

# MUC1 as a biomarker in primary sclerosing cholangitis and cholangiocarcinoma

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## Abstract

**Background** MUC1 is a glycoprotein expressed at low levels in fully glycosylated form on healthy epithelial cells. In inflammation and malignancy, MUC1 becomes overexpressed and hypoglycosylated. We aimed to describe patterns of MUC1 expression in bile-duct tissue of patients with primary sclerosing cholangitis (PSC), PSC/cholangiocarcinoma, cholangiocarcinoma, and healthy controls.

**Methods** In this proof-of-concept pilot study, archived human liver tissue samples were identified and stained using anti-MUC1 antibodies. Staining for all MUC1, and specifically hypoglycosylated MUC1, was performed.

**Results** Tissue from controls minimally expressed the hypoglycosylated/abnormal MUC1. Tissue from patients with PSC demonstrated moderate expression. Very high levels were expressed in sporadic cholangiocarcinoma.

**Conclusion** MUC1 may serve as a biomarker in the identification of bile-duct disease and cancer.

**Keywords** MUC1, primary sclerosing cholangitis, cholangiocarcinoma, bile ducts, biomarker

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## Introduction

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by immune-mediated bile-duct injury of unclear etiology [1]. PSC results in ongoing inflammation, destruction and fibrosis of the intra- and/or extrahepatic bile ducts, and frequently progresses to end-stage liver disease [2]. To date, no medical treatment exists for patients with PSC, and no biomarkers have been identified to predict disease progression. Liver transplantation is the only treatment for patients with PSC who develop decompensated liver disease and cirrhosis, and the median time between PSC diagnosis and liver transplantation is 10-12 years [3]. Unfortunately, PSC recurrence after liver transplantation is common, affecting as many as 20% of patients at 5 years [4].

MUC1 is a transmembrane glycoprotein of the mucins family, which are located on the apical surface of healthy/normal epithelial cells and function as a lubricant and protective physical barrier [5]. MUC1 has a central protein core and oligosaccharide side chains linked O-glycosidically to the protein backbone. In a healthy state, MUC1 is expressed at low levels and in the fully hyperglycosylated form, protecting the apical cell membrane of epithelial cells from a harmful environment [5]. In a diseased state, such as inflammation or cancer, MUC1 loses its apical polarization and becomes overexpressed and hypoglycosylated on the entirety of the cell surface, exposing the tandem repeat MUC1 protein backbone. Importantly, the hypoglycosylated (tumor) MUC1 continues to drive the inflammatory cascade,

as it now provides epitopes that are recognized by the immune system as a danger signal [6-9]. When the MUC1 protein backbone is hypoglycosylated, it is no longer sequestered from the immune system—instead, an immune response ensues, directed against this backbone. MUC1 is also a vital oncogene that plays a significant role in the development of epithelial adenocarcinomas, such as breast, lung, liver, colon, pancreatic and ovarian cancers [10]. In cancer and in chronic inflammation, the loss of sugars (hypoglycosylation) exposes the protein backbone, which acts as an antigen and triggers an immune response directed against abnormal MUC1. The role of MUC1 in carcinoma is connected to its aberrant glycosylation pattern, which has been shown to be the result of deregulation of the glycosyltransferases in cancer cells [11]. Indeed, MUC1 is a well-established biomarker for the early detection of cancers, and may serve as a prognostic biomarker and target for treatment.

A few studies have demonstrated relatively high rates (65.9%) of MUC1-positive staining in human cholangiocarcinoma cancer tissue, especially in those with poor tumor differentiation and those with an advanced tumor stage [12,13]. High MUC1 expression was also associated with shorter median survival [14,15]. These results suggest that MUC1 is a key player in the progression of cholangiocarcinoma, and may serve as a diagnostic marker and prognostic indicator [16]. MUC1 has also been evaluated in studies investigating pathways of PSC carcinogenesis [17].

Our aim was to evaluate the expression of hypoglycosylated MUC1 in the bile ducts of 4 groups of patients: patients with PSC, those with concomitant PSC and cholangiocarcinoma, patients with sporadic cholangiocarcinoma, and a fourth group of controls without bile-duct pathology. We hypothesized that the level of overexpression of hypoglycosylated MUC1 on epithelial bile-duct cells would be elevated in PSC, and would be higher in cholangiocarcinoma, whether with concomitant PSC or sporadic, relative to control patients who present with abnormal liver enzymes unassociated with biliary pathology, in whom no abnormal MUC1 expression would be expected.

