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ORIGINAL RESEARCH

The Prognostic Implications of FIX and FLO Patterns in Mucinous Colon Carcinomas

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Abstract

Purpose Colon mucinous carcinomas (MUCs) have two morphological patterns: (i) glands lined by mucinous epithelium with direct contact to the stroma (FIX) and (ii) carcinoma cells floating in mucin (FLO). In this study, we evaluated the prognostic value of these patterns.

Methods Digital images were captured from the 38 MUC's tissue sections. A grid with 140 points was laid over the computer screen. Totally, 100 points, falling on tumor cells floating in mucin (FLO patterned cells) or on cells contacting stroma (FIX patterned cells), were counted. Tumors were grouped according to the median value of the FIX patterned cells. Cases with more than this value were grouped as FIX and less were grouped as FLO cases. The prognostic value of FIX and FLO pattern was evaluated.

Results The median for FIX patterned cells was 66%, and the cases with lower values than this were grouped as FLO ($N = 18$; 47.37%), while the rest were grouped as FIX cases. There was no significant difference between FIX and FLO cases for overall survival cases ($p = 0.167$). For FIX cases, 62.7 and 51.3% of the patients were alive at second and third years, while this was 78.9 and 72.4% for the FLO group, respectively.

Conclusions This is the first study using a quantitative methodology depending on count pointing to evaluate FIX/FLO feature of MUCs to the best of our knowledge, although we could not observed any prognostic and clinicopathologic relationship statistically. This distinctive feature should be studied in larger cohorts with prognostic information, with a quantitative method, like the one that was applied in this study, in order to achieve strict conclusions.

Keywords Mucinous carcinoma · Colon · Prognosis · FIX · FLO

Introduction

Mucinous carcinoma (MUC) is an adenocarcinoma, characterized by abundant extracellular mucin production. By definition, tumor cells in MUC secrete abundant extracellular mucin that accounts for at least 50% of the tumor volume [1–5]. This histologic subtype accounts for 4.5–15% of all colorectal carcinomas (CRC) [6]. MUCs have distinct clinicopathologic features and the prognosis is worse than usual adenocarcinomas [3]. Age [7, 8], sex [8], tumor location [8], extension outside of the bowel wall [9], peritoneal dissemination [10], lymph node metastasis [10], absence of Crohn-like infiltrate [7], and higher tumor stage [7, 8, 11] are previously reported prognostic factors in colorectal MUCs. Colorectal MUCs are encountered usually as big mass lesions at late stage, and they are frequently located at proximal colon [12]. Moreover, some colorectal MUCs are related to the Lynch syndrome [13].

Statement: The data was presented at the European Congress of Pathology in 2013 as a poster presentation (The prognostic implications of percentage of tumor cells contacting surrounding stroma in mucinous colorectal carcinomas; Virchows Archiv volume 463 issue 2 pages 200–206) achieving bursary award from the congress organization. This study was also presented as a poster at Turkish Congress of Pathology (19–23 November 2014, Trabzon Turkey) and received the "Best poster presentation award."

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The importance of tumor microenvironment for the tumor growth is well recognized. Signals like growth factors secreted by the stromal cells are important for tumor cell maintenance and, stroma is the major supplier of nutrients and oxygen which are required for tumor growth as well [14]. Yakirevich et al. presented stromal interactions of the mucinous salivary gland tumor cells as a valuable histopathological feature [15]. Colorectal MUCs also have similar types of interaction with the stroma in which some of them are predominantly comprised of cells completely floating in the mucin (FLO pattern), while others are composed of cells which have contact with stroma and producing abundant mucin at the same time (FIX pattern). These patterns were included in the 1989 World Health Organization classification, but not included at the more recent classifications [16]. Previously, in a series of colorectal MUCs, Ikeda et al. [17] evaluated the prognostic value of FIX and FLO patterns; however, those cases were classified only by conventional morphological evaluation. The FLO pattern was identified as a poor prognostic factor. We evaluated the prognostic value of FIX and FLO pattern in a series of colon MUC cases with manual point counting method for the first time to the best of our knowledge, for identifying the tumor cell-stroma interaction.

