Case Report

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

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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 diagnosis. 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

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 prominent 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 colorectum. 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-
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).
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 malignant 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.

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Disclosure of conflict of interest

None.

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References


