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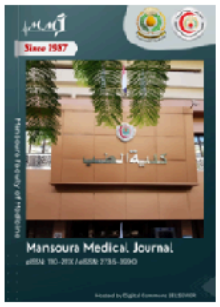

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SOME HISTOCHEMICAL STROMAL CHARACTERIZATIONS IN COLORECTAL EPITHELIAL TUMORS

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ABSTRACT

Stroml changes may play an important role in progression, invasion and prognosis of colorectal tumors. Biopsy specimens of colorectal tumors were evaluated for stromal characterizations. Section stained with Haematoxyline and eosin (H&E) were examined as regard the presence of tumor infiltrating lymphocytes (TILs) and tumor associated eosinophilic infiltrate (TE). The number of TILs were larger in earliest stages of colorectal cancers and decreased with the presence of metastasis. The prognosis of carcinomas was better for those with higher eosinophilic infiltration.

Expression of gelatinaseA type of Matrix Metalloproteinases (MMP2) was assessed histochemically in both adenomas and carcinomas. Cytoplasmic expression of MMP-2 is significantly high in colorectal carcinomas (56%) compared to adenomas (20%).{ p=0.006}. A positive relationship between MMP-2 expression and tumor grade, Dukes' stage and nodal status was reported.. The staining intensity of MMP-2 in adenomas was either moderate (50%) or weak (50%). On the other hand, 28.6% of carcinomas were strongly stained, 39.3% were moderately stained and 32.1% were weakly stained.

INTRODUCTION

In Egypt, colorectal cancer constitutes about 6.1 % of all malignant tumors (1). While the neoplastic cells have been always in the center of interest in cancer research, recently more attention have been paid to the tumor stroma which is known to play

an important role in tumor progression [2]. The ECM provides not only a structural framework for the tumor but also plays an important regulatory role in cell proliferation, apoptosis, migration and differentiation [3]. Matrix metalloproteinases (MMPs) are a group of enzymes thought to be responsible for both normal connective tissue matrix remodeling and accelerated breakdown associated with neoplasm development [4].

Invasion occurs within a tumor–host microenvironment where stroma and tumor cells exchange enzymes and cytokines that modify the local extracellular matrix, stimulate migration, and promote proliferation and survival [5].

The presence of inflammatory infiltrate in the interface between the tumor and the neighboring tissue made up of eosinophils, plasma cells, and lymphocytes indicate a better prognosis for some of colorectal tumors [6]. However, Fisher et al. [7] in their study found that inflammatory infiltrate had no bearing with prognosis.

The aim of this work is to study stromal changes in cases of colorectal tumors as regards cellular infiltrate and expression of matrix metalloproteinases, in benign and malignant tumors. Also correlation of stromal findings with the prognostic parameters in malignant tumors.

MATERIALS AND METHODS

Materials from 70 cases of colorectal tumors (twenty cases of colonic adenomas obtained by endoscopic procedures and fifty cases of colorectal carcinoma from resection) were collected from pathological files in Gastroentrology Center (GEC). All of these patients were reviewed as regard history taking and clinical examination. Hematoxylin and eosin-stained (H&E) sections were reviewed for evaluation and detection of the stromal inflammatory cell reaction of the colonic tumors mainly lymphocytic and eosinophilic infiltrate. Then additional 3-5 μ thick sections were cut from the paraffin blocks for immunohistochimstery. A biotin-streptavidin method was used as previously described [8,9] for detection of gelatinase A type of Matrix Metalloproteinases (MMP-2) expression. The primary antibody (Primary mouse monoclonal MMP-2 (72kDa CollagenaseIV) Ab-4 (Clone A-Gel VC2) (Labvision corporation product.cat. #MS-806-B0, B1), recogniz-

ing both active and latent forms of the matrix metalloproteinas type 2 (gelatinase A) enzyme. The monoclone is provided as 200 µg/ml of antibody purified from ascitic fluid)

Evaluation of tumor infiltrating lymphocytes (TILs)

