Altered expression of MUC2 and MUC5AC in progression of colorectal carcinoma

Xiao-Dong Bu, Nan Li, Xiao-Qiang Tian, Li Li, Jin-Song Wang, Xiao-Jin Yu, Pei-Lin Huang

Abstract

AIM: To study the expression profiles of MUC2 and MUC5AC in tumorigenesis of colorectal carcinoma and in its different pathologic types.

METHODS: Formalin-fixed, paraffin-embedded human colorectal tissue specimens were immunostained with antibodies against MUC2 and MUC5AC. Six samples of normal mucosa (NM), 12 samples of hyperplastic polyp (HP), 15 samples of tubular adenoma with low-grade dysplasia (LGD), 14 samples of tubular adenoma with high-grade dysplasia (HGD), 26 samples of conventional colorectal adenocarcinoma (CCA), 15 samples of mucinous carcinoma (MC), and 8 samples of signet-ring cell carcinoma (SRCC) were collected.

RESULTS: MUC2 was the most widely expressed protein in each study group, although the number of MUC2-positive cases was less in CCA group than in other groups ($P < 0.05$). The staining score for MUC2 was significantly decreased in the HP-LGD-HGD-CCA sequence ($r = -0.73436, P < 0.0001$). Among the neoplasms, MC and SRCC were more frequently associated with the high expression of MUC2 ($P < 0.05$) than with that of CCA. MUC5AC expression was detected in all groups but not in NM group. Furthermore, the staining score for MUC5AC was higher in HP, LGD, HGD, MC and SRCC groups than in NM and CCA groups ($P < 0.05$). The frequency of simultaneous expression of MUC proteins was significantly higher in MC and SRCC groups than in CCA group ($P < 0.05$).

CONCLUSION: Alterations in MUC expression occur during colorectal tumorigenesis. The transformation process in MC and SRCC may be different from that in the traditional adenoma-carcinoma sequence.

Key words: Colorectum; Tumorigenesis; MUC2; MUC5AC; Immunohistochemistry

INTRODUCTION

Mucins are high-molecular-weight glycoproteins, which are heavily decorated with a large number of O-linked oligo-
saccharides and a few N-glycan chains, linked to a protein backbone. Mucins are known to play a central role in the protection, lubrication and hydration of the external surface of human epithelial tissue layers lining the intricate network of ducts and passageways. Mucins have also been implicated in the pathogenesis of benign and malignant diseases of secretory epithelial cells. The identification of novel transmembrane mucin MUC21, means that a total of 20 human mucins have now been recognized. According to their structure and function, mucins can be divided into secreted mucins and transmembrane mucins. Secreted mucins can be gel-forming or non-gel-forming, and include MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9 and MUC19. Transmembrane mucins include MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20 and MUC21, and possess a transmembrane domain for anchoring themselves to the plasma membrane of various cells. These mucin proteins are encoded by various MUC genes.

The genes for gel-forming mucins MUC2 and MUC5AC are found in a cluster on chromosome 11p15.5, and synthesis of the proteins is regulated by biologically active molecules, including cytokines, bacterial products, and growth factors. The MUC2 gene codes for a typical secretory mucin, which is predominantly found in colorectal goblet cells. The MUC5AC gene is mainly expressed in gastric and tracheo-bronchial mucosa. Changes in the expression levels and/or distribution profiles of MUC2 and MUC5AC occur in cancers of the lung, gastrointestinal tract, pancreas, hepatobiliary system and reproductive system. For example, it has been found that MUC5AC is down-regulated in peritumoral epithelium and squamous metaplasia of non-small cell lung carcinoma (NSCLC), and MUC5AC expression is reduced in NSCLC, irrespective of their histologic subtype. The expression of sLe\(^x\) is related to MUC5AC protein in NSCLC, and patients with tumors co-expressing both MUC5AC and sLe\(^x\) antigen have the poorest survival. MUC2 appears to act as a protective protein and has been shown to be associated with tumors of mucinous type, both in biliary system and in pancreatic system, which carry a more favorable prognosis. MUC5AC expression in intrabiliary cholangiocarcinoma is associated with a higher incidence of lymph node metastasis and has been identified as an independent prognostic factor by multivariate survival analysis.

