Original Article

Mucin expression profile in Barrett's, dysplasia, adenocarcinoma sequence in the esophagus

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Abstract

BACKGROUND: The molecular events that accompany the progression to adenocarcinoma (ADC) of the esophagus are poorly understood. Aberrant mucin receptor expression can contribute to increased cell growth and metastatic ability. **AIM:** The aim of this study was to establish a pattern for mucin (MUC) gene expression in the esophageal mucosa under normal and pathological conditions. **SETTING:** University Hospital Cancer Center Laboratory. Archived tissue samples studied in a retrospective fashion. **MATERIALS AND METHODS:** Tissue samples were obtained from the archives of patients with histological evidence of Barrett's esophagus (BE) progressing to ADC. Immunohistochemical analysis was performed using mouse monoclonal antibodies for MUC1, MUC2, MUC5AC, MUC6. Semiquantitative scoring of histological staining was performed using a linear scoring system: 0-staining absent; 1-staining in 0-25%; 2-staining in 25-50%; and 3-staining in 50-75% of the epithelium. The Binomial test was used to explore trends and differences in frequency of mucin expression along the pathological sequence. **RESULTS:** Only mild superficial staining of MUC1 was seen in normal squamous epithelium. MUC1 and MUC2 were uniformly expressed in all samples (7/7) of BE and dysplasia (P=0.008). MUC1 expression was upregulated (7/7) in progression to adenocarcinoma (P=0.008). The secretory mucins, MUC5AC and MUC6 showed a decrease in expression with progression from BE to dysplasia to ADC (P<0.05). **CONCLUSIONS:** Downregulation of MUC5AC and MUC6 decreases mucosal protection against gastric acid. Increasing MUC1 expression is associated with progression from dysplasia to ADC. Upregulation of MUC2 reflects intestinal metaplasia in BE.

Key words: Barrett's esophagus, mucin expression

Introduction

The frequency of adenocarcinoma (ADC) of the esophagus in the western world is increasing at a rate surpassing that of any other cancer.[1] This increase (8-15%) is largely the result of the growing prevalence of ADC in Barrett's mucosa. It is unclear as to why even after successful fundoplication, Barrett's often persists, committing patients to periodic surveillance endoscopy. Esophageal ADC develops in gastro esophageal reflux in a sequential fashion from Barrett's through various degrees of dysplasia to malignancy, giving a therapeutic window for intervention.

Mucins are high molecular weight glycoproteins synthesized by a broad range of epithelial tissues and are coded for by MUC genes. Mucins are broadly subdivided into two groups: those, which are secreted and form extracellular gels (MUC2, MUC5AC, MUC5B and MUC6) and membrane bound mucins (MUC1, MUC3 and MUC4). Mucins play a key role in the GI tract where the gel not only constitutes a

physical barrier and lubricant but also generates a protective diffusion barrier for the underlying epithelium. Altered expression of mucin epitopes have been described in Colon and Stomach cancers and correlated with decreased survival.[2-4] Similarly aberrant mucin expression may contribute to continued epithelial damage, increased cell growth and metastatic ability in the esophagus.[5] Jass was the first to suggest that a pattern of mucin staining in Barrett's esophagus may be associated with a greater risk of progression to adenocarcinoma.[6] This study was designed to address the lack of consensus on the role of mucins in the pathological sequence that follows Barrett's disease. We sought to establish a mucin expression pattern that would be valuable diagnostically in the setting of Barrett's disease.

Materials and Methods

Twenty archived paraffin embedded sections of the esophagus from seven patients with documented histological progression from Barrett's to ADC through the stage of dysplasia were chosen for the study. Barrett's was defined as biopsy proven intestinal metaplasia of any length. The biopsy specimens were collected over a three-year period from January 2001 to December 2003. Only tissue samples with contiguous evidence of Barrett's, dysplasia and ADC in each cross-section were selected to allow for comparison of MUC gene expression across the pathological sequence. Tissue sections had been stained previously in a standard fashion with hematoxylin and eosin to confirm the diagnosis. ADC was deemed to be arising from the Barrett's esophagus, if, on histological examination, there was intestinal metaplasia in proximity to the tumor.

