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Coexpression of MUC1 with p53 or MUC2 correlates with Lymph Node Metastasis in Colorectal Carcinomas

The alteration of the mucin profile have been known to play a role in colorectal carcinogenesis. MUC1 is up-regulated and MUC2 is down-regulated in colorectal carcinomas. Overexpression of p53 is frequently noted in colorectal carcinomas with deep invasion or lymph node metastasis. However, there have been few reports about the association between MUC1, MUC2, and p53 expression with respect to the metastatic potential. This study was aimed to investigate the relationship of MUC1, MUC2, and protein p53 expressions with clinicopathological factors in colorectal carcinomas. Expressions of MUC1, MUC2, and p53 protein were examined immunohistochemically. Of total 97 cancers, 44 (45%) were MUC1 positive, 39 (40%) were MUC2 positive and 58 (59%) showed a p53 overexpression. Coexpression of MUC1 with p53 and dual expression of MUC1 with MUC2 were associated with a higher frequency of lymph node metastasis ($p<0.05$). The right colon showed a higher MUC1 positivity and frequent lymph node metastasis than the left colon ($p<0.05$). These results suggest that the coexpression of MUC 1 with p53 or MUC2 are involved in regional lymph node metastasis in colorectal carcinomas. The high expression of MUC1 in the right colon cancer was revealed to relate with lymph node metastasis.

Key Words : *Mucins; Protein p53; Colorectal Neoplasms*

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INTRODUCTION

Mucins, the major epithelial luminal surface glycoprotein with a high molecular weight, are oligosaccharides attached to serine or threonine residues of the apomucin protein backbone by O-glycosidic linkages (1, 2). During the past several years, a number of human mucins (MUC1-MUC9) have been identified (3-7). MUC1 is a membrane-associated glycoprotein with an extracellular domain consisting of a variable number of highly conserved tandem repeats of 20 amino acids (8, 9). MUC1 expression is up-regulated in a variety of cancers including colorectal cancers and breast cancers (10, 11). MUC2 glycoprotein is a secretory mucin, containing two distinct regions with a high degree of internal homology. Region 1 consists mostly of 48-bp repeats and region 2 is composed of 69-bp tandem repeats (12). MUC2 apomucin is expressed by adenomas and mucinous carcinomas of the colorectum, but the expression of MUC2 may be associated with less aggressive biological properties in non-mucinous adenocarcinoma of the colorectum (13, 14).

It is widely accepted that multiple genetic alterations underlie colorectal carcinogenesis. The *p53* mutation is common in human cancers and overexpression of its products is detected in many colorectal cancers. Thus, the immunohistochemical detection of the overexpression of p53 is a useful marker for the diagnosis of carcinoma (15, 16). However, the relationship between the p53 overexpression and metastasis in colorectal cancers is still controversial (17, 18). There have been few reports about the relation between the p53 overexpression and MUC1, MUC2 expression in colorectal cancers (19).

In this study, we examined the relationship between the expression of MUC1, MUC2, and p53 in colorectal carcinomas with special reference to regional lymph node metastasis.

MATERIALS AND METHODS

Representative samples of paraffin-embedded tissues were obtained from the files of 97 colorectal cancer patients undergoing tumor resection at the Department of Surgery, Hallym University, Sacred Heart Hospital and Hangang Sacred Heart Hospital. Fifty-three were male and the remaining 44 were female. The mean age was 65 yr with a range of 33 to 89 yr. The tumor sites were the cecum in 12, ascending colon in 14, descending colon in one, sigmoid colon in 28, and rectum in 42 patients. The depth of tumor invasion was submucosal (pT1) in 12, muscularis propria (pT2) in

