Advances in Brief

Prognostic Significance of Circulating Matrix Metalloproteinase-2 to Tissue Inhibitor of Metalloproteinases-2 Ratio in Recurrence of Urothelial Cancer after Complete Resection

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Abstract

The relationship between the serum matrix metalloproteinase-2 (MMP-2):tissue inhibitor of metalloproteinases-2 (TIMP-2) ratio and disease recurrence was examined in 53 urothelial cancer patients with muscular invasion or with lymph node metastasis who underwent complete resection. The mean MMP-2:TIMP-2 ratio in patients with recurrence was significantly higher than that in 22 patients without recurrence (P < 0.05). Disease-free survival of patients with high MMP-2:TIMP-2 ratios was extremely poor compared with that of patients with lower ratios (P < 0.01). Cox's multivariate analysis suggests that the serum MMP-2:TIMP-2 ratio would be a new independent prognostic indicator of urothelial cancer recurrence.

Introduction

MMP-2 (gelatinase A, a 72-kDa type IV collagenase) degrades vascular basement membrane components, such as type IV collagen, and is implicated in tumor angiogenesis and metastasis (1, 2). Many investigators have demonstrated that metastatic malignant cells produce a large amount of MMP-2 but that nonmetastatic cells do not (1, 2). Moreover, Garbisa et al. (3) have reported that serum levels of MMP-2 correlate with the extent of human lung cancer invasion and metastasis. On the other hand, TIMP-2 inhibits the protease activity of MMP-2 (2, 4) and could consequently suppress invasion, metastasis, neovascularization, and growth of some rodent and human tumors (2, 4-7). Therefore, the balance of MMP-2 and TIMP-2 is considered important in the regulation of tumor metastasis, invasion, angiogenesis, and growth (2). In view of the importance of these findings and the absence of reports regarding the relationship between the ratio of serum MMP-2 to TIMP-2 and metastasis/recurrence, we measured the serum levels of these enzymes and examined this relationship in urothelial cancer patients.

Materials and Methods

Between January 1986 and October 1994, serum had been preoperatively collected from 97 patients who underwent complete resection, which was determined pathologically to have a surgical margin negative for cancer. The pathological stages were determined according to the TNM classification of urothelial cancer. Histology of tumors and the differentiation were determined according to WHO classifications. Pathological diagnosis was as follows: 44 patients with superficial bladder cancer (noninvasive; pTa, 12; pT1, 32); 29 patients with muscular invasion or lymph node metastatic bladder cancer (advanced bladder cancer; pT1, 2; pT2, 12; pT3a, 3; pT3b, 12); 24 patients with muscular invasion or lymph node metastatic upper urinary bladder cancer (pT1, 3; pT2, 11; pT3b, 8; pT4, 2). All superficial bladder cancers were not associated with lymph node metastasis. Among advanced urothelial cancer, tumors at stage pT1 were associated with regional lymph node metastases. Among 53 patients with advanced urothelial cancer, 14 patients had lymph node metastases, and 30 patients had lymphovascular involvement. The superficial bladder cancers were histologically diagnosed as transitional cell carcinomas (19 grade 1, 19 grade 2, and 6 grade 3). The histology of advanced urothelial cancer was as follows: 49 transitional cell carcinomas (2 grade 1, 20 grade 2, and 27 grade 3); 3 squamous cell carcinomas; and 1 adenosquamous. The normal MMP-2:TIMP-2 value was determined by analyzing healthy controls: 85 males and 42 females between 18 and 69 years old (median, 59 years old).

The serum was stored at −80°C until use. Informed consent was obtained from the patients for measuring serum MMP-2 and TIMP-2. The concentration of serum MMP-2 was measured by the one-step sandwich EIA system using monoclonal antibodies against human MMP-2 as reported previously (8). The sensitivity of this EIA for MMP-2 is 2.4 pg/assay (0.24 ng/ml), and a linearity is obtained between 10 and 5,000 pg/assay (1.0—500 ng/ml). The concentration of serum TIMP-2 was similarly determined using monoclonal antibodies against human TIMP-2 as described previously (9). The sensitivity of this EIA is 16 pg/assay (1.6 ng/ml), and a linearity is obtained between 63 and 500 pg/assay (6.3—50 ng/ml). The assays were carried out in triplicate. The serum MMP-2:TIMP-2 ratios were determined in healthy controls and urothelial cancer patients.