## Patients and methods

### Tissue identification and medical records review

Through a search of our pathology database of liver transplants and liver resections, we identified patients who had either PSC or cholangiocarcinoma, confirmed by pathological examination of their liver surgical resection specimens. After these patients had been identified, the medical records were manually reviewed to confirm each patient's diagnosis, and to confirm that the PSC specifically involved the large ducts. Patients were then separated into 3 groups: 1) large-duct PSC; 2) large-duct PSC and concomitant cholangiocarcinoma; and 3) sporadic cholangiocarcinoma with no PSC. Archived tissue from the surgically resected liver specimens of all included patients was obtained: those with PSC or cholangiocarcinoma, and those with concomitant PSC and cholangiocarcinoma.

In addition, liver biopsies that were performed in patients for further evaluation of abnormal liver enzymes and found no biliary pathology, and liver biopsies that were reported as completely normal, served as controls.

### Microscopic evaluation of MUC-1 expression

Tissue staining was performed at Mayo Clinic Florida Cancer Biology Histology Shared Resource. Slides were deparaffinized and rehydrated, followed by antigen retrieval in a citrate buffer (Agilent, Santa Clara, CA) and blocking with Protein Block Serum-Free blocking buffer (Agilent). Immunohistochemistry was performed using primary antibodies MUC1\_VU4H5 (Cell Signaling, Dancers MA) at 1:200 and MUC1-HMPV (BD Sciences, Franklin Lakes, NJ) at 1:2000 for 1 hour at room temperature. Slides were incubated with Secondary EnVision Plus System HRP Anti-Mouse (Agilent) and counterstained with Gills I Hematoxylin (Eprelia, Kalamazoo, MI). Slides were scanned on Aperio AT2 and viewed using Aperio ImageScope Software (Leica Biosystems, Nusslock, Germany).

Tissue sections of all included patients were stained using 2 different anti-MUC1 antibodies: 1) HMPV, which recognizes all forms of MUC1; and 2) 4H5, which only recognizes the abnormal hypoglycosylated form of MUC1. Each slide was scored based on the quantity and intensity of staining. Quantity was scored between 0 and 4, where: 0, no stain; 1, 1-10%; 2, 11-25%; 3, 26-40%; and 4,  $\geq 41\%$ . These ranges represent the percentage of cells staining positive relative to the total number of cells in the area scored. Intensity was also scored between 0 and 4, based on stain intensity, where 0, no stain; 1, light brown; and incrementally up to 4, dark brown. The average quantity score and average intensity score for each slide were calculated. Their average was then used to calculate the total MUC1 expression score for each case [9]. Each of the slides was scored by 3 members of the team who were blinded to the clinical information. The slides were independently scored by 3 blinded investigators and there was very little interobserver variability. When variability in scoring existed, discordance was resolved by 2 independent members of the team.

### Statistical analysis

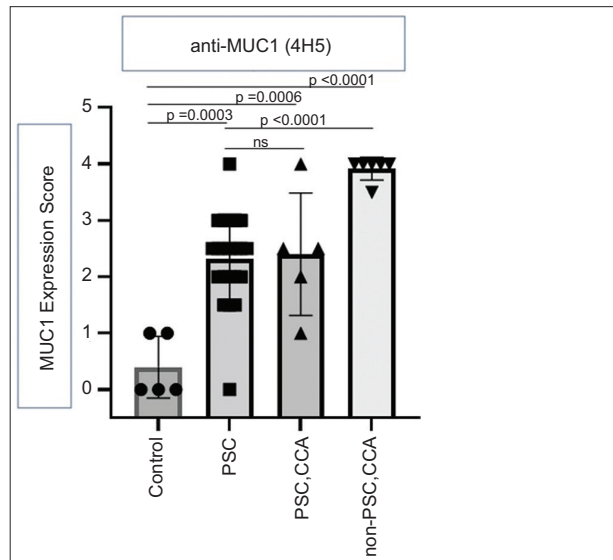
Analysis was performed using Prism, v. 10.0.0 (GraphPad, San Diego, CA). For comparisons, a 2-tailed, unpaired Student's *t*-test was used, and P-values less than 0.05 were considered statistically significant. Data are represented as mean  $\pm$  standard deviation.

