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Prognostic Relevance of MMP-2 (72-kD Collagenase IV) in Gastric Cancer

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Abstract
The association of MMP-2 (matrix metalloproteinase 2, 72-kD collagenase IV) with invasive and metastatic capacity of tumor cells has implicated a potential role in the prognosis for cancer patients. However, no larger study has been done to prove this hypothesis. The present study was therefore designed to investigate the prognostic impact of MMP-2 in a prospective series of 203 gastric cancer patients. MMP-2 expression was measured immunohistochemically and scored semiquantitatively (score 0–3) in carcinoma cells, and results were correlated with clinicopathological tumor parameters and parameters of the urokinase-type plasminogen activator (uPA) system. Survival analyses were done using the Kaplan-Meier method (log-rank statistics) and multivariate Cox analysis. Significant correlations were found for MMP-2 and Laurén's classification, M stage and proteases/inhibitors of the uPA system in the primary tumor. Kaplan-Meier analysis revealed an association of increasing MMP-2 expression with worse prognosis. This was especially seen in patients with a parallel high expression of uPA receptor. However, differences in survival probabilities between low and high MMP-2 levels were not significant. In a separate analysis of diffuse-type cancers, MMP-2 was significantly associated with disease-free ($p = 0.0056$) and overall survival ($p = 0.0426$). Multivariately, MMP-2 was not an independent parameter. Our results demonstrate that there is an association of immunohistochemical detection of MMP-2 with prognosis of cancer patients. For diffuse gastric cancers, it is a significant prognostic parameter, however, not of independent impact. The study further suggests that consideration of interrelated tumor-associated proteases like uPA receptor in combination with MMP-2 may improve its prognostic power.

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Introduction

Tumor cell invasion and metastasis is biologically coherent with proteolytic destruction of surrounding matrix, which evidently seems to be achieved by a series of tumor-associated serine, aspartic, cysteine, threonine and metalloproteinases [1–3]. One decisive step in metastasis is lysis of collagen IV [4], a basic element of constitutive basement membranes of blood vessels, to enable invasion of tumor cells into the systemic circulation. MMP-2 (72-kD collagenase type IV), a member of the zink-dependent matrix metalloproteinases (MMPs), is an enzyme which is able to degrade collagen IV as well as collagen type V, gelatins, laminin and fibronectin [5] and thus is more and more discussed as being an essential tool of metastasizing tumor cells. As other MMPs, MMP-2 is secreted as an inactive proenzyme and activated by N-terminal proteolytic cleavage [5, 6]. As potential activators, other tumor-associated proteases such as urokinase-type plasminogen activator (uPA) [7, 8] and a recently identified membrane-associated metalloproteinase (MT-MMP) are discussed [9]; however, mechanisms and involved factors of activation remain to be elucidated. In addition, the activity of MMP-2 seems to be modulated by tissue inhibitors of metalloproteinases (TIMPs) and especially TIMP-2 [5, 10, 11]. Besides an inhibiting effect, complexing with TIMP-2 at C-terminal pro-MMP-2 diminishes the proteolytic activity of MMP-2 but prolongs its half-life and localizes it to a specific cellular binding site [11]. This is comparable to the interaction of uPA and plasminogen activator inhibitor type 1 (PAI-1) at the uPA receptor (uPA-R) which has already been shown for the uPA system [12], where a similar complexing of uPA, PAI-1 and uPA-R leads to a concentration of the proteolytic effect and dynamic recycling of the protagonists [12].

In human cancers, expression of MMP-2 has been localized to stromal cells but also tumor cells on the DNA, mRNA and protein level [13–19]. In breast cancer, activity of MMP-2 was significantly associated with malignancy [20], and elevated MMP-2/TIMP-2 levels measured by RT-PCR correlated with lymph node involvement [19]. For gastric cancer, MMP-2 was even postulated as a characteristic of the malignant phenotype in contrast to MMP-9, the 92-kD form of collagenase IV [21], which can be activated by MMP-2 [22]. MMP-2 has been detected preferentially on advanced gastric carcinoma cells by immunohistochemistry and was correlated with vascular invasion [15]. Higher percentages of MMP-2-positive tumor cells studied immunohistochemically had been found in patients who died from primary gastric cancer [23]. Thus, an association of MMP-2 with poor prognosis of cancer patients has been suggested [17, 23]. However, a large clinical study on a potential prognostic impact of MMP-2 in human cancer to test this hypothesis has not been performed to date.

