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# Immunoexpression of matrix metalloproteinase-2 (MMP-2) in epithelial ovarian cancers (EOCs)

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ABSTRACT

**Objective:** To evaluate the relation between matrix metalloproteinase-2 (MMP-2) expression and the clinical and/or pathological parameters of the epithelial ovarian cancers (EOCs). **Methods:** Forty-two (42) patients with EOCs diagnosed after histopathological examination of the specimens were included in this study. The pathological specimens were additionally stained by immunoperoxidase technique for MMP-2 using a monoclonal antibody against activated MMP 2. The staining intensity of MMP-2 was correlated with the clinical and pathological parameters of the studied cases, including patient's age, surgical stage, histological grade, omental, and lymph node metastasis. **Results:** The studied cases of EOCs were classified according to the intensity or the degree of MMP-2 expression, as; seven cases (16.7%) negative, eighteen cases (42.8%) weak, seven cases (16.7%) moderate and ten cases (23.8%) intense for MMP-2 staining. There was a significant positive correlation between MMP-2 expression and the histological grades and the surgical stages of the studied EOC ($r$ <1, $P$<0.05), while, there was no significant relation between MMP-2 expression and the histopathological types of the studied EOCs. MMP-2 expression was significantly high in EOCs with ascites, omental, distant and uterine metastasis, while, there was no significant relation between MMP-2 expressions and lymph node metastasis or bilaterality of the EOCs. **Conclusions:** MMP-2 expression was associated with advanced, aggressive EOCs and there was direct relation between expression of MMP-2 and degree of invasiveness and metastasis of EOCs.

## 1. Introduction

Epithelial ovarian cancers (EOCs) are the most common ovarian malignancies. Ovarian cancer represents a major surgical challenge, requires intensive and often complex therapies[1]. Initiation of invasive and metastatic cascades is triggered by degradation of basement membrane components by specific proteinases[2,3]. Understanding the molecular mechanisms of metastasis is crucial for the design and use of novel therapeutic strategies to combat metastasis[4,5]. The degradation of extra cellular matrix (ECM) is an important step of the metastatic cascade which needs active proteolytic enzymes as, serine proteases, cysteine proteases and matrix metalloproteinases (MMPs). MMPs are a family of zinc dependent endopeptidases with broad spectrum proteolytic activity for most classes of ECM proteins. They are secreted as latent form which needs activation by proteolytic cleavage. According to substrate specificity and structure, they are classified into [4] major subgroups including 17 family members which are produced by different genes. The net activity of MMPs is determined by the amount of proenzyme expressed, the extent to which the proenzyme is activated and the local concentration of specific inhibitor (tissue inhibitors of metalloproteinases = TIMPs)[4]. The balance between MMPs and TIMPs is critical in maintaining the integrity of ECM

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and its regulatory role in organ development, cell growth and differentiation[6].

MMPs are key regulators of growth of tumours at both primary and metastatic sites and are important in creating and maintaining an environment that supports the initiation and maintenance of growth of primary and metastatic tumours[4]. The ability of MMPs to degrade ECM proteins and to promote tumour development has been thought to be due to the proteolytic breakdown of tissue barriers[2]. More recently the identification of specific matrix and non matrix substrates for MMPs indicates that perhaps MMPs should be viewed more as playing a complicated role in modulating normal cellular behaviour, cell cell communication, tumour initiation and progression[7]. Matrix metalloproteinases 2 (MMP 2) is a member of the MMPs family (72Kda) which is able to degrade type IV collagen, fibronectin and components of all basement membranes, thereby facilitating stromal and vascular invasion by tumour cells. MMP-2 is strongly implicated in ECM remodelling[8]. Expression of MMP2 appears to be crucial for tumour angiogenesis, which is an important requirement for cancer growth and dissemination[2,9]. Numerous studies have shown that levels of activated MMPs are significantly higher in certain malignant tumour tissues compared with their benign or borderline counterparts[10,11]. Preventing MMPs activation or inhibiting activated MMPs in ovarian carcinomas is therefore a potential strategy for controlling tumour progression. ECM degradation in tumour biology is attributed to an imbalance in local MMPs and TIMPs activity, resulting in the over expression of MMPs[11-14]. So, this study was designed to evaluate the relation between MMP-2 expression and the clinical and/or pathological parameters of the epithelial ovarian cancers (EOCs).

