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Original Research Paper

Medicine

EVALUATİON OF E-CADHERİN , MMP-2 İN COLORECTAL CARCİNOMA

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ABSTRACT

Background : Colorectal cancer pogression is a multistep process, in which many genes and proteins are involved. Matrix metalloproteinase and loss of-E-Cadherin has been considered to be associated with cancer invasion and metastasis. In our study, we aim to evaluate the expressions of E-Cadherin and MMP-2 in colorectal carcinoma .

Material and Methods: Expressions of E- Cadherin and MMP-2 in specimens from 48 colorectal adenocarcinomas (24 classic adenocarcinomas and 24 mucinous adenocarcinomas) were detected by immunohistochemistry.

Results: MMP-2 expression was found 45,84% in classic adenocarcinoma while E-Cadherin was found 33,33% in mucinous carcinomas.

Conclusion: Our findings demonstrate that E-cadherin and MMP-2 expression contributes to tumor development. It sems that MMP-2 expression was more associated with classic adenocarcinomas while E-Cadherin was related with mucinous adenocarcinomas .Further studies are need to find the correlations between E-cadherin ,MMP-2 and epithelial-mesenchymal transition.

KEYWORDS : colorectal cancer, E-Cadherin, MMP-2

INTRODUCTİON:

Colorectal cancer (CRC) is the third most common cancer and nearly one million new cases has been diagnosed annually(1) Colorectal cancer pogression is a multistep process, in which many genes and proteins are involved. The function of extracellular matrix in many kinds of cancers have been revealed and their role of the clinical significance in colorectal cancer is under investigation.

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are involved in degrading extracellular matrix and facilitating tumor invasion(2,3) MMP-9 and MMP-2 are gelatinases of MMP family and degrade substrates of type IV collagen and gelatin. Previous studies have demonstrated that MMP-2 is associated with invasion and metastasis in colorectal carcinoma (4,5,6)

E-cadherin is a key mediator of cell–cell adhesions in epithelial tissues, and loss of E-cadherin can promote invasive and metastatic behaviour in many epithelial tumours, also (7). The cytoplasmic domain of E-Cadherin interacts with catenin molecules, including β-catenin.

β-catenin which is major mediator cadherin-associated protein play important role in WNT signalling pathway. Mutations in adenomatous polyposis coli (APC) which is a tumour suppresser gen leads to enhances β-catenin gene expression. (8). When β-catenin accumulates ,it translocates to the nucleus, and associates with TCF/LEF, leading to its binding to DNA and subsequent transcription of genes associated with CRC development. Wnt/β-catenin signaling pathway is aberrantly activated and responsible for upregulating MMPs expression (9,10) .

Disturbance of the E-cadherin/catenin complex during tumor progression is correlated with the onset of an invasive phenotype (11) . In addition , studies suggested that the matrix metalloproteinase leads directly or indirectly to shedding of the E-cadherin (12) .

In our study, we aim to investigate the expression of E-Cadherin and MMP-2 in colorectal cancer and their associations with clinical pathological features.

MATERİAL AND METHODS:

Tissue samples and patients

The study was designed as retrospectively and due to retrospective nature of the study informed consent was not required. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Our study consisted of 48 patients with colorectal cancer who had undergone surgical resections at Baskent University Hospital between 2014 and 2018 . The study group consisted of 24 classic adenocarcinomas and 24 mucinous adenocarcinomas . Tumor staging and histological classifications were assessed according to World Health Organization classification (13).

Of the patients, 35 were men (62,867%) and 13 were women (37,14%). , The patients ranged in age from 30–84 years (mean, 57,52 ± 14,98 years).The investigated tumors were classified as pT1 tumor was observed in (n:3) 6,25 % of patients, pT2 tumor in (n:20) 41,66 % of patients, pT3 tumor in (n:18) 37,59 % of patients and pT4 tumor in (n:7)14,585% of patients . Among them (n:4) 8,33 %were located at right colon and (n:44) 91,67% were located at left colon. The control group consisted of 20 normal colon mucosa. The clicopathologic features of cases has been listed in Table I .

