

Case Report

Biological role of epithelial-mesenchymal transition markers in the pathogenesis of mural nodules of anaplastic carcinoma in ovarian mucinous cystadenocarcinoma

Qiong Feng^{1,5*}, Jun Deng^{2*}, Long-Jin Xie^{3*}, Jin-Ping Hu^{4*}, Jian Sun³, Xiao-Liang Lou³, Xue-Feng Yu³, Nong-Rong Wang³, Cong Xu³, Xiao-Ni Han³, Lv Zhou⁴, Meng-Meng Wang³, Huan Deng^{3,4,5}

¹Department of Pathology, The Second Affiliated Hospital of Nanchang University, Nanchang, China; ²Department of Emergency, The First Affiliated Hospital of Nanchang University, Nanchang, China; ³Molecular Medicine and Genetics Center, The Fourth Affiliated Hospital of Nanchang University, Nanchang, China; ⁴Department of Pathology, The Fourth Affiliated Hospital of Nanchang University, Nanchang, China; ⁵Renmin Institute of Forensic Medicine, Nanchang, China. *Equal contributors.

Received November 30, 2015; Accepted January 31, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: Ovarian mucinous tumors with mural nodules (MNs) are extremely rare and categorized as follows: sarcomas, sarcoma-like mural nodules (SLMNs), and anaplastic carcinoma. Because the prognosis of each subtype differs essentially, it is urgent to establish a definite diagnosis before the application of tailored therapeutic strategies. Although accumulating evidence improves our ability to classify MNs, the pathogenesis of MNs is largely unknown. We reported the first case of MNs of anaplastic carcinoma expressing the typical epithelial-mesenchymal transition (EMT) immunoprofile in a 69-year-old woman. The lesion was composed of diffused rhabdoid cells with abundant acidophilic cytoplasm on a background of inflammation. Immunohistochemistry was employed to explore the underlying mechanisms. Most of malignant cells exhibited incompletely or negatively membranous staining of E-cadherin. Consistently, the aberrant cytoplasmic and nuclear accumulating of β -catenin, a central component of cadherin-catenin complex, indicated the loss of epithelial features. Consequently, we confirmed the strong-diffuse positivity for Snail and Twist, two pivotal elements of EMT-associated signaling. In addition to cytokeratin, rhabdoid cells co-expressed vimentin indicating the acquisition of mesenchymal characteristics. Immunopositivity for calretinin supported the hypothesis that MNs may derive from mesothelium. Our results led to the conclusion that EMT markers may serve as useful indicators for the differential diagnosis of MNs and help to reveal novel mechanisms in tumorigenesis.

Keywords: Mural nodules, epithelial-mesenchymal transition, mucinous cystadenocarcinoma, mesothelium, immunohistochemistry

Introduction

Ovarian mucinous tumors with mural nodules (MNs) are rare. MNs have been divided into three major types: sarcomas, sarcoma-like mural nodules (SLMNs), and foci of anaplastic carcinoma [1]. Distinction of these three subtypes is not always easy but is important, since each of them carries a different prognosis. SLMNs may represent a kind of reactive lesion rather than true tumor and are associated with favorable clinical behavior [1].

The pathogenesis of MNs is still a matter of debate, in spite of recent advances in diagno-

sis. One of distinguished features of MNs is the heterogenous cell composition. Although the proportions may be different, both epitheloid and sarcomatoid cells can be observed in each type of MNs [1-4]. Immunohistochemical results further demonstrate that the boundary between a SLMN and a nodule of malignant tumor is blurred. SLMNs can co-express cytokeratins and vimentin, indicating that they may originate from submesothelial mesenchymal cells, which undergo partial transformation [1]. In mural nodules of anaplastic carcinoma, cytokeratin reaction is positive in spindle tumorous cells although sometimes only focally [2, 3].

