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ORIGINAL ARTICLE

Prognostic significance of EGFR and COX-2 expression in colorectal cancer and their association. A study in Greek population

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Summary

Purpose: *Colon cancer is one of the most common malignancies. Various prognostic markers have been proposed and individualized treatment strategies have been adapted according to tumor molecular and genetic characteristics. The purpose of the present study was to retrospectively analyze a possible association between the expression of COX-2 and EGFR and clinical and histopathological factors of patients undergoing colon surgery in a Greek population.*

Methods: *Data from our department's prospectively collected database were retrieved for a total of 100 consecutive colectomies that were performed in our department. We examined patient age, sex, tumor stage and location of the tumor. Histological data were also retrieved concerning major tumor diameter, histological grade and immunohistochemical expression of cyclooxygenase (COX)-2 and epithermal growth factor receptor (EGFR).*

Results: *There was no difference between tumors of different differentiation in the expression of EGFR (p=0.146), while there was statistically significant difference in the expression of COX-2 between these groups (p=0.001). There was no difference between these patients in the expression of EGFR (p=0.136), while a statistically significant difference was found in the expression of COX-2 between the same patient groups (p<0.005).*

Conclusion: *These data are quite important in order to certify that colorectal cancer molecular and genetic diversity between different study populations is not a confounding factor in the application and clinical implementation of trending individualized decision making in oncological treatments.*

Key words: *colorectal cancer, COX-2, EGFR*

Introduction

Colon cancer is one of the most frequent cancers worldwide and consists of the ranks as second most common cause of death in the western world. A lot of research has been conducted concerning the natural history of this disease and a clear path of molecular events leading from the appearance of adenomatous polyps to the development of dysplasia, invasiveness and metastatic potential.

Several factors have been linked with the aggressiveness of colon cancer and others have been implicated in the prediction of decreased response to treatment. An effort to individualize treatment for each patient and find patients that can respond to different kinds of treatments is a common place in oncology.

COX-2 has been found to have a major role in the development of colon cancer and increased

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expression has been associated with aggressive tumors and potentially decreased survival [1]. Recent studies have also examined possible benefits of COX inhibitor therapy in combination with chemotherapy in tumors that have lost chemosensitivity [2].

On the other hand, increased expression of EGFR has been reported in many malignancies. It is a member of the receptor tyrosine kinase family which has been linked with tumor cell motility, adhesion and metastatic potential [3]. This has led researchers to develop and use EGFR inhibitors in order to treat patients with metastastic colon cancer [4,5].

The purpose of the present study was to retrospectively analyze a possible association between the expression of COX-2 and EGFR and clinical and histopathological factors of patients undergoing colon surgery in our department in a Greek population.

Methods

Data collection

Data from our department's prospectively collected database were retrieved for a total of 100 consecutive colectomies that were performed in our department. We examined patient age, sex, tumor stage and tumor location. Histological data were also retrieved concerning major tumor diameter, histological grade and immunohistochemical expression of COX-2 and EGFR.

Patients with a past medical history of other malignancy were excluded from the study. In addition, we excluded patients with hereditary colon cancer and polyposis syndromes and also those with inflammatory bowel disease.

The study was approved by our Departments ethics committee for the conduction of studies in human specimens.

Immunohistochemical protocol

Tissue paraffin blocks were retrieved from the Pathology department of our hospital concerning patients that met the inclusion criteria of our study. Thin sections of 5μm were cut from paraffin blocks and were placed in oven at 60°C for 15 min. The sections were dewaxed with xylol and ethanol, re-hydrated in distilled H_2O and immersed in tris-buffered saline (TBS) buffer (pH 7.6).

Sections were stained using the semi-automatic Ventana method. Anti-EGFR (clone 31G7, Zymed, USA) and anti-COX-2 (N-20, SomfaGyz, USA) antibodies were used for staining. Immunostaining was blindy evaluated by an expert pathologist using the semi-quantitive method. Staining was characterized as negative when <10% of cells were positive, positive when >10% of cells were positive and intensely positive when >30% of cells were positive.

Statistics

Statistical analysis was performed using Pearson's x^2 test in order to find differences between qualitative variables and contingency coefficient was used in order to reveal possible correlations. The level of statistical significance was set at $p<0.05$. All statistical analyses were performed using SPSS 24 for Windows.

