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Original article

The expression of MUC mucin in cholangiocarcinoma

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This paper is dedicated to the memory of the late Professor Pauline de la Motte Hall.

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ABSTRACT

Cholangiocarcinoma (CC) is a highly malignant epithelial cancer of the biliary tract, the cellular and molecular pathogenesis of which remains unclear. Malignant transformation of glandular epithelial cells is associated with the altered expression of mucin. We investigated the type of mucins expressed in CC.

Twenty-six patients with histologically confirmed CC were included in this study. The expression of mucin was studied by immunohistochemistry using antibodies to MUC1, MUC1 core, MUC2, MUC3, MUC4, MUC5AC, and MUC6.

There was extensive (>50%) expression of mucin, mainly MUC1 in 11/25 and MUC5AC in 12/26 cases. In the case of MUC3, 6/26 cases expressed mucin extensively, whilst only 1/26 had MUC2, MUC4, and MUC6 expression. Well-differentiated tumors significantly expressed MUC3 extensively compared to poor or moderately differentiated tumors ($p = 0.003$). Fifteen of 25 cases had metastatic disease. MUC1 was extensively expressed in 9/15 cases with metastatic disease. In contrast, MUC1 expression was present in 2/10 cases where metastases were absent. Hilar lesions were less likely to express MUC1, but this was not statistically significant. Fifteen of 25 cases had metastatic disease.

Extensive MUC3 expression was significantly associated with well-differentiated tumors, whilst there was an approaching significance between the extensive expression of MUC1 and metastasis in cholangiocarcinoma.

Introduction

Mucins are high molecular-weight, heavily O-glycosylated glycoproteins that fall into three broad categories: the gel-forming secreted type which confers gel-forming properties to mucus gels that protect epithelial surfaces in the internal tracts of the body [1], the secreted but non-gel forming type, and the trans-membrane mucins [2,3].

Mucin genes are highly polymorphic due to the presence of long stretches of a variable number of tandem repeats (VNTRs) that are heavily glycosylated. Thus far, five secreted gel-forming (MUC2, MUC5AC, MUC5B, MUC6, MUC19) and three secreted non gel-forming (MUC7, MUC8, MUC9) mucins are known [2]. In recent times, a host of trans-membrane mucins (MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC16, MUC17, MUC20) have been described [2], some of which have been found to function as tumor cell modulators affecting tumor cell phenotype, making them potentially useful clinically [4]. One such mucin is MUC4, a trans-membrane mucin, the gene of which was first identified by Porchet et al. [5]. Mucin gene expression is relatively organ-specific, and an extensive loss of normal mucin gene regulation may occur with malignancy [6–8].

Some ambiguity exists with regard to the type of mucin expression in normal biliary tract and its alteration in disease. MUC3 is the major mucin gene expressed in gallbladder and in large intrahepatic bile ducts [9,10], and human bile has been reported to contain MUC1 and MUC3 [11] and MUC5B [12]. Sasaki et al. [10] reported that MUC3 and MUC6 were constantly and focally expressed, respectively, in the epithelial cells in the large intrahepatic bile ducts in histologically normal livers and those with extrahepatic biliary obstruction obtained from cadavers and used as controls. MUC3 and MUC6 are also constantly and focally expressed in the biliary epithelial cells in hepatolithiasis [10]. However, in hepatolithiasis, MUC1, MUC2, and MUC5 apomucins were also focally expressed in the large intrahepatic bile ducts examined, whereas such expression was infrequent in normal livers and in those with

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extrahepatic biliary obstruction [10]. MUC4 and MUC5AC have very recently been reported to be highly specific tumor-associated mucins in biliary tract cancer [13]. MUC6 apomucin, on the other hand, is not expressed in "bile ductules" of normal liver, whereas proliferating bile ductular cells and intralobular small ductal cells express it in chronic viral hepatitis [14].

Cholangiocarcinomas (CCs) have been studied to a limited extent and, not surprisingly, have generally been found to express the same mucins as biliary epithelial cells. Sasaki et al. [15] also found that CCs extensively expressed MUC1 apomucin and focally expressed MUC2 apomucin. In CCs of the hilar type, MUC3 apomucin is frequently expressed. This is in contrast to peripheral CCs, in which MUC3 is not expressed [15]. MUC5/6 expression is characteristic of well-differentiated CCs [15]. Aishima et al. [16], in a study that looked at the expression profile of MUC2, MUC5AC, and MUC6 in a 100 cases of intrahepatic cholangiocarcinoma, reported that the gastric mucin phenotype, particularly the gastric foveolar type, was associated with tumor progression and poor prognosis. Furthermore, Sasaki et al. [17] reported that MUC3 and MUC5AC apomucin were expressed in CC not associated with cirrhosis, whilst MUC6 apomucin was expressed in CC with cirrhosis and in two separate tumors (mixed HCC/CC). Variations in the ratios of MUC1 and MUC2 helped differentiate between invasive cholangiocarcinomas with a poor outcome and bile duct cystadenocarcinomas with a better prognosis [18]. MUC4 has been reported to be a novel prognostic factor in intra-hepatic cholangiocarcinoma of the mass-forming type [19].

