Virchows Archiv (2019) 475:121–125
https://doi.org/10.1007/s00428-019-02551-7

BRIEF REPORT

Gestational trophoblastic neoplasms (GTNs) do not display epithelial-to-mesenchymal transition (EMT) features

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Received: 21 December 2018 / Revised: 11 February 2019 / Accepted: 25 February 2019 / Published online: 8 March 2019

Abstract

Although epithelial-to-mesenchymal transition (EMT) has been described in the development of complete hydatidiform moles and the invasion of the maternal decidua by trophoblasts during normal human placentation, its implication in gestational trophoblastic neoplasm (GTN) without villi is totally unknown. We studied the immunoexpression of EMT transcription factors (TWIST1, ZEB1, ZEB2), E-cadherin, and vimentin in 18 trophoblastic tumors and pseudo-tumors. Weak nuclear TWIST1 immunostaining was seen in 5% to 10% of all trophoblastic cells, without ZEB1 and ZEB2 nuclear staining. Trophoblastic cells did not express vimentin, and the expression of E-cadherin was maintained in all cases, indicating the absence of EMT features in GTN.

Keywords EMT · ZEB1 · TWIST1 · Trophoblastic tumors · Gestational trophoblastic neoplasia · PSTT

Introduction

Ng et al. [1] demonstrated that TWIST1 promotes the invasion of the maternal decidua by trophoblasts during normal human placentation, while Luchini et al. [2] reported that this transcription factor (TF) is involved in the development of complete hydatidiform moles, a common type of gestational trophoblastic neoplasm (GTN). This invasive capacity of extravillous trophoblasts (EVTs) is analogous to the epithelial-to-mesenchymal transition (EMT) in cancer progression and metastasis, which is mediated by TFs such as ZEB1, ZEB2, and TWIST1, by inhibiting the expression of E-cadherin proteins [3]. Since significantly lower E-cadherin expression levels have also been described in placenta accreta EVTs [4] and in several gestational diseases including invasive molar pregnancy and choriocarcinomas (CCs) [5, 6], we hypothesized that EMT-related processes may be implicated in GTNs. However, to our knowledge, the expression of TWIST1 and other EMT TFs has not yet been studied in trophoblastic tumors and pseudo-tumors. To this end, we investigated whether the expression of TWIST1, ZEB1, and ZEB2, as well as the epithelial (E-cadherin) and mesenchymal (vimentin) markers, affected benign tumor-like trophoblastic lesions or trophoblastic pseudo-tumors and trophoblastic tumors.

Methods

Eighteen gestational trophoblastic lesions including benign tumor-like trophoblastic lesions or trophoblastic pseudo-tumors ($n = 7$; 5 placental site nodules (PSNs) and 2 exaggerated placental site reactions (EPSRs)) and trophoblastic tumors ($n = 11$; 3 CCs, 4 placental site trophoblastic tumors (PSTTs), and 4 epithelioid trophoblastic tumors (ETTs)) were retrieved from the file of the Department of Pathology, Hospices Civils de Lyon. Specimens were obtained by uterine curettage or surgical hysterectomy. Immunohistochemical analyses were performed on formalin-fixed paraffin-

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Table 1 Epithelial-to-mesenchymal transition (EMT) factor immunohistochemical staining results

Case	Diagnosis	Twist			Zeb1	Zeb2			E-cadherin			Vimentin
		Intensity/%	Type of cells	Localization	%	Intensity/%	Type of cells	Localization	Intensity/%	Type of cells	Localization	%
1	PSN	1+/5	IT	n/P	0	2+/90	IT	c/D	2+/80	IT	m/D	0
2	PSN	1+/10	IT	n/P	0	1+/90	IT	c/D	2+/100	IT	m/D	0
3	PSN	1+/10	IT	n/P	0	2+/90	IT	c/D	2+/70	IT	m/D	0
4	PSN	1+/10	IT	n/P	0	1–2+/90	IT	c/D	2+/100	IT	m/D	0
5	PSN	1+/10	IT	n/P	0	1–2+/90	IT	c/D	2+/100	IT	m/D	0
6	EPSR	1+/10	IT	n/P	0	3+/100	IT	c/D	2+/90	IT	m/D	0
7	EPSR	1+/10	IT	n/P	0	3+/100	IT	c/D	2+/90	IT	m/D	0
8	CC	1+/10	ST > CT/IT	n/P	0	3+/90	ST-CT-IT	c/D	3+/100	ST-CT-IT	m/D	0
9	CC	1+/10	ST > CT/IT	n/P	0	3+/90	ST-CT-IT	c/D	3+/100	ST-CT-IT	m/D	0
10	CC	1+/5	ST > CT/IT	n/P	0	2+/70	ST-CT-IT	c/D	3+/100	ST-CT-IT	m/D	0
11	PSTT	1+/10	IT	n/P	0	3+/90	IT	c/D	2+ à 3+/90	IT	m/D	0
12	PSTT	1+/10	IT	n/P	0	3+/100	IT	c/D	2+/30	IT	m/pv	0
13	PSTT	1+/10	IT	n/P	0	3+/100	IT	c/D	2+/80	IT	m/pv	0
14	PSTT	1+/10	IT	n/P	1+/10	1+/20	IT	c/D	2+/90	IT	m/D	0
15	ETT	1+/10	IT	n/P	0	2+/100	IT	c/D	3+/100	IT	m/D	0
16	ETT	1+/10	IT	n/P	0	2+/100	IT	c/D	1–2+/70	IT	m/D	0
17	ETT	1+/10	IT	n/P	0	2–3+/100	IT	c/D	2–3+/100	IT	m/D	0
18	ETT	1+/10	IT	n/P	0	3+/100	IT	c/D	2+/90	IT	m/D	0

