Mucin Profile Expression in Gastric Adenocarcinoma

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ABSTRACT

BACKGROUND

The process of neoplastic transformation in the stomach has been reported to be associated with decreased expression of normal mucins of the gastric mucosa and denovo expression of mucins that are normally expressed in other organs. This association may be used as a means to bring new insights into biologic behavior and genetic pathways in the development of gastric cancer. There are controversial reports about differences in the genetic pathway and behavior of gastric cancer in younger patients in comparison with older ones. This study aims to determine if there is any difference in mucin profiles between different age groups with gastric cancer.

METHODS

Over a five-year (2003-2008) period, 43 cases of gastric cancer (≤50) years were referred to our center. Of these, 40 had adequate tissue for additional study, whereas three cases lacked a sufficient amount of tumor tissue for immunohistochemistry (IHC) analysis. A group of 40 gastric cancer patients above the age of 50 years were gender-matched with the first group. Expressions of MUC-1, MUC-2, MUC-5AC, and MUC-6 were evaluated by IHC for the total 80 gastric cancer cases.

RESULTS

The expressions of the mucins did not show a significant difference between the two age groups.

CONCLUSION

Gastric cancer in both young and old age adults was not significantly different in terms of mucin profiles. Our results have shown that younger age is not predictive of gastric cancer phenotype, which can be an indicator of the lack of difference in the genetic pathways and molecular alterations in these two age groups.

KEYWORDS

Mucin expression; Age; Gastric carcinoma

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INTRODUCTION

Mucins are high molecular weight glycoproteins which constitute the major component of the mucus layer that protects the gastric epithelium from chemical and mechanical aggression.[1] Normal gastric

mucosa expresses mucins MUC-1, MUC-5AC, and MUC-6. MUC-1 and MUC-5AC are expressed in the superficial foveolar epithelium, whereas MUC-6 is expressed in the mucus neck cells of the body and deeper glands of the antrum.[2]

The process of neoplastic transformation in the stomach has been reported to be associated with decreased expression of normal mucins of the gastric mucosa and denovo expression of mucins that are normally expressed in other organs (MUC-2).[3] The mucin expression pattern of gastric cancer is heterogeneous. This heterogeneity may provide new insights into the differentiation pathways of gastric cancer enabling its use as a clue to bring new insights into biologic behavior of gastric cancer.[1]

Controversy exists regarding the differences in clinicopathological characteristics of gastric cancer between young and old patients.[4,5] Young people are presumed to develop carcinoma by a molecular genetics profile that is distinct from that of sporadic carcinomas, which occur at a later age.[6]

In the present study we attempted to compare the clinicopathologic features between two age groups of patients (≤50 and >50 years of age) with the intent to determine whether differences existed between these two groups of patients in the expression of markers considered to be important in gastric carcinogenesis.

MATERIALS AND METHODS

In this retrospective study conducted over a five-year (2003-2008) period, medical charts of all patients with histologically proven diagnoses of gastric adenocarcinoma who underwent surgery in hospitals affiliated with Shiraz University of Medical Sciences were reviewed.

During this time period, there were 43 patients ≤50 years of age that were diagnosed with gastric adenocarcinoma who comprised the first group. For the second group, we selected the same numbers of patients, matched for gender, who were >50 years of age. Patients were categorized as group one (younger) and group two (older). For each case, we reviewed all the H&E slides and selected a good tumor paraffin block. In three patients from the younger age group, we were unable to obtain an adequate amount of tumor tissue for immunohistochemistry (IHC), therefore we decreased the number of cases in each group to 40.

All clinicopathological features including gastric tumor location, gross configuration, lymph node status, and depth of invasion were retrieved from each patient's medical and pathologic records.

Formalin-fixed paraffin embedded tissue blocks of the cases were retrieved and five sections prepared, one for H&E and four, for the IHC stain. For immunostaining, sections were deparaffinized in xylene and rehydrated in alcohol. Endogenous peroxidase activity was inhibited by incubating the sections in 3% H_2O_2 for 20 minutes. For antigen retrieval, specimens were boiled in a citrate solution (pH=6) for 20 minutes. All the sections were incubated in goat serum for 20 minutes, after which slides were incubated overnight with primary monoclonal antibodies at 4˚C. Sections were rinsed and incubated for 30 minutes with secondary antibody, horseradish peroxidase conjugated anti-mouse and rabbit immunoglobulin. Then, sections were rinsed and incubated with DAB for 5-7 minutes and counterstained with hematoxylin. Table 1 shows the characteristics of each antibody used for IHC.