Therefore, the present study was designed to examine the role of MMP-2 evaluation in the prognostic analysis of cancer. In a large prospective series of 203 patients with gastric cancer, MMP-2 was investigated immunohistochemically in tumor cells. As potential interactions or activation mechanisms have been speculated for the uPA system [7, 8], respectively, coexpression of parameters of this system (uPA, uPA-R, PAI-1 and PAI-2) [24] was also considered in our analysis.

Patients and Methods

Patients

A consecutive prospective series of 247 patients was operated on for primary gastric cancer between February 1989 and October 1991; 203 of them were tumor resected. The mean age was 63.8 years (SD 10.4, range 22–87), the male/female ratio was 1.14 (108/95). Of 203 patients, 70% (143) could be resected curatively with radical lymph node dissection including compartments I and II. Of the remaining 30% with palliative resection, 22 revealed microscopic, 38 macroscopic tumor residues.

TNM classification of resected tumors revealed 14.8% UICC stage Ia, 12.8% stage Ib, 14.3% stage II, 19.2% stage IIIa, 13.8% stage IIIb and 25.1% stage IV. According to Laurén's classification, 108 tumors (53.2%) were intestinal, 86 (42.4%) diffuse and 9 (4.4%) mixed-type carcinomas.

Twelve patients were given intraoperative radiation therapy (all curatively resected, 28 Gy). Neoadjuvant chemotherapy was applied to 3, adjuvant chemotherapy to 11 patients. Five patients received chemotherapy after noncurative resection.

Follow-up was done prospectively 6, 12, 18, 24 months after operation and at 1-year intervals thereafter. It consisted of physical examination, abdominal ultrasound, gastroscopy, chest X-ray, hematology and blood chemistry and screening for the tumor markers CEA, Ca 19–9 and Ca 72–4. In case of suspicion of recurrence, confirmation by biopsy or second-look operation was recommended. If this could not be achieved, imaging procedures were accepted for diagnosis of recurrence. Causes of death were diagnosed clinically.

Immunohistochemical Staining

For immunohistochemistry, tumors were formalin-fixed and paraffin-embedded immediately, cut into 4-μm serial sections, deparaffinized and inactivated for endogenous peroxidase (0.5% hydrogen peroxide, 20 min). Then slides were rehydrated.

Staining was performed at room temperature and each incubation step was followed by thorough washing in 0.001% Brij/PBS (Sigma, Deissenhofen, Germany). Slides were preincubated with 5% horse serum/PBS for 20 min. Monoclonal antibody No. 14-4012-10004 (Paesel-Lorei, Frankfurt/Main, Germany, IgG1) against 72kD-collagenase (MMP-2) was incubated for 60 min (1.5 μg/ml). Staining was continued using a highly sensitive avidin-biotin elite kit

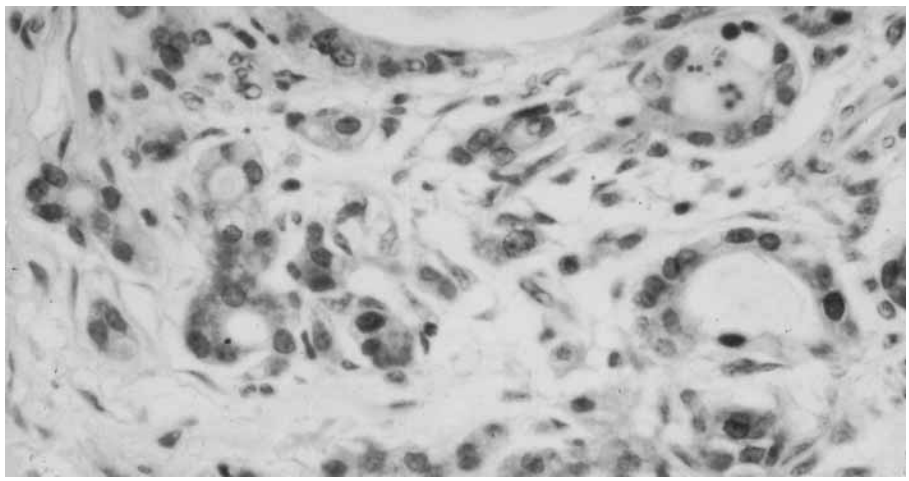

Fig. 1. Immunohistochemical staining for MMP-2 in a gastric carcinoma of the intestinal type (G_2), score 2.