TILs were evaluated in the center and periphery of the tumor and around the invasive carcinoma cells. The TIL level was quantified from ten microscopic fields (x 400) and the mean TIL value of these 10 fields were calculated. TILs was graded as absent (grade 0) when less than ten lymphocyte were observed per high power field (HPF); weak (grade 1) when TILs level is of 10-50 per high power field. The TIL density was dense (grade 3) when the tumor margins and stroma contained a dense lymphocytic infiltrate, with a TIL level more than 100 per HPF. TIL density was moderate (grade 2) when the peri- and/or intratumoral lymphocytic infiltrate was intermediate between grades 1 and 3, with a TIL level of 50-100 per HPF. They found a linear correlation between Dukes' stage and TILs. They were absent or weak in the stroma of a large invasive Dukes' C and D tumors (10)

Evaluation of Tumor associated eosinophilia:

Tumor associated eosinophilia is characterized by the presence of eosinophils as a component of peri- and intratumoral inflammatory infiltrate (11)

Evaluation of MMP-2 expression:

1) Staining positivity: the cases were divided into two groups according to the width of immunopositive staining area: negative (0-10% staining area); and positive (> 10%) (12)
2) Staining inensity : was classified as weak, moderate or strong (13)
3) Cellular localization: Expression of MMP-2 was also evaluated as regard immunostaining of the tumor cell cytoplasm and the stromal cells (12)

Clinicopathological correlations:

were performed by reference to histological type and degree of dysplasia in cases of adenoma. Age, sex, anatomical distribution, Dukes' staging (LN and distant metastasis), tumor grade, histological type, nodal status, depth of tumor invasion, venous and perineural invasion, and margin growth pattern in cases of carcinoma (14).

Statistical analysis : Data were analyzed using SPSS (statistical packaging for social sciences) version 10. Quantitative data were presented as number and percentage. Chi Square or Fisher's exact tests were used for comparison between groups, as appropriate; Spearman (rank) correlation coefficient was used to calculate correlation between variables. $P \leq 0.05$ was considered statistically significant.

RESULTS

I) Clinical data :

Age of patients included in this study ranged between 22 and 76 years. They were divided into two groups: adenoma group (20 patients; 11 males and 9 females) and carcinoma group (50 patients; 27 males and 23 females).The age and sex difference between adenomas and carcinomas was statistically insignificant (X^2=0.06, X^2=0.84 respectively). According to the anatomical distribution, thirty percent of adenomas were right sided, and 50% were left sided, while 38% of carcinomas were right sided and 40% were left sided. The rectum showed an incidence of 20% and 22% for adenomas and carcinomas respectively. The anatomical distribution difference between adenomas and carcinomas was statistically insignificant (X^2=0.4) (table-1).

II) Histopathological characters :

In Adenoma group, 60% of cases were of tubular type (figure-1), 25% of cases were of villous type and 15% of tubulovillous type. 60% of adenomas (12 cases) exhibited low-grade dysplasia and 40% of them (8 cases) exhibited high grade dysplasia (Table-2)

In the studied carcinoma cases; 34 (68%) adenocarcinomas, 14 (28%) mucoid adenocarcinomas and 2 (4%) signet ring carcinomas. Degree of differentiation of adenocarcinomas, Dukes' staging, nodal status, depth of tumor invasion, venous invasion, perineural invasion and growth pattern were shown in table (3)

III) Stromal criteria :

The evaluated stromal criteria included the followings:

A) Tumor infiltrating lymphocytes (TILs) in carcinomas :

Table (4) illustrates the positivity, intensity of tumor infiltrating lymphocytes and the relation between Dukes' stage and TILs in 50 colorectal carcinoma cases. Statistical correlations re-

vealed inverse and significant correlation between TILs, grading and Dukes' stage in colorectal carcinomas ($r = -0.428$, $p=0.002$). (figure-2)

B) Tissue eosinophilia (TE) in colorectal tumors :

Table (5) compares between tissue eosinophilia in adenomas and carcinomas. 65% of adenomas were negative for TE and 35% were positive. 48% of carcinomas were negative for TE and 52% were positive (figure-3). The relation between Dukes' stage and tissue eosinophilia (TE) in colorectal carcinoma was statistically highly significant (A&B vs. C&D: _2=17.11, p=0.000). Our results revealed reduced frequency and intensity of eosinophilic infiltration with increased depth of tumor invasion with no bilharzial infestation (no bilharzial ova or warm).