Table 1: Characteristics of MUC antibodies used in this study.

Antibody	Clone	Dilution	Company
MUC-1	Ma695	1/400	Novocastra
MUC-2	Ccp58	1/400	Novocastra
MUC-5AC	CLH2	1/25	Novocastra
MUC-6	CLH5	1/400	Novocastra

We classified IHC reactions for mucin antibodies as: i) negative (negative IHC or reactivity in less than 10% of the tumor); ii) positive (positive reactivity in more than 10% of the cells); and iii) normal mucosa that was used as a positive control.

The chi-squared test was used to compare both groups.

RESULTS

Of the 80 patients with gastric adenocarcinoma, 58 (72.5%) were male and 22 (27.5%) were female. Group

one was comprised of 40 patients ≤50 years of age, whose ages range was: 24-50 years (41.8±7.245%). In group two, there were 40 gender-matched patients >50 years of age, whose ages ranged from 53-80 years (66.70 ±8.245).

Table 2 shows the clinicopathologic characteristics of these 80 patients according to age. There was no significant difference between two age groups regarding the different clinicopathologic findings.

Table 3 shows the percentage of gastric carcinoma in both groups according to mucin profile expression by IHC. As the table shows, there is no statistically significant difference in mucinantigen reactivity in the two groups. However, as expected in both groups, MUC-1 and MUC-5AC were the most common reactive antigens by IHC (gastric phenotype).

The different phenotypes of gastric adenocarcinoma classified according to age group are listed in Table 4. This table also emphasizes the absence of any difference between the two groups in the phenotype of gastric carcinoma (p=0.627). The gastric phenotypes expressed MUC-1, MUC-5AC, and MUC-6. Intestinal phenotypes expressed MUC-2 and gastrointestinal phenotypes expressedall the gastric and intestinal phenotypes. There were unclassified cases that had no mucin expression according to IHC analysis (Figures 1-2).

Tables 5 and 6 show the mucin profile of the patients according to location as well as Lauren's classification of the tumor type. There was no significant difference in the different locations and mucin profile, nor was a difference noted between tumor type according to Lauren's classification and mucin profile.

DISCUSSION

Gastric cancer is a disease of older patients, with a reported mean age of 50-60 years.[7]

There are numerous controversial reports as to whether gastric cancer in young patients differs from older patients.[5]

Table 2: Comparison of clinicopathologic characteristics between patients with gastric cancer, according to age.

Variable	Category	≤50 years-old	>50 years-old	Total
Tumor Location	Antrum	20 (50%)	17 (42.5%)	37 (46.25%)
	Body	16 (40%)	14 (35%)	30 (37.5%)
	Cardia	4 (10%)	9 (22.5%)	13 (16.25%)
Tumor size	1-5 cm	22 (55%)	17 (42.5%)	39 (48.75%)
	5-10 cm	15 (37.5%)	17 (42.5%)	32 (40%)
	> 10 cm	3 (7.5%)	6 (15%)	9 (11.25%)
LN Metastasis	Negative	11 (27.5%)	13 (32.5%)	24 (30%)
	N1	13 (32.5%)	14 (35%)	27 (33.75%)
	N2	11 (27.5%)	8 (20%)	19 (23.75%)
	N3	5 (12.5%)	5 (12.5%)	10 (12.5%)
Depth of Invasion	T1	4 (10%)	0	4 (5%)
	T2	5 (12.5%)	5 (12.5%)	10 (12.5%)
	T3	27 (67.5%)	33 (82.5%)	60 (75%)
	T4	4 (10%)	2 (5%)	6 (7.5%)

Table 3: Frequency of mucin antigen reactivity.

Mucins	Age group		Total	*p*-value
	≤50 years	>50 years		
MUC-1	20 (50%)	22 (52.5%)	42 (52.5%)	0.152
MUC-2	14 (35%)	9 (22.5%)	23 (28%)	0.162
MUC-5AC	21 (52%)	23 (57%)	44 (55%)	0.411
MUC-6	3 (7%)	7 (17.5%)	10 (12.5%)	0.155

Table 4: Frequencies of different phenotypes of gastric cancer between groups.