## 2. Materials and methods

Patients with ovarian tumours were included in this retrospective study if their histopathological examination revealed EOCs after informed consent and approval of the study by the institute ethical committee. Forty-two (42) patients with different grades and stages of EOCs were enrolled in this study and their pathological specimens were additionally examined for Matrix MMP-2. All patients were operated and underwent surgical staging by sampling of peritoneal fluid, ascites, omentum, retroperitoneal lymph nodes and excision of the uterus and both ovaries. Other surgical details were left to the operating surgeons. All tissue specimens have been already fixed in neutral buffered formalin for a period not more than 24 hours. After fixation, the specimens were dehydrated, cleared and embedded in paraffin blocks according to routine processing. Paraffin sections were cut at 4 microns and subjected to routine haematoxylin and eosin staining and immunohistochemical staining for MMP 2. Slides stained with haematoxylin and eosins were first examined to evaluate the histological types and grades of the EOCs. Immunostaining procedure for MMP-2 was done at room temperature according to Hsu *et al*, using the immunostainer (Shandon Sequenza immunostainer)[15]. Reagents used included: (a) Primary antibody, which is a liquid monoclonal mouse anti-human MMP-2 protein (Santacruz laboratories), (b) Universal Kits: a supersensitive immunodetection system from Biogenex Laboratories, (c) Blocking reagent: block the endogenous peroxidase activity (Biogenex), (d) Ready to use antigen retrieval citra (Biogenex). Sections of invasive breast carcinoma were included in each run as a positive control to judge the effectiveness of the technique. Negative control slides were processed as previous steps, but the negative control serum was used instead of the primary antibody. Staining intensity for MMP-2 was evaluated semi-quantitatively, using a score of 0-3. Negative staining was scored as 0 or (-), whereas scores of 1 or (+), 2 or (++) and 3 or (+++), represented weak, moderate and intense staining, respectively. The intensity of the MMP-2 staining was evaluated by two expert pathologists blinded to the patients' clinical data.

Statistical analysis were done by using Statistical Package for Social Sciences (SPSS); computer software version 15. Numerical variables were presented as mean and standard deviation (±SD), while categorical variables were presented as number of cases and percentage. Chi-square ($\chi^2$) test and Fisher exact were used for count variables. One way ANOVA test was used in assessment of the difference between continuous variable. Spearman's rho correlation was used to explore the association between histological grade, surgical stage of the studied cases and intensity of MMP-2 staining. A difference with P value <0.05 was considered statistically significant between groups.

## 3. Results

Forty-two (42) cases of ovarian epithelial cancers (EOCs) were included in this study and the histopathological types were mentioned in Table 1. The studied cases of EOC were

**Table 1**
The histopathological types and histological grades of the studied EOCs cases.

| Histopathological types of EOCs | Total number (*n*=40) | Grade I (*n*,%) | Grade II (*n*,%) | Grade III (*n*,%) |
|---|---|---|---|---|
| Serous carcinoma | 15 | 3(20.0) | 8(53.3) | 4(26.7) |
| Mucinous carcinoma | 11 | 4(36.4) | 5(45.4) | 2(18.2) |
| Endometrioid carcinoma | 8 | 1(12.5) | 5(62.5) | 2(25.0) |
| Malignant brenner | 3 | 1(33.3) | 2(66.7) | 0(0.0) |
| Mixed cell tumor | 3 | 1(33.3) | 1(33.3) | 1(33.3) |
| Total | 40 | 10(25.0) | 21(52.5) | 9(22.5) |

Data are presented as number (*n*) and percentage (%), Clear cell carcinoma has no histological grade; two cases were included in the study.

categorized according to FIGO staging into: thirteen (31.0%) cases stage I (Ia = 6 cases, Ib = 4 cases and Ic = 3 cases), five (11.9%) cases stage II (IIa = 1 case, IIb = 1 case, and IIc = 3 cases), thirteen (31.0%) cases stage III (IIIa = 4 cases, IIIb = 4 cases, and IIIc = 5 cases) and eleven (26.1%) cases stage IV (Table 2). The studied cases of EOCs were classified according to the intensity or the degree of MMP 2 expression as: seven cases (16.7%) negative, eighteen cases (42.8%) weak, seven cases (16.7%) moderate and ten cases (23.8%) intense for MMP 2 staining (Table 3). The relation between MMP 2 expression and mean age of the studied population was statistically insignificant, also, the relation between MMP 2 expression and the histopathological types of the studied EOC was statistically insignificant ($P>0.05$), Table 3.

