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Distinct endobronchial expression of matrix-metalloproteinases (MMP) and their endogenous inhibitors in lung cancer[1]

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Summary

Background: Degradation of extracellular matrix (ECM) and basement membranes is required for tumour cell invasion and metastasis. The ECM is degraded by matrix metalloproteinases (MMP) which are counteracted by tissue inhibitors of metalloproteinases (TIMP). In aggressive tumours the balance of proteolysis and antiproteolysis is disrupted, resulting in fast tumour progression and invasiveness. We examined MMP and TIMP expression patterns in bronchial washings of 58 consecutive lung tumour patients and 10 controls. Pathohistological investigations revealed squamous cell carcinoma (n = 23), adenocarcinoma (n = 18), small cell lung carcinoma (n = 9), and pulmonary metastases of extrapulmonary tumours (n = 8). MMP/TIMP expression was correlated to histology, location, or staging of tumours.

Methods: Expression and activity of MMP was identified by zymography and Western blotting. Expression of TIMP-1 and TIMP-2 was analysed by Western blotting and enzyme-linked immunosorbent assays.

Results: We identified MMP-1, MMP-2 and MMP-9, but not MMP-3 or MMP-8 in bronchial washings. All MMPs were expressed in the tumour-affected and the tumour-free parts of the lung. While MMP-1 and MMP-9 were present in all samples, the inactive precursor of MMP-2 was specifically expressed in adenocarcinoma or lung metastases of extrapulmonary tumours. No MMP-2 was found in controls. While TIMP-1 was expressed in all samples, TIMP-2 was not detectable.

Conclusion: The tumour type-specific expression of the MMP-2 precursor in adenocarcinoma and lung metastases suggests that MMP-2 in the absence of TIMP-2 correlates with aggressive tumour progression and may serve as an indicator for poor prognosis.

Keywords: lung tumour; matrix metalloproteinase; tissue inhibitor of matrix metalloproteinase; bronchial washing

Introduction

The malignant potential of cancer depends on the capability of tumour cells to invade and form metastases. This process is facilitated by the tumour cells' ability to degrade their surrounding extracellular matrix (ECM), especially basement membranes lining the blood vessels. Increased degradation of ECM and basement membranes through secretion of ECM-degrading enzymes enables the cells to enter and leave blood vessels [1, 2].

The matrix metalloproteinases (MMP) are the key enzymes exerting ECM degradation and have been shown to be increasingly secreted and activated by tumour cells. This, together with decreased availability of protease inhibitors, renders the tumour cells capable of digesting essential tissue barriers such as basement membranes, fasciae and capsulae, thereby enhancing the cells' motility. Increased expression of MMP has thus been associated with the processes of tumour invasion, metastasis and angiogenesis of malignant tumours of different origin [1–4].

In humans, the MMP family consists of at least 17 different members. All MMP are synthesised as inactive zymogens that need to be activated by proteolytic cleavage of a propeptide sequence capping

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a catalytic zinc binding domain [5–7]. Activation of MMP occurs extracellularly and is controlled by other proteases, such as trypsin, tissue- or urokinase-type plasminogen activator, or by active MMP, or by membrane-anchored matrix metalloproteinases (MT-MMP) [7, 8]. Proteolytic activity of MMP is inhibited by specific inhibitors, the tissue inhibitors of matrix metalloproteinases (TIMP).

TIMP are 20–30 kDa proteins which bind to the catalytic domain of MMP in a 1:1 stochiometry, thereby inhibiting the enzymatic activity of MMP [7–9]. The interaction of TIMP with their corresponding MMP seems to be specific [6, 8, 9]. In this respect, TIMP-1 has been shown to inhibit MMP-9, whereas TIMP-2 seems to be specific for MMP-2. Under physiological conditions the expression of MMP and TIMP is highly coordinated at the level of gene expression [6, 10]. This balanced expression guarantees normal tissue structure and organ function, and prevents both excessive ECM deposition and increased ECM degradation [11–14]. In the lung, distortion of this balance is associated with various inflammatory lung diseases and lung cancer [1, 11, 13]. The proposed role for enhanced MMP expression in lung cancer is based on studies describing high expression levels of distinct MMP, assessed by immunohistochemistry, and *in vitro* observations. In this respect, stromelysin-3 and TIMP-2 have been shown to be associated with increased malignancy of squamous cell carcinomas. However, *in vivo* observations concerning the activity of MMP in bronchial secretions have not yet been published.