C) MMP-2 expression in colorectal tumors :

Table (6) compares between MMP-2 expression in colorectal adenomas and carcinomas. While 56% of carcinomas showed positive immunostaining for MMP-2, only 20% of adenomas were positively immunostained. The MMP-2 expression difference between adenomas and carcinomas was statistically significant ($X^2=7.46$, $p=0.006$).

As shown in table (7), (10%) of adenomas were moderately stained (figure-4&5)) and 10% were weakly stained and none of the adenomas was strongly stained. Whereas in carcinoma group, 16% were strongly stained and 22% were moderately stained while 18% were weakly stained.

As regards cellular localization (figure-5 &6), 5% of adenomas revealed tumor cell staining only, 10% revealed stromal and endothelial cell staining and 5% revealed positive immunostaining for tumor, stromal and endothelial cells. In carcinoma group, 12% revealed tumor cell staining only (figure-7), 6% revealed stromal and endothelial cell staining (figure-8) and 38% revealed positive immunostaining for tumor, stromal and endothelial cells (table-7).

Table (1): Clinical data in cases of colorectal tumors:

Variable	*Adenoma Patients No (%)*	*Carcinoma patients No (%)*
Age:		
≤ 50	13 (65%)	19 (38%)
> 50	7 (35%)	31 (62%)
Sex:		
Female	9 (45%)	23 (46%)
Male	11 (55%)	27 (54%)
Anatomical distribution:		
Right side colon	6 (30%)	19 (38%)
Left side colon	10 (50%)	20 (40%)
Rectum	4 (20%)	11 (22%)
Total	20	50

Table (2): The histopathological characters of cases of colorectal adenomas:

Variable	*Adenoma Patients*	
	No	*%*
Histologic type:		
Tubular	12	60%
Villous	5	25%
Tubulovillous	3	15%
Dysplasia:		
Low grade	12	60%
High grade	8	40%

Table (3): The histopathological characters of cases of colorectal carcinomas:

Variable	*Carcinoma patients*	
	No	*%*
Histologic type:		
Adenocarcinoma	34	68%
Mucoid adenocarcinoma	14	28%
Signet ring carcinoma	2	4%
Differentiation		
(for adenocarcinoma cases)		
Well (grade I)	14	28%
Moderate (grade II)	14	28%
Poor (grade III)	6	12%
Dukes' stage: A	-	0%
B	31	62%
C	17	34%
D	2	4%
Nodal status: Positive	18	36%
Negative	32	64%
Depth of invasion:		
T1	-	-
T2	13	26%
T3	31	62%
T4	6	12%
Venous invasion:		
Positive	5	10%
Negative	45	90%
Peri-neural invasion:		
Positive	6	12%
Negative	44	88%
Growth pattern:		
Expanding	11	22%
Infiltrating	39	78%

Table (4): The TILs positivity, intensity and the relation between Dukes'stage and TILs in colorectal carcinoma:

Dukes' stage	*TILs grade*			
	Grade 0	*Grade 1*	*Grade 2*	*Grade 3*
A	-	-	-	-
B	1 (3.2%)	14 (45.2%)	12 (38.7%)	4 (12.9%)
C	6 (35.3%)	8 (47.1%)	3 (27.6%)	-
D	1 (50%)	1 (50%)	-	-
Total		23 (46%)	15 (30%)	4 (8%)
	Negative: 8 (16%)	*Positive:* 42 (84%)		

Table (5): Comparison of tissue eosinophilia in both adenomas and carcinomas:

Tumor type	*TE grade*		
	0	*Mild to moderate*	*Marked*
Adenomas	13 (65%)	5 (25%)	2 (10%)
Carcinomas	24 (48%)	19 (38%)	7 (14%)

Table (6): MMP-2 expression in colorectal adenomas and carcinomas:

Tumor type	*MMP-2 positive*	*MMP-2 negative*
Adenoma	4(20%)	16(80%)
Carcinoma	28(56%)	22(44%)

Table (7): Staining characters of MMP-2 in adenoma and carcinoma cases:

Staining intensity	*adenoma*		*Carcinoma*	
	No	*%*	*No*	*%*
Strong	-	0%	8	16%
Moderate	2	10%	11	22%
Weak	2	10%	9	18%
Cellular localization				
Tumor cells	1	5%	6	12%
Stromal & endothelial cells	2	10%	3	6%
All cell types	1	5%	19	38%