(Vectastain, Burlingame, Calif., USA). Slides were incubated with horse-derived bridging antibody (7.5 μg/ml) for 30 min, followed by Vectastain ABC elite complex for 30 min. After washing in PBS, aminoethylcarbazole (Sigma) was added for 15 min as enzyme substrate. Finally, slides were counterstained with hematoxylin.

One section of each tumor treated with antibody MLG/7S (Nordic, Tilburg, the Netherlands) against murine IgG instead of the primary antibody in equimolar protein concentration served as negative, a routinely processed tumor with known strong MMP-2 expression as positive control.

All slides were coded and evaluated without knowledge of patient and clinical status by an experienced pathologist (R.B.). Scoring was exclusively restricted to tumor cell staining, and stromal staining was not considered. Staining results were classified semiquantitatively into four groups according to the number of positively stained tumor cells: score 0 = negative; score 1 = lower or equal to 30% positive tumor cells; score 2 = 30–70% positive cells; score 3 = greater or equal to 70% positive tumor cells.

For staining regarding uPA, uPA-R, PAI-1 and PAI-2, see the methodology section in Heiss et al. [24].

Statistical Analysis

χ^2 analysis was performed to determine correlations between expected and detected frequencies. Parameters for χ^2 analysis were considered as follows: MMP-2, uPA, uPA-R, PAI-1 and PAI-2 were classified into semiquantitative groups (score 0–3), Laurén's classification as intestinal versus diffuse/mixed, lymphangiosis and vessel infiltration as presence versus absence and pT, pN, M, UICC, G and Borrmann as established. Kaplan-Meier analysis evaluated group-oriented life-table curves and was confirmed by Mantel-Cox log-rank statistics [25, 26]. The Cox proportional hazard model was performed for multivariate analysis, considering established risk factors in gastric cancer [27]. Parameters for multivariate analysis were considered as stated for χ^2 analysis; intended surgical curability (curative/not curative) and operative procedure (extended/not extended) were dichotomized. All statistics were done two-sided at a significance level of $p = 0.05$.

Results

Of 203 patients, 189 (139 curatively resected) could be followed every 6 months postoperatively (14 died in hospital); 3 patients were lost to follow-up. The median time of follow-up was 31 months, the range 9–56. Ninety-three patients died, 82 with malignant disease, but one not causative.

In curatively resected patients, 47 recurrences were observed (10 as peritoneal carcinosis, 24 as locoregional recurrence, 13 as distant metastasis, of which 7 liver, 2 bone, 1 brain and 1 generalized metastasis).

Immunohistochemical staining (example in fig. 1) was also seen in surrounding fibroblasts and endothelia. However, stromal staining was not considered in scoring.

Correlation with Clinicopathological Tumor Parameters

The intensity of MMP-2 staining in tumor epithelia did neither correlate with pT ($p = 0.6598$), pN ($p = 0.8420$), UICC classification ($p = 0.3282$), grading ($p = 0.4298$), Borrmann classification ($p = 0.2712$) nor with lymphangiosis carcinomatosa ($p = 0.9566$) or vessel infiltration ($p = 0.1815$). A significant correlation (table 1) was seen, however, for M stage ($p = 0.0212$) and Laurén's classification ($p = 0.0115$), where higher staining scores were associated with diffuse types.

Correlations with Proteases/Inhibitors of the uPA System

Expression of uPA system parameters had been investigated immunohistochemically; results have been shown previously [24]. The intensity of uPA staining did posi-

