RESEARCH ARTICLE

Lack of Relationships between FGF19 Staining Pattern, Lymph Node Metastasis and Locally Invasive Characteristics of the Tumor in Colorectal Cancers

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Abstract

Introduction: Colorectal cancers are in the top of the cancer-related causes of death in the world and lymph node metastasis is accepted as the primary prognostic factor. In this study, correlations of FGF19 staining pattern with local invasion and lymph node metastasis in a series of colorectal cancers were investigated. Methods: This studyincluded 81 colorectal cancer patients who underwent surgery in our hospital with no evidence of preoperative radiological distant metastasis. Routine pathological examination of the resection material was performed in order to identify vascular, perineural and serosal infiltration, regional lymph node metastasis and the degree of differentiation. Tumor tissue samples were stained with an immunohistochemistry method for FGF 19 evaluation and the staining pattern was statistically compared with the above mentioned characteristics of the tumors. Results: The patient population consisted of 47 females and 34 males with a median age of 70 years. In 40 patients regional lymph nodes were positive and 51%, 32% and 38% had serosal, perineural and vascular invasion. While 64 cases were moderately-differentiated, 11 cases were well-differentiated and 6 poorly-differentiated, there was no association with FGF 19 staining, including intensity. Conclusion: No evidence of significant statistically correlation was found between FGF 19 staining pattern and serosal, perineural, vascular invasion, lymph node involvement and degree of differentiation.

Keywords: Colorectal cancer - FGF staining - lymph node metastasis

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Introduction

Colorectal cancer is among the top of the most common invasive cancers and is ranking the second in cancer-related cause of death (Jemal et al., 2011). One of the main factors indicative for both prognosis and the treatment planning in colorectal cancer (CRC) patients is the status of regional lymph node metastasis (Compton et al., 2000; Johnson et al., 2006; Rosenberg et al., 2008; Wang et al., 2008; Royston et al., 2009; Ainsworth et al., 2010). Therefore, markers predicting the lymph node involvement are widely investigated in order to assist the surgical and medical treatment planning of these patients (Karamitopoulou et al., 2011).

The interaction of the microenvironment of the tumor and the host has a key role in colon cancer metastases (Gout and Huot, 2008). Various pathways interact with microenvironment and play a key role in the spread and progression pattern of the tumor. One of these pathways is the fibroblast growth factor (FGF) pathway. FGF family is comprised 22 polypeptides, having a similar structure but different biological effects and acts over the fibroblast growth factor receptors 1-4 (FGFR 1-4) (Ornitz, 2005). These polypeptides in general act through the autocrine and paracrine pathways, whereas FGF19 also has hormonal effects and shows this effect by binding to FGFR4 (Xie et al., 1999). While FGF19 is a major metabolism regulator under normal physiological conditions, it contributes to the development and progression of cancer (Lin et al., 2012). FGF19 signaling pathway has proliferative and chemotactic effects on cancer cells and increases motility, extends survival, stimulates angiogenesis and also increases adhesion (Nicholes et al., 2002; Miyake et al., 2005; Engstrom et al., 2006; Desnoyers et al., 2008; Pai et al., 2008; Siffroi-Fernandez et al., 2008; Wu et al., 2010). Likewise, it is known that the tumor is more aggressive, in cases where FGF 19 is overexpressed (Feng et al, 2013; Hyeon et al., 2013). This study designed in the light of the foregoing facts and aimed to assess the correlation between the local invasiveness of the tumor and the regional lymph node metastasis with FGF19 staining patterns of the colorectal cancer tissue samples.

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Materials and Methods

This study was approved by Baskent University Institutional Review Board and Ethics Committee (Project no: KA13/12) and supported by Baskent University Research Fund.

Records of the patients, operated in our hospital between 2008 and 2012 upon colorectal cancer diagnosis, were reviewed in terms of eligibility for inclusion in the study. Patients, having distant organ metastasis detected by preoperative radiological tests, were not included in the study.

The vascular, perineural and serosal invasion and degree of differentiation (well, moderate, poor) of the primary tumor were noted during the pathological evaluation. Colorectal cancer patients, who underwent colon resection, were divided into two groups on the basis of their regional lymph node involvement. Patients, identified to have tumor involvement in at least one of the regional lymph nodes, were included to the lymph node positive group and those identified to have no lymph node involvement, in to the lymph node negative group. Immunohistochemical polyclonal antibody FGF19 (ab119336, 1:500; abcam, England) staining was performed for both of the groups on the cross-sections prepared from the paraffin blocks of the tumor, which was fixed in 10% formaldehyde solution. Presence of cells demonstrating cytoplasmic staining for FGF 19 was investigated with the light microscopy. The proportion of stained tumor cells was determined semi-quantitatively and each sample was scored on a scale of 0-2 (0; 0-5%, 1; 5-50%, 2; over 50% positive staining of the tumor cell). Normal gall bladder epithelium was used as the positive control. The correlation between FGF 19 staining pattern and the lymph node status, local infiltration parameters (vascular, perineural and serosal infiltration) and the degree of differentiation of the tumor were statistically evaluated.

