Prognostic value of matrix metalloproteinase-9 (gelatinase-B) expression in epithelial ovarian tumors

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Summary

Purpose of investigation: To determine the expression of matrix metalloproteinase-9 (MMP-9) expression in malignant and borderline ovarian tumors and its correlation to prognosis.

Methods: Forty-five patients with primary epithelial ovarian tumors were enrolled in this retrospective study from 1988 to 2002. Only malignant (n = 30) and borderline (n = 15) ovarian tumors constituted the study group. All cases were surgically staged according to FIGO criteria. Patient characteristics and clinico-pathological findings were obtained from hospital records. Paraffin-embedded tissue blocks were treated with MMP-9 immunohistochemical stain. The percentage of the total number of tumors staining positively was categorised and awarded a score of 0 to 4: < 5% as 0, ≤ 6-25% as 1, 26-50% as 2, 51-75% as 3 and 76-100% as 4. The intensity of immunostaining was scored on a 3–point scale: 1, weak; 2, moderate and 3, intense. A weighed score for each tumor specimen was produced by multiplying the percentage score with the intensity score and was defined as the 'epithelial *MMP-9 score*'. Stromal staining was also assessed as weak, moderate and intense. Cases with final epithelial MMP-9 scores ≤ 6 and > 6 were then recategorised into two groups, accordingly. Based on degree of stromal staining, cases were recategorised into two final groups as mildly stained and intense or moderately stained. Tumor stages were regrouped as early (Stage I-II) and late (Stage III-IV), respectively.

Results: Mean ages of cases with malignant and borderline ovarian tumors were 57.2 ± 3.1 and 49.7 ± 2.1 years, respectively. Epithelial MMP-9 scores were higher in malignant tumors compared to borderline tumors (p = 0.014). However, with regard to stromal MMP-9 staining, no significant difference was observed among malignant and borderline tumors (p = 0.113). Among malignant ovarian tumors, epithelial MMP-9 scores did not differ between early versus late-staged and well versus poorly differentiated tumors. Median survival time of cases with epithelial MMP-9 scores ≤ 6 and > 6 were 24 months and 32 months, respectively (log-rank: 0.93, p = 0.335). Cases with weak stromal MMP-9 staining had a longer median survival (48 months) compared to cases with moderate or intense stromal MMP-9 staining (24 months, log-rank: 4.46, p = 0.03).

Conclusion: Epithelial MMP-9 expression generally appears in the malignant form of ovarian tumors compared to borderline tumors. MMP-9 expression in the stroma but not in the epithelium contributes to poor survival in ovarian cancers.

Key words: Matrix metalloproteinase-9; Epithelial ovarian cancer; Prognosis.

Introduction

Invasion and metastasis of tumor cells require the disruption of several collagen-endowed tissue barriers. The basement membrane that lines the vascular endothelium constitutes a continuous physical obstacle to tumor metastasis [1, 2]. One requisite for invasion and metastasis of ovarian cancer cells is the expression of proteolytic enzymes that degrade the components of the basement membrane and extracellular matrix [3].

Matrix metalloproteinases (MMPs) belong to a family of zinc-requiring endopeptidases [4]. MMP-9, or gelatinase B, has a great preference to degrade type IV collagen which is the major component of basement membranes. Current opinion holds that MMPs putatively participate in tumor invasion, metastasis and angiogenesis [5].

This study was performed to elucidate the degree of MMP-9 expression in borderline and invasive epithelial ovarian tumors and finally, to assess the prognostic impact of its expression on survival.

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Materials and Methods

Forty-five patients with primary epithelial ovarian tumors were enrolled in this nested case-control study from 1988 to 2002. Only malignant and borderline ovarian tumors constituted the study group. All cases were surgically staged according to FIGO criteria. Patient characteristics and clinico-pathological findings were obtained from hospital records.

Paraffin-embedded tissue blocks were treated with MMP-9 stain (Neomarkers, Fremont, CA, USA) with the avidin-biotin complex technique. Paraffinised blocks of 4 μ in thickness were then kept in a microwave oven at 70°C for one hour for deparaffinisation and rehydration. Following this procedure, they were treated twice with xylol solution for 15 minutes and thereafter, with acetone-alcohol solution (1:1) for five minutes and consequently, with 96% alcohol solution for five minutes and rewashed. They were then kept in a microwave oven for ten minutes and washed with TBS buffer for three minutes. Hydrogen peroxide and TBS buffer solutions were applied and the blocks were then treated with MMP-9 stain for 30 minutes. Having been washed with buffer solution for three minutes, the blocks were mixed with Link solution (Lab vision, Fremont, CA, USA) for ten minutes. After that, they were treated with streptavidin (Lab vision, Fremont, CA, USA) for ten minutes and consequently, with buffer solution for three minutes. AEC

