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Expression of MUC1 in primary and metastatic human epithelial ovarian cancer and its therapeutic significance

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Abstract

Background. MUC1 is associated with cellular transformation and tumorigenicity and is considered as an important tumor-associated antigen (TAA) for cancer therapy. The objective of this study was to evaluate the patterns of MUC1 expression in primary tumors and metastatic lesions in the advanced stages of epithelial ovarian cancers (EOCs) and correlate the expression with clinicopathological features.

Methods. The expression of MUC1 was examined on frozen tissue sections from primary EOC ($n=42$), the matched metastatic lesions ($n=30$) and paraffin-embedded tissue sections from primary EOC ($n=60$), normal ovarian tissues ($n=20$) using immunohistochemistry (IHC) by monoclonal antibody (MAb) C595.

Results. The expression of MUC1 was found in 92% (39/42) of EOC and 90% (27/30) of the matched metastatic lesions in frozen tissue sections respectively while the expression of MUC1 was found in 95% (57/60) of EOC and 5% (1/20) of normal ovarian tissues in paraffin-embedded sections respectively. Most of the tumors showed moderate to strong intensity staining while normal ovarian tissues only showed weak intensity staining. The overexpression of MUC1 was significantly associated with various progression parameters such as tumor stage, grade, residual disease status and presence of ascites ($P<0.05$).

Conclusions. MUC1 is overexpressed in above 90% of late stage of EOC and of metastatic lesions but not in normal ovarian tissues, and the high expression of MUC1 is correlated with EOC progression. MUC1 antigen may be a useful therapeutic target to prevent the development of incurable, recurrent metastatic EOC.

Keywords: Monoclonal antibody; Epithelial ovarian cancer; MUC1; Tumor-associated antigen; Metastasis

Introduction

Ovarian cancer is the most fatal malignancy of the female genital tract in industrialized countries [1], and the second most common cause of death of the gynecological malignancies in China [2]. The overall rate of ovarian cancer in women from China has been increasing [3,4]. The high mortality rate is usually ascribed to late diagnosis of this tumor, which lacks early symptoms. Despite advances in surgery and chemotherapy over the past 20 years, overall survival has not changed significantly in patients with ovarian cancer. The current treatment for advanced stage disease includes debulking surgery followed by platinum-based chemotherapy, polychemotherapy, hormonal therapy and signal transduction inhibitors [5]. Although these approaches have yielded responses in 60–80% of patients with advanced-stage disease, the majority of ovarian cancer patients eventually relapse and become refractory to additional treatment.

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Conventional cancer chemotherapy often results in severe side effects, owing to its non-specific modes of action. Until recently, there was no satisfactory adjuvant treatment following surgery and chemotherapy. Therefore, there is an urgent need to develop new approaches for improving the long-term survival of ovarian cancer patients.

The progression of epithelial ovarian cancer (EOC) from primary to metastatic disease is associated with a number of molecular and genetic changes. A variety of changes in genomic structure, growth factor receptors, proto-oncogens and tumor suppression genes have been identified in ovarian cancer [6]. These changes can affect the expression of specific tumor-associated antigens (TAAs) or receptors on the cell surface. Identification of molecular aspects of ovarian cancer growth and the enhancement of cancer cell motility, detachment from the primary tumor, attachment to the peritoneum and invasion of subperitoneal tissue have become the central focus in the development of molecular-targeted therapy for ovarian cancer [7,8]. Targeting cancer surface TAAs with a targeting vector or a vaccine is a new developing area and may have a promising future for control of the late stage and recurrent EOC.

MUC1 is a highly glycosylated type I transmembrane glycoprotein that is aberrantly overexpressed on the cell surface of multiple carcinomas. Cancer-associated MUC1 is structurally different from normal MUC1 in that the former has shorter and less dense O-glycan chains, exposing novel regions of the protein core [9]. This reduced glycosylation permits the immune system to access the peptide core of the tumor-associated underglycosylated MUC1 antigen (uMUC1) and reveal epitopes that are normally masked. This feature allows the design of an antibody that discriminates between normal cells and adenocarcinoma cells. The biological function of MUC1 may be in part due to its large size and the extended rigid structure. It has been proposed that enhanced levels of MUC1 expression by cancer cells may mask extracellular domains from immune surveillance, confer a survival advantage on malignant cells and play an important role in the ability of tumors to invade and metastasize [10].