21, subserosa (pT3) in 61 and serosa exposed (pT4) in 3. Forty-four cases had lymph node metastasis, and the remaining 53 cases had no lymph node metastasis. For MUC1, MUC2, and p53 immunostaining, paraffin-embedded sections were placed on poly-L-lysine-coated glass slides and air-dried at room temperature. Deparaffinized and rehydrated sections were heated in a microwave oven for 5-min twice in citrate buffer to retrieve antigenic activity and cooled for 60 min at room temperature. Endogenous peroxidase activity was inhibited by incubation with 1.5% hydrogen peroxide for 10 min at room temperature. After blocking nonspecific reactions with 10% normal rabbit serum for 20 min, the sections were first incubated with MUC1 antibody (mouse monoclonal antibody Ma552; Novocastra Laboratories, New Castle, U.K.) at a dilution of 1:100, MUC2 antibody (mouse monoclonal antibody Ccp58; Novocastra) at a dilution of 1:100, and p53 antibody (mouse monoclonal antibody BP53. 12; Zymed, San Francisco, U.S.A.) at a dilution of 1:100. The sections were then incubated with biotinylated rabbit antimouse immunoglobulin for 30 min, followed by streptavidin-peroxidase complex (DAKO, U.S.A.) for 30 min. The sections were carefully rinsed with several changes of phosphate-buffered saline (PBS) between each step of the procedure. The color was developed with diaminobenzidine. The sections were lightly counterstained with hematoxylin and mounted. The extent of MUC1 and MUC2 expression was graded semiquantitatively as follows: 0, no positive cells; 1, positive in less than 5% of cells; 2, positive in 5-30% of cells; 3, positive in 30-60% of cells; 4, positive in more than 60% of cells. For the purpose of relating MUC1 and MUC2 reactivity with pathological variables, cancers were regarded as positive when the score was at least 3, according to the previous reports (20). The p53 positivity was defined as cells with brown staining on the nuclei, regardless of the staining intensity, but cells with very weak equivocal staining were considered negative. Staining pattern of a diffuse type (cells with positive nuclear staining present diffusely in most areas of the tumor) or nested type (>20 positive cells aggregated in a part of the tumor) was regarded as having p53 protein overexpression, according to previous reports (21). χ^2 test was used to analyze the data with respect to clinicopathological parameters, such as T stage, lymph node metastasis, and tumor site. *P* values less than 0.05 was considered significant.

RESULTS

MUC1 was expressed intraluminally with the glycocalyx and in intracytoplasmic lumina in colorectal carcinomas (Fig. 1). Of 97 cancers, 45% (44/97) had a MUC1 score of 3 or more. MUC1 positivity was observed in 17% (2/12) of pT1, 24% (5/21) of pT2, 57% (35/61) of pT3, and 67% (2/3) of pT4 cancers. There was a significantly high frequency of MUC1 positivity with respect to depth of tumor invasion (Table 1, p=0.04). In 44 cases with lymph node metastasis, MUC1 positivity was 57% (25/44). MUC1 positivity was high in the group with lymph node metastasis (57% vs 32%, p=0.04) (Table 1).

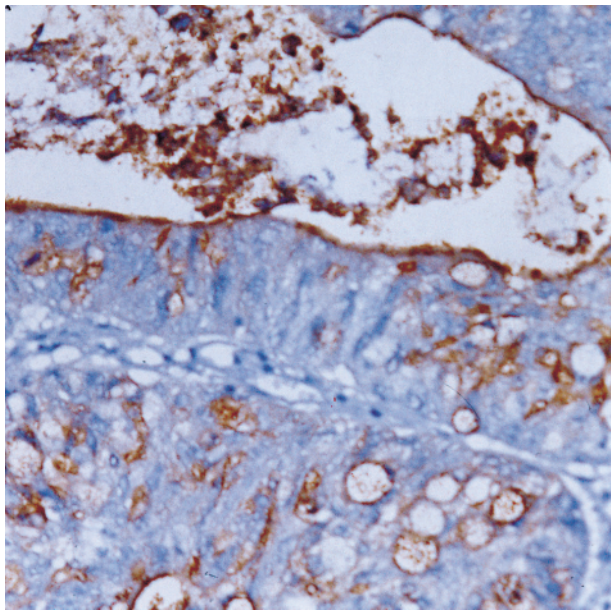

Fig. 1. In immunostaining, MUC1 was expressed in the intraluminal border of colorectal carcinoma (×200).