chromogen (Lab vision, Fremont, CA, USA) was added to the blocks, left for seven minutes, and then tissue blocks were washed with distilled water. Hemotoxylen-eosin staining was performed and tissue blocks were then mounted onto slides. An immunohistochemical technique was performed based on the principle of Sinicrope *et al.* [6] and scored accordingly. Based on this staining procedure, immunohistochemical results were scored quantitatively by co-author pathologists (UO and SK). Results of immunoreactivity were quantifed via weighed scores decribed by Sinicrope *et al.* First, the percentage of the total number of tumors staining positively was categorised and awarded a score of 0 to 4: < 5%, 0; ≤ 6-25%, 1; 26-50%, 2; 51-75%, 3 and 76-100%, 4. The intensity of immunostaining was scored on a 3–point scale: 1, weak; 2, moderate and 3, intense. A weighed score for each tumor specimen was produced by multiplying the percentual score with the intensity score and was defined as the 'epithelial *MMP-9 score*'. Stromal staining was also assessed as poor, moderate and intense MMP-9 stain. Cases with a final epithelial MMP-9 score ≤ 6 and > 6 were then recategorised into two groups, accordingly. Based on degree of stromal staining, cases were recategorised into two final groups as mild and moderate or intense MMP-9 staining. Tumor stages were regrouped as early (Stage I-II) and late (Stage III-IV) stages , respectively.

Statistical analysis was performed using a statistical package software programme, SPSS (SPSS 11.0 Inc., Chicago IL, USA). Normal distribution of the data was tested by the one-sample Kolmogorov-Smirnov test. For skewed and non-skewed data distribution, median, mean rank and mean ± standard error of mean (SEM), respectively, were used. Non-parametric data were analyzed according to the Mann-Whitney U-test, and Fisher's exact chi-square test. The unpaired Student's *t*-test was applied for parametric data analysis. Kaplan Meier life curves were constructed for survival analysis (log-rank). Statistical significance was set at $p < 0.05$.

Results

Forty-five ovarian tumor cases were composed of 30 malignant tumors including 26 serous cystadenocarcinomas, three mucinous cystadenocarcinomas and one undifferentiated carcinoma, and 15 borderline tumors, including eight serous and seven mucinous epithelial ovarian tumors. Mean ages of cases with malignant and borderline ovarian tumors were 57.2 ± 3.1 and 49.7 ± 2.1 years, respectively. Among malignant ovarian tumors, early (Stage I-II) and late (Stage III-IV) stages, according to FIGO staging criteria, consisted of five and 25 cases, respectively. All tumor epithelia were stained and all stroma, except in one case, were stained with MMP-9. In serous borderline tumors, apical luminal surfaces, luminal secretions and cytoplasm were stained and were non-homogeneous and granular in appearance. In mucinous borderline tumors, staining was less dense compared to serous tumors and with a nuclear-cytoplasmic stain distribution especially in the basal part of the cells.

Mean ages of cases with epithelial MMP-9 scores ≤ 6 and > 6 were 58.2 ± 2.1 and 57.4 ± 1.9 years, respectively ($p = 0.55$). In malignant ovarian tumors, mean rank values of epithelial MMP-9 scores did not differ among early (n = 5, mean rank: 16.30) and late stages (n = 25, mean rank: 15.32, U score: 58, $z = 0.23$, $p = 0.8$). Among well or moderate and poorly differentiated tumors, epithelial MMP-9 scores did not differ either (mean ranks, 15.88 and 15.01, respectively, U score: 27, $z = -0.74$, $p = 0.4$). In tumors with moderate or intense stromal MMP-9 stain, epithelial MMP-9 scores had a higher mean rank value compared to those with mild stromal MMP-9 stain (mean ranks 11.60 and 18.47, respectively, U score: 60, $z = -2.20$, $p = 0.02$) (Table 1).

Epithelial MMP-9 expression is shown in Table 2. As clearly outlined in Table 2, malignant tumors had higher epithelial mean rank scores compared to their borderline counterparts (OR: 4.25; 95% CI: 1.12-16.0, $p = 0.014$). This statistical difference was also apparent between the two groups in mild stromal MMP-9 stained cases (U score: 21, $z = -2.93$, $p = 0.003$) (Table 3). However, among moderate or intense stromal MMP-9 stained cases, malignant and borderline tumors did not differ in regard to epithelial MMP-9 score (U score: 18, $z = -1.49$, $p = 0.13$).