This study evaluates the endobronchial expression pattern of MMP and TIMP in 58 pulmonary malignancies of varying histology and 10 controls. The expression of MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, TIMP-1, and TIMP–2 was determined in bronchial washings of all probands. We found strong expression of MMP-1, MMP-9 and TIMP-1 in all samples, irrespective of tumour type or grade, and also in the control collective. In contrast, high levels of MMP-2 were determined only in bronchial washings of all patients with adenocarcinomas or lung metastases. MMP-3, MMP-8 and TIMP-2 could not be detected in any of the samples.

Methods

Materials and reagents

Calcium chloride, β-casein, ethylene-diamine-tetra-acetic acid (EDTA), gelatine, glycerol, phenyl methyl-sulfonyl fluoride (PMSF), sodium chloride, Tris-HCL, Triton X-100, bromphenol blue, and Tween-20 were purchased from Sigma Chemicals (Emmenbrücke, Switzerland). Brij-35, Dithiothreitol (DTT), Pefabloc®, and peroxidase-coupled secondary antibodies were obtained from Boehringer Mannheim (Rotkreuz, Switzerland). Gradient 4-15% SDS-PAGE gels, pre-stained standard proteins, and Coomasie brilliant blue R-250 were from BIO-RAD (Zürich, Switzerland). Hybond-ECL nitrocellulose membranes and ECL solution are products of Amersham (Zürich, Switzerland). Purified human MMP-1, MMP-2, and MMP-9 and polyclonal antibodies to human MMP-1, and MMP-2 were provided by ANAWA Trading SA (Wangen, Switzerland). Recombinant human TIMP-1 and TIMP-2 and monoclonal antibodies specific to human TIMP-1, TIMP-2, and MMP-9 were supplied by Oncogene Science (Paris, France). Gel drying films are a product of Pr0mega Corporation (Zürich, Switzerland).

Patients and controls

Tumour patients: We analysed bronchial washings from 58 patients who underwent diagnostic bronchoscopy for suspected pulmonary malignancy. None of the patients had previously received chemotherapy or irradiation therapy for treatment of the malignancy. The tumour type was classified according to standardised cytology and/or histology on tissue samples obtained at bronchoscopy. Only patients with a definite diagnosis of pulmonary malignancy were included in the study. Each patient gave informed consent to the use of material in this study and the protocol was approved by the local ethics committee.

Controls: Bronchial washings obtained from ten patients without lung disease were used as controls. Control patients underwent elective surgery.

Collection and preparation of bronchial fluid

A total of 116 bronchial washings were obtained from 58 patients. Prior to diagnostic procedures (biopsy, etc.), bronchial fluid was obtained by instillation and suction of 10 ml pyrogen-free 0.9% NaCl solution. The first washing was performed in the radiologically unaffected lung site and the second washing at the tumour-bearing lung site. Bronchial fluids were collected in siliconised tubes and mixed with ETDA (final conc. 25 mM) to prevent autodigestion of MMP or TIMP. Tubes were centrifuged at 3,500 × g (30 min. 4 °C) and stored at –70 °C. After collection of the samples the tracheobronchial tree was inspected and routine diagnostic procedures were performed including bronchial brushing, needle aspiration and biopsies. Tumours were classified according to established pathological criteria.

