# TIMP1, and TIMP2 Immunohistochemical staining along with different histopathological parameters of colorectal adenocarcinoma

Ibrahim Abdul Majeed Mustafa* ,Khalid Tariq Al-Naib**and Luay E. Al-Khuri***

**الخلاصة**

تقدم السرطان هو عمليه معقده، متعدده الخطوات؛ هنالك خطوتين مهمتين في نمو وأنتشار السرطان وهما: عمليه تحطيم المكونات البروتينيه الخارج خلويه والأخرى تكوين أوعيه دمويه جديده، وذلك لتجهيز الورم بالدم. أن أنزيمات Matrix Metalloproteinase هي مجموعة من ألانزيمات المحلله للمواد البروتينيه الخارج خلويه، وتعمل على أعادة هيكلة النسيج وأنتشار السرطان، بينما Tissue inhibitor of MMP هي المانعات النسيجيه للـ MMPs,مثبطات لفعالية أنزيمات الـMMPs وتحافظ على تكامل المكونات الخارج خلويه ، وبالتالي تثبط هجره الورم.

على الرغم من أن هنالك دراسا ت عديده وعلى أنواع مختلفه من ألاورام السرطانيه للانسان التي بينت دور الMMPs في أنتشار النقائل الورميه، لكن يبقى دورها كمؤشر على مآل سرطان القولون و المستقيم غير واضح . الأكثر أهميه من ذلك الدور المتناقض للـ TIMPs في هذه الاورام لم يفسر لحد الآن.

أن هدف هذه ألدراسه هو لتحديد أي من( TIMP-1, & 2 ,) يملك أهميه مرضيه-سريريه خلال تقدم سرطان القولون و المستقيم.

من أجل بلوغ هذه ألاهداف ٣٥ عينه نسيجيه مطموره بالشمع نظرت من مرضى سرطان القولون و المستقيم في العراق، بالاضافه الى حافه النسيج السرطاني المأخوذه من نفس المريض. وقد تم الحصول على العينات من مختبر الدكتور لؤي أدور الخوري للتحليلات ألنسيجيه / بغداد.

أعتماداً على التصبيغ المناعي النسيجي الكيميائي، وجد أن هنالك زياده معنويه في التعبير الخلوي لكل من TIMP-1 و TIMP-2 عندما تمت المقارنه بين النسيج السرطاني ($P<0.001$ ، $P<0.001$ على التوالي). بقي ان نذكر ان هذه الزياده في كل من TIMP-1 و TIMP-2 ، هي مؤقته و تعكس استجابه الجسم لموازنه تحليل النسيج الموقعي.

عندما قسمت العينات السرطانيه بالاعتماد على التغيرات النسيجيه المرضيه، على الرغم من ان التعبير الخلوي لكل من TIMP-1و TIMP-2 يظهر علاقه عكسيه مع المتغيرات النسيجيه المرضيه، لكن يبقى TIMP-2 وحده يملك فرقا معنويا بالمقارنه مع كل من مرحله الورم، وانتشاره للعقد اللمفاويه ($r_S = -0.463$ ،$p<0.01$ ; و $r_S = -0.48$ , $p<0.01$).

كانت إستنتاجاتنا من هذه الدراسة MMP-2 يظهران أهمية سريرية أكثر من TIMP-1 في سرطان القولون و المستقيم .

*Medical Microbiology/ Immunology Al-Qadisiaya University/ College of medicine
**Medical Microbiology/ Immunology Al-Nahrain University/ College of medicine
***Pathology Baghdad University/ College of medicine

**Abstract**

Cancer progression is a complex multi-step process. Two critical steps in tumor growth and invasion: are the proteolytic processing of the extracellular matrix environment, and the angiogenic switch enabling blood supply into the tumor. Matrix metalloproteases (MMPs) are a group of proteolytic enzymes that degrade components of the extracellular matrix and are implicated in tissue remodeling and tumor infiltration, while Tissue inhibitor of metalloproteinases (TIMPs) inhibit activity of the MMP family and preserve stromal integrity, as a consequence, inhibiting tumor migration. More importantly, the recently documented paradoxical functions of TIMPs have not been characterized in these neoplasms.