Median survival of cases with epithelial MMP-9 scores ≤ 6 and > 6 were 24 months and 32 months, respectively

Table 1. — *Mean rank value distribution of epithelial MMP-9 scores among different stages, degree of tumor differentiation and degree of stromal MMP-9 staining (* Mann-Whitney U–test).*

Variables	n	mean rank	p*
Stages			
early (Stage I-II)	5	16.30	0.816
late (Stage III-IV)	25	15.35	
Degree of tumor differentiation			
well or moderate	17	15.88	0.458
poor	13	15.01	
Degree of stromal MMP-9 stain			
mild	13	11.60	0.027
moderate or intense	17	18.47	

Table 2. — *Epithelial MMP-9 score and degree of stromal MMP-9 staining among malignant and borderline tumors (Fisher's exact χ^2 test).*

	Invasive	Borderline	p
Epithelial MMP-9 score			
≤ 6	13 (50)	13 (50)	0.01
>6	17 (89.5)	2 (10.5)	
Degree of stromal MMP-9 stain			
mildly	13 (54.2)	11 (45.8)	0.11
moderate or intense	17 (81)	4 (19)	

(parentheses are percentages).

Table 3. — *Epithelial MMP-9 score distribution among malignant and borderline tumors based on degree of stromal MMP-9 staining (*statistically significant).*

	n	Epithelial MMP-9 score (mean) rank	p
Mild stromal MMP-9 stain			
- malignant	13	1.63	0.003*
- borderline	11	7.91	
Moderate or intense stromal MMP-9 stain			
- malignant	17	11.94	0.136
- borderline	4	7.00	

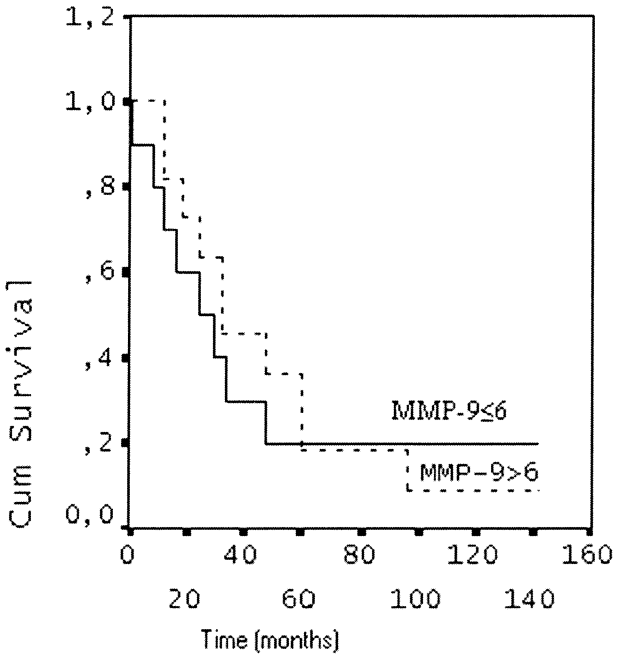

Figure 1. — Kaplan-Meier survival curves among malignant tumors with epithelial MMP-9 score ≤ 6 (n = 13) and > 6 (n = 17). (Log-rank: 0.93, p = 0.335).

(log-rank: 0.93, p = 0.335) (Figure 1). However, with regard to degree of stromal MMP-9 staining, cases with mild MMP-9 staining had a longer median survival (48 months) compared to moderate or intensely stained stromal cases (24 months, log-rank: 4.46, p = 0.03), (Figure 2).

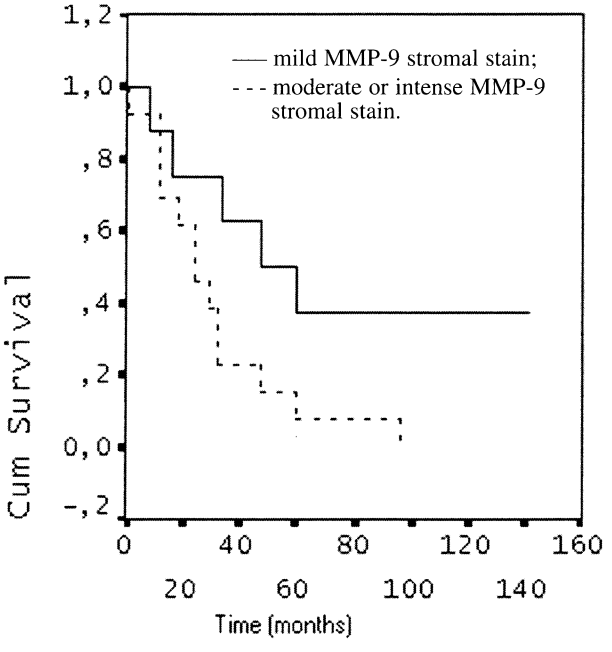

Figure 2. — Kaplan-Meier survival curves among malignant tumors with regard to degree of stromal MMP-9 staining (mild n = 17, moderate or intense n = 13, log-rank: 4.46, p = 0.034).

