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# Prognostic Value of Matrix Metalloproteinase-2 (MMP-2) Expression in Endometrial Endometrioid Adenocarcinoma

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**Abstract.** *Matrix metalloproteinase-2 (MMP-2), a member of the zinc-dependent metalloproteinase gene family, plays an important role in cancer invasion and metastasis. The current study aimed to evaluate whether the expression of MMP-2 is associated with survival in patients with endometrial endometrioid adenocarcinoma. The MMP-2 immunoreactive protein was evaluated from endometrioid adenocarcinoma of the endometrium in 112 patients treated at Oulu University Hospital, Finland. The median follow-up time was 88 months. The expression of MMP-2 was studied immunohistochemically in paraffin-embedded tissue samples from the primary tumours by using a specific monoclonal antibody to MMP-2. The MMP-2 protein was found in 80% of the primary tumours, including all histological grades. All grade 3 tumors were MMP-2-positive. At the end of the study period, 21 of the 22 (95%) patients presenting MMP-2-negative immunostaining were alive, whereas the corresponding figure for those with MMP-2-positive tumours was 78 out of 90 (87%). These data suggest that MMP-2 immunostaining negativity might be linked with a favourable prognosis in endometrial endometrioid adenocarcinoma.*

Endometrial carcinoma is one of the most common malignancies of the female genital tract in Western countries. Endometrioid adenocarcinoma accounts for 60-80% of the histological types of endometrial cancers, and the depth of myometrial invasion is one of the most important prognostic factors in this disease. It is generally considered a relatively low-risk malignancy because the majority of cases are diagnosed in the International Federation of Gynecology and Obstetrics (FIGO) stage I and have a favourable prognosis.

Tumour invasion and metastasis are the major causes of treatment failure or death among carcinoma patients. The role of matrix metalloproteinases (MMPs) in tumour invasion and metastasis, as well as in tumour angiogenesis, is important. Matrix metalloproteinase-2 (MMP-2, gelatinase A, type IV collagenase) belongs to a family of zinc-dependent metalloendoproteinases that degrade matrix proteins as well as type IV collagen and other extracellular macromolecules. MMP-2 expression has been linked to invasiveness and metastasis in several human neoplasias, such as ovarian (1, 2), breast and colon carcinoma (3) and melanoma (4, 5). In those carcinoma types, the expression of MMP-2 protein, or its mRNA, predicts a poor prognosis. The literature regarding the expression and prognostic role of MMP-2 in endometrial endometrioid adenocarcinoma is, however, rather scant.

The present study was designed to assess whether the expression of MMP-2 in endometrial endometrioid adenocarcinoma predicts the patient outcome and to compare MMP-2 immunostaining with conventional clinical and histopathological parameters, especially the depth of invasion.

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## Patients and Methods

The series consisted of 112 stage I – IV endometrial endometrioid adenocarcinoma patients treated in the Department of Obstetrics and Gynecology, Oulu University Hospital, Finland, between 1993 and 1997. Formalin-fixed, paraffin-embedded endometrial tissue samples from the primary tumours were taken from their files in the Department of Pathology.

The median age of the patients was 66 years (range 37-86). According to the FIGO criteria (6), there were 84 stage I, 12 stage II, 14 stage III and 2 stage IV cancers. Fifty-eight tumours were well- (grade 1), 43 moderately- (grade 2) and 11 poorly-differentiated (grade 3). The median follow-up time was 88 months (range 0-124).

Extrafascial hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy were the primary treatments in most cases. One patient received preoperative chemotherapy, and 24 patients received postoperative adjuvant chemotherapy (cyclophosphamide, cisplatin and epidoxorubicin). Nineteen patients had postoperative vaginal cuff brachytherapy and 40 patients postoperative external whole pelvic irradiation. Eleven patients were treated postoperatively with combined vaginal cuff brachytherapy and external whole pelvic irradiation.