Materials and Methods

All colon MUC cases with follow-up information which were diagnosed consecutively between 2000 and 2011 in our institute were re-evaluated from the slides of surgical resection specimens. Cases with more than 50% of the extracellular mucin production were included; however, if the signet ring cell component was exceeding 50% of the neoplastic cells, the cases were excluded from the series. Cases that received neoadjuvant chemoradiotherapy were also excluded for avoiding posttreatment mucinous changes. Finally, 38 patients with colon MUCs were selected for the study. The patients were treated with radical surgery and/or adjuvant chemotherapy.

Demographic features, tumor localization, operation type, pathological tumor and lymph node stage (pT and pN, respectively), grade, lymphatic, venous, perineural invasion, tubular and radial margin, local recurrence, distant metastasis, and oncologic outcome (alive/dead) were determined from the medical records of the patients. Time of disease-related or all-cause death and date of the last follow-up time were recorded.

Histopathologic Evaluation

Thirty-eight colon MUC cases with prognostic information were included in this study. Images were obtained from the H&E stained slides from superficial to deep tumor zones by a 3-CCD color video camera (Olympus DP70, Olympus Optical Co. Ltd., Tokyo, Japan) connected to a light microscope (Olympus BX51, Olympus Optical Co. Ltd., Tokyo, Japan) at ×10 original magnification and saved to a personal computer. A transparent and A4-sized paper grid with equally spaced (2 cm) points which had 10 horizontal points and 14 vertical points was laid over the tumor images. Points, falling on floating tumor cells in mucin (Fig. 1), and tumor cells contacting surrounding stroma (Fig. 2) were counted separately till a total count of 100 cells were achieved. The number of the microscopic fields counted, till having 100 carcinoma cells falling on the grid points, was also noted for each case (Fig. 3), as an indirect reflection of cellularity; more cellular tumors were expected to require less number of microscopic image fields. The percentage of tumor cells contacting stroma (PTCCS) was analyzed statistically along with the clinicopathological prognostic parameters.

Statistical Analysis

Statistical analysis was conducted by Scientific Package for Social Sciences (SPSS Version 24). Non-parametric tests such as chi-squared test and independent-sample *t* test were applied for comparison of these groups. Survival curves were plotted by the Kaplan-Meier method.

The project was approved by the Dokuz Eylul University Ethics Committee at 25 January 2014 (no.: 873-GOA).

Results

Fourteen (36.8%) female and 24 (63.2%) male patients were included in this study. The median age was 65 (range 37–96) years. The clinicopathologic and prognostic features are summarized in Table 1. We divided the cases as a high and low pT stage according to the existence of visceral peritoneal or solid organ invasion. Pathologic T stages were pT2–3 for 17 (44.7%) and pT4 for 21 (55.3%) patients. There were not any pT1 cases in the study. There were 10 (26.3%) patients without lymph node metastasis (pN0) alongside of 14 (36.8%) pN1 and pN2 cases. Lymphatic and perineural invasion were observed in 13 (34.2%) and 9 (23.7%) patients, respectively. The local and distant recurrence were identified in four (10.5%) and eight (21.1%) patients, respectively. Ten (26.3%) patients had synchronous distant metastasis. Median follow-up time was 39 months. There were only 22 (57.9%) patients alive at the end of the follow-up.