Results

One hundred patients that underwent colon surgery in our Department were included in the study. All patients were operated during 2007.

The patient mean age was 64.5±years and 54% were male. Most of the tumors were moderately differentiated (grade II), and in an early disease stage (Dukes A).

In total, 52% of the tumors were negative for EGFR and 16% were negative for COX-2 (Figures 1 and 2). Patient demographics and tumor characteristics are shown in Table 1.

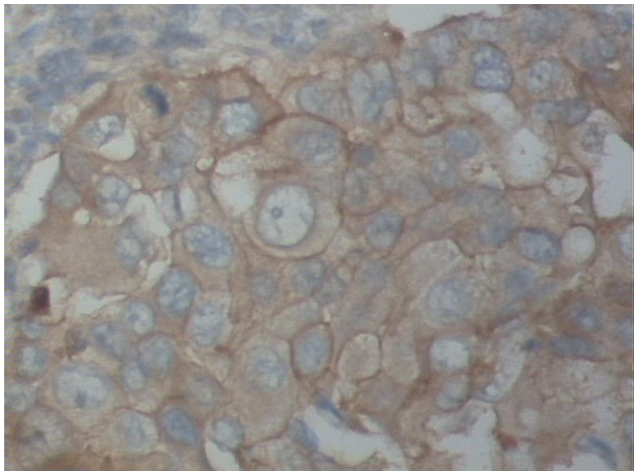

Figure 1. Immunohistochemical staining of EGFR located in the cytoplasmic membrane of malignant epithelial cell (brownish stain in the circumference of the cells (original magnification x240).

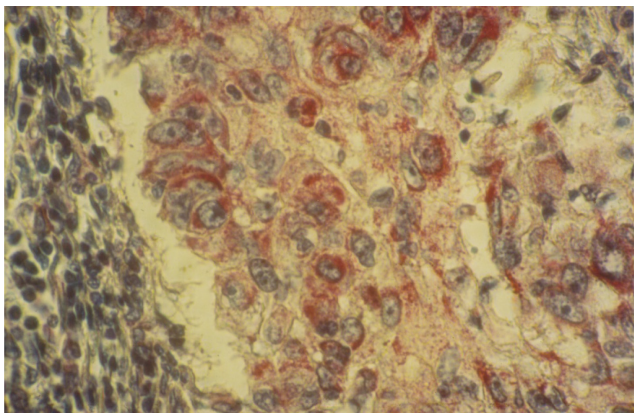

Figure 2. Immunohistochemical staining of COX-2 with cytoplasmic brown-red granulous localization in malignant epithelial cells (original magnification x120).

EGFR positive tumors were significantly smaller than tumors with negative EGFR expression (p<0.05) and there was a trend towards positive expression in patients of older age that did not though reach statistical significance (p=0.07). In addition, positive EGFR expression was significantly associated with advanced nodal stage (p<0.05). There was no difference between tumors of different differentiation, presence of metastases, Dukes and TNM stage in the expression of EGFR.

Table 1. Patient population demographics and histopathological characteristics

Characteristics	*n (%)*
Age (years), mean±SD	64.5 ± 9.9
Timor size (cm), mean±SD	5.3 ± 1.5
Sex	
Male	54 (54)
Female	46 (46)
Dukes stage	
A	27 (27)
B	45 (45)
C	18 (18)
D	10 (10)
TNM stage	
I	16 (16)
II	56 (56)
III	18 (18)
IV	10 (10)
N status	
0	72 (72)
1	19 (19)
2	9 (9)
Metastases	
Absent	90 (90)
Present	10 (10)
Tumor size (cm)	
<5	53 (53)
>5	47 (47)
Location of tumor	
Right colon	16 (16)
Left colon	36 (36)
Rectosigmoid	48 (48)
Grade of tumor	
A	25 (25)
B	62 (62)
C	13 (13)
EGFR expression	
Negative	52 (52)
Positive	48 (48)
COX-2 expression	
Negative	16 (16)
Positive	84 (84)

Finally, EGFR positive tumors where more likely to be negative for COX-2 expression (p<0.05).