Discussion

The local growth of solid tumors as well as distant metastasis is dependent on a controlled degradation of components of the extracellular matrix [7, 8]. Malignant cells produce a wide spectrum of matrix–degrading proteinases as latent precursors or *zymogen* forms and then are activated in the extracellular matrix [9]. Of special importance are the type IV collagen gelatinases gelatinase A or MMP-2 (72-kDa type IV collagenase) and gelatinase B or MMP-9 (92-kDa type IV collagenase), because of their preference to degrade type IV collagen which is the major component of basement membranes. This degradation is essential in tumor invasion [10]. Recent studies have addressed the value of MMP expression in tumor progression by facilitating the angiogenetic process [11-13].

In this series, malignant ovarian tumors highly expressed epithelial MMP-9 expression. In regard to the stromal part, although borderline tumors showed a more mild MMP-9 staining pattern, there was no difference between the malignant and borderline group based on the intensity of MMP-9 stromal staining. This finding may be due to the small number of cases limiting the detection of any statistically important differences.

In this study, early and late-staged ovarian cancers did not differ in epithelial MMP-9 score expression. In the literature, especially in advanced epithelial ovarian tumors, epithelial pro-MMP-9 expression or active MMP-9 expression have been suggested to predict survival and tumor progression [14, 15]. We failed to analyze MMP-9 as a pro- or active form so as to reach the above conclusion. Hence, in our study total MMP-9 expression was assessed. Nevertheless, another important aspect of this study appears to be the the fact that epithelial, but not stromal MMP-9 expression did differ among malignant and borderline ovarian tumors, although the number of cases was small. In our study, epithelial MMP-9 expression did not differ through differrent tumor stages and grade of the primary tumor, as was also observed by Westerlund *et al.* [13].

Davidson *et al.* [16], through their nested case-control study on paraffin-embedded blocks from 70 primary and 34 metastatic ovarian cancers, found that epithelial MMP-9 expression together with a stromal tissue inhibitor of MMP-2 (TIMMP-2) retained their predictive value for survival. In our series, although the degree of stromal MMP-9 expression did not differ between malignant and borderline tumors, stromal MMP-9 expression in malignant tumors was correlated with survival (Figure 2). Sahakat *et al.* [17] demonstrated that overexpression of epithelial MMP-2, MMP-9 and down-regulation of TIMMP-2 contributed to enhanced ovarian tumor growth.

Proteolysis and matrix degradation mediated by metalloproteinase can also be enhanced by various mediators secreted by the microenvironment of the tumor, more specifically, surrounding fibroblasts, some examples of which are trypsin, transforming growth factor–beta or tissue inhibitors of MMPs, interleukin-I, and growth factors such as platelet-derived growth factor, epidermal

growth factor and the integrin and E-cadherin expressions. These mediators have recently been named as *extracellular matrix-metalloproteinase inducers or* 'EMMPRIN's' [18-21]. Therefore, the degradation of the extracellular matrix occurs not only by proteases synthesized by the malignant cells themselves, but also the surrounding fibroblasts are induced to produce proteases by the action of several mediators, EMMPRIN [22]. Another recent important finding in regard to matrix metalloproteinase expresssion is the fact that MMP-expressed tumor cells are less sensitive to apoptosis and are less easily removed from immune surveillance, which further contributes to the invasiveness [23]. Therefore, matrix metalloproteinases facilitate the selection of apoptosis-resistant tumor subpopulations.

As a conclusion, this study has shown that stromal, but not the epithelial MMP-9 expression has a predictive value for survival. Hopefully this study together with future studies may clarify the exact pathophysiogic interrelations of matrix metalloproteinases with the surrounding tissues and EMMPRIN system and may lead to the development of novel and better therapeutic strategies to cope with the progression and metastatic capacity of ovarian tumors. Although matrix metalloproteinases have been heralded as a target for cancer therapy, results of recent in vivo studies using synthetic MMP inhibitors have been disappointing [24]. However, by better understanding the exact pathophysiology and mechanisms of tumor invasion and tumor-host interactions, we believe that new synthetic derivatives of tissue inhibitors of MMPs in the future will have a therapeutic value.

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