Interestingly, altered expression of MUC in colorectal cancer may be significantly correlated with histologic type, sensitivity to chemotherapeutic drugs, and prognosis of colorectal cancer. Colorectal cancer constitutes a suitable model for studying the mechanisms of carcinogenesis and tumor progression in the well-established adenoma-carcinoma sequence. It is possible to observe a dynamic progression from benign adenomatous polyp to adenoma with varying degree of dysplasia, to intramuscular and invasive carcinoma. Moreover, a pathway involving a hyperplastic polyph-adenoma-carcinoma sequence has also been introduced. Alterations in the expression of mucin proteins and genes have been observed in colorectal adenoma and carcinoma, although their significance in neoplastic transformation of the colorectal epithelium is yet to be determined. The present study therefore aimed to study the expression profiles of MUC2 and MUC5AC during tumorigenesis and in different pathologic types of colorectal carcinoma, using immunohistochemical staining.

**MATERIALS AND METHODS**

**Tissue samples**

Formalin-fixed, paraffin-embedded human colorectal tissue specimens were obtained from Department of Pathology, Nanjing First Hospital of Nanjing Medical University. Six samples of normal mucosa (NM), 12 samples of hyperplastic polyps (HP), 15 samples of tubular adenoma with low-grade dysplasia (LGD), 14 samples of tubular adenoma with high-grade dysplasia (HGD), 26 samples of conventional colorectal adenocarcinoma (CCA), 15 samples of mucinous carcinoma (MC), and 8 samples of signet-ring cell carcinoma (SRCC) were analyzed in this study. HP was diagnosed when a serrated polyp with no overt cytological atypia showed narrowed crypt bases, predominantly lined with immature cells. Adenoma was further classified as low or high grade based on the degree of glandular intraepithelial neoplasia (dysplasia), according to the World Health Organization classification. Colorectal cancer was defined as mucinous carcinoma if more than 50% of the lesion contained a mucin lake. Cancer where more than 50% of the tumor cells were signet-ring cells was defined as SRCC. Histologically normal mucosa from margins of the specimens served as control tissue. All tissue samples were diagnosed and classified by two pathologists.

**Immunohistochemistry**

Paraffin-embedded blocks of different tissues were cut into 4-μm thick sections. Slides were deparaffinized in xylene and rehydrated using a graded ethanol series. Antigen was retrieved by boiling the slides in a microwave oven for 15 min in 0.01 mol/L citrate buffer (pH 6.0). Endogenous peroxidase was blocked with a 3% H\(_2\)O\(_2\)-methanol solution, and the slides were incubated in 10% normal goat serum for 30 min to prevent nonspecific staining. The tissue sections were then incubated overnight at 4°C with primary antibody (MUC2 or MUC5AC, 1:100; Santa Cruz, CA). The standard biotin-streptavidin-peroxidase method was then used, and the sections were lightly counterstained with hematoxylin. Histologically normal colon mucosa and gastric biopsies were used as positive controls for MUC2 and MUC5AC, respectively. The sections incubated with phosphate-buffered saline (0.01 mol/L, pH 7.4) instead of primary antibody were used as negative controls.

**Analysis of immunohistochemical data**

Both goblet and non-goblet columnar cells of normal colon and hyperplastic polyps were evaluated. MUC staining was only scored in neoplastic cells of tissues containing either dysplastic epithelium or carcinoma. The range of cytoplasmic staining: 0% (0%-5%); 1: 6%-30%; 2: 31%-60%; and 3: 61%-100% and the intensity of staining: 0: no stain; 1: weak staining; 2: intermediate staining; and 3:
strong staining) were assessed in at least 8 high-power fields by two observers, and averages of the grades were taken. The final staining score was defined as the product of scores for the range and intensity of cytoplasmic staining. Staining was designated as negative if the staining score was 0 or 1, intermediate for 2, 3, or 4, and high for 6 or 9. All specimens were scored blindly.