There was no difference in age, sex, tumor size, tumor location, nodal status, presence of metastasis in tumors with positive COX-2 staining, while there was statistically significant difference in the positive expression of COX-2 in advanced stage of disease stratified by the Dukes staging system (p<0.05) and a trend towards significance when stratified according to the TNM system (p=0.09). COX-2 staining was also positive in dedifferentiated tumors (p<0.01).

Differences in the expression of COX-2 and EGFR are summarized in Table 2.

Discussion

Colorectal cancer is one of the most common malignancies and an severe cause of cancer related death in the western world [3,4]. Extensive research has been conducted and has elucidated many aspects of this cancer, from its natural history to genetic alterations that change the course of the disease, as well as information that permit targeted and individualized treatment [4]. The significance of molecular prognostic factors that are associated with the course of disease and may alter treatment in individual patients is still under research. Recent studies [1] have shown that COX-2 is expressed quite commonly in colorectal cancer cells, as well as in benign lesions of the colon and rectum. Increased expression of COX-2 is found in 50% of benign adenomatous polyps and in up to 85% of sporadic adenocarcinomas, localized in the cytoplasm of the neoplastic epithelial cells [1,6-11]. COX-2 is an enzyme that has a key role in arachidonic acid metabolism to prostaglandins and, apart from its known role in inflammation, it has been implicated in carcinogenesis as well. Studies have shown that COX-2 expression is associated with the development of adenomatous polyps, as well as with their progression to adenocarcinomas [6]. Deletion of the COX-2 gene has been shown to decrease tumor formation and progression in adenomatous polyposis coli (APC) mutation animal models [7]. These data, as well as data regarding the lower incidence of colorectal carcinomas in patients under NSAIDS have led to the clinical introduction of COX-2 inhibitors as chemoprevention of colorectal cancer [7,8]. Furthermore, recent data have also shown that chemotherapeutic agents and radiation therapy can induce COX-2 expression in cancer cells, a fact that leads to chemoresistance [6]. In turn, animal models mimicking colon cancer have been used in order to see if COX-2 inhibitors could potentially

Table 2. Differences in EGFR and COX-2 expression between patients according to clinicopathological characteristics

Characteristics	*EGFR expression*		*p value*	*COX-2 expression*		*p value*
	Positive n (%)	*Negative n (%)*		*Positive n (%)*	*Negative n (%)*	
Age (years)	66.3±9	62.8±10.4	0.075	64.1±10.1	66.1±8.5	0.460
Size (cm)	4.8±1.5	5.7±1.4	**0.004**	5.2±1.5	5.6±1.6	0.426
Sex			0.664			0.369
Male, mean±SD	27 (50)	27 (50)		47 (87)	7 (13)	
Female, mean±SD	21 (45.7)	25 (54.3)		37 (80.4)	9 (19.6)	
Dukes stage			0.136			**0.005**
A	9 (33.3)	18 (66.6)		17 (63)	10 (37)	
B	21 (46.7)	24 (53.3)		40 (88.9)	5 (11.1)	
C	11 (61.1)	7 (38.9)		17 (94.4)	1 (5.1)	
D	7 (70)	3 (30)		10 (100)	0 (0)	
TNM stage			0.214			0.099
I	6 (37.5)	10 (62.5)		11 (68.8)	5 (31.3)	
II	24 (42.9)	32 (57.1)		46 (82.1)	10 (17.9)	
III	11 (61.1)	7 (38.9)		17 (94.4)	1 (5.6)	
IV	7 (70)	3 (30)		10 (100)	0 (0)	
N status			**0.025**			0.100
0	30 (41.7)	42 (58.3)		57 (79.2)	15 (20.8)	
1	10 (52.6)	9 (47.4)		18 (94.7)	1 (5.3)	
2	8 (88.9)	1 (11.1)		9 (100)	0 (0)	
Metastases			0.142			0.146
Absent	41(45.6)	49 (54.4)		74 (82.2)	16 (17.8)	
Present	7 (70)	3 (30)		10 (100)	0 (0)	
Tumor size (cm)			**0.026**			0.419
<5cm	31 (58.5)	22 (41.5)		46 (86.8)	7 (13.2)	
>5cm	17 (36.2)	30 (63.8)		38 (80.9%)	9 (19.1)	
Tumor location			0.337			0.481
Right colon	10 (67.5)	6 (32.5)		15 (93.8)	1 (6.2)	
Left colon	18 (50)	18 (50)		29 (80.6)	7 (19.4)	
Rectosigmoid	20 (41.7)	28 (58.3)		40 (83.3)	8 (16.7)	
Tumor grade			0.146			**0.001**
A	8 (32)	17 (68)		15 (60)	10 (40)	
B	32 (51.6)	30 (48.4)		56 (93.3)	6 (6.7)	
C	8 (61.5)	5 (38.5)		13 (100)	0 (100)	
EGFR expression			N/A			
Positive	N/A	N/A		45 (93.8)	3 (6.2)	**0.011**
Negative	N/A	N/A		39 (75)	13 (25)	
COX-2 expression			**0.011**			N/A
Positive	45 (53.6)	39 (46.7)		N/A	N/A	
Negative	3 (18.8)	13 (81.3)		N/A	N/A	