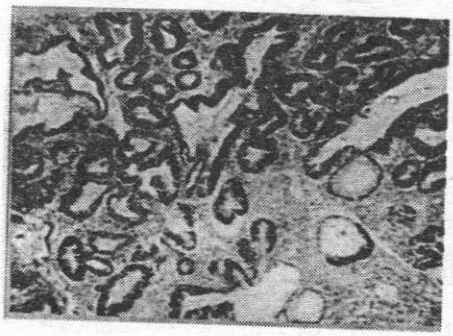

Figure (1) : Tubular adenoma formed purely of glands (Hx&E x 40)

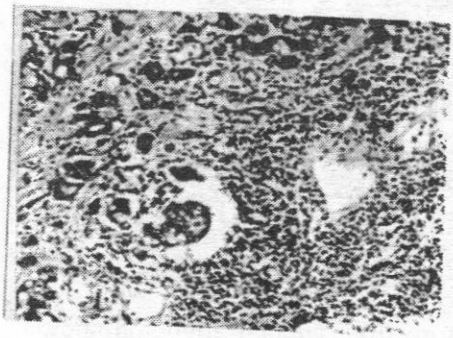

Figure (2) : Moderatly diifferentiated adenocarcinoma showing dense tumour infiltrating lymphcytic reaction (Hx&Ex 40)

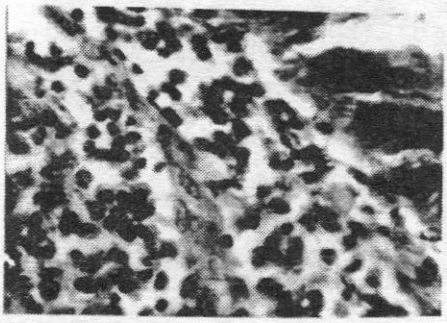

Figure (3) : Marked TE with concentration of eosinophils in the transitional zone of invasive carcinoma (Hx&E x 400)

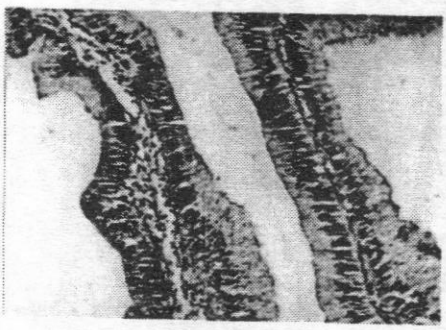

Figure (4) : Villous adenoma showing moderate cytoplasmic staining intensity of tumor and stromal cells for MMR-2 (immunoperoxidase x 200)

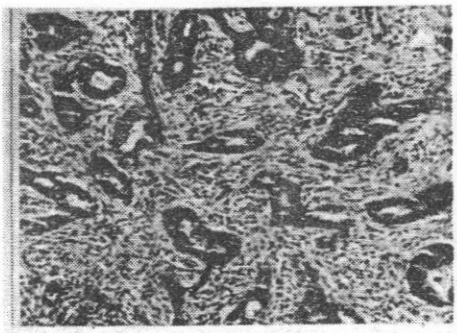

Figure (5) : Tubular adenoma showing moderate cytoplasmic staining intensity of tumor and stromal cells for MMR-2 (immunoperoxidase x 40)

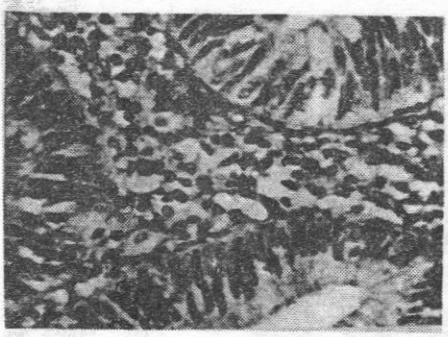

Figure (6) : Tubular adenoma showing moderate cytoplasmic staining intensity of stromal-cells for MMR-2 (immunoperoxidase x 400)

