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

Kazu Gohji, Noboru Fujimoto, Akio Fujii, Toyozo Komiyama, Jiro Okawa, and Motowo Nakajima

Department of Urology, Kobe University School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650 [K. G.]; Biopharmaceutical Department, Fuji Chemical Industries, Takaoka 933 [N. F.]; Departments of Urology [A. F.], Radiology [T. K.], and Pathology [J. O.], Hyogo Medical Center for Adults, Akashi 673; and Bio-organic Research Department, International Research Laboratories, Ciba-Geigy Japan, Takaoka 665 [M. N.], Japan

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 31 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 urothelial 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 ratio 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 (6.3–50ng/ml). The assays were carried out in triplicate. The serum MMP-2:TIMP-2 ratios were determined in healthy controls and urothelial cancer patients.

The distributions of subjects with predicted low, intermediate, and high levels of activity and ORs (95% CI) of lung cancer and 95% CIs in relation to EPHX polymorphisms and predicted mEH activity were calculated by unconditional logistic regression analyses. Three predicted mEH enzymatic activity levels were assessed by the likelihood ratio tests to compare the goodness of fit of the models with- and without the interaction term, taking into account the above-mentioned adjusting factors. For that purpose, the average daily consumption of smoking-related variables were studied to test the equality of the effect of mEH activity on the risk of lung cancer.

Table 2 ORs (95% CI) of lung cancer in relation to predicted mEH activity by duration of smoking and by GSTM1 genotype. Predicted enzymatic activity levels and the CYP1A1 MspI genotype (homozygous site-absent/others), the GSTMJ genotype (homozygous null/others), and the CYPJAI lie-Va! genotype (Ile-Ile./others), and the GSTMJ genotype (homozygous site-absent/others), the

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

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 has antitumor effects in vivo, in addition to their therapeutic antiresorptive properties. Bisphosphonates can inhibit proliferation and cause apoptosis in human myeloma cells in vitro, as demonstrated by the induction of apoptosis in JJN-3 myeloma cells and inhibition of cell proliferation. Incadronate and mevastatin (a known inhibitor of the MVA pathway) can be prevented by the addition of substrates for the isoprenylation of small GTP-binding proteins such as farnesyl PP1 (FOH), farnesol (FAR), and geranylgeraniol (GGOH). Geranylgeraniol and farnesol prevented incadronate-induced apoptosis and inhibited cell proliferation.

Introduction

Multiple myeloma is characterized by the development of bone disease associated with tumor-associated bone disease, including multiple myeloma. The Bisphosphonate Incadronate (YM175), induce apoptosis in macrophages in vitro by inhibiting the formation or utilization of farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), which are key intermediates of the pathway required for protein isoprenylation. This article demonstrates that incadronate-induced apoptosis in human myeloma cells in vitro is the result of inhibition of the MVA pathway. Incadronate and mevastatin (a known inhibitor of the MVA pathway) can be prevented by the addition of FOH, FARM, and GGOH. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Materials and Methods

Cell Culture. The human myeloma cell line JJN-3 was kindly provided by Prof. I. Franklin (University of Glasgow, Glasgow, United Kingdom) and Dr. R. Graham G. Russell (University of Aberdeen Medical School, Polwarth Building, Foresterhill, Aberdeen AB25 2W, United Kingdom). Myeloma cells were cultured in RPMI 1640 (Life Technologies, Inc., Paisley, United Kingdom) containing 10% FCS, 1 msi glutamine, 1 msi sodium pyruvate, and 1X MEM nonessential amino acids (Gibco Laboratories, Grand Island, NY). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO2.

Chemicals. Cycloheptylaminomethylene-l,l-bisphosphonate (incadronate) was a gift from Procter and Gamble Pharmaceuticals (Cincinnati, OH). A stock solution of incadronate was prepared in PBS (pH 7.4) and filter-sterilized using a 0.22μm filter. Incadronate and mevastatin were dissolved in dry ethanol and diluted in MEM. FOH and GGOH were purified as described previously. FPP (Sigma Chemical Co.) and GGPP (Sigma Chemical Co.) were dissolved in dry ethanol and diluted in MEM.

Methods. The human myeloma cell line JJN-3 was cultured in RPMI 1640 (Life Technologies, Inc., Paisley, United Kingdom) containing 10% FCS, 1 msi glutamine, 1 msi sodium pyruvate, and 1X MEM nonessential amino acids (Gibco Laboratories, Grand Island, NY). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO2. JJN-3 cells were treated with either 100μM incadronate or 20μM mevastatin in the presence or absence of 1 mM FOH, 20μM GGOH, or 500μM MMVA. The presence of apoptotic cells was assessed by flow cytometric analysis after the incorporation of fluorescence labeled-UTP into DNA strand breaks, as described previously. These experiments were performed in triplicate.

Results

Incorporation of [3H]thymidine was determined by liquid scintillation counting. Analysis of DNA content was performed by flow cytometry. JJN-3 cells were cultured as for the in situ nick translation. After treatment with 100μM incadronate or 30μM mevastatin in the presence or absence of 1 mM FOH, 20μM GGOH, or 500μM MMVA, the presence of apoptotic cells was assessed by flow cytometric analysis. Apoptotic cells were identified by DNA fragmentation and cell cycle analysis. The proportion of apoptotic cells was determined by the percentage of cells in the sub-G1 phase of the cell cycle. Analysis was carried out on two separate experiments.

Discussion

This study demonstrates that incadronate-induced apoptosis in human myeloma cells in vitro is the result of inhibition of the MVA pathway. Incadronate and mevastatin (a known inhibitor of the MVA pathway) can be prevented by the addition of substrates for the isoprenylation of small GTP-binding proteins such as FOH, FARM, and GGOH. Geranylgeraniol and farnesol prevented incadronate-induced apoptosis and inhibited cell proliferation. This article demonstrates that incadronate-induced apoptosis in human myeloma cells in vitro is the result of inhibition of the MVA pathway. Incadronate and mevastatin (a known inhibitor of the MVA pathway) can be prevented by the addition of substrates for the isoprenylation of small GTP-binding proteins such as FOH, FARM, and GGOH. Geranylgeraniol and farnesol prevented incadronate-induced apoptosis and inhibited cell proliferation.

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