Role of Immunohistochemical Overexpression of Matrix Metalloproteinases MMP-2 and MMP-11 in the Prognosis of Death by Ovarian Cancer

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Abstract

Matrix metalloproteinases (MMPs) are enzymes thought to be involved in tumor invasion. We hypothesized that MMP-2 and MMP-11 overexpression was associated with the aggressiveness of ovarian carcinoma. This study was performed on samples from 100 patients with stage III ovarian carcinomas treated surgically between 1990 and 2000. Immunohistochemical staining was performed on ovarian tumors and peritoneal implants using monoclonal antibodies. Overexpression was defined as more than 10% of cells expressing the marker. Multivariate analyses showed that only MMP-2 overexpression by cancer cells in peritoneal implants was associated with a significant risk of death by disease (hazard ratio, 2.65; 95% confidence interval, 1.41-4.97; P = .003). MMP-11 overexpression was not predictive of survival. These results suggest that MMP-2 overexpression by cancer cells in peritoneal implants and not in the primary ovarian cancer is predictive of ovarian cancer prognosis and more likely reflects the presence of particularly aggressive clones of cancer cells.

Ovarian carcinoma is the leading cause of death of all gynecologic tumors. It is usually diagnosed at a late stage because of its anatomic location and its relative asymptomatic occurrence.1 Despite highly efficient first-line chemotherapy, no more than 10% to 30% of patients with advanced disease experience long-term survival.2 Until now, few markers were found to predict tumor response to chemotherapy and prognosis in ovarian cancer.3,5

Matrix metalloproteinases (MMPs) belong to a family of more than 20 zinc-dependent endopeptidases capable of degrading extracellular matrix and basement membrane components.6 They are thought to be involved in tumor growth, invasion, and metastasis.7,9 In most malignant tumors, stromal cells are the primary source of MMPs.10 Of them, MMP-2 (gelatinase A), a mediator of type IV collagen degradation, and MMP-11, also known as stromelysin-3, are involved in tumor remodeling.11 MMP-2 is secreted as a pro-form and activated extracellularly by other MMPs and membrane-type MMPs.12 MMP-11 and membrane-type MMPs are activated intracellularly before secretion.12 MMPs activity is inhibited by tissue inhibitors of metalloproteinases (TIMPs). TIMPs are also paradoxically necessary for the formation of the MMP-2–membrane-type 1 MMP complex, which activates MMP-2.13 MMP-2 and MMP-11 overexpression was found to confer a poor prognosis in certain carcinomas, such as breast14,15 and prostate cancer,16 but their prognostic significance in ovarian cancer is unclear. This prompted us to test the hypothesis that MMP-2 and MMP-11 overexpression by the primary ovarian cancer and/or peritoneal implants is associated with the aggressiveness of ovarian carcinoma.
Materials and Methods

Patients whose samples were included in this study were treated surgically between January 1990 and December 2000. The inclusion criteria were as follows: (1) International Federation of Gynecology and Obstetrics stage III serous papillary carcinoma; (2) receipt of adjuvant chemotherapy with known dates for treatment start and end. The paraffin blocks from the ovarian carcinoma were available in all cases, and blocks from the peritoneal implants were available in most cases.

A representative block from the ovarian cancer and peritoneal implants was selected for analyses. Immunohistochemical staining was performed using the avidin-biotin complex method and monoclonal antibodies on ovarian tumors and peritoneal implants. Briefly, 1 representative 5-µm tissue section was cut from paraffin-embedded tumors. Sections were deparaffinized and rehydrated in graded alcohols and then incubated with normal goat serum for 20 minutes. Sections were incubated at room temperature for 1 hour with murine monoclonal antibodies against MMP-2 (MMP-2 VC2, dilution 1/50; Neomarkers, Fremont, CA) and MMP-11 (clone 5ST-4A9, gift from the late Paul Basset, PhD, Strasbourg, France). Afterward, sections were incubated with a biotinylated secondary antibody (DAKO, Carpinteria, CA) and then exposed to a streptavidin complex (DAKO). Complete reaction was revealed with 3-3′ dianobenzidine, and slides were then counterstained with hematoxylin. Breast cancer specimens known for their MMP-2 (cancer cells and stromal cells) and MMP-11 (stromal cells) overexpression were used as positive control samples. Negative control samples consisted of tissue sections incubated with phosphate-buffered saline (0.16 mol/L, pH ~7.5) instead of the primary antibody.