## Results

A total of 38 patients were included and had their archived bile-duct tissue successfully stained. The 38 patients comprised 5 controls, 22 patients with PSC, 5 with PSC and concomitant cholangiocarcinoma, and 6 with sporadic cholangiocarcinoma.

Table 1 summarizes the clinical characteristics of the included patients.

Expression of the hypoglycosylated, abnormal form of MUC1 was measured in bile-duct epithelial tissue of all included patients (Figs. 1 and 2). Bile-duct tissue from controls



**Figure 1** Total MUC1 expression score of abnormal hypoglycosylated MUC1 on bile-duct epithelial tissue of patients with normal bile ducts, those with PSC, PSC and concomitant cholangiocarcinoma, and sporadic cholangiocarcinoma, using 4H5 antibody, which stains the hypoglycosylated (abnormal) form of MUC1. Each dot represents a single patient PSC, primary sclerosing cholangitis; CCA, cholangiocarcinoma

showed no to minimal expression of the hypoglycosylated form of MUC1 ( $0.4 \pm 0.55$ ) whereas tissue from patients with sporadic cholangiocarcinoma expressed very high levels of this abnormal hypoglycosylated MUC1 ( $3.92 \pm 0.20$ ;  $P < 0.001$ ). Bile-duct tissue of patients with PSC demonstrated moderate levels of expression of hypoglycosylated MUC1 ( $2.32 \pm 0.8$ ), which was significantly higher than the expression in normal liver biopsies ( $P < 0.001$ ) and significantly lower than in cases with sporadic cholangiocarcinoma ( $P < 0.001$ ). Biopsies from patients with both PSC and cholangiocarcinoma ( $2.4 \pm 1.08$ ) showed moderately elevated MUC1 expression, both the abnormal and normal forms (Figs. 1 and 3).

## Discussion

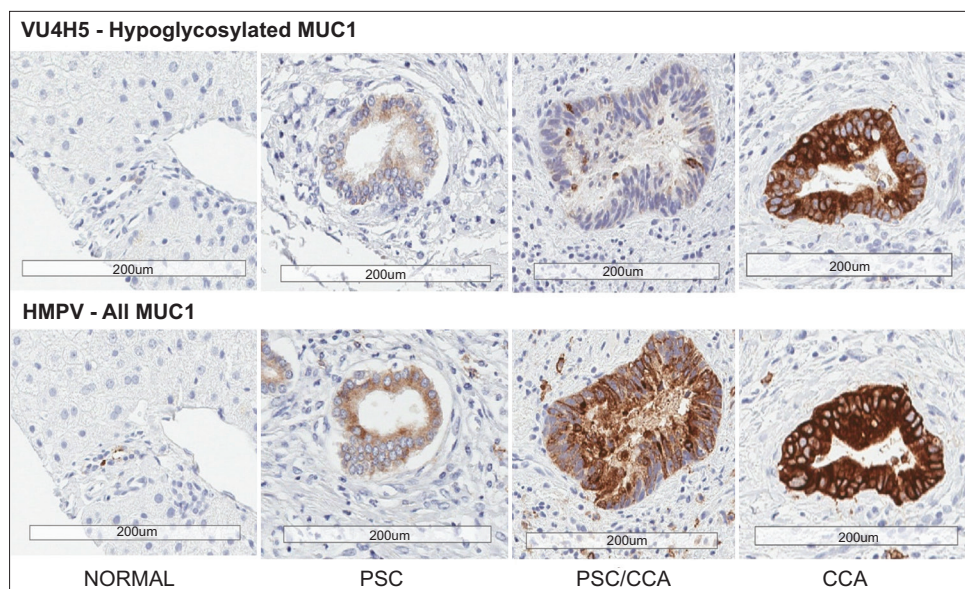
Our study is the first in the literature to measure and document the expression of the hypoglycosylated abnormal MUC1 form in the bile-duct tissue of patients with PSC and cholangiocarcinoma. While other studies have demonstrated high levels of MUC1 expression, they did not specifically evaluate this proinflammatory abnormal hypoglycosylated form. Our results show moderate overexpression of the hypoglycosylated MUC1 in tissue obtained from patients with PSC, and very high overexpression in tissue of patients with sporadic cholangiocarcinoma. Normal bile ducts express no to minimal abnormal MUC1. In addition to hypoglycosylated MUC1's ability to drive chronic inflammation and subsequent cancer development, it has been shown to facilitate cellular