C595 is an IgG_3, murine MAb raised against the protein core of human MUC1 (urinary epithelial mucin1) [11]. Epitope mapping has shown that C595 recognizes a tetrapeptide motif (RPAP) within the protein core of MUC1 mucin that contains a large domain of multiples of a highly conserved 20-amino-acid-repeat sequence (PDTRPAPGSTAPPAHGVTSA) [11,12]. The reactivity of the C595 MAb with synthetic peptides (a recombinant diabody fragment) containing this motif permits efficient antibody purification using peptide-epitope affinity chromatography, which, unlike other methodologies, enables exclusion recovery of functionally active antibody [13]. The purpose of this study was to examine MUC1 expression on primary EOC and its metastatic lesions in both frozen and paraffin sections using immunohistochemistry (IHC) with C595 MAb and investigate the association of staining patterns with clinicopathologic features. The results may have implications in the treatment of recurrent EOC in late stage and for developing new therapies.

Materials and methods

Patients

For fresh surgical specimens, the study population consisted of 42 primary EOC and 30 corresponding intraperitoneal metastatic lesions. Of 42 patients, 30 were serous, 5 mucinous, 3 endometrioid and 4 undifferentiated ovarian carcinomas. The mean age was 53±15 years (range, 45–71). Four tumors were in stage II, 32 tumors were in stage III, and 6 tumors were in stage IV. 95% of patients had ascites. All samples were collected at Henan Tumor Hospital from 2003 to 2006.

For paraffin-embedded sections, the study population consisted of 60 patients diagnosed with EOC from the surgical pathology files of the Department of Pathology from 2001 to 2006. Of 60 patients, 46 were serous carcinomas, 4 mucinous carcinomas, 2 endometrioid carcinomas and 8 undifferentiated carcinomas. The mean age was 54±17 years (range, 42–73). Five tumors were in stage I, 6 tumors were in stage II, 47 tumors were in stage III and 2 tumors were in stage IV. 92% of patients had ascites. Twenty specimens of normal ovarian tissues (control) were obtained from cervical cancer (early stage) patients with a mean age of 51±14 years (range, 40–70), who underwent operation during the same period.

All patients received primary surgery at the Department of Gynecological Oncology, Henan Tumor Hospital, China. None of the patients received chemotherapy before operation. Clinical data were obtained by retrospective review of the medical records. Institutional Review Board approval had been obtained prior to the initiation of this study. Tumors were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) criteria [14]. The details of patients' characteristics are summarized in Table 1.

Tissue specimens

For frozen sections, surgical specimens were collected within 30 min of surgery. Approximately 4 mm diameter samples of each tissue type were snap frozen in liquid nitrogen and stored at −70 °C. Tissues for frozen sectioning were cryoprotected in OCT (Tissue-Tek, Torrance, CA, USA), mounted on a cryostat specimen holder in liquid nitrogen and 5 μm serial sections were cut using a Zeiss Micron cryostat. Sections were collected on Superfrost Plus slides and air-dried overnight (o/n) at room temperature (RT). One slide was processed immediately for routine hematoxylin and eosin (H&E) staining while other

Table 1
Patient characteristics for primary EOP from paraffin and frozen sections

Tissue type	Paraffin sections	Frozen sections
Number of patients	60	42
Age mean±SD (years)	54±17 years	53±15 years
Range	42–73	45–71
Clinical stage (FIGO)	No. %	
I	5 (8%)	
II	6 (10%)	4 (10%)
III	47 (78%)	32 (76%)
IV	2 (4%)	6 (14%)
Ascites		
No	5 (8%)	2 (5%)
Yes	55 (92%)	40 (95%)
Histology		
Serous	46 (77%)	30 (71%)
Mucinous	4 (7%)	5 (12%)
Endometrioid	2 (3%)	3 (7%)
Undifferentiated	8 (13%)	4 (10%)
Grading		
Grade 1	6 (10%)	3 (7%)
Grade 2	17 (28%)	7 (17%)
Grade 3	37 (62%)	32 (76%)
Residual tumor after first surgery		
No	8 (13%)	4 (10%)
Yes	52 (87%)	38 (90%)