The aims of the current study were to determine whether TIMP-1 and TIMP-2 has any histopathological significance during colorectal adenocarcinoma progression.

Accordingly, 35 colorectal adenocarcinoma paraffin embedded sections prepared from Iraqi patients, in addition to their respective resection margins were retrospectively collected from (liver and gastrointestinal hospital)/Baghdad. Based on immunohistochemical staining, it was found that there were a significant increase in the cellular expression of TIMP-1, and TIMP-2 in all of the 35 tumor samples compared to their respective resection margins ($p<0.001$, and $p<0.001$ respectively). Keeping in mind this up regulation of TIMP-1, and TIMP-2, was transient and reflect a host responses to the remodeling stimuli to balance the local tissue degradation process. moreover, when these 35 paraffin embedded sections were broken down according to their various histopathplogical variables, both TIMP-1, and TIMP-2, appear to negatively correlated with tumor histopathplogical variables, still only TIMP-2 revealed a significant negative correlation with both tumor stage and lymph node involvement ($r_S = -0.463$, $p<0.01$; and $r_S = -0.482$, $p<0.01$, respectively).

In conclusion, TIMP-2 are seem to be more interesting clinical tool compared to (TIMP-1) in CRC, since, it was found to have the play maker role during Duke's stage progression, and L.N

involvement, thus they could be used as targets for therapeutic management of patients with primary colorectal adenocarcinoma.

## Introduction

Colorectal adenocarcinomas are malignant neoplasms of epithelial cells origin affecting the large bowel. They are common types of malignancies that affect gastrointestinal tract and they are of favorable prognosis provided that they are diagnosed and treated in early stage (1).

## Role of TIMPs in tumor angiogenesis, invasion, and metastasis.

A number of studies have demonstrated the expression of TIMPs in tumor stroma and tumor cells. In general, there is convincing evidence that overexpression of TIMPs by cancer cells or by the host reduces invasive and
metastatic capacity of tumor cells. In cutaneous and oral SCCs expression of TIMP-1, TIMP-2, and TIMP-3 is detected in stromal cells adjacent to the
tumor (2, 3). Suggesting that their expression represents a host attempt to limit tumor invasion and tumor-induced angiogenesis. This notion is supported by observations indicating that the presence of TIMP-1 and TIMP-2 in SCCs correlates with less aggressive growth (4). However, in breast cancer TIMP-2 expression correlates with tumor recurrence (5) and in cervical carcinomas TIMP-2 expression correlates with poor prognosis (6). Similarly, in malignant breast cancer TIMP-1 expression is enhanced, as compared to non malignant breast tumor (7). Cancer cell invasion can be inhibited by recombinant TIMPs or by over expression of either TIMPs using a variety of gene delivery vehicles. TIMP-2 inhibited the invasion of HT-1080 fibrosarcoma cells in vitro (4, 8), over expression of TIMP-1 reduced metastasis of gastric carcinoma cells (9, 10) The ability of TIMP-1 to inhibit tumor development at different stages has been demonstrated by transgenic mouse models. Constitutive overexpression of TIMP-1 in the liver suppressed tumor initiation, growth and angiogenesis in transgenic mice, which develop hepatocellular carcinomas as a result of SV40 T antigen expression (11) These observations were

also supported by a recent study, in which TIMP-1 overexpression in the brain prevented tumor formation (12). Evidence for the role of MMPs and TIMPs in angiogenesis comes from a number of studies.

Nanomolar concentration of TIMP-2 will block the angiogenic response to bFGF, a principal angiogenic cytokine produced by vascularized human tumors, in the chick chorioallantoic membrane assay (13). TIMP-1 has also been shown to inhibit endothelial cell invasion of human amniotic membranes *in vitro* (14). Cartilage-derived inhibitor (CDI), a TIMP-related protein isolated from bovine articular cartilage, blocks endothelial cell proliferation and angiogenesis (15, 16). In addition, TIMP-1 and TIMP-2 inhibit chick yolk sac vessel morphogenesis in response to polyamines (17).