MUC2 expression was demonstrated along the tumor cell membrane border (Fig. 2). In 97 cancers, 39 (40%) had a MUC2 score of 3 or more. MUC2 was not evident in extracellular mucin. No difference was observed in the frequency of MUC2 positivity with respect to depth of tumor invasion and lymph node metastasis (Table 1).

The p53 overexpression was noted only in the nuclei (Fig. 3).

Table 1. MUC 1, MUC 2, and p53 immunostaining and clinicopathological factors

	MUC 1		MUC 2	p53
Depth of invasion				
T1	2/12 (17%)	*	5/12 (42%)	3/12 (25%)
T2	5/21 (24%)		7/21 (33%)	13/21 (61%)
T3	35/61 (57%)		26/61 (43%)	40/61 (66%)
T4	2/3 (67%)		1/3 (33%)	2/3 (67%)
Regional lymph node				
N0	17/53 (32%)		20/53 (38%)	32/53 (60%)
N1, 2	25/44 (57%)†		19/44 (43%)	25/44 (57%)
Tumor site				
Left	24/72 (33%)		27/72 (38%)	43/72 (60%)
Right	18/25 (72%)‡		12/25 (48%)	15/25 (60%)

*: MUC1 positivity was higher in high T stage (p=0.04). †: MUC1 positivity was higher in lymph node metastasis group (N1, 2) (p=0.04). ‡: The right colon cancers showed a higher MUC1 positivity than the left colon cancers (p=0.001).

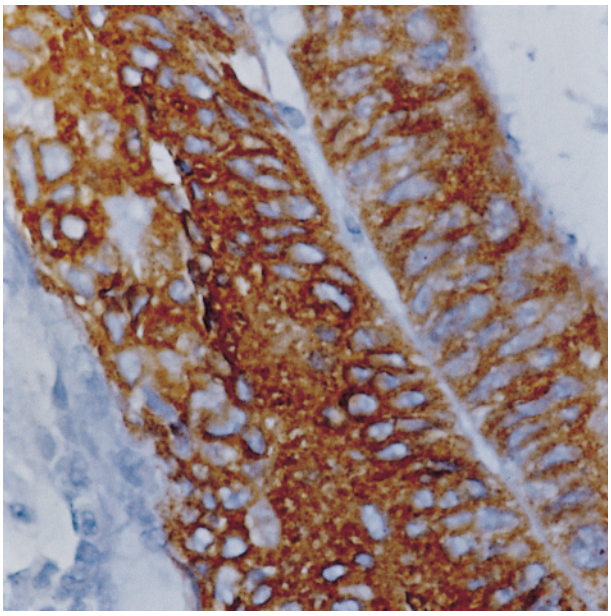

Fig. 2. In immunostaining, MUC2 was expressed in perinuclear cell membrane border (×200).

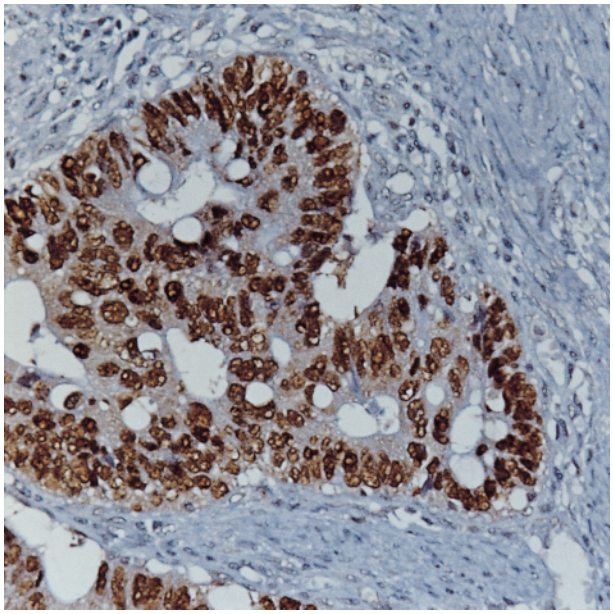

Fig. 3. In immunostainig, overexpression of p53 was noted in nuclei of colorectal carcinoma cells (×100).

