

IMMUNOHISTOCHEMICAL STUDY OF MUCINS IN COLORECTAL CARCINOMA: COULD IT BE A GUIDE FOR BIOLOGICAL BEHAVIOR?

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ABSTRACT

Objective To find out the expression pattern of MUC1, MUC2 and MUC5AC and its relationship to the site, histological differentiation and stage of colorectal adenocarcinoma.

Study design Descriptive study.

Place & Duration of study Jinnah Postgraduate Medical Center Karachi, from June 2008 to December 2008.

Patients and Methods The expression of MUC1, MUC2 and MUC5AC was investigated immunohistochemically in twenty selected cases of colorectal adenocarcinoma. Data regarding site, histological differentiation, and stage was collected from the files. Tumors were assigned to right colon, left colon and rectum. They were differentiated into well-differentiated, moderately-differentiated and poorly-differentiated tumors and were further grouped into nMUC adenocarcinoma, MUC adenocarcinoma and signet ring cell carcinoma. Tumors were staged according to Dukes' classification.

Results All normal colorectal mucosa samples expressed MUC2 but did not show MUC1 and MUC5AC expression. MUC1 expression was found in 15% of tumors originating mostly from proximal colon and were Dukes' stage C lesions. Less frequent expression was seen in MUC adenocarcinoma (25%), with no expression in signet ring cell carcinoma. MUC2 expression was found in 55% of tumors mostly of rectal origin and mostly Dukes' stage B lesions. MUC2 expression was more frequent in MUC adenocarcinoma (75%) and signet ring cell carcinoma (100%). MUC5AC was expressed in 30% of tumors mostly arising from rectum and were Dukes' stage C lesions. Its frequency of staining MUC adenocarcinoma (83%) was more or less the same as that of MUC2.

Conclusions MUC1, MUC2, and MUC5AC appear to be associated with development of colorectal adenocarcinoma and may help in predicting its biological behavior. MUC2 and MUC5AC expression seen mostly in MUC adenocarcinoma may be particularly helpful in predicting the clinical outcome of this particular type of colorectal adenocarcinoma.

Key words Colorectal adenocarcinoma, Mucin protein expression, Immunohistochemistry, Biological behavior.

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INTRODUCTION:

A hallmark of colorectal carcinomas is their ability to synthesize mucin. It is yet to be determined whether the amount and type of mucin produced by colorectal cancers could reflect their biological behavior. Recently many mucin carbohydrate associated antigens and

mucin core protein antigens have been described. Individual mucin genes have distinct patterns of expression within mucin producing tissues, suggesting that the various mucin gene products play functional roles. Epithelial mucins have not only the unique function of protecting and lubricating epithelial surfaces but have also been implicated in growth, epithelial renewal and differentiation, epithelial integrity, carcinogenesis and metastasis.¹

They can be broadly classified into secretory mucins and membrane bound mucins.²⁻⁵ MUC1 gene codes for membrane-associated mucin, and is highly expressed on apical membrane of bronchus, salivary gland, pancreas, prostate and uterus and is sparsely expressed in gastric surface cells, gall bladder, small intestine and colonic epithelium.⁵ Recognized by DF3 antibody, MUC1 is a transmembrane glycoprotein with an extracellular domain, a transmembrane domain and a cytoplasmic tail. Over-expression of MUC 1 by cultured cells inhibits their aggregation, possibly because of its large, extended and rigid structure.⁶

Secretory mucin (MUC2 and MUC3) gene expression was primarily restricted to the intestinal tract.^{4,5} MUC2 was highly expressed in normal jejunum, ileum, colon and gall bladder. MUC5AC gene was mainly expressed in gastric and tracheobronchial mucosae. The surface mucus cells of the cardia, fundus and antrum in the stomach show MUC5AC mucin expression. It was also found in fetal and precancerous colonic mucosa, and in less than 20% of normal colonic mucosa.^{3,7-9}

The aim of our present work was to study the pattern of expression of MUC1, MUC2 and MUC5AC and to find out if there was any relationship between their expression, site, histological differentiation and stage of colorectal adenocarcinoma.