Gelatine zymography

Substrate-specific zymography for determination of gelatinolytic activity of MMP-2 and MMP-9 was performed as previously described [15]. Briefly, based on total protein as determined by Bradford assay, samples were adjusted to equal protein content and 25 µl aliquots were mixed 1:1 with 2 × loading buffer [400 mM Tris-HCl (pH 6.8) 5% SDS, 20% glycerol, 0.006% bromphenol blue], and 25 ìl aliquots were applied onto an 8% polyacrylamide gel containing 0.4% SDS and 0.1% gelatine. Proteins were separated by electrophoresis at 25 mA constant current for 2 hrs at room temperature (RT). To remove the SDS, the gel was incubated in a solution of 2.5% Triton X-100 (1 hour), followed by incubation in enzyme buffer [50 mM Tris-HCl (pH 7.3) 200 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij-35] at 37 °C for 18 hours to activate gelatinolytic activity of MMP. Similar caseinolytic activity for MMP-1, MMP-3, and MMP-8 was assessed. Instead of gelatine, 0.4% casein was added to the polyacrylamide gel [15, 16].

Enzymatic bands were visualised by negative staining of the gel with an aqueous solution of 0.5% Coomassie brilliant blue dye (in: 50% methanol, 10% acetic acid). Gels were de-stained for 2 × 20 min. in a mixture consisting of 20% methanol, 10% acetic acid, and 70% deionised water. Bands displaying enzymatic activity were identified by comparison to pre-stained standard proteins, and to purified MMP-2 or MMP-9 protein applied to the same gel. Enzymatic activities of MMP-bands were ensured by incubating identical zymograms incubated in enzyme buffer with the addition of either 10 mM EDTA, a selective MMP inhibitor; or with 0.1 mg/ml PMSF, a serine protease inhibitor; or in 2 mM Pefabloc, an irreversible serine protease inhibitor [15, 16].

Western-blot analysis

Protein expression of the different MMP and TIMP was detected by Western-blot analysis under reducing conditions [15, 16]. Based on total protein, samples were adjusted to equal protein content and 25 µl aliquots were mixed 1:1 with 2 × loading buffer [400 mM TRIS-HCl (pH 6.8) 4% SDS, 200mM DTT, 20% glycerol, 0.0002% bromphenol blue]. Probes were boiled (5 min.) before application onto 4-15% gradient SDS-PAGE gels (BioRad, Zürich, Switzerland) and electrophoresis was performed as described earlier [15, 16]. Size-fractionated proteins were transferred onto a nitro-cellulose membrane by semi-dry electro-blotting technique (60 min., constant current 1 mA/cm^2). To prevent unspecific binding of antibodies, the membrane was blocked by incubation in 5% skimmed milk in TBS-Tween [10 mM Tris (pH 8.0), 150 mM NaCl, 0.05% Tween 20] for 1 hour at room temperature (RT), followed by overnight incubation (4 °C) with one of the specific antibodies (MMP-1 at a titre of 1:500, MMP-2, MMP-9 1:1000 each, TIMP-1, or TIMP-2 1:200). After washing with skimmed milk solution, the membrane was incubated with a second, peroxidase-coupled, antibody (1 hour, RT), then washed twice in TBS-Tween, and specific bands were visualised using the ECL system (Amersham) according to the manufacturer's instructions.