Of 97 cancers, 58 (60%) showed a p53 overexpression. Neither the depth of tumor invasion nor the lymph node metastasis had a positive correlation with p53 overexpression (Table 1). In 58 cases with p53 overexpression, 26 cases showed MUC1 positivity. In these 26 cases with coexpression of p53 and MUC1, lymph node meatastasis was higher than those 35 cases with p53 overexpression and negative MUC1 (61% vs 31%, p=0.04) (Table 2). MUC2 expression had no positive correlation with p53 overexpression with respect to tumor invasion and lymph node metastasis. But combined with MUC1 expression, subgroup of MUC1$^+$/MUC2$^+$ showed a significant high frequency of lymph node metastasis than subgroup of MUC1$^+$/MUC2$^-$ (75% vs 37%, p=0.002) (Table 3).

The frequency of MUC1-positive cells in right colon cancers was significantly higher than that in left colon cancers (72% vs 33%; p=0.001) (Table 1). In contrast, the frequency of MUC2- or p53-positive cells showed no difference according to the tumor site.

Table 2. Relationship of p53 and MUC1 with lymph node metastasis

	p53 (+)	
	MUC1 (-)	MUC1 (+)
N0	22 (69%)	10 (39%)
N1, 2	10 (31%)	16 (61%)*

*: In cases with p53 overexpression, coexpression of MUC1 was related to lymph node metastasis (p=0.04).

Table 3. Relation between MUC phenotypes and lymph node metastasis

	MUC1+/MUC2-	MUC1+/MUC2+
N0	15 (62%)	4 (25%)
N1, 2	9 (38%)	12 (75%)*

*: Colon cancers with dual expression of MUC1 and MUC2 were related to lymph node metastasis (p=0.002).

DISCUSSION

The present study showed that the MUC1 expression had a significant correlation with the depth of tumor invasion and lymph node metastasis in colorectal carcinomas. This result is consistent with previous reports about up-regulation of MUC1 expression in colorectal carcinomas (19, 20). MUC2 apomucin is commonly expressed in mucinous carcinomas of the colon, pancreas, breast and ovary (22). But, MUC2 apomucin is down-regulated in non-mucinous adenocarcinoma of the colorectum (13, 14). In this study, down-regulation of MUC2 apomucin has no correlation with tumor invasion, lymph node metastasis and tumor site, but combined with MUC1 expression, dual expression of MUC1 and MUC2 was related with regional lymph node metastasis. Colon cancers with mucin phenotype of MUC1$^+$/MUC2$^-$ has been reported to be associated with peritumoral lymphocytic infiltration, thus it is suggested that MUC2 apomucin have immunosuppressant effect (20). MUC2 apomucins bear the sialosyl-Tn antigen, which is known to mediate the inhibition of natural killer cell cytotoxicity (23).

It is suggested that these immunosuppressant effect of MUC2 apomucin seems to be related with high frequency of regional lymph node metastasis in colorectal non-mucinous adenocarcinomas of MUC1 expression cases.

Overexpression of p53 is frequently noted in a variety of malignant neoplasms and metastatic tumors including colorectal carcinomas. Whether the p53 overexpression is a prognostic indicator or not remains controversial (16, 17). In this study, the frequency of p53 overexpression showed no difference in tumor invasion and lymph node metastasis. However the cancers with coexpression of MUC1 with p53 or MUC2 could be considered a high-risk group for lymph node metastasis.

An interesting result of the present study was the significantly high frequency of MUC1 positivity in the right side colon cancers. There have been few reports about high frequency of MUC1 expression in right colon cancers. It has been reported that most mucin-carbohydrate component consists of sulfomucin in the left side colon, and sialomucin in the right side colon (24, 25). Tumor cells expressing sialomucin have been shown to be less sensitive to cytolysis by human lymphokine-activated killer lymphocytes (23, 26). Thus the high level of cell surface sialomucin of the right side colon cancers may contribute to the escape from the immunological attack, resulting in an increased metastatic colonization of cancer cells (27, 28). In this study, right colon cancers showed a significantly high frequency of lymph node metastasis than left colon cancers. Thus up-regulation of sialylated MUC1 mucin in right colon cancers is suggested to relate with high frequency of regional lymph node metastasis. In cholangiocarcinomas of the liver, it has been reported that the expression of sialylated MUC1 mucin is strongly correlated with a poor outcome of patient (29).