Each case was evaluated by 2 observers without knowledge of clinical information (I.H. and M.B.) and finally reviewed by another observer (M.P.) who made the final interpretation in divergent cases. The percentage of cells stained was evaluated semiquantitatively in cancer and stromal cells for both antibodies. As described before, protease overexpression was defined as the presence of more than 10% of cells expressing the marker. Tumor grade was evaluated according to the criteria of Silverberg. Statistical Analyses

The relationship between MMP-11 and MMP-2 overexpression and patient age, tumor grade, tumor size, and the number of chemotherapy cycles received was evaluated by using the Student t test and the χ2 test. Kaplan-Meier survival curves were used to compare overall survival between patients with different levels of MMP-11 and MMP-2 expression in tumors. Cox regression models were performed to estimate the hazard ratios (HRs) for death according to MMP expression. HRs and 95% confidence intervals (CIs) were computed based on univariate and multivariate Cox proportional hazard models, controlling for potential confounding factors.

Results

There were 100 cases eligible for the study. Patient age ranged from 28 to 88 years (median, 64 years). All patients had stage III ovarian serous carcinoma and were treated by surgery and chemotherapy. Of the 100 tumors, 41 had a histologic grade of 1 or 2 and 59 were grade 3 [Table 1]. The median follow-up until death was 1.58 years.

Eight cases of ovarian tumors were excluded because the blocks were not available or MMP-2 immunostaining was not interpretable. Of the 92 cases left, MMP-2 overexpression (>10% cells staining) by cancer cells and stromal cells was found in 41 (45%) [Image 1] and 33 (36%) [Image 2], respectively. MMP-11 immunostaining was available in 95 ovarian tumors, and overexpression by cancer cells and stromal cells was detected in 6 (6%) and 31 (33%) [Image 3], respectively.

In peritoneal implants, MMP-2 and MMP-11 immunostaining was available in 84 cases. MMP-2 overexpression by cancer cells and stromal cells was the same, 38 (45%), in the peritoneal implants. MMP-11 overexpression by cancer cells and stromal cells was detected in 3 (4%) and 50 (60%), respectively. Immunostaining for MMP-2 and MMP-11 was always cytoplasmic in cancer and stromal cells.

Patients with MMP-2 overexpression (>10%) by cancer cells in peritoneal implants were significantly younger (mean age, 58.1 years; median age, 59.5 years; 95% CI, 53.74-62.42; P = .02) than patients with low or no expression (0%-10%) (mean age, 63.9 years; median, 64.5 years; 95% CI, 61.19-66.55).

Table 1 shows the results of univariate and multivariate analyses for predicting time to death for selected clinical factors and MMP-2 and MMP-11 overexpression. Multivariate analyses were adjusted for potential confounding factors such as age, tumor grade, and number of chemotherapy cycles. Age was significantly associated with MMP-2 overexpression by peritoneal implants, grade showed a trend for predicting time to death in univariate analysis (P = .08), and the number of chemotherapy cycles was significantly associated with time to death (P = .004). Results for MMP-11 overexpression by cancer cells were not analyzed because it was found in only 6 ovarian tumors and 3 peritoneal implants.

