Different Expression Of MUC1 In The Gallbladder Disease

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Citation

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Abstract

Background: We demonstrated that the status of MUC1 (a high molecular glycoprotein) expression correlated with the post-surgical prognosis of patients with gallbladder cancer, but the correlation with non-cancerous lesions remains unclear. Therefore, we examined MUC1 in the gallbladder not only cancer, but also non-cancerous tissues to clarify the significance immunohistochemically

Materials and Methods: A monoclonal antibody (CA15-3) was applied to stain surgical specimens.

Results: MUC1 expression is significantly high ($p<0.0001$) in gallbladder cancer (69/88,78.4%), while in contrast the expression is very trace in normal and inflammatory tissues. The MUC1 expression rate is significantly lower ($p<0.0001$) when cancer does not penetrate the proper muscle layer, corresponding to the T1 category in TNM criteria, than when cancers do extend beyond that layer. The rate was 29% (4/14) in T1 tumors, while it was ranged from 79% to 93 % in the advanced T categories: 79% (11/14) in T4, 89% (40/45) in T3, and 93% (14/15) in T2, respectively. The polarized and depolarized staining patterns of MUC1 were recognized in the epithelial or cancer cells. Every cell of normal, inflammatory epithelia, including 8 xanthogranulomatous cholecystitis and T1 cancers had the polarized pattern. The depolarized pattern was dominant in cancer cells from the advanced categories -- T2, T3 and T4. That is, 60% (45/74) of cancer cells from the epithelial layer and 78 % (58/74) of penetrating cancer cells from deeper layers had the depolarized pattern.

Conclusion: MUC1 expression was significantly higher in gallbladder cancer cells than in normal and inflammatory gallbladder cells. In cancer cells, the depolarized staining pattern was dominant, while in non-cancerous tissues the polarized pattern was dominant.

INTRODUCTION

Recently, several reports demonstrated that MUC1 expression is strongly related to tumor progression not only within gastrointestinal cancers([1,2,3,4,5]) but also in gallbladder cancer([6]).

The normal epithelial cells secrete a variety of different mucins (high molecular weight glycoproteins); these mucins serve protective and lubricating roles in the normal epithelium of gastrointestinal organs ([7]). However, no comparative study has been found concerning MUC1 expression among normal, chronic inflammatory and cancer cells of the gallbladder. Therefore, we studied the status of MUC1 expression in normal gallbladder tissue, chronic cholecystitis (including Xanthogranulomatous cholecystitis, which mimics cancerous tumors) and gallbladder cancers by immunohistochemistry.

MATERIALS AND METHODS

TISSUE SPECIMEN

We examined 88 surgical specimens of gallbladder cancer, 45 chronic cholecystitis specimens (including 7 Xanthogranulomatous cholecystities (XGC)) and 5 normal gallbladder specimens obtained at the time of surgical treatment for gastrointestinal disease. Three pathologists, who are the co-authors of this study, independently performed pathologic evaluations of the specimens and disclosed their the findings of their individual determinations.

For the 88 cancer specimens, the primary tumors (T category) have been classified according to the TNM criteria

([8]) as follows: T1, Tumor limited lamina propriae or muscle layer; T1a, Tumor limited in the mucosa, T1b, Tumor extended to muscle layer. T2: Tumor invades perimuscular connective tissue, but no extension beyond the serosa or into the liver, T3: Tumor perforates the serosa (visceral peritoneum) or directly invades one adjacent organ, or both (extension 2 cm or less into the liver), T4: Tumor extends more than 2 cm into the liver, and/or into two or more adjacent organs (stomach, duodenum, colon, pancreas, omentum, extrahepatic bile ducts, any involvement of the liver). Out of the 88-gallbladder cancers, 14 tumors were T1, 15 were T2, 45 were T3 and the remaining 14 were T4.

IMMUNOHISTOCHEMISTRY

All specimens were fixed in 10% formalin, embedded in paraffin, serially sectioned into 4 μm thick cuts and mounted on polysine coated slides. The slides were immersed for 20 min in 0.3% hydrogen peroxide in methanol to deplete the endogenous peroxidase. After washing, they were incubated with a protein blocking agent for 5 minutes. Primary antibodies against MUC1 (monoclonal antibody, mouse anti-human CA 15-3, clone DF3 (IgG1, DAKO corporation) were used at a dilution of 1:50. For the negative controls, the primary antibody was replaced with PBS. The slides were incubated with primary antibody in a humid chamber for 1 hour, washed with PBS for 15 minutes, and underwent changing of the buffer 3 times. Then, biotinylated secondary antibodies were applied for 10 minutes at room temperature. After washing, streptavidin peroxidase reagent was applied and the samples were incubated for 10 minutes at room temperature. Lastly, the slides were visualized by incubation within solution containing 0.3% hydrogen peroxide and diaminobenzidine tetrahydrochloride in PBS. Counterstaining was perfored with haematoxylin, prior to mounting in crystal.