The biological role of EMT markers in MNs

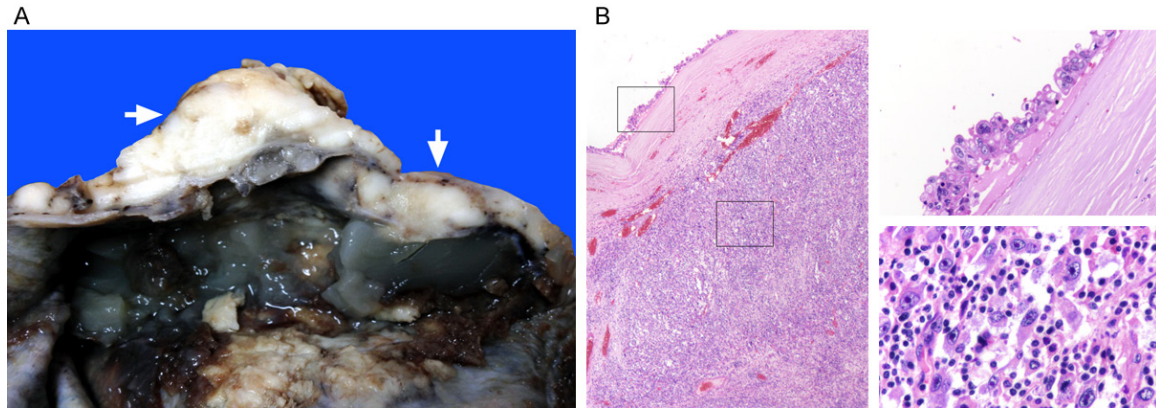


Figure 1. Ovarian mucinous carcinoma with MNs of anaplastic carcinoma. A. Several protruding nodules located on the surface of mucinous carcinoma. B. The significant atypia of lining cells favored the diagnosis of ovarian mucinous carcinoma. The MNs consisted of malignant rhabdoid cells with abundant eosinophilic cytoplasm. The nuclei with prominent nucleoli were eccentric. There was a fibrous septum between mucinous carcinoma and MNs (original magnification $\times 100$, inserts $\times 400$).

Table 1. Summary of primary antibodies used for immunohistochemistry

Antibody	Clone	Supplier	Dilution
Twist	Twist2C1a	Abcam	1:50
Snail	ab180714	Abcam	1:100
Cytokeratin 7	OV-TL 12/30	DAKO	1:100
E-Cadherin	NCH-38	DAKO	1:150
β -Catenin	β -Catenin-1	DAKO	1:150
ER	1D5	DAKO	1:100
PR	PgR 636	DAKO	1:100
Vimentin	V9	DAKO	1:100
Cytokeratin	AE1/AE3	DAKO	1:100
CEA	II-7	DAKO	1:150
CK20	Ks20.8	DAKO	1:100
Desmin	D33	DAKO	1:150
Actin	1A4	DAKO	1:150
Calretinin	DAK-Calret 1	DAKO	1:100

Epithelial-mesenchymal transition (EMT) is commonly known for its transient nature. During EMT, epithelial cells undergo a change in the signaling programs that define cell morphology and reprogram gene expression. Key targets of the pathways include the adherens junction components E-cadherin and β -catenin. In addition to being transcriptionally downregulated and epigenetically switched off, E-cadherin can be proteolytically cleaved for degradation. Consequently, loss of E-cadherin can increase the free pool of β -catenin, which then enters the cytoplasm and even the nucleus. Meanwhile, extensive cytoskeleton remodeling occurs, including switching from a promi-

nent cytokeratin to a vimentin-rich intermediate filament network [5]. Theoretically, the transitioning cells during a given period may coexpress epithelial and mesenchymal markers.

In the present study, we reported a patient with MNs of anaplastic carcinoma in ovarian mucinous cystadenocarcinoma. To gain more insights into the molecular mechanisms underlying MNs, we employed immunohistochemistry to analyze the gene expression profile and provided preliminary data on the involvement of EMT in the pathogenesis of SLMNs.

Case presentation

A 69-year-old female patient presented with lower abdominal pain for three months. Ultrasonography revealed a solid and cystic right ovarian mass occupying almost the entire pelvis, approximately 14 cm in greatest diameter. Preoperative investigations included blood examination, urea, electrolytes, electrocardiogram and liver function, all of which were normal. Serum CA125 was elevated at 144 u/mL (normal < 40 u/mL). Endoscopic examination did not find any tumorous lesion in stomach and colon. The lesion was diagnosed as a carcinoma on frozen sections. Consequently, hysterectomy and unilateral salpingo-oophorectomy were performed.