**Table 1** Clinical characteristics of patients

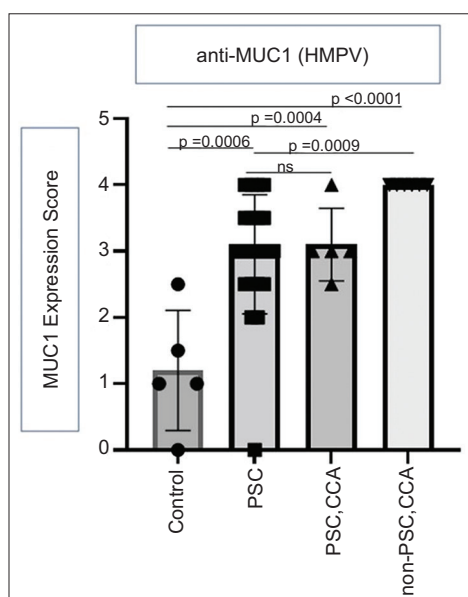
Disease type	Patients	Age (years) mean $\pm$ SD	White race	Male sex	Duration of PSC prior to liver transplant (years) mean $\pm$ SD	CA 19-9 (U/mL) mean $\pm$ SD	AFP (ng/mL) mean $\pm$ SD
Normal	5	41.6 $\pm$ 10.8	100%	40%	N/A	N/A	N/A
PSC	22	53.4 $\pm$ 15.5	73%	64%	9.4 $\pm$ 7.0	68.0 $\pm$ 58.2	3.7 $\pm$ 2.4
PSC and cholangiocarcinoma	5	33.6 $\pm$ 8.2	100%	100%	1.0 $\pm$ 0.0	183.5 $\pm$ 275.8	3.0 $\pm$ 0.7
Cholangiocarcinoma	6	64.8 $\pm$ 7.0	67%	67%	N/A	251.0 $\pm$ 407.3	5.5 $\pm$ 4.7
Disease type	Alkaline phosphatase mean $\pm$ SD range	Total bilirubin mean $\pm$ SD	Total protein mean $\pm$ SD	AST mean $\pm$ SD range	ALT mean $\pm$ SD range	PSC recurrence % (years)	Presence of concomitant IBD; and subtype
Normal	101.8 $\pm$ 28.6 61-136	0.6 $\pm$ 0.6	7.3 $\pm$ 0.6	52.0 $\pm$ 47.0 23-135	66.6 $\pm$ 66.2 23-184	N/A	0%
PSC	618.1 $\pm$ 445.7 127-1627	11.8 $\pm$ 12.2	6.5 $\pm$ 1.3	209.2 $\pm$ 231.9 41-1070	157.0 $\pm$ 188.5 24-690	32% (1-27)	77%; 14 UC, 3 CD
PSC and cholangiocarcinoma	292.0 $\pm$ 152.0 131-433	1.6 $\pm$ 1.7	7.5 $\pm$ 0.7	306.3 $\pm$ 418.0 60-789	248.3 $\pm$ 233.9 90-517	0%	40%; 2 UC
Cholangiocarcinoma	397.7 $\pm$ 285.6 76-810	2.4 $\pm$ 3.1	6.8 $\pm$ 1.5	295.5 $\pm$ 419.7 38-1092	597.0 $\pm$ 118.9 35-2856	N/A	0%

Normal CA19-9 value  $< 5.0$  U/mL; normal AFP value  $< 6.0$  ng/mL

CA, cancer antigen; AFP, alpha-fetoprotein; AST, aspartate transferase; ALT, alanine transaminase; PSC, primary sclerosing cholangitis; SD, standard deviation; N/A, not available; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease



**Figure 2** Expression of hypoglycosylated MUC1 and all MUC1 on bile-duct epithelial tissue using 4H5 and HMPV antibody, respectively PSC, primary sclerosing cholangitis; CCA, cholangiocarcinoma