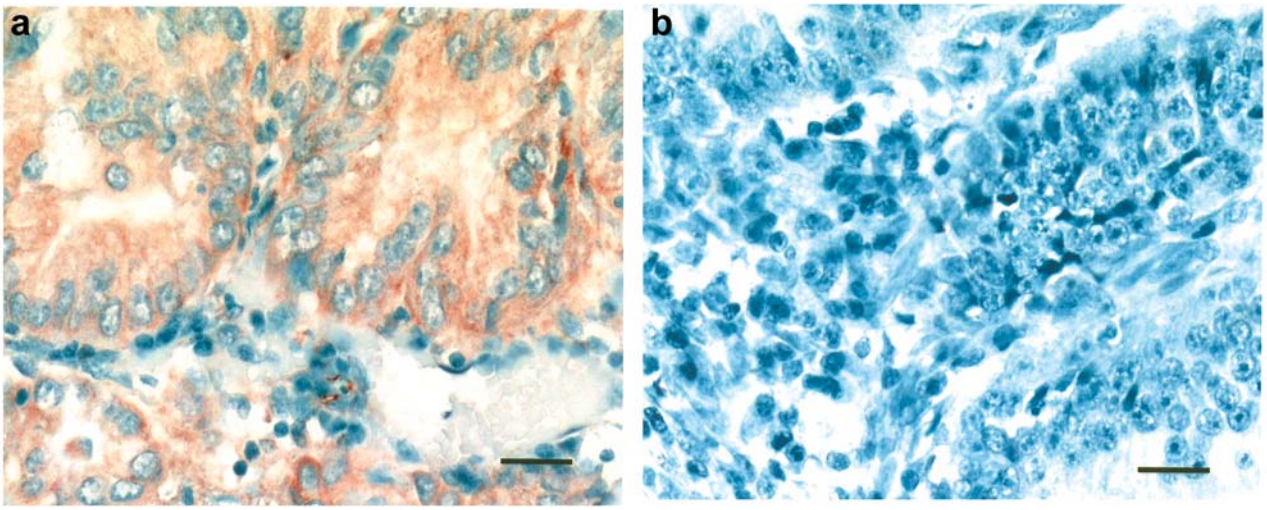


Figure 1. *Cytoplasmic immunostaining of matrix metalloproteinase-2 (MMP-2) in endometrioid adenocarcinoma. Immunostaining was performed as described in Patients and Methods by using a monoclonal antibody for MMP-2. A positive case (1a) and a negative case (1b).*

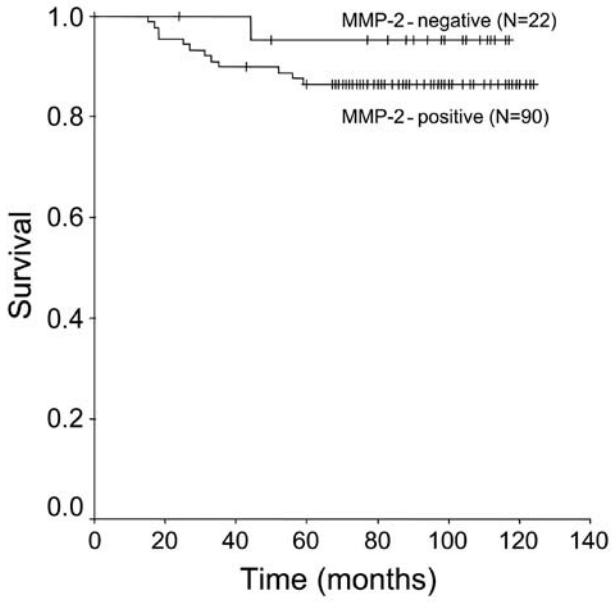


Figure 2. *Overall survival analysis (Kaplan-Meier) of endometrial endometrioid adenocarcinoma.*

*Immunohistochemistry.* The avidin-biotin-peroxidase method was used in the immunostainings. Paraffin sections 4-μm thick were incubated for 12 h at 37˚C, dewaxed in Histo-Clear (National Diagnostics, Atlanta, GA, USA) and hydrated. The specimens were treated with 0.4% pepsin (Sigma, St Louis, MO, USA) for 20 min at 37˚C. Incubation of the slides in 3% hydrogen peroxide in absolute methanol for 15 min blocked endogenous peroxidase activity, and non-specific binding was blocked with 10% goat serum for 15 min.

A mouse monoclonal antibody (CA-4001, Diabor Ltd., Oulu, Finland) against MMP-2 (7-9) was used as the primary antibody (1.5 μg/ml in 0.01 M phosphate buffer, 0.9% NaCl (pH 7.5) with 1% bovine serum albumin. The antibody recognises the aminoterminal end of the latent MMP-2 (inactive), both as a free enzyme and complexed with the tissue inhibitor of metalloproteinase-2. Specifity was confirmed by Western blot analysis (10).