**Table 2**

FIGO staging, nodal status and the result of immunohistochemical staining of the studied EOCs cases

| FIGO Stage | Number | Percentage(%) |
|---|---|---|
| Stage I | 13 | 31.0 |
| Ia | 6 | 14.4 |
| Ib | 4 | 9.5 |
| Ic | 3 | 7.1 |
| Stage II | 5 | 11.9 |
| IIa | 1 | 2.4 |
| IIb | 1 | 2.4 |
| IIc | 3 | 7.1 |
| Stage III | 13 | 31.0 |
| IIIa | 4 | 9.5 |
| IIIb | 4 | 9.5 |
| IIIc | 5 | 12.0 |
| Stage IV | 11 | 26.1 |

There was a significant positive correlation between MMP 2 expression and the histological grades of the studied EOCs ($r<1$, $P<0.05$) (Table 4) also, there was a significant positive correlation between MMP 2 expression and the surgical stages of the studied EOCs ($r<1$, $P<0.05$) (Table 5). MMP-2 expression was significantly high in EOCs with ascites, omental metastasis, distant or uterine metastasis and there was no significant relation between MMP-2 expressions and the lymph node metastasis or bilaterality of the ovarian tumours (Table 6).

## 4. Discussion

MMPs are a family of zinc dependent proteolytic enzymes with a central role in ECM remodelling in a variety of physiological and pathological conditions. They seem to be involved in complex interactions with other cellular and extracellular proteins, thereby assuming an important role in normal cellular function, as well as in tumour invasion and metastasis[16,17]. MMP 2 is one of the matrix metalloproteinase families which are able to degrade type IV collagen. MMP 2 is released in a preform, which can be activated by membrane bound metalloproteinases[18]. MMP 2 over expression and activation has been associated with the invasive potential of ovarian, breast, lung and cervical carcinomas. The presence of MMP 2 mRNA appears to be associated with aggressive tumours and is a marker of poor survival[19]. Normal ovarian epithelial cells don't express MMP 2 and its expression by ovarian carcinomas may promote more aggressive invasion[20,21].

Forty-two (42) cases of EOCs were included in this study, 24 of them (57.1%) were staged as FIGO III/IV, also, 41 (68%) patients FIGO IIIC/IV were included in Barbara Schmalfeldt *et al.* study, while, 16 (75%) patients FIGO IIIC/IV were included in Sakata et al study and 72 (80 %) patients FIGO III/IV were included in Aparna and colleagues study[6,21,22].

The higher percentage of the advanced ovarian cancers (FIGO III/IV) in Sakata *et al.* and Aparna et al. studies, compared with this study and Barbara Schmalfeldt *et al.*

**Table 3**

The relation between MMP 2 expression and the histopathological types of the studied EOCs cases (*n*,%).

| The histopathological types of EOC | Negative | Weak (+) | Moderate (++) | Intense (+++) | Total |
|---|---|---|---|---|---|
| Serous carcinoma | 2(28.6) | 9(50) | 2(28.6) | 2(20) | 15 |
| Mucinous carcinoma | 2(28.6) | 2(11.1) | 4(57.2) | 3(30) | 11 |
| Endometrioid carcinoma | 1(14.2) | 5(27.8) | 1(14.2) | 1(10) | 8 |
| Clear cell carcinoma | 0(0) | 0(0) | 0(0) | 2(20) | 2 |
| Others* | 2(28.6) | 2(11.1%) | 0(0) | 2(20) | 6 |
| Total | 7(16.7%) | 18(42.8%) | 7(16.7%) | 10(23.8%) | 42 |

* Mixed cell tumor and malignant Brenner tumor. Data are presented as number (*n*) & percentage (%). Analysis was done using *Chi* square.