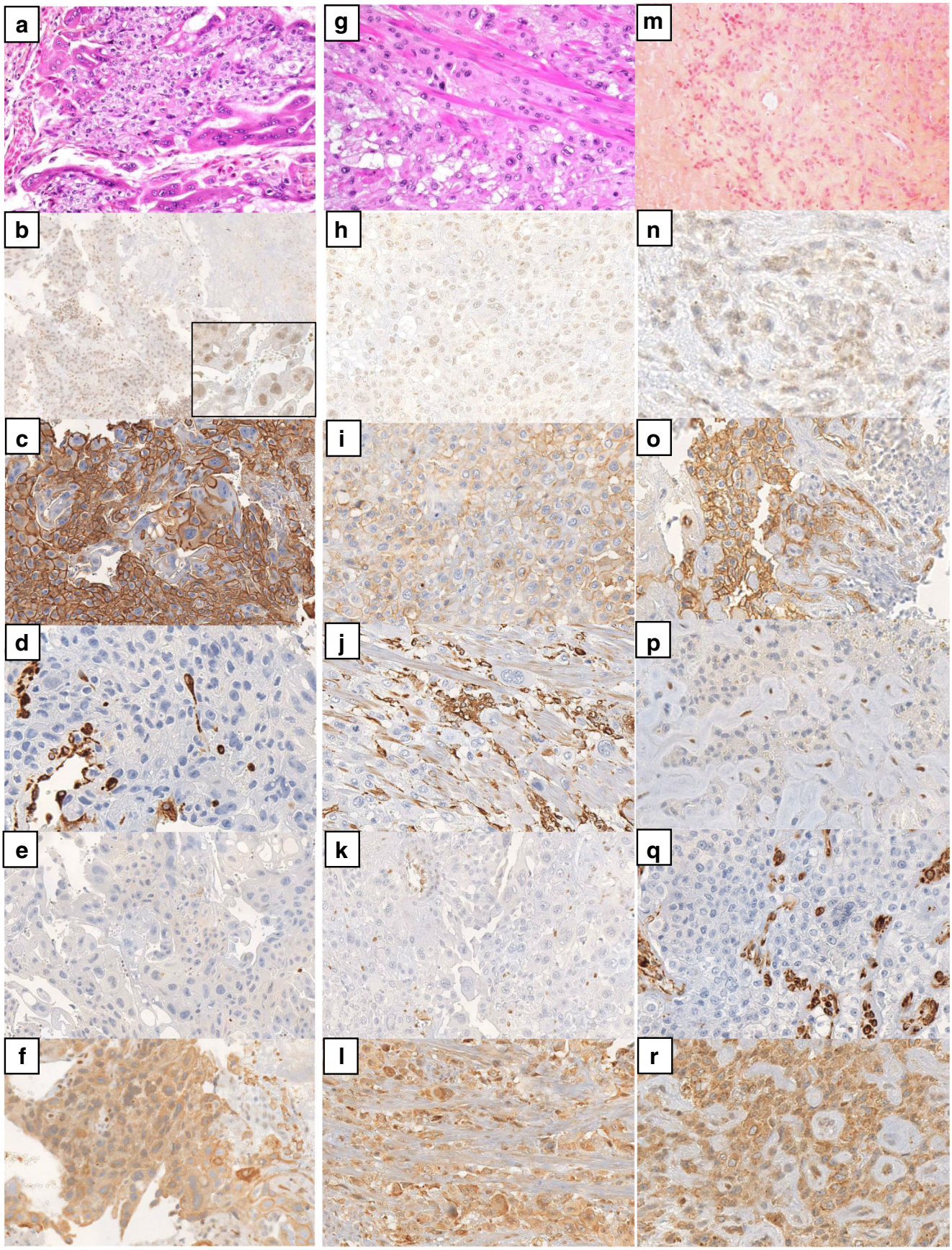
CC choriocarcinoma, *ETT* epithelioid trophoblastic tumor, *PSTT* placental site trophoblastic tumor, *EPSR* exaggerated placental site reaction, *PSN* placental site nodule, *IT* intermediate trophoblast, *ST* syncytiotrophoblast, *CT* cytotrophoblast, *n* nuclear, *c* cytoplasmic, *m* membranous, *P* patchy, *D* diffuse, *pv* perivascular

