Human Pathology (2006) 37, 160–167

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Human
PATHOLOGY

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Prognostic value of mucins in the classification of ampullary carcinomas

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Received 23 June 2005; revised 30 September 2005; accepted 6 October 2005

Keywords:
Hepatopancreatic ampulla;
Major duodenal papilla;
MUC;
Prognostic factor;
Secretion

Summary The ampulla of Vater is of high clinical relevance with regard to influx of chyme, ascending inflammation, intubation during diagnostic and therapeutic endoscopic investigation, therapeutic papillotomy, and especially to malignant transformation. Little is known about the distribution of mucins in the ampulla. In this study, we have investigated the mucin distribution in the normal ampulla of Vater and compared it to duodenal mucosa and Brunner glands. Expression of mucins in the ampulla of Vater and duodenum was monitored by reverse transcription–polymerase chain reaction and localization of the products by immunohistochemistry. The samples investigated originated from 30 autopsy cases. Mucins MUC1, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, and MUC8 were expressed in the ampulla of Vater. Immunohistochemistry revealed production of MUC4, MUC5AC, MUC5B, and MUC6. The mucin composition varied in comparison with the duodenum referring to MUC2, MUC7, and MUC8. Detected mucins contribute to innate immunity, epithelial restitution, and protection against the aggressive secretions of the liver, gall bladder, and pancreas. By cross-linking, they influence the rheological properties of the secretions in the ampulla and facilitate unidirectional flow into the duodenum. Knowledge of their pattern of expression has prognostic value with regard to the detection of malignancy. The observed differences in the mucin distribution between the duodenum and the ampulla of Vater support the use of MUC2, MUC7, and MUC8 as useful tool in the classification of ampullary carcinomas.

1. Introduction

The ampulla of Vater, also known as the hepatopancreatic ampulla, is a dilation of the duodenal papilla that forms the opening of the juncture of the common bile duct and the main pancreatic duct. It is a complex anatomical structure comprising distinct muscle fibers and special neuronal elements that serve to regulate the flow of bile and pancreatic juice [1-3]. However, the mechanisms preventing reflux from the duodenum into the common pancreatic and biliary duct are not completely understood. It

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doi:10.1016/j.humpath.2005.10.001

has been suggested that the mucosal surface may contribute to this function as it is composed of mucosal folds filling nearly the whole lumen of the ampulla [4-8]. In this context, epithelial secretions such as the mucins, which have a major influence on the rheology of mucus gels, are of considerable importance.

Each region of the gastrointestinal tract has characteristic functional requirements, and the properties of the mucus produced at each site are adapted to cope with these functions [9-11]. Mucins, the major components of mucus, are high-molecular-weight epithelial glycoproteins with clustered *O*-glycans linked to threonine-, serine-, proline-rich tandem repeat peptide domains. Two structurally and functionally distinct classes of mucins have been identified: the secreted type, represented typically in man by MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, and MUC19, and the membrane-associated type, including MUC1, MUC3, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, and MUC20 [10-13]. Secreted and membrane-associated forms are found as extracellular, secreted viscous fluids or viscoelastic polymer gels, or located as membrane-anchored molecules in the glycocalyx [11,12,14,15]. A variety of functions are now ascribed to mucins including the viscoelastic properties of the secreted mucous barrier. Some membrane-associated mucins have been shown to function in signaling and may be important as a sensor mechanism in response to invasion or damage of epithelia [16,17]. Other mucins are able to act as an antiapoptotic agent or participate in bacterial adhesion [18-22]. Mucin genes are expressed throughout the human gastrointestinal tract in a site-specific manner [9-11,23]. This pattern of expression has been studied for the entire gut with the exception of the ampulla of Vater.

The presumable impact of mucins on the rheological properties of the secretions of liver, gall bladder, and pancreas and their physiological functions relevant to the surface integrity of mucous epithelia led us to a detailed analysis of mucins MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, and MUC8 in human epithelial cells of the ampulla of Vater.

2. Materials and methods

2.1. Collection of tissue samples from cadavers

Thirty biopsy specimens each of ampulla of Vater and duodenum from a 6-month period in 2002 were collected at the Institute of Legal Medicine, University of Hamburg, Germany. Duodenal specimens were taken from an area located nearly 1.5 cm orally to the major duodenal papilla. Tissue specimens were obtained from nonconsecutive autopsy cases (16 men, 14 women; aged 36-78 years; mean age, 56 years) whose deaths were due to various natural and pathological causes (myocardial infarction, n = 5; cerebral hemorrhage, n = 3; drowning; n = 2; hanging, n = 2; trauma/polytrauma, n = 13; gunshot wounds, n = 3; pulmonary embolism, n = 1; heroin intoxication, n = 1). All fatalities occurred outside the hospital, and none of these individuals had a medical history of gastrointestinal disease before death. After myocardial infarction, cerebral hemorrhage, drowning, hanging, trauma/polytrauma, gunshot wounding, pulmonary embolism, or intoxication with heroin, the affected people lived for a few seconds up to 1.5 hours until they died finally. In each case, preexisting pathological conditions of the gastrointestinal tract were ruled out by histological examination of various gastrointestinal (stomach, small intestine, liver, pancreas) tissue sections, and no other disease was found at autopsy except for the cause of death. The mean length of the postmortem interval of the subjects included in this study group was 24.9 ± 2.4 hours.

2.2. Specimens

For molecular biological assays, 10 tissue samples of both ampulla of Vater and duodenum were prepared, freed from surrounding tissue, placed in phosphate-buffered saline solution, and frozen immediately after collection at −80°C. For immunohistochemistry, 20 tissue samples of both ampulla of Vater and duodenum were fixed in 4% paraformaldehyde and embedded in paraffin.

2.3. Controls

Control tissues taken from the pancreas, duodenum, conjunctiva, efferent tear ducts, gastric fundus, lacrimal gland, and ethmoid with previously described MUC gene expression patterns and protein production [24-27] were included with each batch of investigation.

2.4. Total RNA purification and complementary DNA synthesis

For conventional reverse transcription–polymerase chain reaction (RT-PCR), a part of each frozen sample was crushed in an agate mortar under liquid nitrogen, then homogenized in 5 mL peqgold RNA Pure solution (peqLab Biotechnologie, Erlangen, Germany) with a Polytron homogenizer, and the insoluble material removed by centrifugation (12 000*g*, 5 minutes, 4°C). RNA was isolated as described by the manufacturer (phenol-guanidinium thiocyanate method). Crude RNA was purified with isopropanol and repeated ethanol precipitation, and contaminating DNA was destroyed by digestion with RNase-free DNaseI (27.27 Kunitz units; 20 minutes, 25°C; Boehringer, Mannheim, Germany). This enzyme was heat-inactivated for 15 minutes at 65°C. RNA (500 ng) was used for each reaction: complementary DNA (cDNA) was generated with 50 ng/μL (20 pmol) oligo$(dT)_{15}$ primer (Amersham Pharmacia Biotech, Uppsala, Sweden) and 0.8 μL superscript RNase H^- reverse transcriptase (100 U; Gibco, Paisley, UK) for 60 minutes at 37°C. Integrity of RNA was controlled by RT-PCR of glyceraldehyde 3-phosphate