EVALUATION OF MUC1 EXPRESSION

Three pathologists examined the expression of MUC1 under a light microscope independently. The MUC1 expression was judged as positive when more than 5% of cells were stained.

STATISTIC

Statistical analysis was conducted via the Chi-square test and p values of less than $p<0.05$ were regarded as significant.

RESULTS

MUC1 EXPRESSION AMONG NORMAL, INFLAMMATION AND CANCER CELLS

MUC1 expression in epithelial cells and cancer cells in the gallbladders was estimated separately in the epithelial layer and the layers deeper than sub-mucosal layer, which consisted of the sub-mucosal layer, the muscle layer, sub-muscular connective tissue and serous layer. Two types of staining patterns were recognized: polarized and depolarized. In the polarized type, MUC1 expression is restricted predominantly in the apical membrane of the cell (Fig 1-A~D). In the depolarized type, the membrane and cytoplasm of the cell, as well as the stroma adjacent to the basal membrane of the malignant glands were stained (Fig 1-E).

In the normal gallbladder tissue, the expression rate of MUC1 was 20% (1/5) in the epithelial layer, with a polarized staining pattern. There was no MUC1 expression in the sub-mucosal layer (Fig 1-A). In the chronic inflammatory gallbladder tissue samples, only 2 of 45 specimens (4.4%) expressed MUC1 in the epithelial layer and the staining pattern was of the polarized type (Fig 1-B&C). There was no statistical difference in the rate and type of MUC1 expression in the epithelial and sub-mucosal layers between normal and chronic inflammation tissues.

On the other hand, in the gallbladder cancer samples, the epithelial layer had a significantly ($p<0.0001$) higher expression of MUC1 -- 78.4% (69/88) -- than in non-cancerous (normal and chronic inflammation) tissues (Table 1). The staining pattern of MUC1 was exclusively of the polarized type in the epithelial layer of the non-cancerous gallbladder samples. However, in the gallbladder cancer samples, both staining patterns were marked: 34.8% (24) for the polarized type and 65.2% (45) for the depolarized type. In 74 specimens with cancer cell invasion to the layers deeper than sub-mucosal layer, the MUC1 expressed in 89% (66/74) of them and 88% of MUC1 expressed specimens (58/66) showed depolarized type and only 12% (8/66) demonstrated polarised type.

Figure 1

Table 1: MUC1 expression in the epithelium of different gallbladder lesions

Epithelium (Number of specimen)	No. of MUC1 Positive specimen (Positive rate)
Cancer (88)	69 (78.4%)
Chronic Cholecystitis (45)	2 (4.4%)
Normal Gallbladder (5)	1 (20%)

(Chi-square test: P<0.0001)
Value of a is significantly lower than b. P<0.0001

MUC1 EXPRESSION IN CANCER WITH RELATION TO HISTOPATHOLOGY AND INVASION

According to the TNM classification, 82 specimens were adenocarcinomas and two were adenosquamous carcinomas, while two showed signet ring cell carcinoma features and the remaining two were mucinous carcinomas. Adenosquamous and mucinous carcinomas expressed MUC1 with a depolarized staining pattern, while the signet ring cells did not show MUC1 expression. Out of 88 carcinomas, 51 were classified as well-differentiated, 21 were classified as moderately differentiated and 16 were classified as poorly differentiated carcinomas. The MUC1 expression rates in the cancer cells on the epithelial layer were as follows: 73% in well, 81% in moderate and 94% in poorly differentiated carcinomas, respectively. In the sub-mucosal layer, it was 86%, 90%, 94% in well, moderate and poorly differentiated carcinomas, respectively. These results suggested no correlation in MUC1 expression rate and cancer differentiation.