To limit subjective bias inherent in immunohistochemical measurements at least two samples were obtained from each patient and were studied by two different pathologists. Positive control tissues from gastric fundus, ileum, colon and breast with previously described MUC gene expression patterns, were included with each batch of sections for immunohistochemistry.[7] The primary antibody was omitted as a negative control to test the specificity of the antimucin antibodies for each section. Monoclonal mouse antibodies were used for MUC1, MUC2, MUC5AC and MUC6. Paraffin embedded tissue sections were baked in an oven at 60 degree celsius for thirty minutes and then deparaffinised through three changes of xylene and then rehydrated through a series of decreasing concentrations of ethanol solutions to distilled water. Antigen retrieval was performed by microwave cooking at 121°C for 20 minutes in 10 mM citrate buffer, pH 6.0 and then left to cool at room temperature for 60 minutes. Endogenous peroxidase activity was blocked in 1% hydrogen peroxide in methanol for thirty minutes at room temperature and washed in phosphate buffered solution (PBS) for ten minutes. After blocking nonspecific antibody binding with 10% normal goat serum in PBS for one hour the slides were incubated overnight with a 1:200 dilution of the primary antibody at 4°C. Sections were washed three times for ten minutes in PBS and incubated with secondary antibody, goat anti mouse, Vector BA-1000, 1:500 diluted by 5% normal goat serum in PBS for one hour. Slides were washed three times in PBS. Development was in 0.6 mg/ml 3-3 diaminobenzidine (DAB)/ 0.03% hydrogen peroxide in PBS. Sections were rinsed in water, counterstained with hematoxylin, dipped in saturated lithium carbonate, dehydrated through a series of increasing concentrations of ethanol solutions and mounted under cover-slips. The binomial test was used to explore trends and differences in frequency of mucin and estrogen receptor expression along the pathological sequence. The binomial distribution can be defined as the number of successes produced in a succession of n independent trials, P being the probability for a success in each trial.

Results

Expression of the different mucin genes across the pathological sequence in each of the seven patients is outlined in Table 1. A high degree of concordance was

Table 1: Staining pattern for the mucin gene in the transformation from metaplasia to malignancy

	Squamous	Barrett's	Dysplasia	Adenocarcinoma	*P*
MUC I	7/7 (100%)	7/7	7/7	7/7	0.008*
MUC II	0/7	7/7	7/7	5/7	NS
MUC 5AC	0/7	7/7	5/6 (84%)	2/6 (33%)	<0.05
MUC 6	0/7	7/7	5/7 (72%)	3/7 (42%)	<0.05

*Intensity of MUC1 expression: MUC I expression was noted in 0-25% of Barrett's, 25-50% of dysplastic epithelium and 50-75% of adenocarcinoma.
TABLE I Shows declining secretory mucin (MUC 5AC, MUC 6) expression as one progresses towards malignancy and persistent expression of membrane bound mucin (MUC I) although of increasing intensity.

noted in the interpretation of the stains by each of the pathologists. Results of MUC gene expression in control tissues were similar to previously published data.[8] There was no evidence of nonspecific staining by primary antibodies in the negative controls and no positive signals were seen in non epithelial cells for any of the mucin stains.

Results for MUC gene expression were divided into secretory and membrane bound mucins. In the normal squamous epithelium MUC1, a membrane bound mucin, was expressed along the surface in a superficial manner. With progression along the pathological sequence the intensity of expression in the linear scale increased. There was staining in 0-25% in normal epithelium to 25-50% in dysplasia and 50-75% staining in adenocarcinoma (P=0.008) [Figures 1-3]. Secretory mucins were not found in the normal squamous epithelium. In metaplastic cells where it was expressed

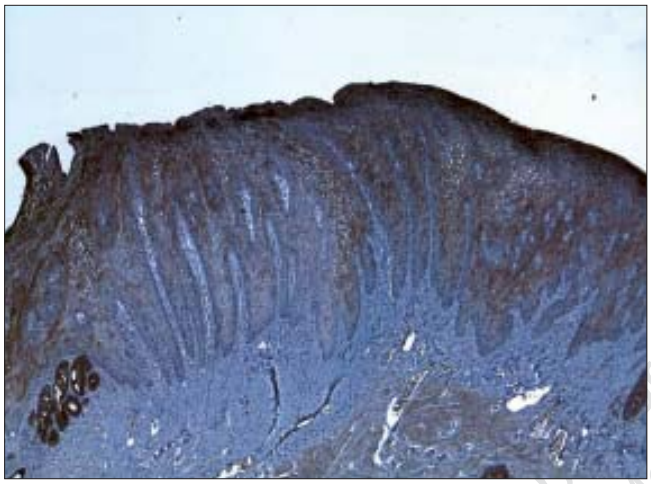

Figure 1: Superficial staining with MUC1 in Barrett's. Immunohistochemistry stains magnification: x20