Phenotype	Age group		Total
	≤50 years	>50 years	
Gastric	24 (60%)	29 (72.5%)	53 (66.2%)
Intestinal	6 (15%)	3 (7%)	9 (11.2%)
Gastric-intestinal	8 (20%)	6 (15%)	14 (17.5%)
Unclassifiable	2 (5%)	2 (5%)	4 (5%)

Table 5: Tumor mucin profiles in study patients according to tumor location.

Location	MUC-1	MUC-2	MUC-5AC	MUC-6
Antrum	17 (42%)	11 (27.5%)	20 (50%)	3 (7.5%)
Body	18 (56.2%)	11 (34.3%)	22 (68.75%)	5 (15.6%)
Cardia	7 (87.5%)	1 (12.5%)	2 (25%)	2 (25%)

Table6: Tumor mucin profiles in study patients according to Lauren's classification.

Lauren's classification	MUC-1	MUC-2	MUC-5AC	MUC-6
Intestinal	20 (58.8%)	13 (38.2%)	19 (55.8%)	4 (11.8%)
Diffuse	20 (50%)	7 (17.5%)	20 (50%)	6 (15%)
Mixed	2 (33.3%)	3 (50%)	5 (83%)	0

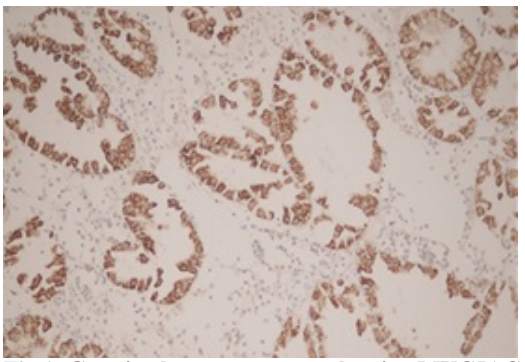

Fig.1: Gastric phenotype cancer showing MUC5AC expression. (400x)

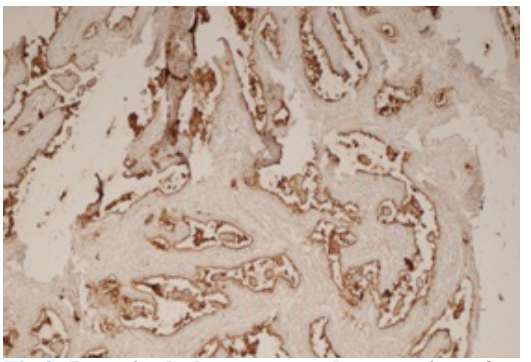

Fig.2: Intestinal phenotype and expression of MUC6. (400x)

Some reports have indicated that gastric cancer in young adults is more aggressive and others have reported a worse prognosis.[7,8]

There are also reports about the close relation of gastric cancer phenotype (determined by type of mucin expression) with tumor invasion and genetic alteration.[9]

Meanwhile, differences in mucin expression in the neoplastic compared to the normal stomach suggest a possible regulatory role for these mucins in gastric epithelial cell proliferation and differentiation.[10]

In this study we attempted to evaluate the mucin expression profile in 80 gastric cancer patients by IHC with monoclonal antibodies to MUC-1, MUC-2, MUC-6, and MUC-5AC. We compared the mucin expression in two different age groups, ≤50 and >50 years of age, but there was no statistically significant difference in these two

age groups in the current study.

In some reports, the relationship between mucin expression and clinicopathologic behavior of gastric cancer has been discussed, and a close relationship between tumor differentiation phenotype and invasion was shown.[9] Pinto-de-Sousa et al.[1] have reported an association between tumor type and location with mucin expression.

Silva et al. in a study from Brazil3showed higher MUC-2, MUC-5AC, and MUC-6 expression in young patients compared with the older group, but they did not find significant difference between the positivity rates of MUC-1 in the two groups.

Another study from Japan[11] showed that MUC-1 expression was influenced by age, with higher expression in the older group, however MUC-2 expression did not correlate with patient age.

Our results showed no significant difference between gastric cancer in terms of mucin expression. This finding was in accordance with our previous study[12] which has noted an absence of any relationship between age and cell adhesion molecule markers. These results can be indicative of the lack of difference in genetic pathways and cancer progression in these two age groups.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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