In order to more completely define the apomucins expressed by cholangiocarcinomas, we performed an immunohistochemical study using antibodies against core peptides of mucins MUC1, MUC1 apoprotein (core) (MUC1c), MUC2, MUC3, MUC4, MUC5AC, and MUC6.

Materials and methods

This study was approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (REC REF 119/2008). Twenty-six patients admitted to the Hepatobiliary Clinic of Groote Schuur Hospital in Cape Town with a diagnosis of cholangiocarcinoma were included in this study. The paraffin wax-embedded blocks were retrieved from the archives of the Division of Anatomical Pathology.

The original hematoxylin and eosin-stained sections of the tumors and adjacent hepatic tissue were reviewed. The features that were assessed in these sections included tumor differentiation, local or metastatic spread, and presence or absence of cirrhosis. Special mucin stains including periodic acid Schiff (PAS) (which detects neutral mucin), alcian blue (AB) (which detects sialo-mucin), and high iron diamine (HID) (which detects sulphated mucin) were performed on the sections.

Sections were immunohistochemically stained for MUC1 apoprotein and MUC1 to MUC6 using the streptavidin–biotin–peroxidase complex. Antibodies to MUC1 apoprotein (core) (MUC1c), MUC1, MUC5AC, and MUC6 were obtained from Novocastra Laboratories (Newcastle-Upon-Tyne, UK). The other antibodies MUC2, MUC3, MUC4, and MUC5AC (M5P-B1) were provided by Professor Sam B Ho (Ho), and used as previously described [6,20,21] (Table 1). The immunohistochemically stained slides were examined by a pathologist. MUC expression was classified into four groups on the basis of the percentage of positive tumor cells: negative; 0–5%, 1+; 5–25%, 2+; 26–50%, 3+; 51–75%, 4+; >75%. For purposes of analysis in this study, we used low ($\leq$50%) and extensive (>50%) expression. For positive controls, breast cancer tissue was stained with MUC1, normal colon with MUC2, small intestine with MUC3, colon with MUC4, and stomach with MUC5AC and MUC6. The primary antibody was omitted in the negative reagent control.

Briefly, after dewaxing and rehydrating through graded alcohols to 96% ethanol, endogenous peroxidase activity was quenched in a 1% H_2O_2 methanol solution. Heat-mediated antigen retrieval was performed in 0.01 M citrate buffer pH 6 in a Presto pressure cooker for 2 min at full pressure.

A standard 3 step peroxidase-conjugated avidin–biotin method was employed. Non-specific binding was blocked with the application of the appropriate non-immune serum, followed by optimally diluted primary antibody, respective secondary antibody, and finally with HRP-conjugated avidin (all for 30 min at room temperature). Peroxidase activity was detected by using 3,3′-diaminobenzidine as a chromagen. Sections were then counterstained with hematoxylin, blued in Scotts Tap Water Substitute, dehydrated through graded ethanols to xylol, and mounted in synthetic resin.

Statistical analysis

Data were analyzed using Stata version 10.0 (Stata Corporation, College Station, Texas, USA). Proportions of cases with and without metastases expressing different mucins were compared using Fisher's exact tests; all tests were 2-sided at alpha = 0.05.

Results

Demographics

Of the 26 patients, 15 were males and 11 females, with an average age of 57.6 years (range: 30–83 years) and 45.6 years (range: 21–70 years), respectively. Specimens were obtained by surgical resection, open surgical biopsy, needle biopsy, or post-mortem.

Clinical and histologic assessment

Obstructive jaundice was the presenting complaint in 19 patients. The clinical records revealed that only 11.5% (3/26) of the patients had cirrhosis at presentation, 42.3% (11/26) had no cirrhosis as judged in the biopsy, surgically resected and post mortem specimens, whilst in 46.1% (12/26), it was unknown because non-

Table 1
A list of antibodies used in this study and their sources.

Mucin Ab	Ab clone	Ab type	Dilution	Positive control
MUC1 core	NCL-MUC-1-core	Mouse mAb	1/100	Breast cancer
MUC1	NCL-MUC-1	Mouse mAb	1/100	Breast cancer
MUC2	MRP	Rabbit pAb	1/500	Normal colon
MUC3	M3P	Rabbit pAb	1/1000	Normal small intestine
MUC4	M4P(ho-9)	Chicken pAb	1/1000	Normal bronchus, colon
MUC5AC	NCL-MUC-5AC	Mouse mAb	1/100	Normal stomach
MUC5AC (Ho)	M5P(B1)	Rabbit pAb	1/100	Normal stomach
MUC6	NCL-MUC-6	Mouse mAb	1/100	Normal stomach

Monoclonal antibody, mAb; polyclonal antibody, pAb.