**Statistical analysis**

Statistical comparison of immunohistochemical staining was performed using SAS software version 9.0 (SAS Institute, Cary, NC). The rank-sum test and Spearman’s rank correlation analysis were used to determine differences between the groups and to evaluate correlations, respectively. The T approximation test in Wilcoxon’s rank-sum test and Fisher’s exact test were used to compare differences between CCA and other groups. 𝑃 < 0.05 was considered statistically significant.

**RESULTS**

**Immunohistochemical localization of MUC2 and MUC5AC**

The expression of MUC2 and MUC5AC proteins differed among the study groups, which was prominently characterized by perinuclear and diffuse cytoplasmic staining. The MUC2 was expressed in perinuclear cytoplasm of partial goblet cells in NM group (Figure 1A). The MUC2 labeling was generally increased in cytoplasm of columnar cells and goblet cells in HP group (Figure 1B), and the positive signals were also observed in apical cytoplasm of columnar cells, especially in LGD and HGD groups (Figure 1C and D). MUC2 expression was positive in the cytoplasm of cancerous cells, while the extracellular mucin remained unstained (Figure 1E-G). The staining pattern for MUC5AC was largely similar to that for MUC2 in all groups but not to that in NM group, and positive signals were found in extracellular mucin (Figure 2).

**Immunohistochemical analysis of MUC2 and MUC5AC**

The frequency of MUC protein expression in different groups was examined with immunohistochemical staining, and the results are summarized in Table 1. MUC2 was the most widely expressed antigen in all groups, but the number of MUC2-positive cases (46.15%) was less in CCA group than in other groups. Both the expression frequency and staining intensity of MUC2 were significantly decreased in the HP-LGD-HGD-CCA sequence ( 𝑟 = -0.73436, 𝑃 < 0.0001). The frequency of MUC2 expression was significantly higher in MC and SRCC groups than in CCA group ( 𝑃 < 0.05). The MUC5AC expression was detected in all groups but not in NM group. Furthermore, the frequency of MUC5AC expression was dramatically lower in CCA group (30.77%) than in other groups with the exception in NM group. The proportion of high staining scores was significantly higher in MC and SRCC groups than in CCA group ( 𝑃 < 0.05), which was similar to that of MUC2 expression. Concordance between MUC2 and MUC5AC expression was also noted in individual specimens from different groups (Table 2). Concordance was defined as positive (intermediate or high) or negative MUC2 and MUC5AC expression. The frequency of simultaneous expression of MUC proteins was significantly higher in MC and SRCC groups than in CCA group ( 𝑃 < 0.05).

**DISCUSSION**

MUC expression has been studied in colorectal carcinoma, but few reports are available on the expression in relation to the hyperplastic polyp-adenoma-carcinoma sequence, or in different pathologic types of colorectal cancer. This study focused on the altered and de novo expression profiles of MUC2 and MUC5AC in the tumorigenic sequence, and in different pathologic types of colorectal cancer.

MUC2 is characteristically expressed in goblet cells of native intestinal epithelium and intestinal metastasis of gastric mucosa, but not in normal gastric epithelium. The results of the present study, with immunohistochemical staining of paraffin-embedded human tissue samples, are consistent with those of previous studies showing reduced MUC2 expression in colorectal adenocarcinoma[16-18]. Decreased MUC2 expression in nonmucinous colon cancer can result from methylation of the MUC2 promoter[1]. Gratchev et al[19] demonstrated that MUC2 promoter methylation is lower in normal goblet cells than in columnar cells and in specimens of mucinous colorectal carcinoma than in those of nonmucinous adenocarcinoma. Loss of functional p53 is also related to the down-regulation of MUC2 expression in colorectal carcinoma. It has been shown that MUC2 expression is transcriptionally regulated by p53 protein in several cell lines[20]. There are two potential p53-binding sites in the MUC2 promoter, each of which contributes to stimulation of promoter activity. It was reported that MUC2 immunoreactivity is inversely correlated with p53 alteration in mucinous carcinoma, i.e. the level of p53 alteration is lower in regions with a high MUC2 expression level[21]. Decreased in vivo expression of MUC2 is related to colon carcinogenesis accompanying increased proliferation, decreased apoptosis, and increased migration of intestinal epithelial cells[22].