Univariate analyses showed that only MMP-2 overexpression by cancer cells in peritoneal implants was associated with a significantly increased risk of death of disease (HR, 2.51; 95% CI, 1.37-4.61; P = .003) [Table 1]. Overexpression of MMP-11 and MMP-2 by stromal cells of ovarian tumors or...
peritoneal implants and overexpression of MMP-2 by cancer cells of ovarian tumors were not associated with survival. In multivariate analyses, MMP-2 overexpression by cancer cells in peritoneal implants was an independent prognostic factor (HR, 2.65; 95% CI, 1.41-4.97; P = .003). Although a majority of cases were positive or negative for MMP-2 in cancer cells of the ovarian tumor and the peritoneal implant (49/79 [62%]), 15 (19%) had MMP-2 overexpression in peritoneal

**Table 1**

Cox Regression Univariate and Multivariate Analysis to Predict Time to Death Due to Ovarian Cancer

| Factor                             | Death/No. of Cases (%) | Univariate Analyses | Multivariate Analyses * |  
|------------------------------------|------------------------|---------------------|-------------------------|---------- 
|                                    |                        | HR                  | 95% CI                  | P        | HR          | 95% CI                  | P        |
| Age (y) <60 60                      | 21/37 (57)             | 1.20                | 0.69-2.08               | .52      | 1.23‡       | 0.61-2.51               | .57      |
| ≥80                                | 33/63 (52)             |                     |                         |          |             |                         |          |
| Histologic grade                   |                        |                     |                         |          |             |                         |          |
| 1 or 2                             | 19/41 (46)             | 1.64                | 0.94-2.89               | .08      | 1.40‡       | 0.72-2.72               | .31      |
| 3                                  | 34/59 (58)             |                     |                         |          |             |                         |          |
| Chemotherapy (cycles) ≥6 ≤5        | 44/86 (51)             | 4.65                | 2.18-9.91               | <.0001   | 4.18‡       | 1.60-10.89              | .004     |
| MMP-2 overexpression in Ovarian stromal cells 0-10 11-100 | 35/59 (59)             | 0.69                | 0.38-1.25               | .22      | 0.60        | 0.32-1.13               | .11      |
| 11-100                             | 16/33 (48)             |                     |                         |          |             |                         |          |
| MMP-2 overexpression in Ovarian cancer cells 0-10 11-100 | 28/61 (55)             | 0.90                | 0.52-1.57               | .72      | 0.80        | 0.45-1.41               | .45      |
| Peritoneal stromal cells 0-10 11-100 | 23/41 (56)             | 0.66                | 0.36-1.21               | .18      | 0.68        | 0.36-1.32               | .22      |
| Peritoneal cancer cells 0-10 11-100 | 27/46 (59)             | 2.51                | 1.37-4.61               | .003     | 2.65        | 1.41-4.97               | .003     |
| MMP-11 overexpression in Ovarian stromal cells 0-10 11-100 | 17/46 (37)             | 1.11                | 0.63-1.95               | .71      | 1.28        | 0.68-2.43               | .45      |
| 11-100                             | 28/38 (74)             |                     |                         |          |             |                         |          |
| MMP-11 overexpression in Ovarian cancer cells 0-10 11-100 | 32/64 (50)             | 1.15                | 0.62-2.14               | .67      | 1.15        | 0.58-2.30               | .69      |
| 11-100                             | 20/31 (65)             |                     |                         |          |             |                         |          |
| MMP-11 overexpression in Peritoneal stromal cells 0-10 11-100 | 15/54 (44)             | 1.15                | 0.62-2.14               | .67      | 1.15        | 0.58-2.30               | .69      |
| 11-100                             | 31/50 (62)             |                     |                         |          |             |                         |          |
| CI, confidence interval; HR, hazard ratio; MMP, matrix metalloproteinase. * Adjusting for age, tumor grade, and number of chemotherapy cycles. ‡ Based on the model that includes MMP-2 expression by peritoneal epithelial cells.

**Image 1** Matrix metalloproteinase (MMP-2) overexpression by cancer cells. (MMP-2 VC2 [Neomarkers, Fremont, CA], ×100).

**Image 2** Matrix metalloproteinase (MMP-2) overexpression by stromal cells. (MMP-2 VC2 [Neomarkers, Fremont, CA], ×100).
implants only and 15 (19%) had overexpression in the ovarian tumor only.

Figure 1 shows the Kaplan-Meier survival curves for time to death according to MMP-2 expression by cancer cells in peritoneal implants. The curves show that MMP-2 overexpression is significantly associated with an increased risk of death.