The median value for PTCCS was 66%, and the cases were divided into low and high PTCCS according to the median value (Figs. 1 and 2). PTCCS high cases were named as FIX, and low cases were named as FLO. There was not any significant difference between the number of images evaluated

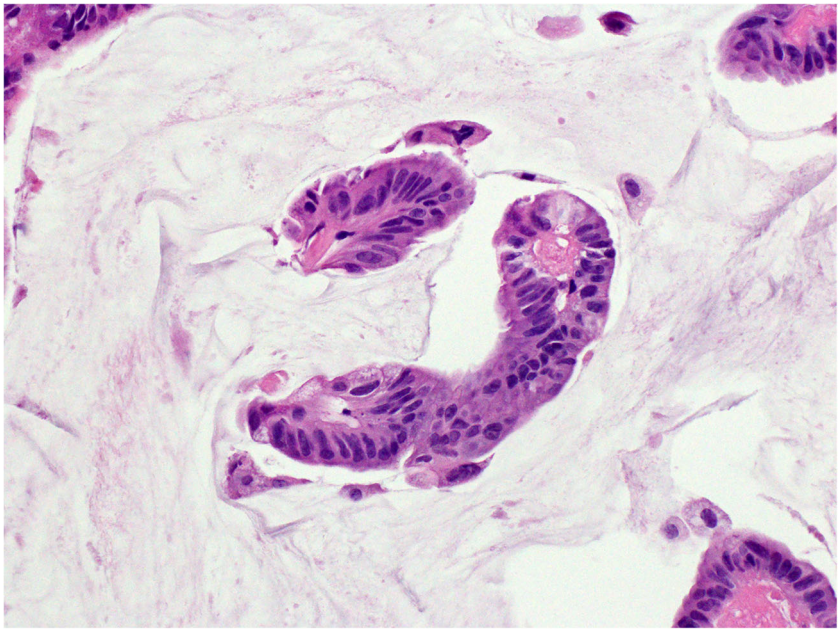

Fig. 1 Tumor cells floating in the mucin

while counting till 100 tumor cells and FIX/FLO feature ($p = 0.501$). There was also no significant difference between the FIX/FLO feature and the pT stage ($p = 0.744$).

For the FIX cases, 62.7% of the patients were alive at second year and 51.3% at the third year, while this was 78.9 and 72.4% for the FIX cases, respectively. We observed that the survival difference increases with time as FLO and FIX survival rates at the end of fifth year were 36.7 and 72.4%, respectively, although there is no significant difference in the Kaplan-Meier survival analysis.

Discussion

MUCs originate from colorectal epithelium, and they are characterized with extracellular mucin production [1–4]. MUCs comprise 4.5 to 15% of all colorectal cancers, and they seem to be different from non-mucinous carcinomas with their different pathogenetic pathways, pathogenetic profiles, and clinicopathological characteristics. MUCs have several distinctive clinicopathological features including, emergence in young age, presentation at late stage, frequent lymph node metastasis,