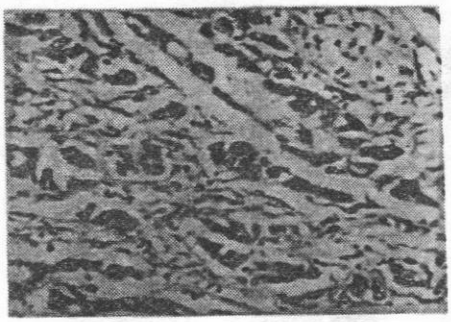

Figure (7) : Poorely differentiated adenocarcinoma showing strong cytoplasmic staining intensity of tumor cells for MMR-2 (immunoperoxidase x 400)

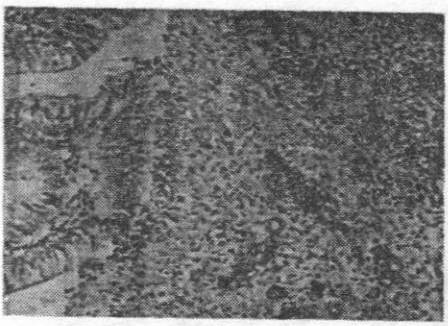

Figure (8) : Grade I adenocarcinoma showing strong cytoplasmic staining intensity of endothelial and stromal cells for MMR-2 (immunoperoxidase x 200)

DISCUSSION

Tumor invasion is a fundamental character of malignant tumor cells and one of their most dangerous properties. Remodeling of the ECM seems to be a necessary step in tumor invasion. Matrix metalloproteinases (MMP-2) are type IV collagen degrading enzymes that have been strongly correlated with tumor growth, invasion and metastasis.

The immune system was known to react against tumors and it has been postulated that tumor-infiltrating lymphocytes (TILs) reflect a tumor related immune response [13] Tumor infiltrating lymphocytes (TILs) were detected in 84% of carcinomas. In agreement with the previous reports [16], the density of TILs was significantly correlated to the Dukes' stage (much higher in Dukes' stage B tumors than stage C and D (96.3%, 64.7%, and 50% respectively).

Also tissue eosinophilia (TE) was detected in 35% of adenomas and 52% of carcinomas. Positive adenoma cases included 25% cases of mild to moderate eosinophilic intensity and 10% of marked eosinophilic intensity. Moezzi et al. [6] in their study of 313 adenomas were reported mild to moderate eosinophilic infiltration in 75% of adenomas and marked infiltration in 13% of adenomas, while 12% of colonic adenomas were negative. This difference may be attributed to the small number of adenomas included in our study.

In this series, 48% carcinomas were negative for TE and 52% were positive. Positive carcinomas included 38% of mild to moderate eosinophilic intensity and 14% of marked eosinophilic intensity. These observations confirmed the results of the previous investigators [17]. The relation between Dukes' stage and tissue eosinophilia (TE) in colorectal carcinoma was statistically highly significant (A&B vs. C&D) ($X2=17.11$, $p=0.000$). Thus, in agreement to Nielsen et al. [18], the high eosinophilic count appeared to predict a good prognosis in a Dukes' dependant manner. However, Fisher et al. [7] in their study found that eosinophilic infiltration had no bearing with prognosis.

Our results revealed reduced frequency and intensity of eosinophilic infiltration with increased depth of tumor invasion. These results are in agreement with Moezzi et al. [6] who found that presence of intense eosin-

ophilic infiltration was prominent at the transitional zone of the less invasive (T1 and T2) carcinomas and its conspicuous absence in more invasive (T3 and T4) carcinomas.

MMP2 revealed higher expression in colorectal carcinomas than in adenomas (56% versus 20%). MMP-2 expression difference between adenomas and carcinomas was statistically significant in favor of carcinoma (X^2=7.46, p=0.006). This was proved by Baker et al., [19].

The staining intensity of MMP2 in adenomas was either moderate (50%) or weak (50%). On the other hand, 28.6% of carcinomas were strongly stained, 39.3% were moderately stained and 32.1% were weakly stained. Liabakk et al. [20] found no MMP-2 overexpression in adenomas and these results conflicts with the results of Parson et al. [21] who detected MMP-2 immunoreactivity in 6.2% of colorectal adenomas.

It has been demonstrated that most of adenoma cases stain weakly for MMP-2 and that the staining appears homogenous and diffuse in the cytoplasm with perinuclear pattern. [14] In this study, 50% of the MMP-2 positive adenomas were moderately stained, while the other cases (50%) were weakly stained .