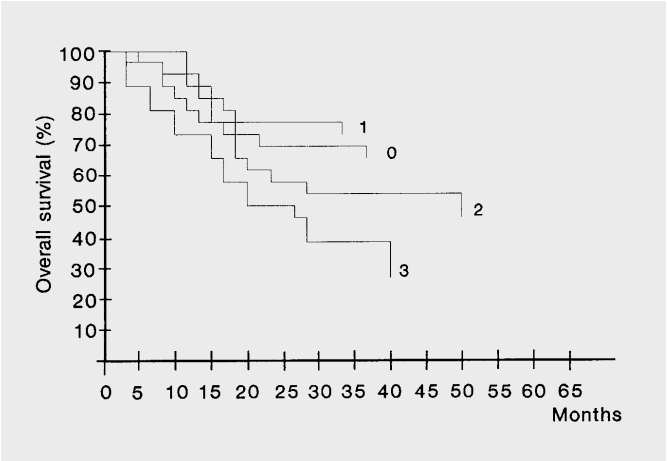

Fig. 2. Overall survival of 139 curatively resected gastric cancer patients according to semiquantitative scoring (0–3) of immunohistochemical MMP-2 detection in tumor cells (Kaplan-Meier analysis); p (Mantel-Cox log-rank test) = 0.2743. Score 0: cases 22, events 8, mean survival time (MST) 43.19 months, standard deviation (SD) 6.22; score 1: cases 44, events 11, MST 44.91 months, SD 5.60; score 2: cases 46, events 15, MST 41.65 months, SD 5.98; score 3: cases 27, events 16, MST 31.58 months, SD 7.06.

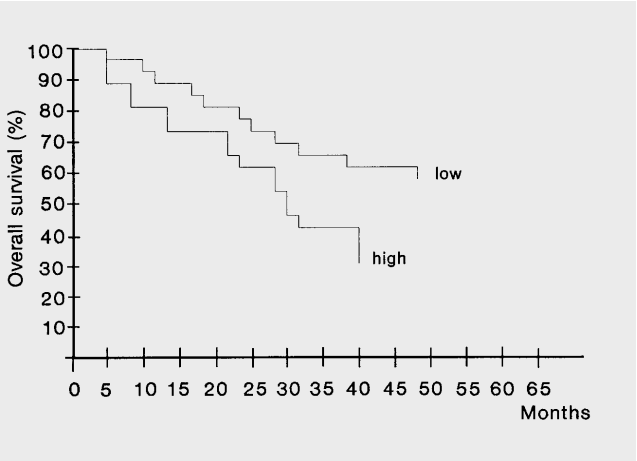

Fig. 3. Overall survival of 139 curatively resected gastric cancer patients according to dichotomization of immunohistochemical MMP-2 detection in tumor cells between low (score 0/1) and high (score 2/3) staining degrees (Kaplan-Meier analysis); p (Mantel-Cox log-rank test) = 0.0709. Low MMP-2 levels: cases 66, events 19, mean survival time (MST) 43.26 months, standard deviation (SD) 4.62; high MMP-2 levels: cases 73, events 31, MST 36.62 months, SD 6.52.

tively correlate with MMP-2 detection (p = 0.0027). There was also a significant positive association with uPA-R (p = 0.0004), PAI-1 (p < 0.0001) and PAI-2 (p = 0.0358; table 1).

Prognostic Analysis of MMP-2 Expression

Kaplan-Meier survival analysis of immunohistochemical MMP-2 detection did not show significant differences in disease-free (p = 0.3903) and overall survival of curatively resected cases (p = 0.2743, fig. 2) and all 189 patients (p = 0.4645). Also, dichotomization of MMP-2-negative versus MMP-2-positive cases did not result in significant prognostic differences (curatively resected patients/disease-free survival: p = 0.6257, overall survival: p = 0.7578, all patients/overall survival: p = 0.5789).

However, dichotomization between low (score 0/1) and high MMP-2 expression (score 2/3) showed a correlation of high MMP-2 levels with worse outcome (overall survival, curatively resected patients: p = 0.0709, fig. 3, all patients: p = 0.1105; disease-free survival: p = 0.4188). As univariate prognostic significance could not be shown for MMP-2, multivariate Cox regression analysis considering established risk factors in gastric cancers was not performed.

Table 1. Overview of significant χ^2 correlations of MMP-2

Significantly correlating variable	p value
M	0.0212
Laurén	0.0115 (high scores in diffuse types)
uPA	0.0027
uPA-R	0.0004
PAI-1	<0.0001
PAI-2	0.0358

MMP-2 and uPA system parameters are classified semiquantitatively (score 0–3), Laurén's classification and M stage were entered as dichotomized variables (diffuse/mixed vs. intestinal, M_0 vs. M_1).

Prognostic Analysis of MMP-2 according to Laurén's Classification

As higher immunohistochemical MMP-2 scores were significantly correlated with Laurén's diffuse/mixed types in χ^2 analysis, Kaplan-Meier survival calculations were performed for intestinal and diffuse/mixed carcinomas separately.