**Materials and Methods**

Patients and Sampling:

Thirty five patients (20 male and 15 females) with colorectal adenocarcinoma, who were confirmed histopathologicaly, were included in this study. Their age were ranged from 25- 80 years.

Paraffin embedded blocks of tumor and resection margins were retrieved along with the histopathological report of each patient from histopathological laboratory. For staging of the cancer, both astler-coller and TNM staging systems were adopted in this study (18, 19). In addition, resection margins were confirmed again to be free of malignancy. Adequate thin paraffin embedded sections (5µm thick) of tumor and resection margins were prepared on positively charged slides for the immunohistochimistrey Technique.

Immunohistochemistry for detection of TIMP-1 and TIMP-2 cellular expression in paraffin embedded sections.

Principle of test:

A primary antibody reacts with an antigen. A biotinylated secondary antibody then reacts with the primary antibody. This is followed by the attachment of an enzyme-conjugated streptavidin

to the biotins on the secondary antibody. The enzyme converts a substrate to a colored reaction product.

**Materials:**

1. Nuclear fast red or fast red B salt (MCB Manufacturing Chemists, Germany).
2. UniMAK Universal Multi-Application Kit (InnoGenex, USA): consist of the following:

a. Biotinylated secondary antibody, which is biotinylated goat anti-mouse immunoglobulin (20X).
b. Alkaline phosphatase-streptavidin conjugate (20X).
c. BCIP/NBT substrate.
d. Dilution/Blocking buffer (10X).
e. Phosphate buffered saline tablets, 1 tablet dissolved in 100 ml deionized distilled water to make 1X phosphate buffered saline (PBS).
f. Activation buffer (10X).

3. Monocolnal Antibodies:

-Anti-TIMP-1 glycoprotein (Chemicon,USA): Clone: 102D1 Isotype: IgG1 to a recombinant human TIMP-1.
-Anti- TIMP-2 protein (Chemichon:USA): Clone: 3A4 Isotype IgG2a to a recombinant haman TIMP-2.

**Immunohistochemistry procedure:**

1. Dewaxing: paraffin embedded sections were placed inside a hot air oven at 65°C overnight, then dipped in xylene and ethanol containing jars in the following order:
2. Slides were washed in distlled water for 5 minutes then drained and blotted gently.
3. 100 µl of a protein-blocking reagent was placed onto the section and incubated for 10 minutes in a humid chamber at room temperature. Then slides were drained and blotted gently.
4. 100 µl of diluted primary antibody was placed onto the section and incubated for 1 hour at 37°C in a humid chamber. After incubation, the slides were drained and blotted gently.
5. Slides were rinsed with PBS-Tween for 5 minutes, then drained and blotted gently.

6. 100 µl of diluted secondary antibody was placed onto the section and incubated for 30 minutes at 37°C in humid chamber. Slides were drained and blotted gently.
7. Slides were rinsed with PBS-Tween for 5 minutes, then drained and blotted gently.
8. 100 µl of diluted streptavidin-alkaline phosphatase conjugate was placed onto the section and incubated for 20 minutes at 37°C in humid chamber. Slides were drained and blotted gently. .
9. Slides were rinsed with PBS-Tween for 5 minutes, then drained and blotted gently.
10. One hundred microliter (100 µl) of the BCIP/NBT substrate was placed onto the section and incubated for 10 minutes at room temperature. .
11. Slides were washed in running water for 5 minutes and then drained and blotted gently.
12. 100 µl of counterstain (nuclear fast red) was placed onto the section and incubated for 1 minute at room temperature. Slides were drained and blotted gently.
13. Slides were washed in distilled water then dehydrated by placing them in ethanol and xylene .
14. A drop of mounting medium (DPX) was placed onto the xylene-wet section by using a xylene-moist cotton swab and the section was quickly covered with a cover slip. Slides were let to dry.
15. Slides were examined by pathologist by light microscope at X400 magnification.

**Results**

Histopathological Data.