As regards cellular localization of MMP-2, the present work demonstrated that 25% of positive adenomas revealed tumor cell staining only, 25% of positive adenomas revealed stromal and endothelial cell staining and 50% revealed positive immunostaining for tumor, stromal and endothelial cells. However, Papadopoulou et al. [14] showed that the expression of MMP-2 was equally distributed among tumor cells, fibroblasts and monocytes of the interstitial region.

In agreement with Ring et al [22], 8 of the MMP-2 positive carcinomas (28.6% of the cases) in our study were stained strongly and 11 carcinomas (39.3% of the cases) were moderately stained, while 9 of the MMP-2 positive carcinomas (32.1% of the cases) were weakly stained.

In a study by Kikuchi et al. [12], positive MMP-2 staining for tumor cells was 20% and for stromal cells was 30%, but 14% were positive for MMP-2 in both the tumor cell and the stroma. However, Papadopoulou et al. [12] showed that the expression of

MMP-2 was equally distributed among cancer cells, fibroblasts and monocytes of the interstitial region. We also observed that 21.4% of positive carcinomas showed tumor cell staining only, 10.7% of showed stromal and endothelial cell staining and 67.9% showed positive immunostaining for tumor, stromal and endothelial cells.

In contrast, the data provided by Ko et al., (23) showed that MMP-2 is exclusively expressed in fibroblasts and not by cancer cells. Identified soluble factor secreted from tumor cells may stimulate MMP-2 production by fibroblasts. (24), positive cytoplasmic staining for MMP-2 was restricted to the tumor epithelium, with no staining of the interstitial stroma or basement membranes. This was explained by the uptake of MMP-2 by the tumor cells after being secreted by the stromal cells.

In this study, we were able to detect MMP-2 positive immunostaining in 8.33% of tubular adenomas, 40% of villous adenomas and 33.3% of tubulovillous adenomas. Also 33.3% of high-grade dysplasia cases were positive and only 16.7% of low grade dysplasia were positive. In a study by Papadopoulou et al. (14), they demonstrated that most of the MMP-2 positive adenomas exhibited high dysplastic changes.

Over expression of MMP-2 in colorectal carcinomas is crucial for invasion and metastasis (25). They found a significant association between MMP-2 expression and the measures of colonic tumor aggressiveness such as Dukes' stage, tumor grade and nodal status. In contrast to Kikuchi et al. (12), demonstrated that MMP-2 expression is not strongly correlated to these factors. In our study there was a tendency for poorly differentiated tumors to be MMP-2 negative more often than moderately and well differentiated tumors. Consequently, the immunohistochemical detection of MMP-2 expression appears not to be an appropriate indicator of invasion and metastasis.

In this work, 66.7% of nodal metastasis positive cases were positive for MMP-2 and 50% of nodal metastasis negative cases were positive for MMP-2. This finding correlates with the results of other studies. (14) who found that 5% of lymph node negative carcinomas were immunoreactive for MMP-2 in contrast

to 64.9% of lymph node positive carcinomas.

In the present study 66.7% of venous invasion positive cases were positive for MMP-2 and 50% of venous invasion negative cases were positive for MMP-2. In addition, 66.7% of peri-neural invasion positive cases were positive for MMP-2 and 54.5% of peri-neural invasion negative cases were positive for MMP-2. Similar were previously obtained by Kikuchi et al. (12) .

Finally, the present work could clearly reveal that stromal changes in cases of colorectal tumors as regards cellular infiltrate and expression of matrix metalloproteinases help to differentiate between benign and malignant tumors and different grades and stages in malignant cases.

REFERENCES

1- **El Bolkiny N. (ed.) (1991) :** General pathology of cancer. Al asdekaa graphics center AGC Cairo: 122.

2- **Wernert N, Hugel A and Locherbach C (1998):** Genetic alterations in the fibroblastic stroma of invasive colon and breast carcinomas. Ver Dtsch Ges Pathol; 82: 317-321

3- **Garbett EA, Reed MW and Brown NJ (1999) :** Proteolysis in colorectal cancer. Molecular Pathol; 52(3): 140-145.