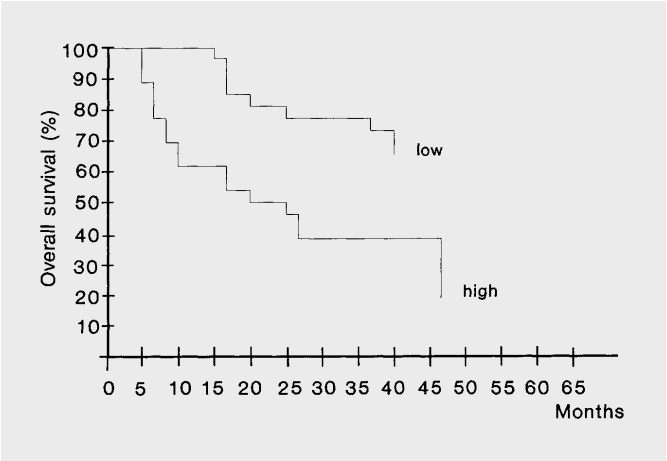

Fig. 4. Overall survival of 54 curatively resected patients with diffuse/mixed gastric cancers according to dichotomization of immunohistochemical MMP-2 detection in tumor cells between low (score 0/1) and high (score 2/3) staining degrees (Kaplan-Meier analysis); p (Mantel-Cox log-rank test) = 0.0056. Low MMP-2 levels: cases 34, events 9, mean survival time (MST) 47.73 months, standard deviation (SD) 4.28; high MMP-2 levels: cases 20, events 13, MST 28.50 months, SD 7.50.

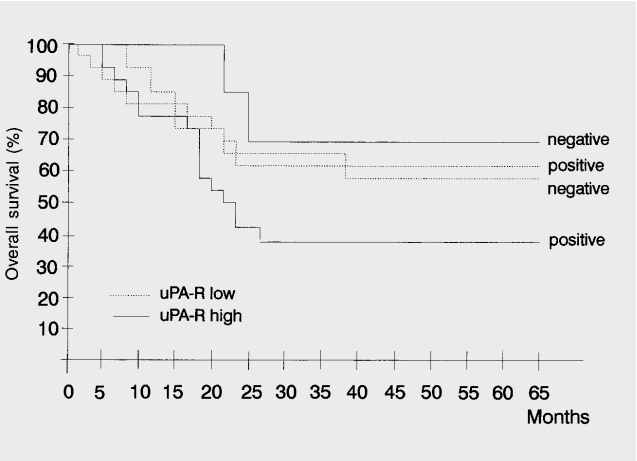

Fig. 5. Overall survival of 33 curatively resected gastric cancer patients with high (score 3) tumor cell evidence of uPA-R according to immunohistochemically detected MMP-2 presence or absence (Kaplan-Meier analysis); p (Mantel-Cox log-rank test) = 0.2598. MMP-2 negative: cases 5, events 2, mean survival time (MST) 47.50 months, standard deviation (SD) 6.50; MMP-2 positive: cases 28, events 13, MST 33.41 months, SD 6.96. In comparison, survival curves for patients with low expression of uPA-R (score 0–2) are indicated by dashed lines, again for MMP-2 presence versus absence; p (Mantel-Cox) = 0.7111. MMP-2 negative: cases 17, events 6, MST 45.00 months, SD 6.00; MMP-2 positive: cases 89, events 13, MST 48.00 months, SD 3.00.

Table 2. Association of immunohistochemical MMP-2 detection in 89 diffuse/mixed gastric cancers (54 curatively resected) with prognosis according to Mantel-Cox log-rank test

	p (Mantel-Cox) for semiquantitative scores 0–3	p (Mantel-Cox) for dichotomization 0/1 and 2/3
Disease-free survival (curatively resected patients)	0.2318	0.0426
Overall survival (curatively resected patients)	0.0448	0.0056
Overall survival (all patients)	0.1884	0.1092

Analysis for semiquantitative scores 0–3 and dichotomization between scores 0/1 and 2/3.

For intestinal types (n = 100), no significant association with prognosis was seen (curatively resected patients: disease-free survival p = 0.6749, overall survival p = 0.8960; all patients: overall survival p = 0.9665).