The MUC1 expression rate of 29% (4/14) in the epithelial layer of T1 tumors was significantly ($p<0.0001$) less than the rate noted in the advanced T categories; that is, 93% (14/15) in T2, 89% in T3 (40/45) and 79% in T4 tumors (11/14), respectively (Table 2). All of the cells in the MUC1-positive T1 cancers displayed polarized staining. On the other hand, 69 % (45/65) of the MUC1-expressing cancer cells in the advanced T categories -- T2 (14), T3 (40) and T4 (11) -- had a depolarized pattern. In these advanced T categories, cancers penetrating beyond the epithelial layer demonstrated very high MUC1 expression rates: as 100% (15/15), 84.4% (38/45) and 92.8% (13/14) in T2, T3 and T4, respectively. There is no correlation between the positive expression of MUC1 and the staining pattern among T2, T3 and T4 tumors in the both epithelial and sub-mucosal layers.

{image:2}

T category is a criterion based upon the extent of primary tumor invasion classified by TNM criterion

Venous invasion was assessed in 74 advanced cancers (T2~T4) under the microscope and was noted in 32 specimens of these specimens. In these venous invasion-positive specimens, the mucin expression rates are 84% and 94 %, respectively, in the epithelial layer and in the sub-mucosal layer. In the specimens where there is no venous invasion, the mucin expression rates are 77% and 86%, respectively. Therefore, the mucin expression rate has no relation to the degree or presence of venous invasion.

Figure 1A-E:

MUC1 expression detected by the immunoperoxidase method using DAB as a coloring agent (brownish pigment).

{image:3}

Less than 5% of epithelial cells (arrow) expressed MUC1 on the apical surface.

{image:4}

Less than 5% of epithelial cells (arrow) expressed MUC1 in a polarized pattern.

{image:5}

Less than 5% of the epithelial samples (arrow) expressed MUC1 in a polarized pattern.

{image:6}

Cancer cells extensively expressed MUC1 in a polarized pattern.

{image:7}

Muc1 is expressed in the cytoplasm, as well as along the entire surface and within the stroma adjacent to the basal membrane (depolarized pattern).

DISCUSSION

Despite the dismal prognosis of patients with gallbladder cancer, the relevance of biologic characteristics to clinical medicine has not been fully studied yet. Recently, several reports pointed out that the expression of MUC1 may relate closely to prognosis in patients with gastrointestinal cancers([4], [5], [9], [10]) and the greater the increase in expression the more the cells lose their apical polarity([11], [12]). We also previously reported that the depolarised expression of MUC1 in gallbladder cancer would be a potential prognostic predictor ([6]) and Yamashita, et, al. demonstrated that MUC1 expression was significantly related to prognosis in patients with cholangiocarcinomas ([3]). MUC1 and its family of glycoproteins have been reported to regulate immune recognition([13]), cellular adhesion of cancers ([14]) and cellular differentiation in breast cancer([15]). However, the status of MUC1 expression in gallbladder cancer compared to MUC1 expression in normal tissue and inflammatory lesions has not been studied yet and it may lead to an improved understanding of the role of MUC1 in cancer biology.

Therefore, an aim of this study was to clarify the significance of MUC1 expression in cancerous and non-cancerous lesions of the gallbladder.

Our study revealed that the MUC1 expression rate is significantly higher in cancer cells than in non-cancerous cells. The mechanism of high expression of MUC1 has been studied in colon cancer and it has been observed that the activity of GalNAc transferases are increased in cancer, specifically within the rough endoplasmic reticulum (RER). O-glycosyltion usually occurs in golgibodies of normal cell, but Egea et al advocate that it occurred in the swollen RER in spite of golgibodies ([16]). Swelling of the RER may be responsible for cellular stress and neoplastic transformation. In gallbladder cancer, a similar mechanism may occur. We have seen that the depolarised staining pattern is dominant in cancer cells. Egea et al demonstrated in colon cancer that in typical cancers golgi stacks are not observed, probably due to the lack of polarization of the cells and the fact that mucus droplets are also distributed in a non-polarized fashion ([16]). This lack of polarization of the cells may be responsible for the depolarised expression of MUC1 noted in gallbladder cancer.

Xanthogranulomatous cholecystitis mimics gallbladder cancer clinically and radiologically ([17]). Interestingly, there is a significant difference in MUC1 expression and staining pattern between XGC and cancer. However, we did not find any significant difference in MUC1 expression and the staining pattern between normal and XGC tissue. (Less than 5% cells are stained in XGC cases and the staining pattern is also polarized). So, from this study and also from our previous study of COX2 ([18]) expression in gallbladder tissue, we can say that XGC is not a pre-cancerous condition.

CONCLUSION

In conclusion, mucin expression is significantly higher in gallbladder cancer than in normal and inflammatory gallbladder tissue. In cancer cells, the depolarised staining pattern is dominant. Based upon this study and the others referenced above, we further conclude that xanthogranulomatous cholecystitis may not be a pre-cancerous condition.

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