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Original contribution

The clinical role of epithelial-mesenchymal transition and stem cell markers in advanced-stage ovarian serous carcinoma effusions☆

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Summary We recently identified gene signatures that allow classification of ovarian carcinoma into 5 distinct clinically relevant groups. In the present study, we investigated the clinical role of 10 protein products of the discriminating genes, with focus on epithelial-mesenchymal transition and stem cell markers. Expression of E-cadherin, N-cadherin, P-cadherin, Zeb1, HMGA2, Rab25, CD24, NCAM (CD56), Sox11, and vimentin was assessed in 100 advanced-stage (International Federation of Gynecology and Obstetrics stages III-IV) serous ovarian carcinoma effusions using immunohistochemistry. Results were analyzed for association with clinicopathological parameters, including chemotherapy response, and survival. All 10 proteins were frequently expressed in carcinoma cells. HMGA2 expression was related to older age ($P = .015$). HMGA2 and NCAM expression was related to stage III disease ($P = .011$ and $P = .023$, respectively), and NCAM was overexpressed in peritoneal compared with pleural effusions ($P = .001$). Vimentin and Zeb1 expression was significantly related to poor chemotherapy response at diagnosis ($P = .005$ and $P = .017$, respectively). The associations between NCAM and peritoneal localization and of vimentin and poor chemoresponse were retained after Bonferroni correction. NCAM expression was associated with a trend for shorter overall survival in univariate survival analysis ($P = .187$), but emerged as an independent prognosticator in Cox multivariate analysis ($P = .042$). This study identifies vimentin and Zeb1 as markers of poor chemoresponse in metastatic serous ovarian carcinoma effusions and suggests NCAM as potential prognostic marker in metastatic disease. The generally limited prognostic role of the studied markers

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emphasizes the difficulty in applying data obtained in studies of primary ovarian carcinomas to analyses of ovarian carcinoma effusions, reflecting the unique biology of the latter.

1. Introduction

Ovarian cancer, consisting predominantly of ovarian carcinoma (OC), is the most fatal gynecologic malignancy. The combination of aggressive cytoreductive surgery and neoadjuvant or adjuvant platinum/paclitaxel-based chemotherapy has prolonged survival in OC. However, chemotherapy resistance remains a major determinant of treatment failure and unfavorable clinical outcome, and most patients consequently still die of their disease [1,2]. The development of malignant peritoneal, and less commonly, pleural effusions constitutes an almost universal finding in advanced-stage OC [3], and OC cells in effusions have been growingly perceived to represent a chemoresistant population with cancer stem cell characteristics [4,5], highlighting the need to better understand their genotypic and phenotypic characteristics.

Epithelial-mesenchymal transition (EMT) is a process by which epithelial cells assume mesenchymal characteristics, facilitating migration through the extracellular matrix and settlement in areas of new organ formation during embryogenesis. Wound healing represents another form of physiological EMT, whereas pathological EMT occurs in tissue fibrosis and cancer [6–8]. EMT is induced by multiple signals, including growth factors, the Wnt signaling pathway, integrins, Notch transcription factors, prostaglandin E_2, cyclooxygenase-2, and hormones [8].

During EMT, carcinoma cells lose their epithelial characteristics and acquire mesenchymal properties that promote extracellular matrix invasion and distant metastasis. This occurs through down-regulation of E-cadherin, cytokeratins, ZO-1, claudins, occludin, laminin-1, entactin, MUC-1, and the microRNA 200 family, and acquisition of the transcription factors Snail1, Snail2, Twist, Zeb1 and Zeb2/SIP1, E47, KLF8, E2.2, Goosecoid, LEF-1, and FoxC2, as well as N-cadherin, vimentin, fibronectin, miR10b, and miR21 [8–10].

In OC, particularly serous carcinoma, the balance between the epithelial and mesenchymal phenotypes is complex, in part due to the inherent nature of serous carcinoma cells to express mesenchymal markers, such as vimentin and N-cadherin. During OC progression, both EMT and the reverse process, mesenchymal-epithelial transition (MET) occur, and OC cells in effusions up-regulate E-cadherin and repress Snail1 and Snail2 expression compared with primary carcinomas [11–13].

We recently generated a classification system for OC based on the gene expression patterns of 1538 OC, including both public databases and our tumor material. OC could be classified into 5 subgroups—Epi-A, Epi-B, Mes, Stem-A, and Stem-B—of which Epi-B and Stem-A signatures were independent prognosticators of good and poor survival, respectively [14]. In the current study, we evaluated the expression of 10 protein products of genes identified by significance analysis of microarray [15] as differentiators between the above groups. Among these 10 genes, 4 were significantly down-regulated and 6 were significantly up-regulated in either Mes or Stem-A tumors (significance analysis of microarray $q < 10$), the groups that had the worst outcome. Proteins were chosen based on antibody availability and performance from an initial group of 20 markers. In order to minimize the effect of clinical parameters on outcome, we analyzed a homogenous series of 100 prechemotherapy effusions tapped at diagnosis from 100 patients with International Federation of Gynecology and Obstetrics (FIGO) stage III-IV serous OC. All patients received platinum-based chemotherapy. Immunohistochemistry (IHC) results were analyzed for association with clinicopathological parameters, chemoresponse, and survival.

2. Materials and methods

2.1. Patients and material

Specimens and clinical data were obtained from the Department of Gynecologic Oncology, Norwegian Radium Hospital during the years 1998 to 2006. Informed consent was obtained according to institutional and national guidelines. Study approval was given by the Regional Committee for Medical Research Ethics in Norway.

Fresh nonfixed serous carcinoma effusions (n = 100; 72 peritoneal, 28 pleural; 80 OCs, 16 peritoneal carcinomas, 4 tubal carcinomas) were obtained prechemotherapy at diagnosis from 100 patients. Because of their closely linked histogenesis and phenotype, all tumors are referred to as OC henceforth. Fifty-two patients had primary surgery, 36 had secondary debulking, and 12 patients only received chemotherapy. All patients received platinum-based therapy, 82 combined with paclitaxel.

Effusions were submitted for routine diagnostic purposes and processed immediately after tapping. Cell blocks were prepared using the Thrombin clot method. Diagnoses were established using morphology and IHC. Clinicopathological data are detailed in Table 1. Grading was according to FIGO.