**Table 4**

The relation between MMP 2 expression and the histological grades of the studied EOCs cases (*n*,%).

| The histological grades of EOC | Negative (-) | Weak (+) | Moderate (++) | Intense (+++) | Total* |
|---|---|---|---|---|---|
| Grade I | 4(57.2) | 4(22.2) | 1(14.28) | 1(12.5) | 10 |
| Grade II | 2(28.6) | 12(66.7) | 6(85.72) | 1(12.5) | 21 |
| Grade III | 1(14.2) | 2(11.1) | 0(0.00) | 6(75.0) | 9 |
| Total | 7(17.5) | 18(45.0) | 7(17.50) | 8(20.0) | 40 |

*Two cases of clear cell carcinoma which has no histological grade and expression of MMP-2 was intense (removed from the total number and from intense MMP-2 staining). Data are presented as number (*n*) & percentage (%). Analysis was done using correlation coefficient test.

**Table 5**

The relation between MMP 2 expression and the surgical stages of the studied EOCs cases (*n*,%).

| The surgical stages of EOC | Negative | Weak (+) | Moderate (++) | Intense (+++ ) | Total |
|---|---|---|---|---|---|
| Stage I | 5(71.4) | 8(44.45) | 0(0.0) | 0(0.0) | 13 |
| Stage II | 2(28.6) | 2(11.10) | 1(14.2) | 0(0.0) | 5 |
| Stage III | 0(0.0) | 8(44.45) | 3(42.9) | 2(20.0) | 13 |
| Stage IV | 0(0.0) | 0(0.00) | 3(42.9) | 8(80.0) | 11 |
| Total | 7(16.7) | 18(42.80) | 7(16.7) | 10(23.8) | 42 |

Data are presented as number (*n*) & percentage (%). Analysis was done using Correlation coefficient test.

**Table 6**

The relation between MMP 2 expression and factors that determine the stage of EOCs.

| Variables | Negative | Weak (+) | Moderate (++) | Intense ( +++) | *P* value |
|---|---|---|---|---|---|
| Ascites (Significant) | 0(0.0) | 10(55.6) | 7(100.0) | 9(80.0)* | <0.05 |
| Omental metastasis (Significant) | 0(0.0) | 8(44.4) | 7(100.0)* | 10(100.0)* | <0.05 |
| LN metastasis (Non-significant) | 1(14.3) | 3(16.7) | 4(57.1) | 2(20.0) | 0.28 |
| Distant metastasis (Significant) | 0(0.0) | 0(0.0) | 4(57.1)*† | 7(70.0)*† | <0.05 |
| Uterine metastasis (Significant) | 0(0.0) | 2(11.1) | 6(85.7)*† | 7(70.0)*† | <0.05 |
| Bilateral tumor (Non-significant) | 1(14.3%) | 8(44.4%) | 5(71.4%) | 6(60.0%) | 0.4 |
| Total (42) | 7(16.7%) | 18(42.8%) | 7(16.7%) | 10(23.8) | – |

Data are presented as number (*n*) & percentage (%). Analysis was done using Chi square.*$P$<0.05 versus the negative staining group. †$P$<0.05 versus the weak staining group.

study, is due to different patterns of patients' selection during each study. Patients included in this study if their histopathological examination revealed EOCs (irrespective early or advanced EOCs), in Barbara Schmalfeldt *et al.* study, patients with LMP (low malignant potential) and advanced ovarian cancers were selected, while, Sakata *et al.* studied the expression of MMP-2 and -9 by cells isolated from the peritoneal fluid (cancer cells is usually isolated from the peritoneal fluid of advanced ovarian cancers) and patients with invasive ovarian cancers were selected in Aparna *et al.* study[6,21,22].

Forty-two (42) cases of EOCs of different grades and stages were studied by both histopathological examination and immunohistochemistry for MMP 2, to evaluate the relation between MMP-2 expression and the clinical and/or pathological parameters of the EOCs. There was a significant relation between expression of MMP 2 and histological grades of the studied EOCs. Barbara Schmalfeldt & colleagues concluded that MMP-2 and -9, were related to the growing malignant potential and invasiveness of ovarian tumours and Skata *et al.* concluded that the positive expression for MMP-2 was observed in high-grade (grade 2/3) than in low-grade ovarian malignancy (grade 1)[6,23].