All superficial bladder tumors were completely resected endoscopically, and the patients were observed for recurrence over a median period of 30 months (10—67 months). Of 29 advanced bladder cancer patients, 26 underwent radical cystectomy and pelvic lymph node dissection, and 3 underwent partial cystectomy and hemipelvic lymph node dissection. All 24 upper urothelial cancer patients received nephroureterectomy, partial cystectomy, and regional lymph node dissection. The median observation period of the 53 advanced urothelial cancer patients who underwent complete resection was 29 months (3—107 months).

The differences in the serum MMP-2:TIMP-2 ratios were examined by Student's t test. The significance of the recurrence was determined by χ² tests. The disease-free survival was calculated by the Kaplan-Meier method, and the difference was determined by the log rank test. P values <0.05 were regarded as significant. The prognostic significance of recurrence was assessed by Cox's proportional hazards regression.

Results and Discussion

The serum MMP-2:TIMP-2 ratio was not related to sex, age in healthy controls, or primary tumor size in cancer patients (data not shown). The mean ± 2 SD of the serum MMP-2:TIMP-2 ratio of healthy controls was 11.0; any higher value was regarded as "elevated." Of 53 advanced urothelial cancer patients, 19 (36%) exhibited high serum MMP-2:TIMP-2 ratios. A significantly positive correlation (Spearman's) was also observed between this ratio and lymph node metastasis (P = 0.0119; r = 0.349).

The mean MMP-2:TIMP-2 ratio in 31 patients with recurrence...
was significantly higher (11.2 ± 3.43) than in any of the remaining 22 patients without recurrence (8.48 ± 4.13) or in the 44 patients with superficial bladder cancer (7.76 ± 1.55; P < 0.05 and < 0.01, respectively; Fig. 1). The serum MMP-2:TIMP-2 ratios in cases of superficial bladder cancer, none of which developed recurrence, were similar to those of healthy controls. The rate of recurrence in advanced urothelial cancer patients with high MMP-2:TIMP-2 ratios (≥11.0; n = 19) was significantly higher than that in those with normal ratios (<11.0; n = 34; 79 versus 47%; P < 0.05). Although patients' characteristics in the two groups appeared similar, the 1- and 3-year disease-free survival of patients with high MMP-2:TIMP-2 ratios (50 and 12%, respectively) was unfavorable compared with those with normal values (82 and 56%, respectively; P < 0.01; Fig. 2). Multivariate and univariate analyses for recurrence revealed that the serum MMP-2:TIMP-2 ratio could be a new independent predictor comparable with the traditional prognostic factors, such as the depth of invasion, tumor grade, and node involvement (Table 1).

Our results demonstrated that the mean serum MMP-2:TIMP-2 ratio in patients with recurrence was significantly higher than that in patients without recurrence. Moreover, the disease-free survival was unfavorable for the advanced urothelial cancer patients with high MMP-2:TIMP-2 ratios compared with those with normal ratios. As regards recurrence and disease-free survival, patients with elevated serum MMP-2:TIMP-2 ratios were at a significantly unfavorable status compared with patients with normal values. The disease-free survival did not, however, correlate with the serum level of MMP-2 or TIMP-2 alone (data not shown). Thus, the increase in the MMP-2:TIMP-2 ratio (≥11.0) is an important event associated with metastasis formation. Recently, Koop et al. (10) have reported that the decreased metastatic ability of TIMP-overexpressing B16F10 cells is due to the effects of TIMP on tumor growth after tumor cell extravasation in metastatic target organs. We observed that if the serum MMP-2:TIMP-2 ratio had been within the normal range (<11.0), the secondary tumor at any metastatic site did not grow well. In such cases, even when micrometastatic lesions were formed before the operation, they would not develop visible metastasis.

