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Original contribution

Exploring the peritoneal surface malignancy phenotype—a pilot immunohistochemical study of human pseudomyxoma peritonei and derived animal models☆

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Summary Peritoneal surface malignancies are characterized by the propensity for tumor growth on peritoneal surfaces without development of extraperitoneal metastases, but the molecular basis for this phenomenon is incompletely understood. Five human tumors and corresponding orthotopic animal models of human pseudomyxoma peritonei and peritoneal mucinous carcinomatosis from colorectal carcinoma were extensively characterized by immunohistochemical analysis of molecular markers of tissue differentiation (carcinoembryonal antigen, CK20, CK7, and vimentin), proliferation and metastasis (Ki-67, vascular endothelial growth factor, and S100A4), mucins (MUC1, MUC2, MUC4, MUC5AC), and adhesion molecules (E-cadherin, N-cadherin, P-cadherin, claudin 1, claudin 3, and claudin 4). Macro- and microscopic growth patterns of implanted human tissues were preserved through passages in the animals, as were with few exception immunohistochemical staining profiles, supporting the relevance of the models as tools for studying the human disease. Tissue differentiation marker expression was in accordance with previously published results and high Ki-67 score confirmed high proliferative capacity, whereas absence of metastatic capacity was supported by low expression levels of the studied metastasis markers. These mucinous tumors expressed high levels of MUC2 and MUC4, whereas MUC1 was not expressed and MUC5AC expression was variable. Similarly, specific adhesion molecules from the cadherin and claudin families were shown to be of relevance in the investigated samples. The results indicate that mucinous peritoneal surface malignancies of intestinal origin are characterized by the presence of specific molecular markers and represent a step toward understanding the complexity of this intriguing phenotypic entity.

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1. Introduction

The term *peritoneal surface malignancy* is used to describe isolated intraperitoneal (IP) tumor growth without development of extraperitoneal metastases and rests on the conception of the peritoneum as a barrier against disease spread [1]. The peritoneal cavity is then viewed as an isolated compartment, and localized disease may be amenable to potentially curative local treatment strategies, such as complete surgical removal of tumor tissue combined with local (IP) chemotherapy [2,3]. However, in, for instance, peritoneal carcinomatosis (PC) from colorectal cancer (CRC), only a small group of patients actually exhibit isolated peritoneal tumor growth and are candidates for such treatment [4,5]. The predilection for extensive growth on the peritoneal surfaces without metastasizing suggests the existence of a distinct phenotype that might be present in some tumors and absent in others and which might be explored by studying tumor cells growing in the peritoneal cavity.

Pseudomyxoma peritonei (PMP) is a rare, clinically uniform but histologically heterogeneous, progressive malignant disease characterized by the accumulation of mucinous tumor tissue in the peritoneal cavity without metastasis development and, as such, a peritoneal surface malignancy in its purest form [6]. Abdominal distension because of large amounts of extracellular mucin is the main cause of morbidity, and untreated PMP will slowly lead to compression of intraabdominal organs and ultimately, the patients' demise. The primary lesion is in most cases a mucinous tumor of the appendix from which tumor cells are shed to the peritoneal cavity, but in some cases, clinical PMP may originate from mucinous tumors of the colorectum [7,8]. Current standard treatment is cytoreductive surgery combined with hyperthermic IP chemotherapy using the cytostatic agent mitomycin C, although the efficacy of adding IP chemotherapy has not been verified in randomized controlled trials. This treatment strategy has produced excellent long-term results in the most benign histologic variant, disseminated peritoneal adenomucinosis, whereas peritoneal mucinous carcinomatosis (PMCA) responds less well [9].

As clinical studies are challenging to perform in a disease as rare as PMP, the possibility of studying disease characteristics and treatment response in representative model systems is appealing. In the present study, we show that 5 models of PMP, established in nude mice from human tumors, have retained macro- and microscopic growth and similar immunohistochemical expression patterns compared to their human counterparts. Furthermore, a pilot study was performed using immunohistochemistry, examining the reactivity toward a panel of antibodies against markers of differentiation, proliferation and metastasis, mucins, and adhesion molecules, all of potential relevance for the peritoneal surface malignancy phenotype.

2. Materials and methods

2.1. Patients

Tumor tissue was acquired from patients undergoing surgical treatment at The Norwegian Radium Hospital (Oslo, Norway) after obtaining written informed consent. The study was approved by the Regional Committee for Medical Research Ethics of Southern Norway. The clinical presentation in all the patients was that of PMP with abdominal distension as one of the main symptoms, but the primary lesions and histopathologic characteristics varied as detailed below.

2.1.1. Patients 1 (*PMP-1*) and 2 (*PMP-2*)

Both had appendiceal primary lesions and relatively benign histopathologic characteristics of PMCA of intermediate type (PMCA-I type), presented in detail [10].

2.1.2. Patient 3 (*PMCA-1*)

The surgical specimen used to establish the PMCA-1 model was obtained from a 68-year-old woman who received palliative cytoreduction with removal of large amounts of mucinous tumor tissue. Her primary lesion was a rectal carcinoma treated by abdominoperineal resection 4 years previously, followed by a local recurrence that was removed after preoperative radiotherapy with free surgical margins. One year subsequent to surgery for recurrent rectal carcinoma, she presented with monstrously distended abdomen caused by mucinous ascites. Curative surgery was deemed impossible, and repeated palliative debulking procedures were performed.

2.1.3. Patient 4 (*PMCA-2*)

Tissue for the PMCA-2 model was collected from disseminated abdominal mucinous carcinomatosis in a 74-year-old man. The primary lesion was a mucinous adenocarcinoma of the descending colon that was removed by left-sided colectomy 3 years earlier. One year after primary surgery, peritoneal metastases were diagnosed. An attempt at curative surgery was performed followed by 5-fluorouracil and oxaliplatin-based postoperative systemic chemotherapy. Two years after this procedure, a bulky mucinous recurrence was diagnosed and explorative laparotomy revealed extensive disease not amenable to curative cytoreduction, and the current sample was retrieved at this procedure.

2.1.4. Patient 5 (*PMCA-3*)

Patient 5 was a 62-year-old man who, 3 years before the main surgical procedure, had his appendix removed for suspected appendicitis, but the resected specimen was not evaluated by routine histologic examination. In the course of investigations for abdominal pain, computer tomographic scans revealed bulky mucinous tumor in the abdomen,