Thirty five patients with colorectal adenocarcinoma were investigated. The mean age of patients was 52.34 with a range of 25-80 years. The male to female ratio was 4:3. There were only 2 cases of recurrent colorectal carcinoma. According to the histological differentiation, tumors were broken down in to three groups, well differentiated (WD, n=5), moderately differntiated (MD, n=25), and porrly differentiated (PD, n=5), moreover,

patients were further grouped according to their histopathological criteria, as follow: tumor stage (B, n=11, C, n=15, and D, n=9), lymph node involvment (N0, n=11, N1, n=11, and N2, n=13).

Tissue inhibitors of matrix metalloproteinase (TIMP-1), and (TIMP-2) immunohistochemical staining

Tumor sites versus resection margins

Tumor sample from 35 cases were further investigated for determining TIMP-1, and TIMP-2 cytoplasmis expression based on Immunohistochemical staining technique. Their respective resection margins were analyzed as well (TIMP-1 and TIMP-2 typical staining pattern in tumor sections and resection margins are shown in figure 1), interstingly, the mean±standard error (SE) of immunohistochemical staining in both tumor samples and their resection margins were 57.45±3.6, and 40.42±4.52 versus 23.5±1.89 and, 26.8±1.89 for TIMP-1 and TIMP-2, respectively, with a statistically significant differences of $p< 0.001$ and $p< 0.001$, respectively based on $t$ test of analysis (table 3-1).

**Table (3-1). Tissue inhibitors of matrx metalloproteinase 1, and 2, immunohistochemical staining in tumor sites and their resection margins based on t,test**

| PARAMETER | RESECTION MARGINE (NO=35) | TUMOR TISSUE (NO=35) |
|---|---|---|
| ***TIMP-1*** | | |
| Mean ±SE† | **23.5±1.89** | **57.45±3.6,** |
| 99%C.I ‡ | -------- | **25.73-42.18** |
| t-test *p*-value | -------- | *P<0.001* |
| ***TIMP-2*** | | |
| Mean ±SE† | **26.8±1.89** | **40.42±4.52** |
| 95%C.I ‡ | ------- | **3.72-23.53** |
| t-test *p*-value | ------- | ***P<0.001*** |

† Standard error. C.I ‡ confidence interval

*Correlation between TIMP-1 and TIMP-2 immunohistochemical staining with different histopathological variables*

Table (3-2) shows the correlation between expression of TIMP-1, and TIMP-2 , with different histopathological variables. Based on Spearman's correlation test of analysis ($r_S$). The current data showed that in contrast to TIMP-1 cellular expression that demonstrated no significant correlation with respect to the various histopathological variables, on the contrary, TIMP-2 cellular expression, iterstingly, revealed a significant negative correlation with respect to both tumor stage ($r_S$ = - 0.436, $p$<0.01) and lymph node involvment ($r_S$ = - 0.474, $p$<0.01).

**Table (3-2). Correlation between TIMP-1, and TIMP-2, immunostaining with varius histopaphological variables of CRC, based on spearman's correlation ($r_S$).**

| HISTOPATHOLOGICAL VARIABLES | TIMP-1 | TIMP-2 |
|---|---|---|
| **Stage** | -0.167 | **-0.463**** |
| **Grade** | -0.090 | -0.058 |
| **L.N involvement** | -0.074 | **-0.482**** |