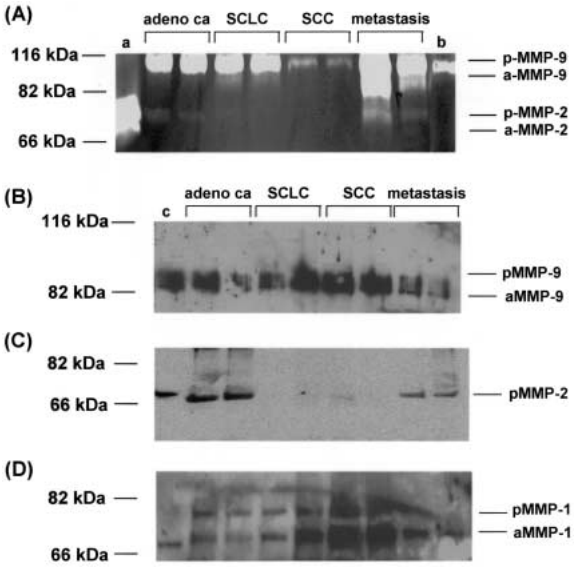
Results

116 bronchial washings obtained from 58 patients with lung tumours and ten from controls were analysed. The group investigated consisted of 18 patients with adenocarcinoma, 23 with squamous cell carcinoma, 9 with small cell carcinoma, and 8 presenting pulmonary metastases of extrapulmonary primary malignant tumours. In 15 patients (26%) with bronchial carcinoma, stage IV disease was present at the time of bronchoscopy. Exophytic endobronchial tumour growth was found in 14 patients. The group of patients with metastases of extrapulmonary primary tumours included carcinomas of the oesophagus (n = 3), breast, larynx, thyroid, kidney, and ovaries.

Enzyme activity of matrix metalloproteinases: Gelatinolytic activity of MMP-2 and MMP-9 in bronchial fluid was analysed by performing gelatine zymography. Two distinct double bands of gelatinolytic activity were detected in the investigated samples (fig. 1). The first gelatinolytic double band was identified at an approximate size of 90 kDa and a similar double band was located at the size of 70 kDa. Addition of EDTA, a selective MMP inhibitor, resulted in loss of the gelatinolytic activity, identifying the observed enzymes as gelatinases. Gelatinolytic bands were further characterised as MMP-9 (92 kDa) and MMP-2 (72 kDa) by comparison with the size of purified human MMP-2 (fig. 1, first lane) and MMP-9 (fig. 1, last lane) protein. Gelatinolytic activity at 92 kDa (MMP-9) was determined in all 126 samples. The presence of the serine protease inhibitors, Pefa-bloc and PMSF, did not affect gelatinolytic activity. PMSF and Pefa-Bloc did not influence gelatinolytic activity whereas the presence of EDTA in the SDS-polyacrylamide gel inhibits gelatinolytic activity of MMP, confirming that the gelatinolytic activity observed is due to

Figure 1

MMP activity and protein expression in bronchial washings obtained from various lung tumours.
(A) Representative gelatine zymography for two adenocarcinomas, two small-cell lung carcinomas (SCLC), two squamous-cell carcinomas (SCC) and two pulmonary metastases of extrapulmonary primary tumours. "a" indicates purified human MMP-2, "b" indicates purified human MMP-9.
(B) Western-blot analysis of the same bronchial washings analysed in panel 1 A. "c" indicates purified human MMP-9.
(C) Western-blot analysis of samples used in panel 1 A for expression of MMP-2 protein, "c" indicates migration of purified human MMP-2.
(D) Protein expression analysis by Western blotting for MMP-1 in the same samples as shown in panel 1 A. "c" indicates the purified human MMP-1.



MMP activity (data not shown). Enzymatic activity based on the digestion of casein, a specific substrate for MMP-1, MMP-3, and MMP-8, indicated the presence of MMP-1 in all 126 samples. MMP-1 was characterised by comparison with the purifed MMP-1 and with its size on the casein PAGE at 55 kDa. Similar results were obtained by Western blot analysis, as demonstrated in Figure 1 C. With regard to MMP-9, the activity of MMP-1 correlated neither with the tumour type nor with the stage or location of the tumour (figure 1 C). A summary of computer-assisted densitometric analysis of MMP-2 and MMP-9 activity is presented in table 1. MMP-3 and MMP-8 could not be detected in any of the samples (data not shown).

Representative gelatine zymographies for the investigated types of lung tumours, including metastases, are presented in figures 1 and 2. Tumour-specific examples of the zymographic activity of MMP-2 and MMP-9 are presented in figure 1 A for each of the lung tumour types investigated: adenocarcinoma, small-cell lung carcinoma, squamous-cell carcinoma, and lung metastases of other primary tumours. In figure 1 B-D we analysed the identical samples, presented as zymographies, by Western blotting and confirmed the data. We identified the precursor of MMP-9 and active MMP-9, which was observed in all investigated washings (figure 1 A, 1 B).