Discussion

The role of MMP-2 in ovarian carcinoma is controversial. Significantly higher rates of MMP-2 overexpression were found in ovarian carcinomas compared with benign and borderline tumors, suggesting that MMPs may have a role in carcinogenesis. However, although certain investigators reported an association between MMP-2 overexpression and prognosis in ovarian cancer, others did not reach such conclusions. Furthermore, of those who found an association, in one study, MMP-2 did not retain its predictive value in multivariate analysis, and, in the other, the activated form of MMP-2 by Western blot only, and not MMP-2 expression by immunohistochemical analysis, was predictive of disease progression. Furthermore, it is worth noting that the analysis was performed on ovarian and metastatic tumor tissue in one study.

Figure 1 Kaplan-Meier survival curves for time to death according to matrix metalloproteinase-2 expression by epithelial cells in peritoneal implants. For 0-10 cells, median survival was 3.6 years and for 11-100 cells, 1.6 years. P = .0021; log-rank test.
Our results show that MMP-2 overexpression by cancer tumor cells in the peritoneal implants, not in the primary ovarian tumor, is a significant risk factor for poor prognosis. This finding suggests that peritoneal implants with MMP-2 overexpression originate from an aggressive ovarian tumor clone, which might explain the poorer outcome for those patients. This is supported by data from the literature: more aggressive clones were identified among ascitic fluid cells. However, we cannot explain why MMP-2 overexpression in peritoneal implants and not ovarian tumors is associated with tumor aggressiveness. This finding may suggest the presence of additional genetic changes or a different balance between aggressiveness and TIMPs in peritoneal implants. Davidson et al found increased MMP-2 and reduced TIMP-2 levels in ovarian carcinoma cells in effusions compared with the primary tumor. Additional studies are needed to investigate this hypothesis.

Our study also showed that patients with MMP-2 overexpression are significantly younger than those with low MMP-2 expression by peritoneal cancer cells, suggesting that younger patients may have more aggressive tumors. However, there is no clear evidence in the literature that age is a factor for a poor prognosis in ovarian cancer.

Few studies explored the prognostic role of MMP-11 expression in ovarian cancer. In a study, MMP-11 overexpression was more prevalent in carcinomas than in borderline ovarian tumors, but no significant prognostic impact was found. We also failed to relate MMP-11 overexpression in ovarian tumors and peritoneal implants with prognosis. MMP-11 was identified by differential analysis of metastatic breast cancer and benign breast tissue and was expressed by reactive stromal cells of malignant tumors only. The role of MMP-11 in oncology is unclear, but it has been established that MMP-11 overexpression favors tumor implantation and has little impact on tumor progression. Indeed, MMP-11 was found to be associated with prognosis in certain tumor types by univariate analyses, but failed to remain significant in multivariate analyses.

The fact that in our study MMP-2 overexpression in implants was associated with a poor prognosis raises the possibility that synthetic MMP inhibitors (MMPIs) may be effective as antineoplastic agents in cases with MMP-2 overexpression. Studies are evaluating such drugs in combination with other anticancer agents in ovarian cancer and other neoplasms. Despite clear evidence of the efficiency of MMPIs in experimental settings, preliminary data from a phase 3 study in patients with advanced ovarian cancer show no impact of such a drug on progression-free and overall survival. The experience with other molecules for which targets have been developed shows that the marker level may influence therapy response. For example, recent literature shows that HER-2 protein overexpression and gene amplification are strongly associated with poor prognosis but are also strong predictors of responsiveness to trastuzumab. However, no immunohistochemical MMP analyses were performed on primary tumors or peritoneal implants in studies involving MMPIs. Therefore, our study suggests that in cases with MMP-2 overexpression in peritoneal implants, in addition to a poor prognosis, a better response to MMPIs might be anticipated.

Our results show that MMP-2 overexpression by cancer cells in implants, but not in the primary ovarian tumor, is an independent prognostic factor in International Federation of Gynecology and Obstetrics stage III serous ovarian carcinoma. Our data suggest that MMP-2 analysis on peritoneal implants in ovarian cancers may help select patients with tumors that would more likely respond to MMPIs.

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