**highly significant correlation, $p$<0.01

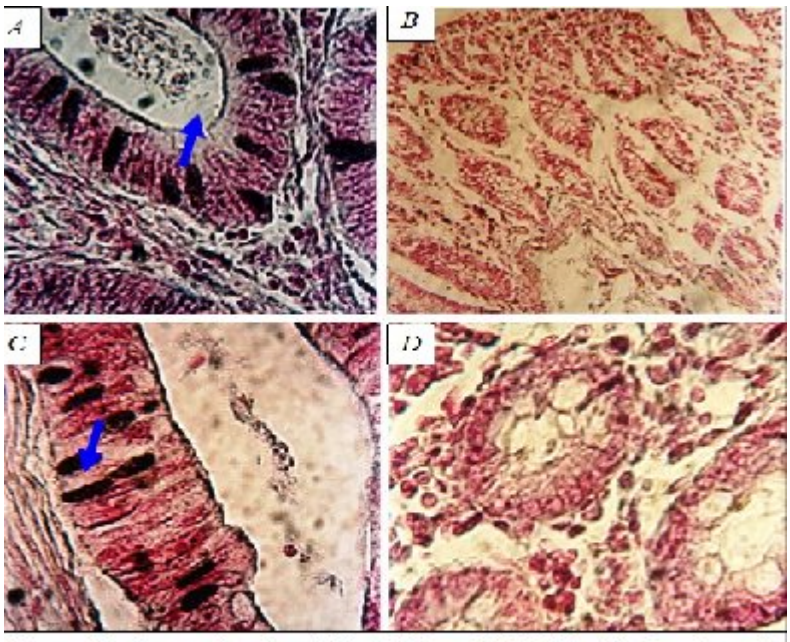


**Figure 1.** Immunohistochemichal staining of TIMP 1, 2, in colorectal adenocarcinoma section and their resection margins by BCIP/NBT (bluish-black) counterstained with nuclear fast red. (A) TIMP-1 cytoplasmic expression, percent of stained cells (60%). (B) Resection margin stained with TIMP-1. (C) TIMP-2 cytoplasmic expression, percent of stained cells (45%). (D) Resection margin stained with TIMP-2. Magnification power from A to F (400X), except B (100X).

**Discussion**

The current results revealed a significant increase in the cellular expression of TIMP-1, and TIMP-2 among the 35 investigated colorectal adenocarcinoma (57.45±3.6, 40.42±4.52, $p<0.001$ and $p<0.001$, respectively, table 3-1) in tumor tissue versus (23.5±1.89, 26.8±1.89, respectively, table 3-2) in resection margins.

These results were come in agreement with previous studies by (20, 21), since they found significant over expression of TIMP-1, and TIMP-2 in human colon cancer compared to their respective resection margins. Although, both TIMP-1 and TIMP-2 cellular expression were elevated in the investigated tumor sections, still, the MMP/TIMP balance might contribute to be in favor of proteolysis. This observation could be explained on the bases of the coordinated regulation of proteases and their corresponding inhibitors or, alternatively the synthesis of inhibitors may be a cellular reaction to the presence of proteases, (22). It should be noted that TIMPs are multifunctional proteins, keeping in mind Both TIMP-1 and TIMP-2 have erythroid-potentiating activity and have been shown to stimulate mitogenesis of several cell types in vitro. (23). Accordingly, it could be suggested that the erythroid-potentiating activity and the MMP-inhibitory property of TIMP-1 are independent and separable activities (24).

The current study had also focused on determining the level of TIMP-1 and TIMP-2 protein expression in CRC, and their resection margins. Here, it was demonstrated that although there was an increase in the protein expressions of both markers exclusively at the tumor tissues, compared to resection margins (table 3-1), however, this up regulation was transient, and thus may reflect one of the subsequent acute host responses to the remodeling stimuli to balance the local tissue degradation process (25). Subsequently, as tumor progress, there was a clear decline in the expression of both inhibitors, here interestingly, TIMP-2 may have the upper hand since, it has been exclusively correlated in negative manner with Duke's stage as well as with lymph node involvement, ($r_S$ = -0.463, $p<0.01$; and $r_S$ = -482, $p<0.01$,

respectively, table (3-2). These negative correlations may concomitant with elevated MMPs expression, which support the notion that in order for tumor cells to invad and metastasized, the levels of MMPs enzymes must be exceed their inhibitors (26). Previous studies that focused on the possible role of TIMP-1 and TIMP-2 during CRC progression are controversial. For example, one study, illustrated that although Strong stromal TIMP-1 immunoreactivity correlated with Dukes' stage, status of L.N metastasis, and poor survival, the degree of immunohistochemical staining of TIMP-2 did not correlate with all clinicopathological variables, (27). While, another study revealed that TIMP-2 significantly correlated with tumor stage and L.N involvement (28). Still, in another two separated studies, TIMP-1 showed a significant increase where as TIMP-2 demonstrated a significant decrease in tumor samples compared to their respective non tumous tissues ( *i.e,* resection margins) (25, 29). There are several reasons that may explain why the current study differs in its out come from those listed above. First, ralatively small sample size with most of them falls within advanced stages. Second, the sensitivity and specificity of the monoclonal antibodies that were used. Lastly, and most important, the constituent of the microenvironment within the tumor that may modulate TIMPs cellular expression had not been determined neither by the current study nor by those previously sited.