Histologic findings

The gross tumor measured 15 \times 10 \times 9 cm in size and contained a unilocular cyst with sev-

The biological role of EMT markers in MNs

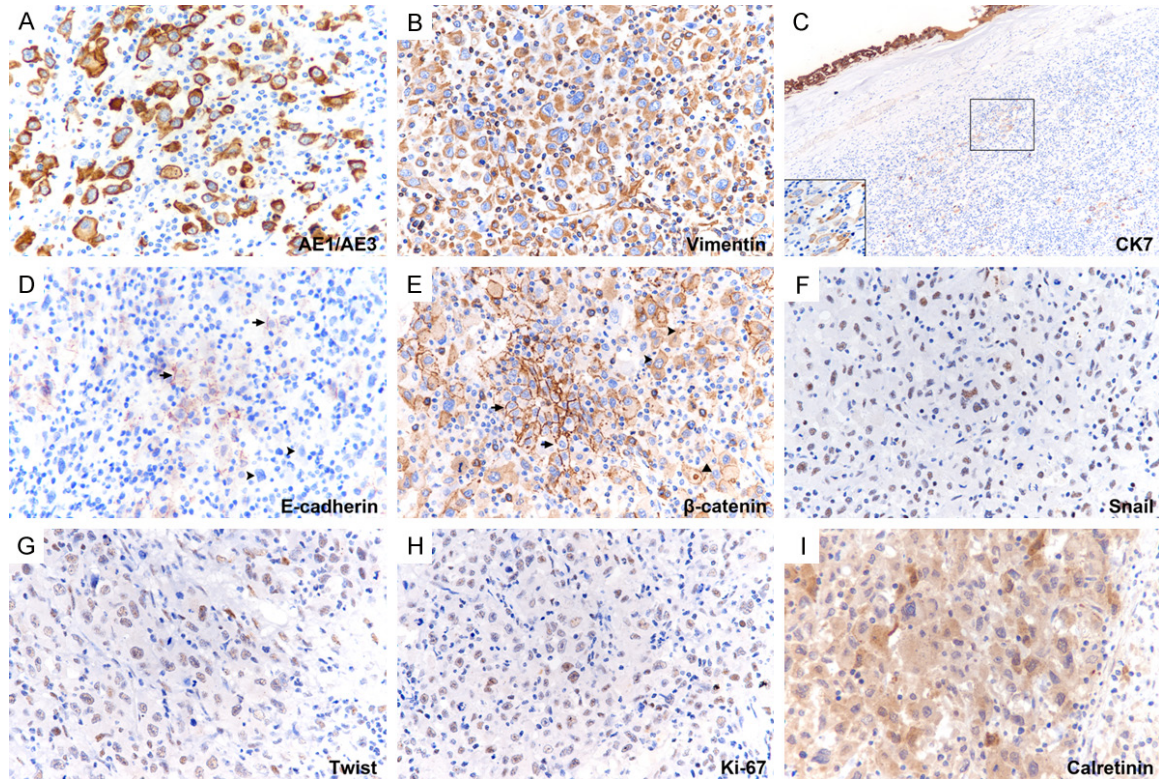


Figure 2. Immunohistochemical profile of MNs. A, B. Malignant cells exhibited strong positivity for both AE1/AE3 and vimentin (original magnification $\times 400$). C. In contrast to the lining cells of ovarian mucinous carcinoma, abdooid cells of MNs showed moderate staining for CK7 (original magnification $\times 100$, inserts $\times 400$). D, E. Incomplete membranous staining for E-cadherin and β -catenin had been observed in most of rabdoid cells (arrow). A few of them showed aberrant cytoplasmic or nuclear accumulation of β -catenin (arrowhead) (original magnification $\times 400$). F, G. Strong and diffuse staining pattern of EMT core elements, Snail and Twist (original magnification $\times 400$). H. The Ki-67 positive malignant cells up to 80% (original magnification $\times 400$). I. The immunopositivity for calretinin indicated the close relationship between MNs and mesothelium (original magnification $\times 400$).