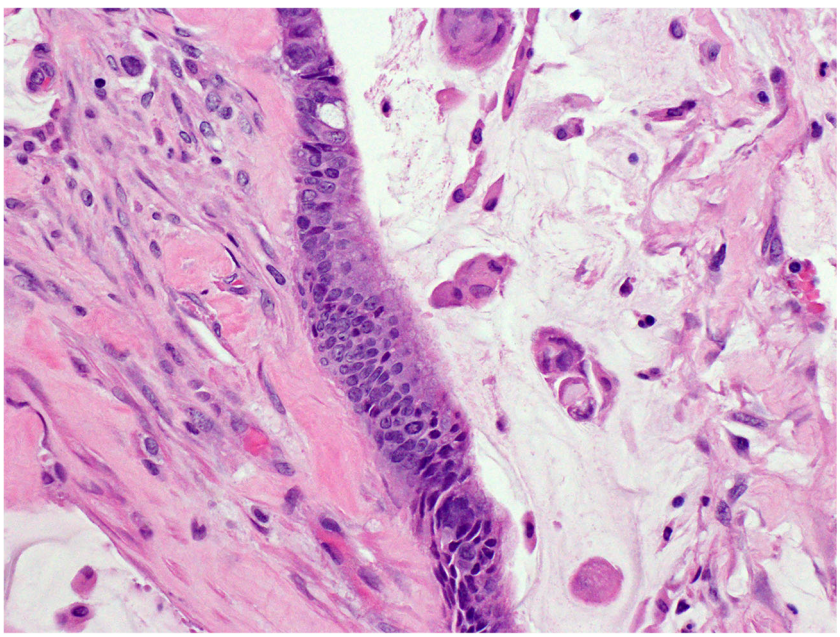

Fig. 2 Tumor cells contacting to the stroma

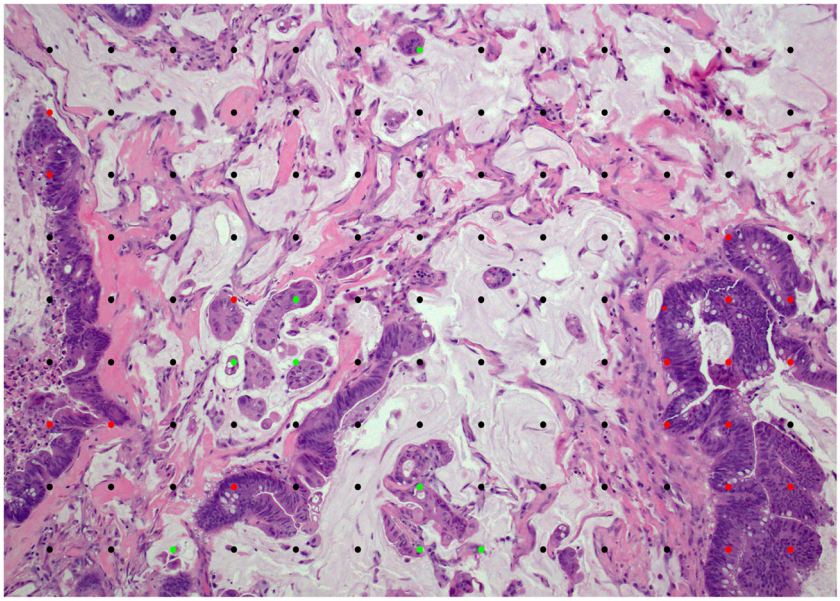

Fig. 3 Illustration of the point counting method. Green dots were used to mark the tumor cells floating in the mucin; red dots are used to mark the tumor cells contacting to the stroma; black dots are marked the remaining non-tumoral area. FIX (red dots) and FLO (green dots) cells are independently counted till a total count of a hundred

proximal colon involvement, peritoneal extension, and lower curative resection rate [4, 6].

There are controversial results about the significance of mucinous histology as a prognostic marker in colorectal cancer [6, 12]. In some former studies, mucinous histology has not been reported as an independent prognostic factor for overall survival [18, 19] and it is not accepted as a predictive factor in the therapeutic algorithm of the National Comprehensive Cancer Network, AJCC, and CAP guidelines [20, 21]. However, there are some studies reporting MUC histology as a poor prognostic marker associated with the other poor prognostic histopathological features [22–24].