Nevertheless, the fact that TIMP-2 but not TIMP-1 demonstrated in the current study to correlate significantly and negatively with various colorectal adenocarcinoma histopathplogical variables might be due to the recent observation revealed by Researchers, they demonstrated a novel growth inhibitory mechanisms exclusively mediated through TIMP-2 , that was independent from MMPs inhibition. This novel growth inhibition was proved experimentally via blocking basic fibroblast growth factor (bFGF), a growth factor that mediate microvascular endothelial cell growth, independent from MMPs inhibitory cascade, resulted in direct termination of infiltrating of primary tumor mass, by new blood vessels as well as tumor cell

dissemination. Accordingly such a growth inhibitory activity mediated by TIMP-2 make it largely acceptable the observed significant down regulation of TIMP-2 revealed by the currently observed study since it may pave the way for tumor invasion and possible metastasis (30).

**References**

1. Cotran R. S., Kumar V. and Collins T., 1999**.** Neoplasia. In Robbins: Pathologic Basis of Disease, 6th ed., pp. (165-209).
2. Airola K, Ahonen M, Johansson N et al., 1998. Human TIMP-3 is expressed during fetal development, hair growth cycle, and cancer progression. J Histochem Cytochem, 46:437-447.
3. Sutinen, M. Kainulainen, T., Hurskainen, T., Vesterlund, E., Alexander, J. P., Overall, C. M. Sorsa, T., and Salo, T., 1998. Expression of matrix metalloproteinases (MMP-1 and -2) and their inhibitors (TIMP-1, -2, and -3) in oral lichen planus, dysplasia, squamous cell carcinoma and lymph node metastasis. Br. J. Cancer, 77: 2239–2245.
4. Reunanen N. and Kähäri V.M., 2002**.** Matrix metalloproteinases in cancer cell invasion. In Cell Invasion, Jyrki Heino and Veli-Matti Kähäri Eds.
5. Visscher DW, Höyhtyä M, Ottosen SK et al., 1994. Enhanced expression of tissue inhibitor of metalloproteinase-2 (TIMP-2) in the stroma of breast carcinomas correlates with tumor recurrence. Int J Cancer, 59:339-344.
6. Nuovo GJ, MacDonnell PB, Simsir A et al., 1995. Correlation of the in situ detection of polymerase chain reaction-amplified metalloproteinase complementary DNAs and their inhibitors with prognosis in cervical carcinoma. Cancer Res, 55:267-275.
7. Yoshiji H, Gomez DE, Thorgeirsson UP., 1996. Enhanced RNA expression of tissue inhibitor of metalloproteinases-1 (TIMP-1) in human breast cancer. Int J Cancer, 69:131-134.
8. Albini A, Melchiori A, Santi L et al., 1991.Tumor cell invasion inhibited by TIMP-2. J Natl Cancer Inst, 83:775-779.
9. Tsuchiya Y, Sato H, Endo Y et al., 1993. Tissue inhibitor of metalloproteinase-1 is a negative regulator of the metastatic ability of