In diffuse/mixed types (n = 89), however, a significant association with overall survival was revealed for curatively resected patients (p = 0.0448, table 2). With dichotomization between low (score 0/1) and high (score 2/3) MMP-2 detection (table 2), a significant association with prognosis was seen. As an example, Kaplan-Meier analysis for overall survival of curatively resected patients is given in figure 4 (p = 0.0056).

Multivariate analysis performed to correct these results for established risk factors in gastric cancer (pT, pN, M, G, intended surgical curability, tumor localization and diameter, extended vs. not extended operative procedure) and for uPA system parameters did not establish MMP-2 as a new independent prognostic parameter for diffuse gastric cancers (table 3). As the stepwise Hazard model revealed, the univariate impact of MMP-2 was lowered by the strong correlation with PAI-1, uPA and uPA-R (table 1) as confounding variables.

Prognostic Impact of MMP-2 Considering Expression of uPA System Parameters

In view of biological interactions between different tumor-associated protease systems, we asked whether expression of MMP-2 would be modified in prognostic impact when patients with strong evidence of uPA system parameters [24] were separately analyzed.

Table 3. Multivariate analysis of MMP-2 in diffuse/mixed gastric cancers

Variable	Disease-free survival, curatively resected patients (n = 54)			Overall survival, curatively resected patients (n = 54)			Overall survival, all patients (n = 89)		
	p value	odds ratio (relative risk)	95% CI	p value	odds ratio (relative risk)	95% CI	p value	odds ratio (relative risk)	95% CI
Surgical curability	–	–	–	–	–	–	<0.001	6.87	3.11–15.20
pT	0.041	5.22	1.93–14.11	0.043	2.65	1.65–4.26	n.s.	–	–
Tumor diameter	n.s.	–	–	n.s.	–	–	0.038	1.80	1.49–2.18
uPA	<0.001	2.05	1.50–2.79	<0.001	2.42	1.64–3.58	0.002	2.05	1.68–2.48
uPA-R	n.s.	–	–	n.s.	–	–	0.026	1.80	1.56–2.07
PAI-1	0.020	2.78	1.80–4.32	n.s.	–	–	n.s.	–	–
MMP-2	n.s.	–	–	n.s.	–	–	n.s.	–	–

Multivariate analysis of immunohistochemical MMP-2 detection in 89 diffuse/mixed type gastric cancers considering additional risk factors in gastric cancer (pT, pN, M, G, lymphangiosis carcinomatosa, vessel infiltration, surgical curability, extended/not extended resection, tumor localization and diameter, Borrmann, uPA, PAI-1). Parameters were analyzed as stated in the Methods. MMP-2 was dichotomized (score 0/1 vs. 2/3). Level of significance, relative risk estimated by odds ratio and 95% confidence interval (CI) are given in the table for those parameters that were revealed to be independent risk factors (significant p value, relative risk >1). n.s. = Not significant.

In the subgroup of patients with high expression of uPA-R (score 3, 33 curatively resected patients), Kaplan-Meier analysis showed an evident, however not significant association of MMP-2 presence (vs. absence) with poor prognosis (fig. 5; survival curves given in comparison to low uPA-R expression). This was not seen in subgroups of cases with score 3 detection of uPA, PAI-1 or PAI-2.

In patients with low expression of uPA, uPA-R (fig. 5), PAI-1 or PAI-2 (scores 0, 1), there was no association of MMP-2 detection with prognosis.

Discussion

This is the first study which investigated the prognostic relevance of MMP-2 in a large prospective series of cancer patients. It revealed a positive association of high MMP-2 levels detected immunohistochemically with a poorer outcome of gastric cancer patients, however not as a significant clinical prognostic parameter. In contrast, for diffuse cancer types MMP-2 is a significant, but not independent indicator of prognosis. Further consideration of the uPA system as an interactive pattern of proteases for the first time revealed that potentially a more detailed biological prediction of prognosis will be possible if a combination of proteases is considered.