**Figure 3** Total MUC1 expression score of total MUC1 (normal and abnormal) on bile-duct epithelial tissue of patients with normal bile ducts, those with PSC, PSC and concomitant cholangiocarcinoma, and sporadic cholangiocarcinoma, using HMPV antibody, which stains all forms of MUC1. Each dot represents a single patient PSC, primary sclerosing cholangitis; CCA, cholangiocarcinoma

proliferation by increasing nucleotide metabolism, and to promote migration and invasion through its interaction with numerous signaling pathways [18,19]. The importance of our work lies in its translational capability. The expression of hypoglycosylated MUC1 in PSC patients could be used as a marker to determine treatment options. Patients with PSC and cholangiocarcinoma exhibited comparable hypoglycosylated MUC1 expression to that observed in tissue

from patients with PSC. It is possible that this “dampened” expression may be related to scarring and loss of epithelial cells from prior chemoradiation therapy, since all of the patients with cholangiocarcinoma had received therapy prior to the transplant. Since MUC1 is an epithelial glycoprotein, diminishing the epithelial cells with chemoradiation therapy results in lower expression of MUC1 (both normal and abnormal forms), as was seen in our patients (Fig. 3).

To date, there is no diagnostic serum biomarker for PSC. Elevated serum IgG4 has been associated with a more aggressive PSC disease course [1,20]. While atypical perinuclear anti-neutrophil cytoplasmic antibodies (P-ANCA) and anti-glycoprotein 2 (anti-GP2) IgA have been associated with increased risk of cholangiocarcinoma, they lack diagnostic specificity for PSC [21,22]. For cholangiocarcinoma, the most common serum biomarker is the tumor marker CA19-9, which has variable sensitivity and specificity depending on the cutoff value used, and lacks any expression in 10% of patients [23]. Serum-based panels combining additional biomarkers to CA19-9, such as pyruvate kinase M2 (PKM2), cytokeratin 19 fragment (CYFRA21.1), mucin 5AC (MUC5AC), and  $\gamma$ -glutamyl transferase, have improved the sensitivity and specificity for differentiating PSC from cholangiocarcinoma [24]. Other emerging panels, relying on proteomic and metabolomic profiling as well as bile methylation markers, can facilitate the early diagnosis of cholangiocarcinoma in patients with PSC and improve prognostication [25-27].

Strengths of our study include that it is the first to investigate hypoglycosylated MUC1 expression in bile-duct tissue from liver surgical resection specimens of patients with PSC and cholangiocarcinoma. Another strength is the blinded quantification of expression by 3 independent observers blinded to the provenance of the tissues in which they appraised MUC1 expression. However, our study also had a few limitations. First,



our sample size was small despite our center being one of the largest liver transplant centers in the country. This is explained by the rarity of PSC, and the additional need for these patients to have had viable liver tissue available for staining. Additionally, we only used liver resection specimens—rather than tissue from biopsies performed during endoscopic procedures—to ensure that the size of the tissue analyzed was adequate. Another limitation is that the study did not evaluate other forms of cholangitis to assess the specificity of the observed increase in hypoglycosylated MUC1 expression in PSC. The retrospective nature of the study also imposes limitations on the clinical data obtained, especially since some patients were followed-up locally, leading to variability in the available data.

Overall, the current study demonstrates that hypoglycosylated MUC1 may serve as an additional biomarker for the identification of patients with more severe biliary inflammation and cancer. Prospective trials to validate the role of hypoglycosylated MUC1 as a biomarker for more accurate and earlier diagnosis of PSC and cholangiocarcinoma would be critical. Additionally, the results from this current study suggest that MUC1 might be used as a therapeutic target to prevent progression of these biliary diseases.

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### Summary Box

#### What is already known:

- Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by inflammation of the bile ducts
- PSC leads to liver cirrhosis, and is associated with an increased risk of cholangiocarcinoma and other cancers
- MUC1 is an epithelial glycoprotein that functions as a lubricant and protective barrier
- Hypoglycosylated MUC1 further drives inflammation

#### What the new findings are:

- Hypoglycosylated MUC1 was found to be elevated in the bile-duct tissue of patients with PSC, and more so in tissue affected by cholangiocarcinoma
- Hypoglycosylated MUC1 can serve as a biomarker of disease severity
- Our results suggest a need for further research examining MUC1 as a potential therapeutic target to stop the progression of PSC

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