The specimens were incubated for 20 h at room temperature in a humidity chamber, and immunohistological staining was continued using a Histostain-bulk kit (Zymed Laboratory, San Francisco, CA, USA), according to the manufacturer's instructions. Biotinylated anti-mouse immunoglobulin IgG served as a second antibody, and the peroxidase was introduced using a streptavidin conjugate. The slides were washed thoroughly with phosphate-buffered saline between all stages in the procedure. The antibody reaction was visualized using a fresh substrate solution from an aminoethyl carbazole substrate kit (AEC, Sigma). The sections were counterstained with hematoxylin, dehydrated and mounted in glycerol-vinyl-alcohol (GVA mount, Zymed). For negative controls, the primary antibody was replaced with mouse nonimmuno IgG. For positive controls, previously known MMP-2-positive specimens were used.

A section was considered negative or positive depending on the absence or presence of cytoplasmic staining of neoplastic cells. The case was considered positive when >1% of tumour cells showed positive staining. Two independent observers scored the MMP-2 immunostaining.

*Statistical analyses.* Statistical analysis was carried out by using the SPSS program. The relationships between the clinicopathological variables and MMP-2 immunostaining were assessed with Fisher's exact test (2-sided). Postoperative survival rates were assessed by the Kaplan-Meier method. The survival differences between the subgroups were compared by means of a log-rank test. Survival was defined as the time from the primary operation to the date of death or the last control visit. *P*-values <0.05 were considered statistically significant.

Table I. *MMP-2 immunostaining by prognostic indicators of 112 endometrial endometrioid adenocarcinoma patients*

| Prognostic factor | MMP-2 immunostaining* | | |
|---|---|---|---|
| | Total | Positive | Negative |
| Age (years) | | | |
| ≤66 | 55 | 42 | 13 |
| >66 | 57 | 48 | 9 |
| Stage | | | |
| I | 84 | 64 | 20 |
| II | 12 | 11 | 1 |
| III | 14 | 13 | 1 |
| IV | 2 | 2 | 0 |
| Grade | | | |
| 1 | 58 | 45 | 13 |
| 2 | 43 | 34 | 9 |
| 3 | 11 | 11 | 0 |
| Depth of invasion | | | |
| endometrium | 12 | 11 | 1 |
| ≤1/2 | 68 | 52 | 16 |
| >1/2 | 32 | 27 | 5 |
| Peritoneal cytology** | | | |
| classes 1-3 | 95 | 74 | 21 |
| classes 4-5 | 17 | 16 | 1 |

*$P$-value for MMP-2 immunostaining: NS
**Papanicolaou classes

## Results

Expression of MMP-2 immunoreactive protein was found in 80% of the primary tumours (Figure 1a), while 20% were negative (Figure 1b). In carcinoma cells, the immunoreactive protein localized to the cytoplasm (Figure 1 a). MMP-2 staining did not correlate with age, clinical stage, histological grade, depth of invasion, or peritoneal cytology (Table I). All grade 3 tumours were MMP-2-positive.

Thirteen of the 112 patients died of their endometrial endometrioid adenocarcinoma. Only one of the 22 (4.5%) patients presenting MMP-2-negative immunostaining died of the disease during the follow-up. The corresponding figure for the patients presenting MMP-2-positive immunostaining was 12 out of 90 (13%). The Kaplan-Meier analysis showed that the 5-year overall survival of the patients with endometroid adenoarcinoma and MMP-2-positive immunostaining was 87%, whereas 95% of the patients presenting MMP-2-negative primary tumour were alive at that time (Figure 2). The difference did not reach statistical significance.

Kaplan-Meier survival analysis showed the conventional prognostic indicators of endometrial endometrioid adenocarcinoma (FIGO stage, histological grade, depth of myometrial invasion and peritoneal cytology) to be significant prognostic indicators of the patients' clinical outcome. The conventional prognostic indicators were not associated with MMP-2 staining (data not shown).

## Discussion

MMPs are central effectors in endometrial physiology, and ovarian steroids and cytokines regulate their production (11, 12). MMPs also have a function in matrix degradation at menstruation (13). Even though MMPs have long been associated with malignancy, their role in endometrial cancer and other gynaecological malignancies has not been clearly established. Basement membrane-degrading enzymes (including MMP-2) have been given considerable attention for their roles in invasion and metastasis in malignant neoplasms (14). The depth of myometrial invasion is one of the most important prognostic factors in endometrial endometrioid adenocarcinoma, and proteolytic degradation of the endometrial extracellular matrix is assumed to be a prerequisite for tumour invasion into the myometrium.