Bold numbers denote statistical significance. N/A: non-applicable

reverse this chemoresistance of tumors and combined therapy with chemotherapeutic agents and celecoxib was shown to have synergistic effect in inhibiting tumor growth. Also, a recently conducted meta-analysis by Peng et al. showed that COX-2 overexpression was linked with a decrease in survival in patients with colorectal cancer [1]. In view of these data COX-2 expression seems to be an important piece of information for individualized treatment strategies.

EGFR has been detected in many types of malignancy, such as lung, breast, ovarian, bladder, esophageal, cervical and head and neck cancers and has been linked to poor prognosis and progression of disease [12-16]. In particular, EGFR expression has been shown to be associated with poor prog-

nosis in metastatic colorectal cancer patients [9] and to predict poor response in patients with rectal cancer undergoing radiotherapy [10]. Finally, anti-EGFR agents have come to play a significant role in the treatment of chemoresistant and radioresistant cancers [11].

Although data over the expression of EGFR and COX-2 are reported by other studies [1,12-17], there is no data on the expression pattern of EGFR in a Greek population. The purpose of this study was to investigate the expression of these factors in 100 patients undergoing surgery for colorectal carcinoma and to see potential clinical and histological factors that are associated with this expression in a Greek population.

EGFR expression in colorectal cancers is reported to range from 25-77% in various studies [14]. This variability seems to be due to differences in the detection methods used, but is certainly also due to genetic differences of the disease in different populations. In our study expression of EGFR was noted in 48% and of COX-2 in 84% of our specimens.

The expression of COX-2 differed significantly between tumors with various grades of differentiation, showing overexpression in tumors with poor differentiation. In addition, overexpression was also observed in patients with advanced disease stage according to the Dukes staging system. These findings are in concordance with the literature, as other authors have also linked overexpression of COX-2 in colon cancer patients with metastatic disease, lymph node positivity, poorly differentiated tumors, serosal invasion and tumors larger than 5 cm [12].

On the contrary, EGFR was not found to have any significant difference between patients with cancers of different grade or stage. Data from the literature seem not to elucidate this issue [15]. Although EGFR has already been reported to be linked to aggressive disease, increased risk of metastases, advanced tumor stage and advanced T and N stage [15], there are very few studies showing any significant association with the grade of tumor, apart from sporadic studies [9,14,18,19].

In our literature review, results concerning the expression of COX-2 seem to be conflicting and this is often attributed to different methods of marker detection that are used by different research groups. Our findings are in contrast with the findings of Jang et al., where COX-2 overexpression was reported to be associated with favorable clinical and histological characteristics of tumors. However, in their study, a higher percentage of tumors were found to be positive for COX-2 expression when they were poorly differentiated [20].

Conclusions

Identifying the expression of these markers seems to be quite important in clinical practice. On one hand, the expression of these markers seems to be related to tumor aggressiveness and prognosis, while on the other hand, individualized treatment strategies can be developed for each patient, according to tumor characteristics. Although data for EGFR and COX-2 expression are reported in several studies, this is a report of their expression pattern and association with clinical and histopathology factors in a Greek population of colorectal cancer patients. These data are quite important in order to certify that colorectal cancer molecular and genetic diversity between different study populations is not a confounding factor in the application and clinical implementation of trending individualized decision making in oncological treatments.

Conflict of interests

The authors declare no conflict of interests.

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