.

Author's contribution Tatiana Franceschi: writing of the article and immunohistochemical analyses.

Emeline Durieux: immunohistochemical analyses and selection of the cases and of the paraffin blocks.

Anne Pierre Morel: choosing and providing antibodies for the study and critical review of the manuscript.

Pierre de Saint Hilaire: selection of the patients and surgery and providing surgical tumor material for the study.

Isabelle Ray-Coquard: selection of the patients,

Alain Puisieux: designing the study, critical review of the article.

Mojgan Devouassoux-Shisheboran: designing the study and writing of the article.

Compliance with ethical standards

The study was approved by the Ethics Committee of the Medical Board (CHU Lyon). This study was conducted in accordance with the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

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