Table 1 Distribution of the cases according to the median value of PTCCS

PTCCS value		*N*(%) < 66% (FLO)	*N*(%) > 66% (FIX)	Total *N*(%)
pT	Low (2–3)	8 (21.1)	9 (23.7)	17 (44.7)
	High (4)	11 (28.9)	10 (26.3)	21 (55.3)
pN	N0	5 (13.2)	5 (13.2)	10 (26.4)
	N1	5 (13.2)	9 (23.6)	14 (36.8)
	N2	9 (23.6)	5 (13.2)	14 (36.8)
Grade	Low	14 (36.8)	16 (42.1)	30 (78.9)
	High	5 (13.2)	3 (7.9)	8 (21.1)
Lymph vessel invasion	Negative	13 (34.2)	12 (31.6)	25 (65.8)
	Positive	6 (15.8)	7 (18.4)	13 (34.2)
Venous invasion	Negative	13 (34.2)	16 (42.1)	29 (76.3)
	Positive	6 (15.8)	3 (7.9)	9 (23.7)
Perineural invasion	Negative	12 (31.6)	17 (44.7)	29 (76.3)
	Positive	7 (18.4)	2 (5.3)	9 (23.7)
Tubular margin	Negative	17 (44.7)	18 (47.4)	35 (92.1)
	Positive	2 (5.3)	1 (2.6)	3 (7.9)
Local recurrence	Negative	17 (44.7)	17 (44.7)	34 (89.4)
	Positive	2 (5.3)	2 (5.3)	4 (10.6)
Distant recurrence	Negative	17 (44.7)	13 (34.2)	30 (78.9)
	Positive	2 (5.3)	6 (15.8)	8 (21.1)
Sync. distant metastasis	Negative	15 (39.5)	13 (34.2)	28 (73.7)
	Positive	4 (10.5)	6 (15.8)	10 (26.3)
Status (alive/ex)	Alive	14 (36.8)	8 (21.1)	22 (57.9)
	Dead	5 (13.2)	11 (28.9)	16 (42.1)

Recently, an increased percentage of tumor stroma has been described as an important prognostic factor [25–27] in colorectal carcinomas, and this finding was explained with many interesting mechanisms. The composition of the stroma is important in the destiny of both the tumor and the patient. It can even sometimes change the tumor morphology. In their experimental study, Dingemans et al. [28] presented that when colon carcinoma tumor cells were transplanted into a granulation tissue, they grew in an infiltrative pattern; however, they formed encapsulated and well-differentiated tumors at undisturbed stroma. Stroma provides nutrients and tumor growth factors in addition to secreting matrix metalloproteases and their inhibitors, and they might act as a barrier to penetration of the immune response [29–31]. Tumor stromal cells have been shown to contribute to Warburg effect in a reverse fashion increasing the tumor growth advantage [32, 33].

Although the prognostic factors are well studied in colorectal adenocarcinomas, the knowledge about the MUCs is relatively sparse [6–10] and further prognostic and predictive factors are required. FIX and FLO patterns are disregarded for some time, probably due to the difficulty of separation in cases with mixed patterns and no cut point was described previously. Tumor cells show variable features regarding FIX or FLO patterns for most of the MUC cases. Ikeda et al. [17] evaluated the prognostic value of FIX and FLO patterns in a series of 41 colorectal carcinoma cases and found 19 (46.34%) had FLO pattern and these cases had less p53 expression but poor survival. However, they did not describe the methodology during the histopathological evaluation and dichotomous classification of the cases into FIX and FLO types.

Yakirevich et al. [15] carried out a study in mucin producing salivary gland tumors which have morphologic patterns partially resembling to FIX and FLO patterns, and they evaluated the importance of neoplastic cell stroma and mucin contact percentage as a measure for the differential diagnosis of colloid carcinoma and mucinous cystadenocarcinoma. In this study, neoplastic epithelium and stroma relationship was interpreted in five microscopical fields by two observers at ×100 magnification, by semiquantitative methods. In this series, we performed point counting, which is accepted as an objective method [34, 35]. In contrast to Ikeda et al. [17], we observed that the FIX/FLO feature has no effect on overall survival in the Kaplan-Meier survival analysis and there were not any significant relationship between the FIX/FLO feature with the other clinicopathologic factors as well. The main difference between the two studies was probably the method applied for dichotomous classification of the cases into FIX and FLO patterns, which might have led to different prognostic information. However, this is a preliminary study including small number of cases as the series by Ikeda et al. [17] (38 cases and 41 cases, respectively) and this feature should be studied in larger cohorts, with a quantitative method, like the one that was applied in this study, in order to achieve precise results reflecting the prognostic potential of FIX/FLO patterns.

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Compliance with Ethical Standards The project was approved by the Dokuz Eylul University Ethics Committee at 25 January 2014 (no.: 873-GOA).

Conflict of Interest The authors declare that they have no conflict of interest.

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