The aim of this study was to determine the value of MUC1, MUC2, and p53 immunostaining as a marker to predict metastatic potential in colorectal cancers. In conclusion, this study revealed that MUC1 positivity and coexpression with p53 or MUC2 could be useful markers for metastatic potential in colorectal carcinomas.

REFERENCES

1. Kim YS. *Mucin glycoproteins in gastrointestinal malignancies and metastasis. Eur J Gastroenterol Hepatol 1993; 5: 219-25.*
2. Kim YS, Gum JR Jr. *Diversity of mucin genes, structure, function, and expression. Gastroenterology 1995; 109: 999-1001.*
3. Gum JR, Hicks JW, Swallow DM, Lagace RL, Byrd JC, Lamport DT, Siddiki B, Kim YS. *Molecular cloning of cDNAs derived from a novel human intestinal mucin gene. Biochem Biophys Res Commun 1990; 171: 407-15.*
4. Meerzaman D, Charles P, Daskal E, Polymeropoulos MH, Martin BM, Roes MC. *Cloning and analysis of cDNA encoding a major airway glycoprotein, human tracheobronchial mucin (MUC5). J Biol Chem 1994; 269: 12932-9.*
5. Toribara NW, Roberton AM, Ho SB, Kuo WL, Gum E, Hicks JW, Gum JR Jr, Byrd JC, Siddiki B, Kim YS. *Human gastric mucin. Identification of a unique species by expression cloning. J Biol Chem 1993; 268: 5879-85.*
6. Bobek LA, Tsai H, Biesbrock AR, Levine MJ. *Molecular cloning, sequence, and specificity of expression of the gene encoding the low molecular weight human salivary mucin (MUC7). J Biol Chem 1993; 268: 20563-9.*
7. Lapensee L, Paquette Y, Bleau G. *Allelic polymorphism and chromosomal localization of the human oviductin gene (MUC9). Fertil Steril 1997; 68: 702-8.*
8. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J, Pemberton L, Lalani EN, Wilson D. *Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J Biol Chem 1990; 265: 15286-93.*
9. Lan MS, Batra SK, Qi WN, Metzger RS, Hollingsworth MA. *Cloning and sequencing of a human pancreatic mucin cDNA. J Biol Chem 1990; 265: 15294-9.*
10. Nakamori S, Ota DM, Cleary KR, Shirotani K, Irimura T. *MUC1 mucin expression as a marker of pregression and metastasis of human colorectal carcinoma. Gastroenterology 1994; 106: 353-61.*
11. McGuckin MA, Walsh MD, Hohn BG, Ward BG, Wright RG. *Prognostic significance of MUC1 epithelial mucin expression in breast cancer. Human Pathol 1995; 26: 432-9.*
12. Toribara NW, Gum JR Jr, Culhane PJ, Lagace RE, Hicks JW, Petersen GM, Kim YS. *MUC-2 human small intestinal mucin gene structure. Repeated arrays and polymorphism. J Clin Invest 1991; 88: 1005-13.*
13. Blank M, Klussmann E, Kruger-Krasagakes S, Schmitt-Graff A, Stolte M, Bornhoeft G, Stein H, Xing PX, McKenzie IF, Verstijnen CP, Rieken E, Hanski C. *Expression of MUC2-mucin in colorectal adenomas and carcinomas of different histological types. Int J Cancer 1994; 59: 301-6.*
14. Ho SB, Niehans GA, Lyftgot C, Yan PS, Cherwitz DL, Gum ET, Dahiya R, Kim YS. *Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer Res 1993; 53: 641-51.*
15. Purdie CA, O`Grady J, Piris J, Wyllie AH, Bird CC. *p53 expression in colorectal tumors. Am J Pathol 1991; 138: 807-13.*
16. Baas IO, Mulder JW, Offerhaus GJ, Vogelstein B, Hamilton SR. *An evaluation of six antibodies for immunohistochemistry of mutant p53 gene product in archival colorectal neoplasms. J Pathol 1994; 172: 5-12.*
17. Lanza G Jr, Maestri I, Dubini A, Gafa R, Santini A, Ferretti S, Cavazzini L. *p53 expression in colorectal cancer: relation to tumor type, DNA ploidy pattern and short-term survival. Am J Clin Pathol 1996; 105: 604-12.*
18. Houbiers JG, van der Burg SH, van de Watering LM, Tollenaar RA, Brand A, van de Velde CJ, Melief CJ. *Antibodies against p53 are associated with poor prognosis of colorectal cancer. Br J Cancer 1995; 72: 637-41.*
19. Matsuda K, Masaki T, Watanabe T, Kitayama J, Nagawa M, Muto T, Ajioka Y. *Clinical significance of MUC1 and MUC2 mucin and p53 protein expression in colorectal carcinoma. Jpn J Clin Oncol*