embedded tissue sections using a Ventana automate (Ultra-XT Benchmark, Ventana Medical Systems SA, Tucson, AZ, USA). The following antibodies were used: anti-TWIST1 (clone twist2C1, 1/50 dilution, Abcam), anti-ZEB1 (clone H102, 1/1000 dilution, Santa Cruz), anti-ZEB2 (polyclonal, 1/1000 dilution, Tulchinsky) (Sayan et al. 2009), anti-E-cadherin (clone 36 mouse, pre-dilution, Ventana), and anti-vimentin (clone V9, 1/40 dilution, DAKO). For all the markers, the percentage of positive cells (nuclear staining for ZEB1, ZEB2, and TWIST1; cytoplasmic staining for vimentin; and membranous staining for E-cadherin) and the intensity of staining were scored (1+ weak, 2+ moderate, 3+ strong). Localization of the staining, pattern of expression (diffuse, focal, perivascular, tumor invasion front), and type of positive cells were also recorded.

Results and discussion

Nuclear TWIST1 immunostaining was weak in 5% to 10% of all trophoblastic cells, irrespective of their benign or malignant status and of staining intensity or localization (invasive front versus other areas) (Table 1) (Fig. 1). However, in CC, the staining was slightly more intense in syncytiotrophoblasts than in cytotrophoblasts (CTB) and intermediate trophoblasts. Our specimens were devoid of ZEB1 staining, while diffuse ZEB2 staining was exclusively observed in the cytoplasm of all trophoblastic neoplasia. Although trophoblastic cells did not express vimentin, the expression of E-cadherin was maintained in all PSNs, EPSRs, and CCs (strong positive membranous staining in 90% to 100% of cells). Half of the ETTs displayed a weak to moderate E-cadherin staining, and

Fig. 1 Expression of epithelial-to-mesenchymal transition (EMT) factors in gestational trophoblastic neoplasms (GTNs): (**a**–**f**) choriocarcinoma (**a** H&E (× 20): trimorphic population of syncytiotrophoblast, intermediate trophoblast, and cytotrophoblast invading the myometrium; **b** (× 10): positive nuclear Twist1 immunostaining in all cell lines with more intensity in syncytiotrophoblast (right inset × 40); **c** E-cadherin expression (× 20) was maintained in the trimorphic population of tumoral cells; **d** trophoblastic cells did not express either vimentin (× 20), Zeb1 (**e**; × 20), or Zeb2 (absence of nuclear staining) (**f**; × 20); (**g**–**l**) PSTT (**g** H&E (× 20): large atypical intermediate trophoblastic cells infiltrate and dissect existing smooth muscle fibers of the myometrium; **h** (× 20): weak nuclear Twist1 immunostaining in trophoblastic cells; **i** (× 20): decreased E-cadherin staining in tumoral cells without acquired vimentin expression (**j**; × 20) nor Zeb1 (**k**; × 20) or Zeb2 (absence of nuclear staining) (**l**; × 20); (**m**–**r**) ETT (**m** H&E (× 20): nest of uniform mononucleate intermediate trophoblastic cells surrounded by necrosis; **n** (× 40): weak nuclear Twist1 immunostaining in tumoral cells; **o** (× 20): heterogeneous E-cadherin expression with moderate to strong membranous staining in tumoral cells; **p** (× 20): trophoblastic cells did not acquire vimentin expression and were negative for Zeb1 (**q**; × 20) and Zeb2 (absence of nuclear staining) (**r**; × 20)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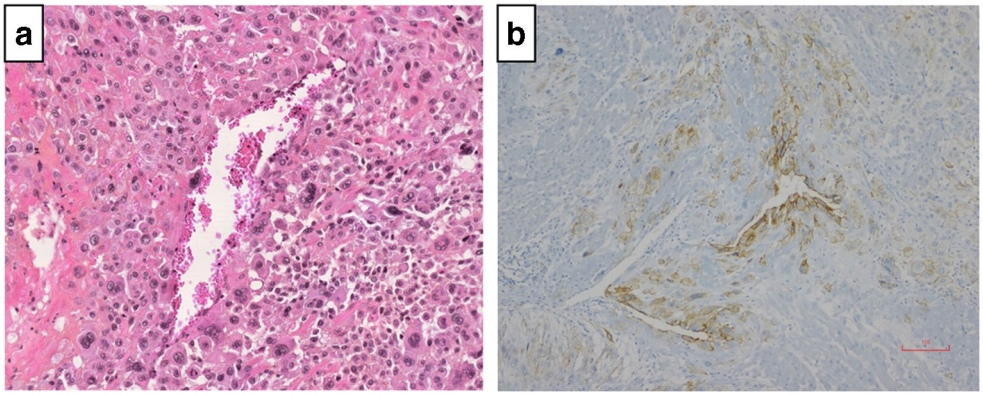