Immunohistochemical Staining:

Immunohistochemistry was performed using antibodies to MMP-2, (Novo Castra, Clone 17C2, 1/100 dilution), E-Cadherin (Biogenex, Clone EP ,1/300 dilution) immuno staining were performed by using manuel method. In manuel method, tissue sections (4 μm thick) were cut, deparaffinized in xylene, dehydrated with graded ethanol and then the sections were placed in 10 mM citrate buffer solution (pH 7,3) and processed in an autoclave for 1,5 minutes at 120°C for antigen retrieval. After washing in distilled water (5 minutes) and in phosphate-buffered saline (PBS) (5 minutes) immersed in methanol with 0,3 % peroxidase for 5 minutes to inhibit endogenous peroxidase activity. Antibody was applied, and incubations were performed 2 hours at room temperature. After washing five times in PBS, slides were incubated with biotinylated second antimouse antibody (Zymed, diluted 1:200) After five washes with washing buffer, the sections were incubated with AEC. The sections were counterstained with Meyer's hematoxylin.

Evaluation of immunostaining:

MMP-2 Expression: MMP-2 staining was restricted to the stromal fibroblasts and inflammatory cell, with no staining of

tumor cells. Staining intensity was graded as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, intense staining.

E-cadherin expression: E-Cadherin immunoreactivity was localized in the cytoplasm and plasma membrane in normal mucosa. Cytoplasmic expression of E-cadherin was recorded when staining was absent from the cancer cell membrane and preserved in cancer cell cytoplasm and nucleus. (0) Negative, no detectable staining. Weak but detectable discontinuous staining graded as (1) ; moderate, but discontinuous staining graded as(2) ; intense and continuous staining graded as (3) .

Statistical analysis

The associations between expressions of the two items with clinicopathological variables were assessed by chi-square test. *P*-values were calculated with significance level <0.05. All calculations were performed with SPSS software package, standard Version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS:

MMP-2 staining:

MMP-2 was localized in stromal fibroblast in colon adenocarcinomas (FigureI) . MMP-2 staining was also weakly observed in inflammatory cells, macrophages, endothelial cells and muscularis mucosa. While MMP-2 was weakly expressed in 21,7% of normal mucosa, higher expression seen in 45,84% of classic adenocarcinomas . In mucinous tumors, the rate of positive cases were similar to normal mucosa . The staining intensity was scored as (range: 0 to 3) and the percentage of positive casess as (range: 0 to 3 [0, {n:13, 54,16%}, 1 {n:8,%33,3%}, 2 {n:2, %8,33%}, 3 {n.1, 4,16%}]). The mean level of MMP-2 was between 0,200,52 and 1,661,41. Staining was increased from normal mucosa (0,621,03) to carcinomas group (0,730,98) however there was no statistical difference . There was no correlation between MMP-2 staining and tumor grade and stage.

Table I shows MMP-2 expression according to staining intensity.

E-Cadherin staining:

All of the classic adenocarcinomas showed the membranous and cytoplasmic expression pattern of E-cadherin (Figure II) . Of the 24 tumors, 12 (50%) were considered as grade 3 staining intensity and 12 (50) were considered as grade 2 staining intensity. There were no decreased in E-Cadherin expression in classic adenocarcinomas. Loss of E-cadherin expression was seen in 33,33% of patients with mucinous tumors (Figure III). Of the 24 tumors, 16 patient (66,67%) were considered as grade 3 staining intensity and 8 patient (33,33%) were considered as grade 0.

Table II shows E-Cadherin expression according to staining intensity.

DİSCUSSİON:

In this study, we evaluated the expressions of E-Cadherin and MMP-2 and their relation with clinocopathologic features. Numerous studies were performed to determine how MMPs contribute to colorectal carcinogenesis. In colorectal cancer, MMP-7(matrilysin), MMP-2(gelatinase A), MMP-9(gelatinase B) and MMP-1 have demonstrated to be overexpressed (4-6,14-16) . MMP-2 has the ability to degrade basement mem brane type IV collagen, which would facilitate tumor cells invasion. The present study showed that the expression of MMP-2 was higher compare to normal mucosa however its correlation with tumor stage and grade was not obtained. Studies on the functions of MMP-2 in cancer progression were quite controversial. Waas and colleagues similarly showed that low stage (I and II) disease showed higher MMP2 expression than more advanced stages (III and IV) (17). Wong et al demonstrated that lack of MMP2 expression was associated with poorer outcomes in stage III rectal cancers, including the risk of local recurrence (18) .

Matrix metalloproteinases are synthesized by neoplastic and stromal cells (19)

In the present stuy the source of MMP-2 was only fibroblasts and inflammatory cells.

Our research has demonstrated that weak expression of MMP-2 in the inflammatory infiltration and a much stronger response in stromal fibroblast in tumor tissue. Groblewska et al found a significant correlation between MMP-2 immunoreactivity in inflammatory cells and the presence of distant metastases. (20)

In the present study MMP-2 expression was mostly seen in classic adenocarcinomas while stromal MMP-2 expression could not obtained in majority of mucinous tumors . We thought that MMP-2 expression coul not pooperly evaluated due to mucinous nature of stroma in mucinous tumors.