There was a significant relation between expression of MMP 2 and surgical stages of the studied EOCs, this finding was in agreement with that found by Sakata *et al*, they found that the expression of MMP 2 and -9 together with low expression of TIMP-1 contribute to lymph node metastasis of ovarian tumours (stage III or IV) and expression of MMP-2 and TIMP-2 within the same tumour seems to play an important role in the progression of ovarian cancers[23]. This was supported by Davidson *et al.* and Barbara Schmalfeldt *et al.* they concluded that the relative level of any individual matrix metalloproteinase tends to increase with advanced stages of EOCs and they also, concluded that MMP 2 have an important role in tumours invasion, progression and metastasis[6,16].

The relation between expression of MMP-2 and presence of ascites in the studied EOCs was significant, denoting that the MMP 2 expression was high in patients with ascites. Sakata & colleagues, concluded that MMP 2 and -9 are frequently over expressed in ovarian cancer cells disseminated in the peritoneal cavity and they also, concluded that detection of cellular MMP-2 and -9 expression could be useful in distinguishing cancer cells from mesothelial cells in peritoneal fluid cytologic specimens from women with EOCs[21].

The relation between MMP 2 expression and presence of omental metastasis in the studied EOCs was highly significant. Wu *et al.* found a significant relationship between activated MMP-2 and invasiveness, metastasis and disease progression in EOCs and they concluded that activated MMP-2 is a potential marker of prognosis, because the positive percentage of active form of MMP-2 in stage III

and IV was significantly higher than that in stage I and II EOCs (81% versus 33%; respectively)[20].

Davidson *et al.* concluded that MMP 2 expression in metastatic lesions is more than that in primary lesions and Kikkawa & colleagues stated that MMP 2 cleaves matrix components and releases polypeptide fragments with new biological properties as well as releasing signalling components embedded within the matrix promotes cell migration[16,24].

The relation between MMP 2 expression and presence of uterine metastasis in the studied EOCs cases was significant, this finding correlates with the ability of metalloproteinases to degrade ECM helping in tumours invasion and metastasis, but the relation between MMP 2 expression and lymph node metastasis of the studied EOCs cases was insignificant, although, Sakata and colleagues concluded that the expression of MMP-9 was significantly higher in ovarian cancers with lymph node metastasis than in those without lymph node metastasis[23]. This was explained by the adhesion of ovarian cancers to type 1 collagen followed by secretion of serine and metalloproteinases as biochemical markers by which the intraperitoneal dissemination of ovarian carcinoma is mediated[25]. Ovarian cancer cells could use MMP 2 to detach from surface epithelium and migrate into the peritoneal cavity, they may also use MMP2 to invade through basement membrane into the ovarian stroma[20,21].

The above data suggests that the MMP-2 expression was associated with advanced, aggressive EOCs and there was direct relation between expression of MMP-2 and degree of invasiveness and metastasis of EOCs. Patients with EOCs should be counselled about the possible survival based on the staining intensity or MMP-2 expression. There was no significant correlation between MMP 2 expression and the clinical (age, bilaterality) or histological types of the studied EOCs. Also, Wu *et al.* concluded that the expression of MMP-2 proteins and MMP-2 mRNA were not significantly related to the clinical or pathological features of the EOCs[20].

This study was a retrospective study and further prospective studies are needed to estimate the relation between MMP2 expression and clinical outcome in patients with EOC including; the progression free survival (PFS) and the overall survival (OS). The above data suggests that drugs with antimetalloproteinase activity may lead to limitation of EOCs growth and metastasis, also, Barbara Schmalfeldt *et al*, concluded that inhibition of MMP-2 activation would be an interesting approach toward a biological therapy of ovarian cancer, because MMP-2 was never converted to its active form in benign ovarian tumours[6]. Batimastat is the first MMP inhibitor evaluated in cancer patients; Batimastat produces significant inhibition of metastasis and suppression of ascites formation in ovarian cancer models[26]. Marlimastat "MAR" also called (BB 2516) is a new cytostatic agent with antimetalloproteinase activity. Combination between Marimastat and cytotoxic agents as Carboplatin or 5 FU (Fluorouracil) in treating patients with EOCs, showed synergistic effect with no additional toxicity[27]. BAY 12 9566 is a novel nonpeptidic matrix metalloproteinase inhibitor (MMPI) that directly inhibits MMP 2, -3 and -9. Preclinical research showed that the BAY 12-9566 is acting by inhibition of angiogenesis and proliferation, leading to reduction in tumour growth and metastatic potential[28]. AG3340 is a nonpeptidic MMP inhibitor that inhibits MMP -2, - 3, -9, and -13, AG3340 can be safely combined with mitoxantrone/prednisone or carboplatin/paclitaxel and these combinations are currently being tested as first-line therapy for patients with hormone-refractory prostate cancer or non-small-cell lung carcinoma[25]. BMS-275291 is an orally bio-available MMP inhibitor, it has potent inhibitory activity against MMP-2 and -9[26]. The tetracycline derivatives inhibit not only the activity but also the production of MMPs and are thus being investigated for the treatment of disorders in which the MMP system becomes amplified. Col-3 (metastat) or the chemically modified tetracyclines (CMTs) inhibit the activity of MMP-2 & -9 in cancer cell lines[26]. Cisplatin alone or in combination with PI3K (phosphoinositide3-kinase) inhibitor LY294002, inhibit the ovarian cancer cell motility via down-regulation of activated MMP-2 and TIMP-2 expression[29].