A significant association of MMP-2 with prognosis could be shown for diffuse gastric cancers. In multivariate analysis this impact was lowered by the strong correlation with uPA, uPA-R and especially PAI-1, a dominant prognostic parameter in gastric cancer [24]. In contrast to other authors [15, 16], we saw a significant correlation of MMP-2 immunostaining with Laurén's classification, higher staining scores being seen in diffuse types, a finding which is corroborated by observations of Grigioni et al. [23]. The discrepancy to David et al. [16] and Nomura et al. [14, 15] can be explained by a higher number of cases investigated (189 in contrast to 87 and 46, respectively). However, diffuse gastric cancers are characterized by spread of cancer cell nests or tumor cells surrounded by extensive stroma [28], and probably MMP-2 is of more biological relevance for cancers with those morphological characteristics. Correspondingly, Höyhtyä et al. [18] reported stronger MMP-2 staining at the periphery of tumor cell nests in gastric, breast and endometrial, ovarian and prostate cancers. For human pancreatic cancer, Gress et al. [13] demonstrated a concentration of MMP-2 levels in tumor areas with strong desmoplastic reactions. These observations agree with our results and support the hypothesis that relevance of MMP-2 may be special for tumors with the morphological features of a low tumor cell/stroma ratio, diffuse tumor cell nests and strong stromal reaction.

The lack of overall prognostic significance seems to be astonishing since collagenase IV as one key enzyme for destruction of basement membranes should be essential for aggressiveness of tumors and thus risk of relapse and metastasis [4]. Moreover, the 72-kD form of collagenase IV as an activator of the 92-kD form of collagenase IV (MMP-9) [22] has been defined as one characteristic of malignant gastric cancer cells [21], and Nomura et al. [15] demonstrated significant correlations of immunohistochemical MMP-2 evidence with advanced gastric cancer stages as well as vascular invasion. This corresponds to the significant association with M stage in our series.

The failure of immunohistochemical MMP-2 analysis to be of significant prognostic impact could have two main reasons. First, immunohistochemistry does not distinguish between activated and latent MMP-2. As Nomura's study indicates, not sole detection, but moreover evidence of activation of MMP-2 in cancers may be decisive [15]. Gelatin zymography revealed that the ratio of active to total MMP-2 was significantly higher in carcinomas than in normal tissue and significantly higher in advanced than in early gastric cancer stages. Another study of the same group indicates an important role of MT-MMP (a metalloproteinase with a transmembrane domain [9]) in combination with MMP-2 analysis. MT-MMP is thought to be a surface-bound activator of MMP-2 in invasive tumor cells [9, 14], and immunohistochemically detected MT-MMP increases with grade of vascular tumor cell invasion in gastric cancer [14]. Tumors with colocalization of MT-MMP and MMP-2 significantly show higher degrees of vessel infiltration, and gelatin zymography demonstrated that active MMP-2 can only be detected in tumor samples with positive evidence for MT-MMP [14]. These results indicate that biological and prognostic relevance of MMP-2 may be a matter not of expression, but of enzyme activity.

Furthermore, not immunohistochemical analysis of MMP-2 alone, but evaluation of interacting factors could be of prognostic importance. The activating role of MT-MMP has already been discussed. Other authors postulate that not MMP-2 expression alone, but more the balance between MMP-2 and its specific inhibitor TIMP-2 [10] may be decisive for prognosis. In RT-in situ-PCR analyses of 23 cases of cervical carcinoma, Nuovo et al. [17] demonstrated a ratio of MMP-2/TIMP-2 of approximately 1 as correlated with good prognosis, whereas increasing ratios favoring MMP-2 were seen in cases with poor outcome. In these cases, preferentially downregulation of TIMP-2, not upregulation of MMP-2 was seen. In parallel, Grigioni et al. [23] observed a higher MMP-2/TIMP-2 ratio in advanced gastric cancers. The balance between the protease and its inhibitor may thus be essential for the event that a tumor cell does metastasize. However, TIMP-2 should not only be seen as an inhibitor of MMP-2. TIMP-2 is able to form complexes with MMP-2 that potentially protect MMP-2 from degradation and enable binding to a membrane-bound MMP receptor, evidence of which has been given by Emonard et al. [29]. Characteristics and cycling of this receptor remain to be elucidated; however, it has been speculated that, similar to the uPA/PAI-1 complex, binding and recycling of MMP-2/TIMP-2 may play a role in tumor growth and neoangiogenesis [11, 12]. Correspondingly, TIMP-2 has been shown as associated with poor prognosis in invasive bladder cancer [30].