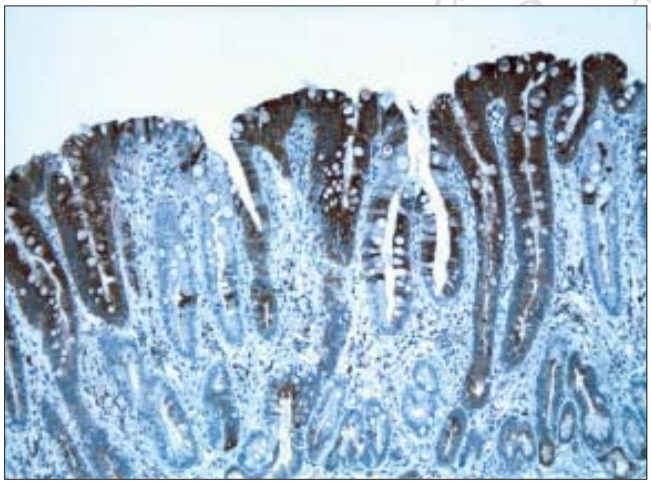

Figure 2: Staining with MUC1 in Barrett's with dysplasia. Immunohistochemistry stains magnification: x20

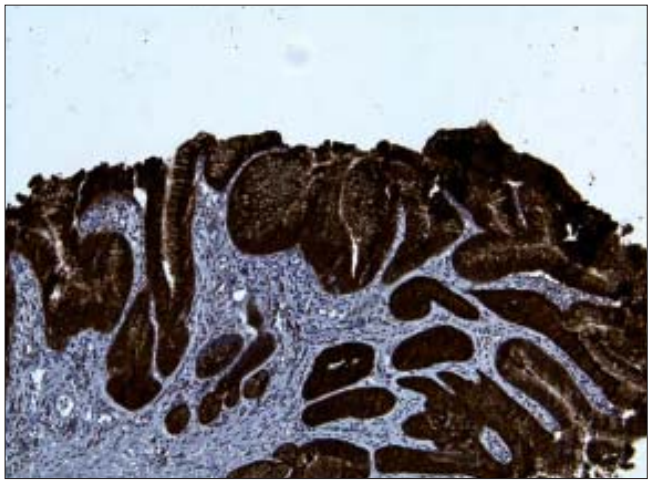

Figure 3: Increased intensity of MUC1 staining in adenocarcinoma. Immunohistochemistry stains magnification: x20

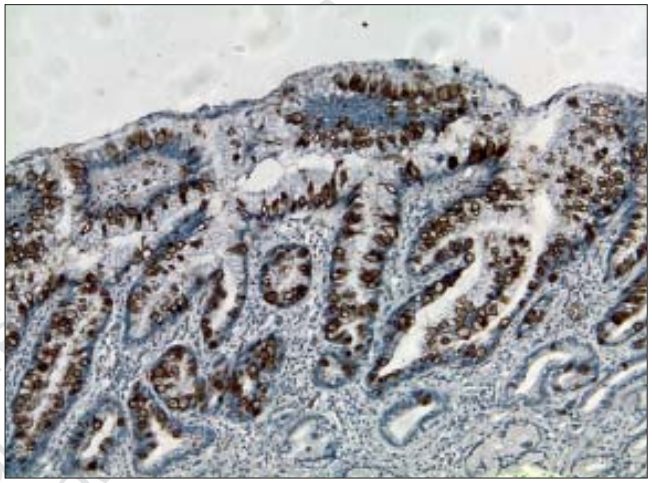

Figure 4: MUC2 staining in goblet cells. Immunohistochemistry stains magnification: x20