4- **Roeb E, Dietrich CG, Winogard R. et al (2001) :** Activity and cellular origin of gelatinases in patients with colon and rectal carcinoma differential activity of matrix metalloproteinase-9. Cancer; 92 (10): 2680-2691

5- **Liotta LA and Kohn EC. (2001) :** The microenvironment of the tumor- host interface. Nature; 411: 375-379.

6- **Moezzi J, Gopalswamy N, Hass RJ et al. (2000) :** Stromal eosinophilia in colonic epithelial neoplasms. Am J Gastroenterol; 95:520-523.

7- **Fisher ER, Paik SM, Rockette H et al. (1989) :** Prognostic significance of eosinophil and mast cells in rectal cancer: finding from the national

surgical adjuvant breast and bowel project. Human Pathol; 20: 159-163.

8- **Bindl JM, Warnke RA. (1986) :** Advantages of detecting monoclonal antibody binding to tissue sections with biotin and avidin reagents in coplin jars . Am J Clin Pathol; 85: 490-496.

9- **Kell DL, Kamel OW, Rouse RV. (1993) :** Immunohistochemical analysis of breast carcinoma estrogen and progesterone receptors in paraffin-embedded tissue: correlation of clone ER1D5 and 1A6 with a cytosol-based hormone receptor assay. Appl Immunohistochem; 1: 275- 281.

10- **Ropponen KM, Eskeline MJ, Lipponen PK et al. (1997) :** Prognostic value of tumor infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol; 182: 318-324.

11- **Dorta RG, Landman G and Kowalski PL (2002) :** Tumor associated tissue eosinophilia as a prognostic factor in oral squamous cell carcinomas. Histopathol; 41: 152-155.

12- **Kikuchi R, Nouguchi T, Takeno S et al. (2000) :** Immunohistochemical detection of membrane-type-1 matrix metalloproteinase in colorectal carcinoma. British J Cancer; 83(2): 215-218.

13- **Johansson N, Airola K, Grénman R et al. (1997) :** Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck. Am J Pathol; 151: 499-508.

14- **Papadopoulou S, Scorilas A, Arnogianaki N et al. (2001) :** Expression of gelatinase-A (MMP-2) in human colon cancer and normal colon mucosa.Tumor Biol; 22: 383-389.

15- **Jass JR, Ajyoka Y and Allen JP (1996) :** Assessment of invasive growth pattern and lymphocytic infiltration in color-

ectal cancer. Histopathol; 28: 543-548.

16- **Ropponen KM, Eskeline MJ, Lipponen PK et al. (1997) :** Prognostic value of tumor infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol; 182: 318-324.

17- **Yoon IL (1995) :** The eosinophil and gastrointestinal carcinoma. Am J surgery; 97:195-200.

18- **Nielsen HJ, Hansen U, Christensen IJ et al. (1999) :** Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. J Pathol; 189: 487-495.

19- **Baker EA, Bergin FG and Leaper DJ (2000) :** Matrix metalloprotienases, their tissue inhibitors and colorectal cancer staging. British J Surg; 87: 1215-1221.

20- **Liabakk NB, Talbot I, Smith AR et al. (1996) :** Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) type IV collagenases in colorectal cancer. Cancer Res; 56: 190-196.

21- **Parson SL, Watson SA, Collins HM et al. (1998) :** Gelatinase (MMP-2,-9) expression in gastrointestinal malignancy. British J Cancer; 78: 1495-1502.

22- **Ring B, Johansson K, Hoyhtya M et al. (1997) :** Expression of tissue inhibitor of metalloproteinases TIMP-2 in human colorectal cancer – a predictor of tumor stage. British J Cancer; 76(6): 805-811.

23- **Ko K, Yazumi S, Yoshikawa K et al. (2000) :** Activation of fibroblast derived matrix metalloproteinase-2 by colon cancer cells in non-contact co-cultures. Int J Cancer; 87: 165-171.

24- **Ornstein DL, Mac Nab J and Chon KH (1999) :** Evidence of tumor –host cooperation in regulating MMP-2 expres-

sion in human colon cancer. Clin Exp Metastasis; 17: 205-212.

25- Stetler-Stevenson WG (1999) : Matrix metalloproteinases in angiogenesis: a *moving target* for therapeutic intervention. J Clin Invest; 103(9): 1237-1241.