In conclusion, our results suggest that the imbalance of serum MMP-2 and TIMP-2 is a critical factor in the recurrence of urothelial cancers. Therefore, the serum MMP-2:TIMP-2 ratio may be a new useful prognostic marker of recurrence and may help us to determine whether or not cases of advanced urothelial cancer need intensive therapy, such as adjuvant chemotherapy, after complete resection.

References


Fig. 1. Distributions of the serum MMP-2:TIMP-2 ratios in 44 superficial bladder cancer and 53 advanced urothelial cancer patients. The mean serum MMP-2:TIMP-2 ratio in advanced urothelial cancer with recurrence was significantly higher than that in advanced urothelial cancer without recurrence and that in superficial bladder cancer (P < 0.05 and P < 0.01, respectively; Student's t test).

Fig. 2. Disease-free survival of 53 advanced urothelial cancer patients after complete resection according to the serum MMP-2:TIMP-2 ratio. The 1- and 3-year disease-free survival in patients (n = 19) with high MMP-2:TIMP-2 (≥11.0) were significantly unfavorable compared with those in patients (n = 34) with lower values (<11.0; P < 0.01; log rank test).

Table 1 Prognostic factors (univariate and multivariate analyses)

<table>
<thead>
<tr>
<th>Disease-free survival</th>
<th>Univariate (hazard ratio)</th>
<th>Multivariate (hazard ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2:TIMP-2 (&lt;11.0 vs. ≥11.0)</td>
<td>0.009 (2.81)</td>
<td>0.041 (2.24)</td>
</tr>
<tr>
<td>Age (&lt;61 vs. ≥61 yr)</td>
<td>0.36 (1.14)</td>
<td>0.09 (1.75)</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>0.30 (0.78)</td>
<td>0.23 (0.68)</td>
</tr>
<tr>
<td>Grade (G1 vs. G2 vs. G3)</td>
<td>0.038 (1.52)</td>
<td>0.048 (1.96)</td>
</tr>
<tr>
<td>Stage (T1 vs. T2 vs. T3 vs. T4)</td>
<td>0.007 (3.71)</td>
<td>0.006 (5.78)</td>
</tr>
<tr>
<td>Lymphovascular involvement (negative vs. positive)</td>
<td>0.11 (1.63)</td>
<td>0.047 (1.05)</td>
</tr>
<tr>
<td>Lymph node metastasis (negative vs. positive)</td>
<td>0.027 (1.79)</td>
<td>0.048 (1.65)</td>
</tr>
<tr>
<td>MMP-2 (&lt;730 vs. ≥730 ng/ml)</td>
<td>0.15 (1.48)</td>
<td>0.39 (1.18)</td>
</tr>
<tr>
<td>TIMP-2 (&lt;94 vs. ≥94 ng/ml)</td>
<td>0.06 (0.37)</td>
<td>0.19 (0.54)</td>
</tr>
</tbody>
</table>
Advances in Brief

Optosis was recently found to be a result of inhibition of the mevalonate (MVA) pathway in human myeloma cells in vitro. In macrophages, bisphosphonate-induced apoptosis in vivo, in addition to their therapeutic antiresorptive effects, may have direct antitumor effects on malignant plasma cells in vivo. This raises the possibility that bisphosphonates may have direct antitumor effects on malignant plasma cells in vivo, thus raising the possibility that bisphosphonates may have direct antitumor effects on malignant plasma cells in vivo.

Introduction

Bisphosphonates can induce apoptosis and cause cell cycle arrest in human myeloma cells in vitro, thus raising the possibility that bisphosphonates may have direct antitumor effects on malignant plasma cells in vivo. This raises the possibility that bisphosphonates may have direct antitumor effects on malignant plasma cells in vivo.

Materials and Methods

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The Bisphosphonate Incadronate (YM175) Causes Apoptosis of Human Myeloma Cells in Vitro by Inhibiting the MVA Pathway

Serum MMP-2:TIMP-2 Ratio in Recurrence

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