Cellular adhesion molecules also plays an important role in the invasion and metastasis of the tumour (21) Loss of E-Cadherin expression was reported to promote cancer cell proliferation and invasion and proved to correlate with cancer progression and unfavorable prognosis in various cancer. (22,23,24) Loss of E-cadherin has been associated with colorectal cancer, before (25). However conflicting results exist on the role of E-cadherin in tumor progression. Authors reported that decreased expression of E-Cadherin correlated with poor prognosis (26,27,28) while unaltered E-Cadherin level has been found in in patients with inflammatory bowel disease (IBD) or early stage colorectal tumors (29).

In this study, loss of E-cadherin was demonstrated to be found more frequently in mucinous tumors. We found that E-Cadherin expression were similar to normal mucosa in majority of classic adenocarcinomas .

The ability of matrix metalloproteinases to enhance tumor cell invasion has been thought to be a result of the degradation of the extracellular matrix. Authors found that that matrix metalloproteinase mediated induction of cellular invasion also can be independent of direct matrix degradation and, instead, a result of the release of E-cadherin fragments (30). In addition, authors found that the increase in the metastatic potential of tumour cells is associated with an increased expression of MMP-2 and a decreased expression of E-cadherin(31). However we did not find the relation betwwen MMP- and E-cadherin , in the present study. We thougt that the present study may indicates that the extracellular matrix transition (EMT) process may be different in classic adenocarcinomas and mucinous tumors.

Recent studies described Wnt signaling pathway is aberrantly upregulated in both MSS and MSI colorectal cancers . The MSI pathway is often associated with, mucinous morphology, proximal colon(32) .

The limitation of our study , study we did not evaluate molecular MSI status and tumor site.

In conclusion our finding prove that MMP-2 and loss of E-cadherin play role in colorectal carcinogenesis however there is no relation between the MMP-2 expression and loss of E-Cadherin. It seems that MMP-2 expression was more related with classic adenocarcinomas while loss of E-Cadherin expression was more related with mucinous adenocarc

inomas. We thought that further studies needs to find the underlying mechanism of extracellular matrix transition in colorectal cancer .

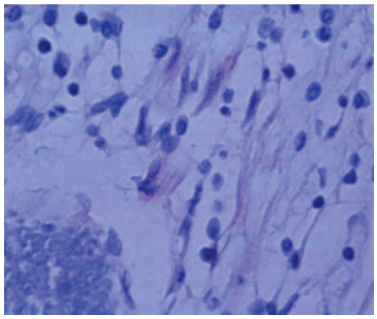
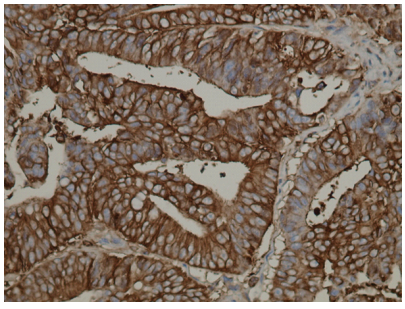


Figure I: Figure shows MMP-2 expression in stromal fibroblast (MMP-2X100)



FigureII: Figure shows grade 3 E –Cadherin expression (E-Cadherin x40)

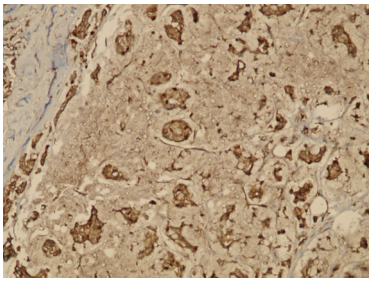


Figure III Figure shows loss of membranous expression in mucinous adenocarciomas (Ecadherin X20)

Table I: The distrubution of MMP2 staining intensity

	0	1	2	3
Classic Adenocarcinoma	n:13 54,16%	n:8 33,3 %	n:2 8,33 %	n:1 4,16 %
Mucinous Adenocarcinoma	n:20 83,33%	n:2 8,33 %	n:2 8,33%	0 0 %

Table II: The distrubution of E-Cadherin staining

	0	1	2	3
Classic Adenocarcinoma	0 0 %	0 0 %	n:12 50%	n:12 50 %
Mucinous Adenocarcinoma	8 33,33%	0 0 %	0 0 %	0 66,67 %

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