eral whitish nodules. The greatest diameter of MNs ranged from 1.5 to 3 cm (**Figure 1A**). Microscopically, atypical mucinous cells were seen lining the wall of the cysts. Stromal invasion indicated the malignant nature of the cystic lesion. Anaplastic nodules consisted of diffused rhabdoid cells with abundant acidophilic cytoplasm. The nuclei were hyperchromatic with irregular membranes, in which one or more prominent nucleoli can be observed, and the mitotic rate was 70 to 80/50 HPF (**Figure 1B**). Scattered multinucleated tumor giant cells were observed on a background of inflammation. Definite lymphovascular invasion, hemorrhage and necrosis of geographic type were seen (**Figure 1B**).

Immunohistochemistry

Tissue sections were cut in 4-mm slices and incubated with primary antibodies overnight at

4°C (**Table 1**). The chromogenic reaction was developed by using EnVision system (DAKO, Glostrup, Denmark). Anaplastic cells co-expressed AE1/AE3 and vimentin (**Figure 2A, 2B**), but were negative for ER, PR, CEA, CK20, MyoD1, myogenin, desmin and actin (data not shown). Mucinous lining cells exhibited strong positivity for CK7 (**Figure 2C**). By contrast, the anaplastic cells showed moderate immunopositivity for CK7 (**Figure 2C**). Although mild and incomplete membranous staining pattern was detected, most of malignant cells did not expressed the cell adhesion molecule E-cadherin (**Figure 2D**). In line with E-cadherin staining, a few of rhabdoid cells exhibited incomplete membrane positivity for β -catenin, which anchors to the intercellular domain of E-cadherin and establish cadherin-catenin complex. Most of them showed aberrant cytoplasmic localization of β -catenin (**Figure 2E**).

The biological role of EMT markers in MNs

Interestingly, both cytoplasmic and nuclear immunopositivity for β -catenin was detected in some scattered malignant cells (**Figure 2E**). Diffuse aggregation of EMT core elements Snail and Twist was detected in the nucleus (**Figure 2F, 2G**). Ki-67 index was up to approximately 80% (**Figure 2H**). Malignant cells also expressed calretinin, a traditional mesothelium marker (**Figure 2I**).

Discussion

Although most patients with malignant MNs received postoperative adjuvant treatment, the mortality rate approaches 50% [6]. Thus, it is urgent to clarify the three major subtypes of MNs. The differential diagnosis is traditionally based on morphological features such as size, circumscription, inflammation reaction background, multinucleated giant cells, and presence of vascular invasion. Unfortunately, the establishment of a definite diagnosis is always difficult because of the overlapping histological characteristics. For example, spindle cells, which have been considered to be a hallmark of SLMN, are also found in anaplastic nodules [2, 3, 7]. Additionally, relatively sharp circumscription can be observed in a carcinomatous nodule [8]. Issues are also raised in tumors containing components of both SLMN and anaplastic nodules [9].

We employed immunohistochemistry to explore whether EMT involved in the pathogenesis of MNs. E-cadherin/ β -catenin complex is one of the most important targets of EMT. The loss of E-cadherin through downregulation or deregulation can lead to the aberrantly cytoplasmic accumulation of β -catenin, which in turn enters nucleus and modulates the consequently transcriptional events of EMT. In this study, most of rhabdoid cells showed aberrant cytoplasmic accumulation of β -catenin, indicating that these epithelium-derived cells may be losing their characteristics. Consistently, the majority of anaplastic cells were negative for E-cadherin, and a few of them exhibited mild and incomplete membrane positivity for E-cadherin. We further demonstrated for the first time that up-regulated expression levels of Snail and Twist, two core transcriptional elements of EMT, were associated with the tumorigenesis of MNs. Hemorrhage and inflammation may serve as powerful drivers of EMT and promote the malig-

nant transdifferentiation of epithelial cells toward mesenchymal cells [10]. Consequently, enhanced expression of pro-EMT elements such as Snail and Twist, and suppression of the junctional cadherin-catenin complexes contribute to extensive cytoskeleton remodeling and morphological changes [11]. Asynchronous activation may result in a heterogenous cell population and different immunoprofiles.