Fig. 2 Placental site trophoblastic tumors (PSTTs) **a** H&E (× 20) Large cells with abundant eosinophilic cytoplasm and convoluted nuclei and marked hyperchromasia replacing the wall of a blood vessel with preservation of the vascular lumen. **b** (× 10) Increased E-cadherin expression in trophoblastic cells that permeate the vascular endothelium vessels

similarly to PSTTs, the immunoreactivity of this latter was weaker and heterogeneous with a varying proportion of positive cells (60–90%). This decrease in the E-cadherin expression in ETTs and in all PSTTs suggests an invasive phenotype of tumor cells. However, a decreased E-cadherin staining was associated neither with acquired vimentin expression nor with stronger TWIST1 staining. Surprisingly, in the two PSTT cases, an increased E-cadherin staining was distinctly observed in trophoblastic cells that permeate the vascular endothelium vessels (Fig. 2). This fluctuation in E-cadherin can possibly be accounted for by the fact that its expression is reversible and can be either up- or downregulated, depending on the stage of tumor progression. Indeed, tumor cells may revert to being more adhesive to form cell aggregates and disseminate into vessels. Tomlinson et al. [7] described this phenomenon in inflammatory breast carcinoma, in which tumor emboli expressed both E-cadherin and β-catenin.

Maybe E-cadherin overexpression in these tumor cells reflects that other mechanisms are implied in the disruption of the cadherin axis. In fact, interaction of E-cadherin with the catenins is required for normal cell adhesive function. This adhesion may be inactivated by mutation of the alpha-catenin gene rather than E-cadherin itself. These alterations were described in prostatic cancer cell lines [8].

With regard to CC, our results diverge from those of a previous study that demonstrated reduced immunoreactivity of E-cadherin in CC compared with that of a normal first-trimester placenta [5]. However, other studies analyzing the expression of E-cadherin in invasive hydatidiform moles and post-molar CCs [9] demonstrated that while CTB cells weakly express E-cadherin in CC, their staining was significantly stronger in infiltrating CCs, in concordance with our findings. This expression could reflect a final state of tumor cell invasion. As mentioned above, ZEB2 was expressed in the cytoplasm of all specimens, corroborating its cellular localization in a variety of tumor tissues, including colorectal cancer, glioma, and renal cell carcinoma, suggesting that cytosolic ZEB2 may have additional EMT-unrelated functions [10, 11].

Our group was the first to examine the expression of EMT TFs in non-molar GTN and benign tumor-like trophoblastic lesions. Based on our findings, it appears that a complex regulation of the E-cadherin expression occurs in the invasive process of GTN. However, the fact that we found no concomitant expression of vimentin, ZEB1, ZEB2, and TWIST1 advocates for its EMT-independent implication. Further work is required to delineate other molecular mechanisms involved in this E-cadherin/GTN axis. Alternatively, metalloproteinases and tissue inhibitors of the metalloproteinases may play a role in GTN progression [12]. Synergistic upregulation of c-Myc, c-erb-2, c-fms, and Bcl-2 oncoproteins has been suggested in the pathogenesis of CC [13], and, similarly, abnormal expression of cell cycle regulatory gene products including cyclins, cyclin-dependent kinases, and p53 have been reported in PSTTs [14]. Finally, our results provide a first step in understanding the pathology of these rare gestational neoplasms, exposing novel unexplored molecular mechanisms.

Contributions of each author Estelle Dubruc: writing of the manuscript, immunohistochemical analyses

Fabienne Allias: immunohistochemical analyses

Anne Pierre Morel: immunohistochemical study, choosing and providing antibodies

François Golfier: selection of the cases

Alain Puisieux: critical review of the manuscript

Mojgan Devouassoux-Shisheboran: design of the study, critical review of the manuscript

Compliance with ethical standards

The study was approved by the ethical committee of Hospices Civils de Lyon.

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

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