a human gastric-cancer cell-line, Kkls, in the chick-embryo. Cancer Res, 53:1397-1402.
10. Watanabe M, Takahashi Y, Ohta T et al., 1996. Inhibition of metastasis in human gastric cancer cells transfected with Tissue inhibitor of metalloproteinase 1 gene in nude mice. Cancer 77:1676-1680.
11. Martin, DC, Ruther U, Sanchez-Sweatman OH et al., 1996. Inhibition of SV40 T antigen-induced hepatocellular carcinoma in TIMP-1 transgenic mice. Oncogene, 13:569-576.
12. Kruger A, Sanchez-Sweatman OH, Martin DC et al., 1998. Host TIMP-1 overexpression confers resistance to experimental brain metastasis of a fibrosarcoma cell line. Oncogene , 16:2419-2423.
13. Ray J.M, Stetler-Stevenson W.G., 1994. The role of matrix metalloproteinases and their inhibitors in tumor invasion, metastasis and angiogenesis. Eur Respir J, 7:2062-2072.
14. Mignatti P, Robbins ER, Rifkin DB., 1986. Tumor invasion through the human amniotic membrane: requirement for a proteinase cascade. Cell, 47: 487–498.
15. Moses MA, Sudhalter J, Langer R., 1990. Identification of an inhibitor of neovascularization from cartilage. Science, 248: 1408–1410.
16. Moses MA, Langer R., 1991. Inhibitiors of angiogenesis. Biotech, 9: 630–634.
17. Takigawa M, Nishida Y, Suzuki F, Kishi J, Yamashita K, Hayakawa T., 1990. Induction of angiogenesis in chick yolk-sac membrane by polyamines and its inhibition by tissue inhibitors of metalloproteinases (TIMP and TIMP-2). Biochem Biophys Res Commun, 171: 1264– 1271.
18. Astler V. B. and Coller F. A., 1954. The prognostic significance of direct extension of carcinoma of the colon and rectum. Ann Surg, 139: 846-847.
19. Beahrs O. H., 1992. Staging of cancer of the colon and rectum. Cancer, 70: 1393-1396.
20. Pesta M, Holubec L Jr, Toplcan O, Cerna M, Rupert K, Hoolubec LS, Treska V., 2005. Quantative estimation of matrix metalloproteinases 1 and 2 (TIMP-1, TNMP-2) in colorectal carcinoma tissue samples. Anticancer Res, 25(5):3387-91. (Abstract).

21. Adachi, Y., Yamamoto, H., Itoh, F., Hinoda, Y., Okada, Y., and Imai, K., 1999. Contribution of matrilysin (MMP-7) to the metastatic pathway of human colorectal cancers. Gut, 45: 252–258.
22. McCarthy K, Maguire T, McGreal G, et al., 1999. High levels of tissue inhibitor of metalloproteinase-1 predict poor outcome in patients with breast cancer. Int J Cancer, 84:44-48.
23. Goss KJ, Brown PD, Matrisian LM., 1998. Differing effects of endogenous and synthetic inhibitors of metalloproteinases on intestinal tumorigenesis. Int J Cancer, 78:629-635.
24. Chesler L, Golde DW, Bersch N, et al., 1995. Metalloproteinase inhibition and erythroid potentiation are independent activities of tissue inhibitor of metalloproteinases-1. Blood, 86:4506-4515.
25. Morán A, Iniesta P, García-Aranda C, Dejuan C, and Díaz-López A., 2005. Clinical relevance of MMP-9, MMP-2, TIMP-1 and TIMP-2 in colorectal cancer. Oncology Reports, 13:115-120.
26. Chang C. and Werb Z., 2001. The many faces of metalloproteinases cell growth, invasion, angiogenesis and metastases. TRENSA in Cell Biology, 11 (11): s37-s43.
27. Joo YE, Seo KS, Kim J, Kim HS, Rew JS, Park CS, Kim SJ., 1999. Role of tissue inhibitors of metalloproteinases (TIMPs) in colorectal carcinoma. J Korean Med Sci, 14(4):417-23. (Abstract).
28. Wang M, Liu YE, Jreene J, etal., 1997. Inhibition of tumor growth and metastasis of human breast cancer cells transfected with tissue inhibitor of metalloproteinase 4, Oncogen, 14:2767-2774.
29. Mukai M, Sadahiro S, Tokunaga N, Ishizu K, Ito I, Kameya T, Ishikawa K, Iwase H, Suzuki T, Ishida H, Tajima T, Makuuchi H., 1999. The expression of MMP-2 and TIMP-2 in patients with primary colorectal adenocarcinoma: correlation with liver metastasis. Oncol Rep, 6(5):969-73. (Abstract).
30. Baker EA, Bergin FG, Leaper DJ., 2000. Matrix metalloproteinases, their tissue inhibitors and colorectal cancer staging. Br J Surg, 87(9):1215-21. (Abstract).
31. Murphy G, Willenbrock F., 1995. Tissue inhibitors of matrix metalloendopeptidases. Methods Enzymol, 248:496–510.