2000; 30: 89-94.

20. Ajioka Y, Allison LJ, Jass JR. *Significance of MUC1 and MUC2 mucin expression in colorectal cancer. J Clin Pathol 1996; 49: 560-4.*
21. Hanski C, Hofmeier M, Schmitt-Graff A, Riede E, Hanski ML, Borchard F, Sieber E, Niedobitek F, Foss HD, Stein H, Riecken EO. *Overexpression or ectopic expression of MUC2 is the common property of mucinous carcinomas of the colon, pancreas, breast, and ovary. J Pathol 1997; 182: 385-91.*
22. Matsuda K, Watanabe H, Ajioka Y, Kobayashi M, Saito H, Sasaki M, Yasuda K, Kuwabara A, Nishikura K, Muto T. *Ulcerative colitis with overexpression of p53 preceding overt histological abnormalities of the epithelium. J Gastroenterol 1996; 31: 860-7.*
23. Ogata S, Maimonis PJ, Itzkowitz SH. *Mucins bearing the cancer-associated sialosyl-Tn antigen mediate inhibition of natural killer cell cytotoxicity. Cancer Res 1992; 52: 4741-6.*
24. Katsuyama T, Ono K, Nakayama J, Akamatsu T, Honda T. *Mucosubstance histochemistry of normal mucosa and carcinoma of the large intestine. Galactose oxidase-Schiff reaction and lectin stainings. Acta Pathol Jpn 1985; 35: 1409-25.*
25. Katsuyama T, Nakayama J, Ota H, Kanai M. *Mucosubstance histochemistry of the human large intestine. Rinsho Byori 1987; 35: 405-12.*
26. Moriarty J, Skelly CM, Bharathan S, Moody CE, Sherblom AP. *Sialomucin and lytic susceptibility of rat mammary tumor ascites cells. Cancer Res 1990; 50: 6800-5.*
27. Irimura T, Mclsaac AM, Carlson DA, Yagita M, Grimm EA, Menter DG, Ota DM, Cleary KR. *Soluble factor in normal tissues that stimulates high-molecular-weight sialoglycoprotein production by human colon carcinoma cells. Cancer Res 1990; 50: 3331-8.*
28. Irimura T, Nakamori S, Matsushita Y, Taniuchi Y, Todoroki N, Tsuji T, Izumi Y, Kawamura Y, Hoff SD, Cleary KR, *et al. Colorectal cancer metastasis determined by carbohydrate-mediated cell adhesion: role of sialyl-Lex antigens. Semin Cancer Biol 1993; 4: 319-24.*
29. Higashi M, Yonezawa S, Ho JJ, Tanaka S, Irimura T, Kim YS, Sato E. *Expression of MUC1 and MUC2 mucin antigens in intrahepatic bile duct tumors: its relationship with a new morphological classification of cholangiocarcinoma. Hepatology 1999; 30: 1347-55.*