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Original contribution

Expression of CEA, CA19-9, CA125, and EpCAM in pseudomyxoma peritonei☆,☆☆

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Summary Pseudomyxoma peritonei is a fatal clinical syndrome with mucinous tumor cells disseminated into peritoneal cavity and secreting abundant mucinous ascites. The serum tumor markers CEA, CA19-9, and CA125 are used to monitor pseudomyxoma peritonei remission, but their expression at tissue level has not been well characterized. Herein, we analyzed expression of these proteins and the adenocarcinoma marker EpCAM in 92 appendix-derived pseudomyxoma peritonei tumors by immunohistochemistry. All tumors were found to ubiquitously express CEA and EpCAM. In the majority of the tumors (94.6%), CEA showed polarized immunostaining, but in 5 aggressive high-grade tumors containing numerous signet ring cells, a nonpolarized staining was detected. We found preoperative CEA serum values to correlate with peritoneal cancer index. However, the serum values of the advanced cases with nonpolarized staining pattern were normal, and the patients died within 5 years after diagnosis. Thus, serum CEA measurements did not reflect aggressiveness of these tumors. CA19-9 showed strong immunopositivity in most of the tumors (91.3%), and mutated enzyme FUT3 was demonstrated from the cases showing negative or weak staining. CA125 was infrequently expressed by tumor cells (focal staining in 6.5% of the cases), but in most of the cases (79.3%), adjacent nonneoplastic mesothelial cells showed immunopositivity. As a conclusion, CEA and EpCAM are invariably expressed by pseudomyxoma peritonei tumor cells and could be exploited to targeted therapies against this malignancy.

Abbreviations CA125, carbohydrate antigen 125; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CRC, colorectal carcinoma; EpCAM, epithelial cell adhesion molecule; FUT3, fucosyl transferase 3; HG, high-grade; LG, low-grade; PCI, peritoneal cancer index; PMP, pseudomyxoma peritonei.

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1. Introduction

PMP is a rare malignancy characterized with mucinous tumor cells growing in the peritoneal cavity. Most often, these cells originate from LG mucinous tumors of the appendix, which leak neoplastic cells into the peritoneal cavity. The number of tumor cells in PMP lesions is usually relatively low, but the abundance of secreted mucinous ascites leads to progressive obstruction, which is fatal. In contrast to the relatively slowly progressing LG tumors, HG tumors are able to invade surrounding tissues and to metastasize, leading to reduced survival [1]. The current standard treatment of PMP is complete cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy [2]. This combinatory therapy is, however, only amenable to 60%-70% of the cases [3,4]. At the moment, no effective targeted therapies are available for the treatment of PMP.

CEA, CA19-9, and CA125 are cell membrane glycoproteins, which are used as serum markers to monitor tumor progression or remission. Furthermore, the preoperative serum levels help in estimation of prognosis. CEA is one of the most widely used tumor markers and is the most commonly used marker of CRC [5]. Serum CEA is elevated in more than 60% of patients with advanced CRC, and patients with high CEA levels have poorer prognosis [6,7]. CA19-9 is a marker commonly used in pancreatic cancer, but it is also elevated in about 45% of patients with advanced CRC [7,8]. Patients having mutated *FUT3* gene (approximately 5% of the population) cannot express CA19-9 because of the deficiency of this biosynthetic enzyme [9,10]. CA125, a repeating peptide epitope of the mucin MUC16 [11], is used as a serum marker in ovarian cancer, where it shows elevated serum levels in more than 80% of the patients [12].

EpCAM is a transmembrane glycoprotein mediating epithelium-specific, homotypic cell-cell adhesion [13,14]. EpCAM is strongly expressed in the majority of epithelial cancers [13,15], and especially CRC nearly invariably shows high EpCAM immunopositivity. Because of this widespread overexpression in epithelial cancers, EpCAM has been studied as a target for antitumor immunotherapy [14].

CEA, CA19-9, and CA125 serum markers are used to monitor the remission of PMP tumors, and their levels have also been related to the prognosis of PMP patients [4,16,17]. The tissue expression patterns of these proteins have, however, not been well characterized in PMP specimens, the previous reports analyzing mostly overall positivity in a limited number of specimens [18–20]. The aim of this study was to investigate by immunohistochemistry the expression of CEA, CA19-9, and CA125 in a larger series of PMP tumors (n = 92) and to evaluate their relevancy as PMP serum markers and their possible prognostic value. Furthermore, we studied expression of the epithelial cell marker and an immunotherapy target EpCAM in PMP.

2. Materials and methods

2.1. Tissue samples

Ninety-two formalin-fixed, paraffin-embedded tumor specimens of appendix-derived PMPs (52 LG and 40 HG) were analyzed using immunohistochemistry. The appendix origin was judged by reviewing the appendix tissue block when possible and otherwise trusting the surgery report. Grading of the tumors was done according to the WHO 2010 classification [21]. All the tissue samples were processed at the Meilahti Pathology Division, HUSLAB, Helsinki University Central Hospital, between 2006 and 2014. This study was approved by the Ethics Committee of the Helsinki University Central Hospital (DNRO 239/13/03/02/2011 and 265/13/03/02/2011).

2.2. Immunohistochemistry

Three-micrometer tissue sections were immunostained with CEA, CA19-9, CA125, EpCAM, and E-cadherin specific mouse monoclonal antibodies CEA31 (Cell Marque, Rocklin, CA), NCL-L-CA19-9 (Leica Biosystems, Nussloch GmbH, Germany), NCL-L-CA125 (Leica Biosystems), VU-1D9 (Abcam, Cambridge, UK), and HECD-1 (Thermo Fischer Scientific, Kalamazoo, MI), respectively. CEA and EpCAM stainings were performed with Ventana BenchMark XT immunostainer (Ventana Medical Systems, Tucson, AZ) using UltraView DABv3 kit (Ventana), and CA19-9, CA125, and E-cadherin stainings were performed with LabVision (Thermo Fisher Scientific) using EnVision kit (Dako, Glostrup, Denmark). The chromogen was 3,3′-diaminobenzidine in all the stainings. Positive controls were colorectal carcinoma for CEA and EpCAM, pancreatic cancer for CA19-9, and ovarian cancer for CA125. To inspect the expression pattern of the used markers in normal appendix epithelium, 5 normal appendix samples were stained.

2.3. CEA serum levels and peritoneal cancer index

The preoperative serum CEA levels were determined immunochemiluminometrically (HUSLAB, Helsinki, Finland) from 91 patients (1 patient had palliative emergency operation without serum measurements). PCI [22], estimating the extent of the disease, was determined for the patients attempted to treat with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (n = 81; PCI range, 5-39).

2.4. Sequencing of the mutational hotspots of *FUT3* gene

Genomic DNA was extracted from the formalin-fixed, paraffin-embedded samples of all CA19-9–negative/low-expressing patients and 3 CA19-9–immunopositive patients as previously reported [23]. To determine the germline *FUT3*