Thus, in view of multiple interactions consideration of additional factors of tumor-associated proteolysis has to be postulated for further prognostic analyses of MMP-2. As a first approach, this has been done in our present study regarding the uPA system. The 55-kD serine protease uPA, its cellular receptor uPA-R and specific inhibitors PAI-1 and PAI-2 have extensively been studied [8, 12, 31]; prognostic relevance of this system is established [32, 33] and has been published by our group examining the same series of gastric cancer patients [24]. As the uPA system is discussed as being able to activate collagenases, thus potentially MMP-2 [7, 8], we proposed that there could be a correlation of expression and modification of the prognostic impact of MMP-2 by this system. In fact, χ^2 analysis showed that uPA, uPA-R, PAI-1 and PAI-2 are significantly positively correlated with MMP-2, which potentially implies a corresponding regulation of those enzymes. uPA, PAI-1 and PAI-2 failed to improve prognostic relevance of MMP-2; however, in cases of high expression of uPA-R, Kaplan-Meier analysis demonstrated evident association of MMP-2 detection with worse prognosis (see curves fig. 5), the low significance level of which in our opinion should be explained by small patient numbers. uPA-R as the membrane-bound center of the uPA system is able to concentrate uPA activity by focusing receptor-bound uPA [31]. Thus, the presence of further enzymes in the proteolytic cascade like MMP-2 may become prognostically relevant as focused potential of their activation is available. Finally, it should be emphasized that immunocytochemical detection of MMP-2 in tumor cells as performed in our study does not necessarily imply that gastric tumor cells do secrete MMP-2 themselves. As other immunohistochemical studies on MMP-2 in gastric cancers [14–16, 18], we correspondingly saw MMP-2 staining also in fibroblasts and

endothelia of the surrounding stroma (which was not considered in scoring). This also implies that cancer cells could capture MMP-2 of surrounding stromal cells by a surface-bound receptor or could even recruit stromal cells for MMP-2 production as similarly postulated for uPA and PAI-1 [19, 34–36]. This is supported by Nomura et al. [15] who report staining of stromal cells in gastric carcinomas in contrast to normal gastric tissue, where stromal cells stained only weakly or were negative for MMP-2. In situ localization studies on DNA and mRNA levels as done for other cancers like pancreatic or bladder cancer [13, 17] are necessary to further investigate potential tumor-stroma interactions.

In summary, our present study demonstrates that immunohistochemical detection of MMP-2 in gastric cancers does show a prognostic association especially in diffuse gastric cancer; however, it is not an independent prognostic parameter. Further investigations on the prognostic value of MMP-2 should be done measuring activated MMP-2 and considering functionally related tumor-associated proteases. As it is indicated by our analysis of patients with additional high expression of uPA-R, this concept could specify the very individual cancer patient's prognosis by means of biological parameter patterns.

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Book Review

V. Diehl (Guest Editor)

Bailliere's Clinical Haematology

Int. Practice and Research – Hodgkin's Disease

Bailliere Tindall, London 1966 GBP
640 pp; £30.–
ISBN 0–7020–2174–1

Hodgkin's disease is a fascinating malignancy that seems to have a distant pathology, epidemiology and therapy. Despite the many uncertainties as to its etiology, much has been learned about its evaluation and treatment so that most patients with this disease can be cured. Dr. Diehl has put together an extensive analysis of the disease in a relative small book on its epidemiology, pathology, immunology, diagnostic evaluation, treatment and outcomes. The eleven chapters are very detailed expositions of the issues and are well referenced. The material is helpful to the clinical oncologist since it compiles information that may not be readily available in the clinical literature. The chapters on the molecular pathology and immunology are excellent examples of new information – detailed and well summarized.

There are a few disappointments in the organization of the book. The excellent chapter providing a historical perspective by Dr. Tubiana is put in the middle of the book. A deficiency that was somewhat disappointing was the organization of the chapters on treatment. They are broken up into several chapters including the management of early, intermediate, late and relapsed patients. It is hard to grasp an overall sense of what the recommendations for management might be. The role of laparotomy on staging was not clearly defined. It was like looking at a painting of pieces of the picture rather than the whole scene. Dr. Tubiana would have been able to present a more cohesive assessment because of her experience.

However, this book is a valuable addition to the library of an academic oncologist as well as those individuals who see many patients. For the investigator in the field, it also serves as a recent overall update of the issues.

Paul P. Carbone, Madison, Wisc.