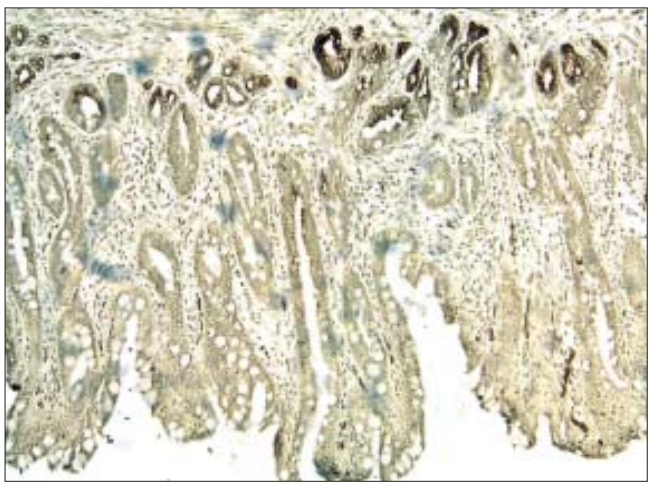

Figure 5: MUC6 staining in deep foveolar glands. Immunohistochemistry stains magnification: x20

the MUC2 gene was confined to the goblet cells [Figure 4]. In Barrett's and dysplasia the expression was 7/7(100%) while in adenocarcinoma the expression proportionately decreased to 5/7(71%). MUC5AC was found in the foveola of superficial epithelium and MUC6 in the deep glands of the metaplastic epithelium [Figure 5]. While there was an expression of 7/7 (100%) of both these gastric secretory mucins in Barrett's, their expression proportionately decreased to 5/6 (84%) and 5/7 (71%) in dysplasia and 2/6 (33%) and 3/7 (42%) respectively in adenocarcinoma ($P<0.05$).

Discussion

A high degree of correlation between mucin mRNA detection by *in situ* hybridization and mucin immunohistochemistry has been established.[9] The latter is quicker and needs a less sophisticated laboratory. Specific and efficient antibody tests have been validated for all apomucins studied in this article allowing us to perform experiments over a reasonable time frame and draw accurate conclusions as compared to previously available tests.

MUC1 expression showed an up-regulation along the metaplasia, dysplasia and adenocarcinoma sequence in this study. In a recent study MUC1 positive Barrett's cases showed a trend toward longer length of Barrett's compared with MUC1 negative cases.[7] The MUC1 peptide core mediates firm adhesion of tumor cells to adjacent cells via binding to intercellular adhesion molecule-1 (ICAM-1) and facilitates metastases.[10] Binding to ICAM-1 also impairs T-lymphocyte surveillance.[11] Abnormal oncogene expression has been reported in Barrett's epithelium, including c-erbB2, p53 and primary cell nuclear antigen. An association between c-erbB2 and MUC1 has been hypothesized.[12] Thus, over expression and abnormal glycosylation of MUC1 sets the stage for increasing cellular instability.

It is generally acknowledged that the "metaplastic epithelium" may reflect an adaptive response to a new luminal environment with MUC5AC and MUC6 offering protection from gastric acid and MUC2 associated with protection from bile. In this study, we noted a significant down-regulation of these secretory mucins as one progressed along the metaplasia-adenocarcinoma sequence. The trefoil peptides (TFF1, TFF2) are associated with mucosal repair and act synergistically with mucins (MUC5AC) to protect epithelial tissues.[13] Downregulation of secretory mucins thus could impair mucosal protection. Loss of MUC2 in previously mucin secreting cells may indicate loss of differentiation in the neoplastic cells as illustrated by a decrease in expression in adenocarcinoma.

In all intestinal metaplasia samples, we found gastric-type MUC gene expression, suggesting a common origin for gastric and intestinal metaplasia. Others have also noted the possibility of activation of multipotent esophageal stem cells following destruction of normal squamous epithelium.[14,15] At present it is however unclear as to whether gastric metaplasia and intestinal metaplasia develop simultaneously or successively. There appears to be a definite order in the appearance and subsequent decrease of the various mucins in the Barrett's-adenocarcinoma sequence. It is still not clear whether this is the primary cause for the persistence of the Barrett's epithelium in-spite of successful anti-reflux surgery, an adaptive response to reflux or if it reflects the genetic instability associated with this preneoplastic condition. Further studies in patients undergoing surveillance for Barrett's and dysplasia will help answer whether mucin gene expression has a diagnostic role in predicting those at risk and does not merely represent an artifact of progression. An open question is whether therapeutic manipulation of MUC gene expression will decrease the risk of malignancy for patients with BE and dysplasia.

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Source of Support: Nil, **Conflict of Interest:** None declared.

Announcement

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