Statistical analysis

Demographic and clinical data of the patients were analyzed with the SPSS 17.0 package program. Since all data did not demonstrate normal distribution, non-parametric tests were used. For this reason, besides the chi-square test, Mann-Whitney U and Kruskal-Wallis tests were used for the analysis of numeric data. P<0.05 value was considered as statistical significance. All tests were run in two-ways.

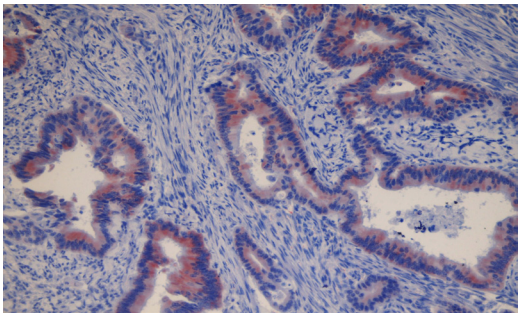

Figure 1. Pattern of Strong Cytoplasmic Stain with Polyclonal Antibody to FGF19 in Tumor Tissue (x200)

Table 1. Patient Characteristics

		All patients (n=81)	Lymph node involvement No (n=41)	Yes (n=40)	P*
Age (year)	Median	70 (36-97)	69.4	70	0.499
Diameter (cm)	Median	4.5 (1.2-13)	4.5	4.5	0.786
No. of lymph nodes removed	Median	16.5 (3-64)	16.5	16.5	0.573
No. of lymph nodes involved	Median	1 (0-24)			
			Yes (%)	No (%)	
Gender	Female	47 (58.0)	56.1	60.0	0.722
	Male	34 (32.0)	43.9	40.0	
Localization	Rectum	21 (25.9)	19.5	32.5	0.158
	Colon	60 (74.1)	80.5	67.5	
Differentiation	Well	11 (13.6)	24.5	2.5	0.008
	Moderate	64 (79.0)	65.9	92.5	
	Poor	6 (7.4)	9.8	5.0	
Perforation	No	74 (91.4)	90.2	92.5	0.718
	Yes	7 (8.6)	9.8	7.5	
Serosal involvement	No	40 (49.4)	56.1	42.5	0.221
	Yes	41 (50.6)	43.9	57.5	
Vascular invasion	No	50 (61.7)	85.4	37.5	<0.001
	Yes	31 (38.3)	14.6	62.5	
Perineural invasion	No	55 (67.9)	87.8	47.5	<0.001
	Yes	26 (32.1)	12.2	52.5	
FGF 19 staining pattern	No	39 (48.1)	48.8	47.5	0.348
	Weak	32 (38.5)	43.9	35.0	
	Strong	10 (12.3)	7.5	17.5	

Results

A total of 81 colorectal cancer patients were included in the study 40 patients had lymph node metastasis 41 and no lymph node metastasis. Patient characteristics are given in Table 1. The median age of the patients was 70 (36-97). 58% of the patients were female and 42% male. In 74 percent of the cases, the tumor was localized in the colon, whereas in 26 percent in the rectum. Moderately-differentiated tumors were the most common (79%). There was tumor perforation in 7 percent of the cases. Serosal infiltration was found positive in 51% of the cases, perineural invasion in 32% and vascular invasion in 38% of the cases. The median number of lymph nodes removed was 16.5 (3-64) and the median number of lymph nodes involved was 1 (0-24).

The age characteristics and the number of lymph nodes removed were similar among the both groups with and without lymph node metastasis (p=0.499 and p=0.573, respectively). Moreover; age, localization, tumor perforation, serosal infiltration were found similar in both groups. Tumor differentiation (p=0.008), vascular invasion (p<0.001), perineural invasion (p<0.001) were significantly different in lymph node positive cases. The presence of perineural invasion and vascular invasion were found to be higher in lymph node positive cases.

While no FGF 19 staining was observed in 48% (n=39) of the cases, weak FGF 19 staining was observed in 40% (n=32) of the cases and strong staining in 12% (n=10) of the cases (Figure 1). FGF 19 staining pattern was similar within the group with and without lymph node involvement (p=0.348). FGF 19 staining pattern showed no difference in terms of gender, localization, differentiation and the presence of perineural and vascular invasion (Table 2). No statistical difference was found for the same parameters in the group with mph node involvement.

