Correlation of Serum Metalloproteinase Levels with Lung Cancer Metastasis and Response to Therapy

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Abstract

Cancer cells elaborate metalloproteinases which may play a role in invasion and metastasis. The serum level of the Mr 72,000 type IV collagenase (MMP-2) was measured in 87 lung cancer patients. Stage IV cancer levels were significantly elevated (P < 0.0001) compared to normal sera. A significant difference (P < 0.01) was found between enzyme levels in the presence versus the absence of distant metastasis. For 29 patients treated with combination chemotherapy, a positive relationship was noted between response failure and elevated enzyme levels. Serum metalloproteinase levels may provide information relevant to prognosis as well as treatment decisions.

Introduction

The role of extracellular matrix turnover in tumor growth, invasion, and metastasis has been extensively studied in recent years, and the basement membrane has been demonstrated to be an important anatomical barrier in this respect. In murine and human models tumor metastatic potential correlates with the degradation of basement membrane collagen (type IV) and the elaboration of metalloproteinases (1). Northern blot analysis of total cellular RNA from human colon tumor tissue has demonstrated a higher expression of the Mr 72,000 type IV collagenase (MMP-2) in tumor tissue compared to adjacent normal mucosa (2). The possibility that this secreted metalloproteinase has potential utility as a serum marker was evaluated by means of recently available antibodies which selectively recognize this member of the metalloprotease enzyme family.

Materials and Methods

Plasma and sera obtained from 87 patients at the time of lung cancer diagnosis and from 10 healthy subjects were stored at -80°C. Aliquots of 1–100 μl were seeded onto gelatin-coated microwells, and the level of MMP-2 was measured by the substrate capture enzyme-linked immunosorbent assay as described previously, using affinity-purified rabbit anti-MMP-2 antibodies (3). Verification of the antigen was accomplished by recovery of the molecule from the enzyme-linked immunosorbent assay well followed by Western blotting (Fig. 1). Each serum sample was analysed ten times, and the mean value was used for analysis.

Pathologic diagnosis was as follows: 13 squamous carcinomas; 19 adenocarcinomas; 6 large cell carcinomas; 36 small cell carcinomas; 3 carcinoids; 3 mesotheliomas; 1 adenosquamous; 6 other. Clinical staging included a complete history and physical examination, blood chemistry analysis, chest radiograph, fiberoptic bronchoscopy, with bronchial (or transbronchial) biopsy, computed tomography of the chest, abdominal ultrasonography, and whole-body bone 99mTc scan. Histologic type was assigned according to WHO criteria (4). Clinical staging was determined according to the newly revised tumor, node, metastases staging system of the American Joint Committee on Cancer (5).

The response status for patients receiving chemotherapy was considered and stratified according to standard criteria (6) for 29 patients treated with the same protocol of cyclophosphamide, doxorubicin, and etoposide: all 10 nonresponders examined had clinically evident metastases. In patients with clinically evident metastases the serum levels of MMP-2 were significantly elevated (P < 0.01) compared to those without metastases (Fig. 2).

Moreover, in patients who subsequently did not respond to chemotherapy and demonstrated tumor progression, the elevation in serum MMP-2 was 2.8-fold higher (P < 0.01) compared to patients in which the therapy was associated with a partial or full response over a period of 1 month (Fig. 2). Data on responsiveness to chemotherapy were available for 29 patients treated with the same protocol of cyclophosphamide, doxorubicin, and etoposide: all 10 nonresponders examined had clinically evident metastases, but only 5 of the 19 responders did. A biochemical linkage between the metastatic phenotype and metalloproteinase expression has been noted in animal and human systems (7–10); therefore, in patients where MMP-2 producing cells...
Fig. 1. MMP-2 (cIVase) levels in sera of normal healthy subjects and of lung tumor patients at different disease staging. The columns represent the mean values of 10 normal subjects (SD 13%), 32 stage II to IIIb (SD 22%), and 35 stage IV (SD 17%) lung tumor patients. The difference between the mean value of normal subjects and stage IV lung tumor patients was significant (P < 0.0001). Inset: Western blot for MMP-2 immobilized on gelatin-coated microwells. Thirty μl of serum from one normal (N) and one lung tumor (T) patient were seeded onto gelatin-coated microwells and incubated overnight at 4°C. After exhaustive washing, 50 μl of sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer with 10% mercaptoethanol have been added, and the sealed microwell was immersed for 3 min in boiling water; the sample was then directly loaded into 8% sodium dodecyl sulfate-polyacrylamide gel, transferred onto Hybond-C extra, and incubated with 1/500 anti-MMP-2 (3), 1/6000 peroxidase-conjugated second antibody, and 4-chloro-1-naphthol color development reagent (Bio Rad).

Fig. 2. Quantitation of MMP-2 (cIVase) in the sera of lung tumor patients with (+M) and without (–M) metastases at the time of diagnosis and with partial (+R) or no (–R) response to therapy. The columns represent the mean values of 52 –M, 35 +M, 19 +R, and 10 –R patients. The SD was <10% of the mean, except for +R (20%). Group +R includes patients showing stationary tumor or partial regression, and group –R includes patients with progressing disease after therapy. The sera were obtained before therapy.

colonized organs outside the lung, higher serum enzyme level could relate to the additional body burden of tumor cells.

Does the response to therapy in lung cancer patients parallel the serum MMP-2 level? We had the opportunity to measure MMP-2 levels in the serum of seven lung tumor patients obtained before, during, and after therapy. All cases were small cell cancers treated with the cyclophosphamide, doxorubicin, and etoposide combination. A representative therapy course is illustrated in Fig. 3. A patient with metastatic disease (+M) and who did not respond to the therapy (–R) was found to have a serum MMP-2 level which did not change following therapy. In a +M +R patient the serum MMP-2 was significantly lowered following the third cycle of chemotherapy. When the response ceased no further decrease in serum MMP-2 level occurred. The time course of 6 of 7 patients exhibited a negative relationship between MMP-2 levels and response.

Monitoring serum metalloproteinase levels in lung cancer patients therefore has potential for forecasting tumor aggressiveness and response to therapy. Additional expanded studies are warranted to evaluate the role of this enzyme as a marker of remission or recurrence during therapy.

Acknowledgments

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References


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