PATIENTS AND METHODS:

This descriptive study was carried out at Jinnah Postgraduate Medical Center, Karachi from June 2008 to December 2008. Formalin fixed, paraffin-embedded tissues from twenty patients with colorectal carcinoma were studied. Tumors were located in the right colon, left colon and rectum. They were classified as well-differentiated, moderately-differentiated, and poor / undifferentiated tumors, which were further grouped into mucinous (MUC) and non-mucinous (nMUC) adenocarcinoma. There were two cases of signet ring cell carcinoma. Tumors were staged according to Duke's classification.

For immunohistochemistry the ABC immunoperoxidase method was followed.¹⁰ Sections were deparaffinized, rehydrated and incubated at room temperature with 0.3% hydrogen peroxide in methanol for thirty minutes to block endogenous peroxidase and washed with 1x phosphate-buffered saline (PBS), pH 7.4. 2% goat serum in PBS was applied for thirty minutes at room temperature to prevent non-specific staining. The sections were incubated with a primary antibody (DF3, 1:500 dilution, anti-MRP, 1:600 dilution and MUC5AC, 1:100 dilution in PBS with 1% bovine serum albumin for sixteen hours at 4°C). The sections were washed three times with PBS, incubated with biotinylated secondary antibodies, and washed again three times with PBS. All sections then were incubated with ABC complex for thirty minutes. After washing with PBS three times, the sections were reacted with diaminobenzidine substrate for 10-30 minutes, rinsed with tap water and counterstained with hematoxylin. Negative controls consisted of substituting PBS or nonimmune rabbit serum for the primary antibodies.

Immunohistochemical analysis was done by observing the whole area of neoplasms by low power (x 10) optical fields.^{3,6,11,12} The sections were graded as follows:

- + less than 5% of neoplastic cells positive
- + 5% to 50% of neoplastic cells positive
- ++ more than 50% of neoplastic cells positive

RESULTS:

Histologically there were fifteen well- to moderately differentiated adenocarcinoma, three poorly-differentiated adenocarcinoma, which were grouped into ten cases of nMUC adenocarcinoma and eight cases of MUC adenocarcinoma. There were two cases of signet ring cell carcinoma. There were eleven stage B and seven stage C tumors. Two biopsies were not staged.

Normal mucosa did not show DF3 reactivity. MUC1 expression was found in six (30%) of twenty tumors with a score of ++ seen in only three (15%) cases. There was intense positivity of secretions present within the glandular lumina which were prominently outlined. The pattern of DF3 expression was essentially the same in all cases. Most of the cases with DF3 expression were Dukes' stage C lesions and originated from proximal colon. Although no significant difference was observed in frequency of MUC1 positivity with regard to histological differentiation, MUC1 expression was less frequent in MUC adenocarcinoma (25%) with no expression seen in signet ring cell carcinoma.

Normal mucosa showed an intense positivity of goblet cells of the intestine. MUC2 expression was found in eleven (55%) of twenty cases of colorectal adenocarcinoma with a score of ++. The tumor cells positive for anti – MRP antibody exhibited cytoplasmic positivity with perinuclear localization in some cases. All cases of signet ring cell carcinoma revealed intra cytoplasmic and supranuclear reactivity of the signet ring cells. The tumors with abundant mucin content revealed staining of the mucin lakes. The staining intensity was however less as compared to the DF3 antibody. Most of the cases with MUC2 expression were Dukes' stage B lesions and originated from rectum. Although no significant difference was observed in frequency of MUC1 positivity with regard to histological differentiation, MUC2 expression was more frequently seen in MUC adenocarcinoma (73%) with both cases of signet ring cell carcinoma staining positive for anti-MRP antibody.

Normal mucosa did not show any significant staining with only some areas exhibiting a focal luminal or intra-cytoplasmic positivity for MUC5AC. Six (30%) of twenty cases showed MUC5AC expression with a score of ++. MUC5AC positivity differed in different tumors. It was more or less luminal, with some cases exhibiting cytoplasmic positivity. Tumors with abundant mucin content showed positive staining of mucin. Most of the cases with MUC5AC expression were Dukes' stage C lesions and originated from rectum. Although no significant difference was observed in frequency of MUC1 positivity with regard to histological differentiation, MUC5AC expression was more frequently seen in MUC adenocarcinoma (83%).