MUC5AC is not expressed in normal colonic epithelium, but de novo expression occurs in adenoma and colorectal cancer. The number of immunoreactive cells and the intensity of MUC5AC staining are greatest in larger adenoma with moderately villous histology and dysplasia, while immunostaining is lower in highly villous polyps with severe dysplasia[23]. However, in the current study, the score for MUC5AC staining was higher in HGD group than in LGD group, in contrast to the expression of MUC2 in the two groups. This discrepancy in MUC5AC expression in the two studies might be due to the use of a different histologic type of adenoma. Although MUC5AC expression correlates with neural invasion and advanced stage of intrahepatic cholangiocarcinoma[24], the relation between MUC5AC expression and progression of colon cancers may be different. Kocer et al[25] found that the expression of MUC5AC in colon cancer is associated with a better...
Figure 1  Expression of MUC2 (SP method, × 200). A: Normal mucosa; B: Hyperplastic polyp; C: Tubular adenoma with low-grade dysplasia; D: Tubular adenoma with high-grade dysplasia; E: Conventional colorectal adenocarcinoma; F: Mucinous carcinoma; G: Signet-ring cell carcinoma.

Figure 2  Expression of MUC5AC (SP method, × 200). A: Normal mucosa; B: Hyperplastic polyp; C: Tubular adenoma with low-grade dysplasia; D: Tubular adenoma with high-grade dysplasia; E: Conventional colorectal adenocarcinoma; F: Mucinous carcinoma; G: Signet-ring cell carcinoma.
Table 1 Frequency of MUC2 and MUC5AC protein expression in colorectal tissues n (%)  

| MUC staining score | NM       | HP      | LGD     | HGD     | CCA     | MC       | SRCC    |
|--------------------|----------|---------|---------|---------|---------|----------|---------|
| MUC2               |          |         |         |         |         |          |         |
| High               | 6 (100.00)| 12 (100.00)| 9 (60.00)| 5 (35.71)| 3 (11.54)| 9 (60.00)| 4 (50.00)|
| Intermediate       | 0 (0.00) | 0 (0.00) | 6 (40.00)| 9 (64.29)| 9 (34.62)| 6 (40.00)| 3 (37.50)|
| Negative           | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 14 (53.85)| 0 (0.00) | 1 (12.50)|
| P                  | 0.0013   | < 0.0001| 0.0003  |          |         | 0.023    | -       | 0.0198  |
| MUC5AC             |          |         |         |         |         |          |         |
| High               | 0 (0.00) | 5 (41.67)| 3 (20.00)| 6 (42.86)| 3 (11.54)| 7 (46.67)| 2 (25.00)|
| Intermediate       | 0 (0.00) | 7 (58.33)| 12 (80.00)| 8 (57.14)| 5 (19.23)| 8 (53.33)| 6 (75.00)|
| Negative           | 6 (100.00)| 0 (0.00) | 0 (0.00) | 0 (0.00) | 18 (69.23)| 0 (0.00) | 0 (0.00) |
| P                  | 0.1444   | 0.0007  | 0.0007  |          |         |          | 0.0002  | 0.0042  |

1MUC2 protein expression is significantly decreased in HP-LGD-HGD-CCA sequence (r = -0.73436, P < 0.0001); 2MUC2 protein expression in CCA vs other groups; 3MUC5AC protein expression in CCA vs other groups. NM: Normal mucosa; HP: Hyperplastic polyp; LGD: Low-grade dysplasia; HGD: High-grade dysplasia; CCA: Conventional colorectal adenocarcinoma; MC: Mucinous carcinoma; SRCC: Signet-ring cell carcinoma.