As for the cellular origin, accumulating evidence indicate that MNs may derive from mesothelium [10]. Of note is the fact that in addition to the close spatial relationship, the strong immunopositivity for calretinin, a typical mesothelium marker, further supported the above hypothesis [10]. Furthermore, ultrastructural features of tumor cells of MNs are similar to that of mesothelium [13].

In summary, we have identified for the first time EMT markers may serve as useful indicators for the differential diagnosis of MNs. Although, we provided preliminary evidence for the involvement of EMT in the pathogenesis of MNs of anaplastic carcinoma, further studies will be required to clarify the exact molecular mechanisms.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (No. 81300347), the Natural Science Foundation of Jiangxi Province, China (No. 20132-BAB205037, 20151BAB215008, 20151BBG-70200), and Foundation of Jiangxi Educational Committee (No. GJJ14192), Foundation of Health and Family Planning Commission of Jiangxi Province (No. 20155592, 20155103, 20161086, 20161093).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Huan Deng, Department of Pathology, The Fourth Affiliated Hospital of Nanchang University, 133 South Guangchang Road, Nanchang 330003, China. E-mail: beandeng@hotmail.com

References

- [1] Bague S, Rodriguez IM and Prat J. Sarcoma-like mural nodules in mucinous cystic tumors

The biological role of EMT markers in MNs

- of the ovary revisited: a clinicopathologic analysis of 10 additional cases. *Am J Surg Pathol* 2002; 26: 1467-1476.
- [2] Provenza C, Young RH and Prat J. Anaplastic carcinoma in mucinous ovarian tumors: a clinicopathologic study of 34 cases emphasizing the crucial impact of stage on prognosis, their histologic spectrum, and overlap with sarcomalike mural nodules. *Am J Surg Pathol* 2008; 32: 383-389.
- [3] Yamazaki H, Matsuzawa A, Shoda T, Iguchi H and Kyushima N. Ovarian mucinous cystic tumor of borderline malignancy with a mural nodule of anaplastic spindle cell carcinoma: a case report. *J Ovarian Res* 2013; 6: 86.
- [4] Prat J and Scully RE. Sarcomas in ovarian mucinous tumors: a report of two cases. *Cancer* 1979; 44: 1327-1331.
- [5] Sleeman JP and Thiery JP. SnapShot: The epithelial-mesenchymal transition. *Cell* 2011; 145: 162, e1.
- [6] Baergen RN and Rutgers JL. Mural nodules in common epithelial tumors of the ovary. *Int J Gynecol Pathol* 1994; 13: 62-72.
- [7] Nichols GE, Mills SE, Ulbright TM, Czernobilsky B and Roth LM. Spindle cell mural nodules in cystic ovarian mucinous tumors. A clinicopathologic and immunohistochemical study of five cases. *Am J Surg Pathol* 1991; 15: 1055-1062.
- [8] Czernobilsky B, Dgani R and Roth LM. Ovarian mucinous cystadenocarcinoma with mural nodule of carcinomatous derivation. A light and electron microscopic study. *Cancer* 1983; 51: 141-148.
- [9] Fujii S, Konishi I, Kobayashi F, Okamura H, Yamabe H and Mori T. Sarcoma-like mural nodules combined with a microfocus of anaplastic carcinoma in mucinous ovarian tumor. *Gynecol Oncol* 1985; 20: 219-233.
- [10] Demirel D, Gun I, Kucukodaci Z, Balta AZ and Ramzy I. Primary retroperitoneal mucinous cystadenoma with a sarcoma-like mural nodule: an immunohistochemical study with histogenetic considerations and literature review. *Int J Gynecol Pathol* 2013; 32: 15-25.
- [11] Lamouille S, Xu J and Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 2014; 15: 178-196.
- [12] Matias-Guiu X, Aranda I and Prat J. Immunohistochemical study of sarcoma-like mural nodules in a mucinous cystadenocarcinoma of the ovary. *Virchows Arch A Pathol Anat Histopathol* 1991; 419: 89-92.
- [13] Fujii S, Konishi I, Okamura H and Mori T. Mucinous cystadenocarcinoma of the retroperitoneum: a light and electron microscopic study. *Gynecol Oncol* 1986; 24: 103-112.