DISCUSSION:

Mucin antigen expression in human cancers has been studied and its relationship with malignancy potential identified.⁶ MUC1, MUC2, and MUC5AC expression was examined in different histologic types of colorectal carcinoma to determine whether they can prove to be useful markers for the development of colorectal carcinoma. An altered pattern of expression of mucin genes may be found in cancer tissue. Normal colorectal mucosa rarely expresses MUC1 and MUC5AC but MUC2 is predominant secreted mucin, abundantly expressed in the cytoplasm of goblet cells and columnar cells.^{3,4,8,13} The present study showed a similar pattern of staining of the normal colonic mucosa and the tumor tissue.

We found only 15% of the cases showing MUC1 and 55% of the cases showing MUC2 expression in our study. Some studies have reported more than 80%

cases of colon cancer as positive,^{3,14,15} while others have shown slight to moderate variation in MUC1 expression,^{12,16-19} and a wide variation for MUC2 expression ranging from 33% - 100%.^{3,12,16-19} In this study MUC5AC was detected in 30% of the cases which is in agreement with some other studies.^{3,20} Although there is a wide variation especially in expression of MUC1 and MUC2 in colorectal adenocarcinoma as reported in literature, all these studies including ours suggest that there is an over-expression or up-regulation of MUC1 expression and there is a down-regulation of MUC2 and MUC5AC expression.

The MUC1 expression did not correlate with histologic differentiation, but a significant association between MUC2 and MUC5AC expression and histological type of colorectal carcinoma was noted in this study. Both these mucins were strongly expressed in mucinous (MUC) rather than non-mucinous (nMUC) adenocarcinoma.²⁰⁻²³ This fact has also been noticed in this study, and it may be said that level of MUC2 and MUC5AC expression is associated with histological type and aggressiveness of tumor.

The frequency of MUC1 positivity in carcinomas with metastasis (Dukes' C) was higher than in carcinomas without metastasis (Dukes' A and B).¹² Most of the MUC1 and MUC5AC positive cases were stage C lesions while MUC2 positive cases were mostly stage B lesions. MUC 1 expressed in tumors may function as an anti-adhesion molecule, which inhibits cell-to-cell adhesion including the release of cells from the tumor. Cells that show high levels of MUC 1 had reduced interaction between integrins and the extracellular matrix. MUC 1 mucin expression may be related with invasion or metastasis of carcinoma cells in this manner.⁶ This is supported by increase in MUC1 expression in cancers which have noncohesive cells with invasive properties. MUC2 expression decreases in cancers due to loss of ability of carcinomatous intestinal epithelium to express native mucin type due to defective glycosylation. MUC5AC may be involved in the early stages of carcinogenesis and their aberrant expression may be due to alteration in their methylation status, transcriptional regulation or aberrant differentiation.³

We divided colorectal tumors into tumors of the proximal colon, distal colon and rectum,^{24 25} due to differences in their embryological development and physiological activity. They can also predict the clinical outcome. Distal colon and rectal tumors have similar molecular and clinical characteristics.

In contrast proximal tumors have specific histopathological characteristics, molecular patterns, and clinical outcomes.²⁴ Most of the tumors of the proximal colon were nMUC adenocarcinoma (75%), while tumors of the distal colon and rectum were MUC adenocarcinoma (70%). Present study consists of a small series of cases. Further studies in larger groups should be done to determine whether prognostic value of expression of MUC1, MUC2 and MUC5AC antigens differ significantly in different sites in colorectum as well as in different histological types

CONCLUSIONS:

MUC1, MUC2, and MUC5AC appear to be associated with development of colorectal adenocarcinoma and may help in predicting its biological behavior. MUC2 and MUC5AC expression seen mostly in MUC adenocarcinoma may be particularly helpful in predicting the clinical outcome of this type.

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