Table 2 Concordance of MUC2 and MUC5AC protein expression in colorectal tissues n (%)  

| Tissue | n | Positive cases | Concordance | P |
|--------|---|----------------|-------------|---|
|        |   | MUC2 | MUC5AC | MUC2 vs MUC5AC |   |
| NM     | 6 | 6 (100.00)| 0 (0.00)| 0 (0.00) | 0.5662 |
| HP     | 12| 12 (100.00)| 12 (100.00)| 12 (100.00) | < 0.0001 |
| LGD    | 15| 15 (100.00)| 15 (100.00)| 15 (100.00) | < 0.0001 |
| HGD    | 14| 14 (100.00)| 14 (100.00)| 14 (100.00) | < 0.0001 |
| CCA    | 26| 8 (30.77)| 4 (15.38)|         | -      |
| MC     | 15| 15 (100.00)| 15 (100.00)| 15 (100.00) | < 0.0001 |
| SRCC   | 8 | 7 (87.50)| 7 (87.50)|         | < 0.0001 |

1Concordance of MUC protein expression in CCA vs other groups. NM: Normal mucosa; HP: Hyperplastic polyp; LGD: Low-grade dysplasia; HGD: High-grade dysplasia; CCA: Conventional colorectal adenocarcinoma; MC: Mucinous carcinoma; SRCC: Signet-ring cell carcinoma.

In conclusion, alterations in MUC expression occur during colorectal tumorigenesis. The transformation process in MC and SRCC may be different from that in the traditional adenoma-carcinoma sequence. De novo expression of MUC5AC can occur in both mucinous and non-mucinous colorectal carcinomas, but its expression is stronger in mucinous colorectal carcinoma than in non-mucinous colorectal carcinoma. Further investigations using molecular biological techniques based on a larger clinical sample size are needed to confirm our findings.

COMMENTS

Background

Although alterations in mucins have been observed in colorectal cancer, little is known about their expression during the development and progression of colorectal tumor. Mucinous and signet-ring cell carcinomas are the two types of colorectal cancer characterized by abundant mucin secretion. However, whether the mechanism underlying the tumorigenesis of mucinous and signet-ring cell carcinomas differs from that of other colorectal cancer remains controversial.

Research frontiers

Mucinous components, such as MUC2 and MUC5AC, are associated with the distinct clinical pathologic features of colorectal cancer and the survival rate of such patients.

Innovations and breakthroughs

The abnormal expression of MUC2 and MUC5AC in mucinous and signet-ring cell carcinomas suggests that a different process may be involved in the tumorigenesis of these types of colorectal cancer.

Terminology

Mucins are high-molecular-weight glycoproteins, which are heavily decorated with a large number of O-linked oligosaccharides and a few N-glycan chains, linked to a protein backbone. Mucins are known to play a central role in the protection, lining the intricate network of ducts and passageways. Mucins have also been implicated in the pathogenesis of benign and malignant diseases of secretory epithelial cells.

In conclusion, alterations in MUC expression occur during colorectal tumorigenesis. The transformation process in MC and SRCC may be different from that in the traditional adenoma-carcinoma sequence. De novo expression of MUC5AC can occur in both mucinous and non-mucinous colorectal carcinomas, but its expression is stronger in mucinous colorectal carcinoma than in non-mucinous colorectal carcinoma. Further investigations using molecular biological techniques based on a larger clinical sample size are needed to confirm our findings.
Applications
The clinical treatment of mucin-secreting tumor may differ from that of other types of colorectal cancer.

Peer review
This is a good paper that provides new data regarding MUC expression profiles in specific types of colorectal carcinoma.

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S-Editor Wang JL  L-Editor Wang XL  E-Editor Lin YP