Table 2. Clinical Characteristics of the Patients Over FGF Staining Pattern

		FGF 19			p
		No staining	Weak	Strong	
Age (year)	Median	69.5	72.5	67.5	0.510
Diameter (cm)	Median	5.0	4.25	4.25	0.081
No. of lymph nodes removed	Median	17.5	15	16	0.239
No. of lymph nodes involved	Median	1	0	2	0.305
		No staining (%)	Weak (%)	Strong (%)	
Gender	Female	40.4	46.8	12.8	0.233
	Male	58.8	29.4	11.8	
Localization	Rectum	33.4	47.6	19.0	0.364
	Colon	53.4	36.6	10.0	
Differentiation	Well	36.4	36.4	27.3	0.227
	Moderate	46.9	42.2	10.9	
	Poor	83.3	16.7	0	
Perforation	No	47.3	40.5	12.2	0.825
	Yes	57.1	28.6	14.3	
Serosal involvement	No	45.0	47.5	7.5	0.229
	Yes	51.2	31.7	17.1	
Vascular invasion	No	44.0	42.0	14.0	0.618
	Yes	54.8	35.5	9.5	
Perineural invasion	No	54.5	38.2	7.3	0.078
	Yes	34.6	42.3	23.1	

Discussion

Colorectal cancer is one of the most common cancer and is ranking the second among the cancer-related causes of death (Jemal et al., 2011). Mortality rates are still very high despite of all efforts. The major factor indicative of the disease prognosis is the stage of the tumor (TNM). N-stage of the tumor is identified through the pathological evaluation surgical specimen. However, in cases were the number of removed lymph nodes is inadequate, the reliability of the N stage would diminish and the patients with the same N stage would demonstrate different clinical courses (Quirke et al., 2007). Therefore, biomarkers to predict the lymph node metastasis prior to the surgery have been widely investigated. Karamitopoulou et al. (2011) studied 21 protein markers acting through the carcinogenetic signaling pathways on the tumor tissues taken from 221 colorectal cancer patients, in order to determine the tumor N stage. As a result of the study, they have concluded that e-cadherin is an efficient and independent marker in predicting N stage in colorectal cancers. E-cadherin is a peptide required for the stabilization of inter-cellular connections.

The first and foremost step in tumor metastasis is the separation of the tumor cell from the primary focus and passing to the circulation. This step may occur with the weakening or breaking of the inter-cellular connections. The interaction of e-cadherin with α and β catenin plays a critical role in ensuring a stable inter-cellular connection. This interaction is regulated with the tyrosine phosphorylation/dephosphorylation of β catenin. Pai et. al. demonstrated that externally administrated FGF 19 enhances the tyrosine phosphorylation of β catenin and breaks the e-cadherin/ β catenin relation (Pai et al., 2008). Disconnection of this relation results in the breaking of inter-cellular connection and eventually the separation of the tumor cells from the primary focus, which is the first step required for metastasis. Likewise, in this study it has been concluded that when FGF19 is blocked with 1A6, therefore blocking the antibodies against FGFR4, the receptor of FGF19, this action of FGF19 is removed. This earlier study thus commented that FGF 19 may play a potential role in tumor progression and metastasis (Pai et al., 2008).

FGF 19 affects proliferation in cancer tissue, cell survival, motility and adhesion (Nicholes et al., 2002; Miyake et al., 2005; Engstrom et al., 2006; Desnoyers et al., 2008; Pai et al., 2008; Siffroi-Fernandez et al., 2008; Wu et al., 2010) and is also densely expressed in the cancer tissue, when compared to the normal tissue (Desnoyers et al., 2008; French et al., 2012). It has been demonstrated that the tumors have a more aggressive progression, as a result of the interaction of FGF 19 in particular with the Wnt pathway (Katoh, 2006). In the light of the foregoing data, we investigated the FGF 19 staining pattern in the tumor tissues of patients without any detected distant organ metastasis. We compared the local invasiveness characteristics of the tumor (vascular invasion, perineural invasion, serosal infiltration, degree of differentiation and local lymph node spread) with the FGF 19 staining pattern.

Our expectation was to observe more intense FGF 19 staining in the locally invasive colon cancers with lymph node involvement, considering the results of the previous studies. However the statistical evaluation, FGF 19 staining pattern were found similar in both the lymph node positive and negative group. Moreover, FGF 19 staining pattern was not different in terms of vascular, perineural, serosal infiltration status and the degree of differentiation. When the lymph node positive group was considered alone, no statistically significant difference was found between the number of lymph nodes involved and the FGF 19 staining pattern. According to the results of our study; the intensity of FGF 19 in the tumor tissue was not correlated with the local invasiveness and lymphatic metastasis capacity of the tumor. We believe that the main reason for this difference with other studies was related to the tissues which we have studied. While previous studies were mainly on colon cancer cell cultures, we used the paraffin blocks. Our purpose was to find a marker, that may be used in clinical practice and which may assist in predicting the prognosis of the disease. In pursuit of this aim, one may criticize the need for including a couple of different extra markers associated with tumor genesis in the study, besides FGF 19. However, taking into account of physiological principles, aim of the study and the study costs, we set FGF 19 as the primary target of investigation.

In conclusion, events occurring in the primary tumor microenvironment play a key role in tumor metastasis and the prognosis of the patient. In view of our study, despite FGF 19, being a major player in the tumor microenvironment, presently is not a favorable marker for use in clinical practice. However, we believe that there is still a need for further studies in order to investigate the clinical implications of FGF 19 with regard to colorectal tumor progression and spread.

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