Tumour types bear an important relationship with survival. Poor prognostic variants of endometrial carcinoma include papillary serous carcinoma, adenosquamous and clear-cell carcinoma. Due to the adverse prognosis and low prevalence of these tumours, they were excluded from the study. The advantage of our study was that the study group was homogeneous and all the patients were operated upon and treated in the same gynaecological oncological unit. Differences in adjuvant treatment did not influence the outcome, since no distinctions were seen between the subgroups of patients.

In line with our previous studies (4, 15-21) and the studies by others (10, 22-27), evidence of an immunoreactive protein positive for MMP-2 was seen in carcinoma cells, but not in fibroblast-like cells. The number of cases displaying positive immunostaining was within the range published previously in various malignant neoplasms (1, 17-21, 28-30). Although neoplastic cells are the main source of MMP-2, some tumours also induce MMP-2 activity in stromal fibroblasts (31). In colorectal (32) and breast carcinomas (33, 34), immunostaining for MMP-2 protein was predominantly localized to neoplastic cells, but the predominant labelling for MMP-2 mRNA was found in stromal fibroblast-like cells.

MMP-2 immunostaining did not correlate with the conventional prognosticators of endometrial endometrioid adenocarcinoma tested here. In the present study, all poorly-differentiated carcinomas were MMP-2-positive, but we found no statistically significant correlation between

histological grade and MMP-2 staining. There was, however, a trend to suggest that MMP-2 positivity among patients with moderately- or poorly-differentiated tumours could be associated with unfavourable prognosis. In a recent study by Di Nezza *et al.* (31), semi-quantitative analysis revealed increases in MMP-2 staining in tumour epithelial cells in the transition from histological grade 1 to grades 2 and 3.

The tissue inhibitors of matrix metalloproteinases (TIMPs) primarily control the proteolytic activity of MMPs. TIMPs are small proteins of 21-28 kDa that specifically block MMP activity by binding to the highly conserved zinc-binding site of active MMPs. TIMP-1, TIMP-2 and TIMP-3 inhibit the activity of most MMPs, including MMP-2 (35). Malignant tumours are often associated with an increase in TIMP production (36). The antibody test used also recognized the aminoterminal end of MMP-2 when it was in complex with TIMP-2. However, a simple balance in the local MMP-2/TIMP concentration cannot explain the lack of association between MMP-2 staining and tumour invasiveness.

New data show membrane-type matrix metalloproteinases (MT-MMPs) to be major modifiers of the pericellular environment and key regulators of tumour cell behaviour. MT-MMPs are a relatively new subfamily of membrane-anchored MMPs, which includes six members (37). MT-MMPs are highly expressed in almost all types of human cancer and, consistent with their high expression, they play an important role in promoting cell migration, invasion, experimental metastasis and angiogenesis. The MT-MMP family includes the major activators of pro-MMP-2, and in the case of MT1-MMP, this process involves the action of TIMP-2. The MT1-MMP/TIMP-2/pro-MMP-2 complex, referred to as the 'ternary complex', facilitates the first cleavage of the pro-MMP-2 prodomain by a neighbouring TIMP-2-free active MT1-MMP. Full activation of pro-MMP-2 is achieved by a second cleavage event, in which the intermediate MMP-2 species is autocatalytically processed to a fully active enzyme. This process only occurs at a low TIMP-2 concentration relative to MT1-MMP, to permit availability of enough inhibitor-free MT1-MMP to initiate pro-MMP2 activation. High levels of TIMP-2 inhibit activation by blocking all free MT1-MMP molecules (37). Thus, the relationship between MMPs, MT-MMPs and TIMPs in malignant tumour is a complex phenomenon. Since the MMP-2 immunostaining used here also detects pro-MMP-2, the potential role of biologically relevant molecules of MMP-2 to promote the degradation of extracellular matrix in endometrioid carcinoma remains to be evaluated.

Death was relatively infrequent in the present study, which means that, when comparisons are made between different prognostic factors, the numbers in each group are small. In Kaplan-Meier survival analysis, however, all conventional prognostic indicators discriminated between high-risk and low-risk patients.

In conclusion, the data suggest that MMP-2 may have some prognostic role in identifying the endometrial endometrioid adenocarcinoma patients with a favourable outcome from those with a poor prognosis. Although the difference in Kaplan-Meier analysis between the groups with positive and negative MMP-2 staining was not significant, the small number of events and the complex relationship between MMPs, MT-MMPs and TIMPs in malignant tumours mean that a potential benefit of the MMP-2 test may have been missed. This indicates a need for further studies with sufficient data and a long follow-up time to evaluate the prognostic value of MMP-2 among endometrial endometrioid adenocarcinoma patients.

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