In this study, the MMP-2 expression was associated with advanced, aggressive EOCs and there was direct relation between expression of MMP-2 and degree of invasiveness and metastasis of EOCs.

## Conflict of interest statement

No actual or potential conflict of interest in relation to this article exists.

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## References

[1] Ozols RF, Rubin SC, Thomas GM. Epithelial ovarian cancer. In: *Principles and practice of gynecologic oncology*. Philadelphia: Lippincott Williams & wilkins; 2000; 34: 983-1044.

[2] Brown MR, Blanchette JO, Kohn EC. Angiogenesis in ovarian cancer. *Clin Obstet Gynecol* 2000; 14: 901-918.

[3] Arnold JM, Cummings M, Purdie D, Chenevix Trench G. Reduced

expression of intercellular adhesion molecule 1 in ovarian adenocarcinomas. *Br J Cancer* 2001; **85**: 1351–1358.

[4] Hossam Kamel, Ibrahim Abdelazim, Sherif M Habib, Mahmoud A El Sbourbagy, Naglaa Samier Ahmed. Immunoexpression of matrix metalloproteinase–2 (MMP–2) in malignant ovarian epithelial tumours. *J Obstet Gynaecol Can* 2010; **32**(6): 580–586.

[5] Aumohle B, Sanches R, Didier E, Bignon YJ. Major oncogenes and tumor suppressor genes involved in epithelial ovarian cancer. *Int J Oncol* 2000; **16**(3): 567–576.

[6] Barbara Schmalfeldt, Dieter Prechtel, Kathrin Härting. Increased expression of matrix metalloproteinases MMP 2, MMP 9 and the urokinase plasminogen activator is associated with progression from benign to advanced ovarian cancer. *Clin cancer Res* 2001; **7**: 2396–2404.

[7] McCawleyu, Matrisian LM. Matrix metalloproteinases, multifunctional contributors to tumor progression. *Molecular Med Today* 2000; **6**: 149–156.

[8] Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases, biologic activity and clinical implications. *J Clin Oncol* 2000; **180**(5): 1135–1149.

[9] Kumaki F, Matsui K, Kawai T, Ozeki Y, Yu ZX, Ferrans VJ, et al. Expression of MMPs in invasive pulmonary adenocarcinoma with bronchoalveolar component and atypical adenomatous hyperplasia. *Am J Pathol* 2001; **159**: 2125–2135.

[10]Vergani V, Garofalo A, Bani MR, Borsotti P, Pelina Parker M, Drudis T, et al. Inhibition of matrix metalloproteinases by over–expression of tissue inhibitor of metalloproteinase–2 inhibits the growth of experimental hemangiomas. *Int J Cancer* 2001; **91**: 241–247.

[11]Mitra A, Chakrabarti J, Chattopadhyay N, Chatterjee A. Membrane associated MMP 2 in human cervical cancer. *J Environ Pathol Toxicol Oncol* 2003; **22**(2): 93–100.

[12]Skiles JW, Gonnella NC, Jeng AV. The design, structure, and therapeutic application of matrix metalloproteinase inhibitors. *Curr Med Chem* 2001; **8**(4): 425–474.

[13]Zhou CY, Yao JF, Chen XD. Expression of matrix metalloproteinase –2, –9 and their inhibitor TIMP 1, 2 in human squamous cell carcinoma of uterine cervix. *Ai Zheng* 2002; **21**: 735–739.

[14]Asha Nair S, Karunagaran D, Nair MB, Sudhakaran PR. Changes in matrix metalloproteinases and their endogenous inhibitors during tumor progression in the uterine cervix. *J Cancer Res Clin Oncol* 2003; **129**(2): 123–131.

[15]Hsu SM, Raine L, Fanger H. A comparative study of the peroxidase antiperoxidase method and an avidin biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J clin Pathol* 1981; **75**(5): 734–738.

[16]Davidson B, Goldberg I, Gotlieb WH, Kopolovic J, Ben–Baruch G, Nesland JM, et al. The prognostic value of metalloproteinases and angiogenic factors in ovarian carcinoma. *Mol Cell Endocrinol* 2002; **187**(1–2): 39–45.

[17]Imard N, Legeais JM, Renard G, Robert L. Effect of hyaluronan on MMP expression and activation. *Cell Biology Int* 2001; **25**(8): 735–739.

[18]Milagros Balbin, Antonio Fueyo, Vera Knauper, Jose M Lopez, Jesus Alvarez. Identification and enzymatic characterization of two diverging murin counterparts of human interstitial collagenase (MMP 1) expressed at sites of embryo implantation. *J Biol Chem* 2001; **276**(13): 10253–10262.

[19]M A Mohamed Naser, S B Ayyad, I K I El–Lamie, M Y Mikhail. Expression of matrix metalloproteinases in preinvasive and invasive carcinoma of the uterine cervix. *Eur J Gynaec Oncol* 2005; **XXVI**(2): 199–202.

[20]Wu X, Li H, Kang L, Li L, Wang W, Shan B. Activated matrix metalloproteinase 2, a potential marker of prognosis for epithelial ovarian cancer. *Gynecol Oncol* 2002; **84**: 126–134.

[21]Sakata K, Shigemasa K, Uebaba Y, Nagai N, Ohama K. Expression of MMP 2 and MMP 9 by cells isolated from peritoneal fluid of women with ovarian carcinoma. *Acta Cytol* 2002; **46**(4): 697–703.

[22]Aparna A Kamat, Mavis Fletcher, Lynn M Gruman, Peter Mueller, Adriana Lopez, Charles N Landen, et al. The clinical relevance of stromal matrix metalloproteinase expression in ovarian cancer. *Clin Cancer Res* 2006; **12**(6): 1707–1714.

[23]Sakata K, Shigemasa K, Nagai N, Ohama K. Expression of matrix metalloproteinases –2, –9, (MMP –2 & –9) and their inhibitors (TIMP–1, TIMP 2) in common epithelial tumors of the ovary. *Int J Oncol* 2000; **17**(4): 763–780.

[24]Kikkawa F, Tamakoshi K, Nawa A, Shibata K, Yamagata S, Yamagata T, et al. Positive correlation between inhibitors of matrix metalloproteinase –1 and matrix metalloproteinases in malignant ovarian tumors. *Cancer Lett* 1997; **120**: 109–115.

[25]Moser TL, Pizzo SV, Bafetti LM, Fishman DA, Stack MS. Evidence for preferential adhesion of ovarian epithelial carcinoma cells to type 1 collagen. *Int J Cancer* 1996; **67**(5): 695–701.

[26]Manuel Hidalgo, S Gail Eckhardt. Development of matrix metalloproteinase inhibitors in cancer therapy. *J Nat Cancer Inst* 2001; **93**(3): 178–193.

[27]O'Reilly S, Mani S, Ratain MJ, Brown K Elz, Johnson S, Vogelzang NJ, et al. Schedules of 5FU and the matrix metalloproteinase inhibitor Marimstat (MAR). Am Society of Clin Annual Meeting 1998; Abstract No 839.

[28]Rowinsky E, Hammond L, Aylesworth C, Humphrey R, Siu L, Smith L, et al. Prolonged administration of Bay 12 9566, an oral matrix metalloproteinase inhibitor. ASCO: American Society of Clinical Oncology Meeting 1998; Abstract No 836.

[29]Amer K Karam, Chintda Santiskulvong, Mirela Fekete, Carol Eng, Oliver Dorigo. Cisplatin and PI3Kinase Inhibition decrease invasion and migration of human ovarian carcinoma cells and regulate Matrix–Metalloproteinase expression. *Cytoskeleton* (Hoboken) 2010; **67**(8): 535–544.