In contrast, MMP-2 was observed only in trace amounts in washings obtained from patients with adenocarcinoma or with pulmonary metastases of other primary tumours (figure 1 A, 1 C). No MMP-2 was observed in washings obtained from controls. Both methods revealed that MMP-2 protein was present in the 36 samples obtained from the 18 patients with adenocarcinoma and in all samples but one from patients with pulmonary metastases (n = 13), as shown in figure 2. Most MMP-2 positive samples contained only the inac-

Table 1

Densitometric analysis of gelatine zymograms grouped by lung tumour types. The optical density of MMP bands was quantified defining the optical density generated by zymography of 1 ng/ml purified human MMP-2 or MMP-9 as one standard unit. The data represent the mean ± S.E. of the respective number of zymograms. Active MMP-2 was not detectable in any of the bronchial washings investigated.

	adenocarcinoma	small-cell lung carcinoma	squamous cell carcinoma	lung metastases	control
Pro-MMP-2	3.6 ± 0.9	0.7 ± 0.004	0.02 ± 0.008	2.8 ± 0.6	0.09 ± 0.002
Active MMP-2	0.06 ± 0.003	nd	nd	0.1 ± 0.02	nd
Pro MMP-9	14.6 ± 3.7	12.2 ± 3.7	11.9 ± 2.2	16.5 ± 4.9	13.8 ± 3.6
Active MMP-9	26.5 ± 9.7	26.1 ± 7.4	18.7 ± 4.1	1.4. ± 0.06	3.1 ± 1.2

Figure 2

Representative gelatine zymography for six characteristic bronchial washings obtained from patients with extrapulmonary primary tumours.

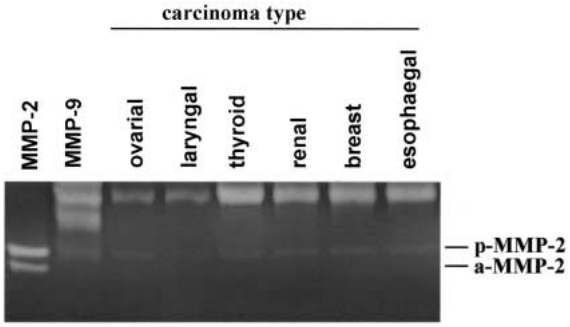
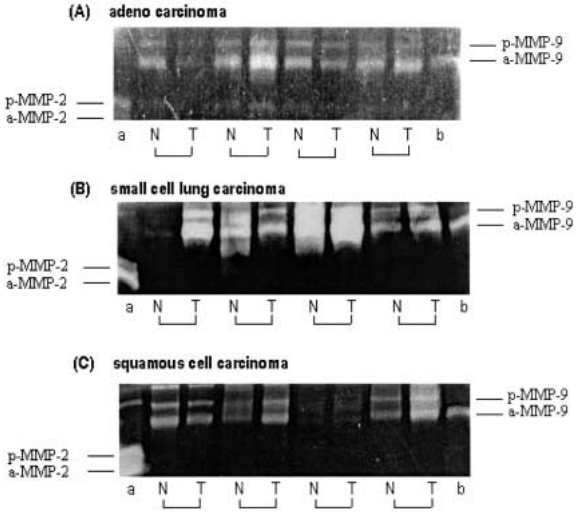


Figure 3

Comparison of MMP activity in tumour-free and tumour-affected lung. "T" indicates tumour-affected site, "N" indicates tumour-free lung site, "a" indicates purified human MMP-2, "b" indicates purified human MMP-9. (A) representative zymography for four adenocarcinomas, (B) representative zymography for four small-cell lung carcinomas, and (C) representative zymography for four squamous-cell lung carcinomas.



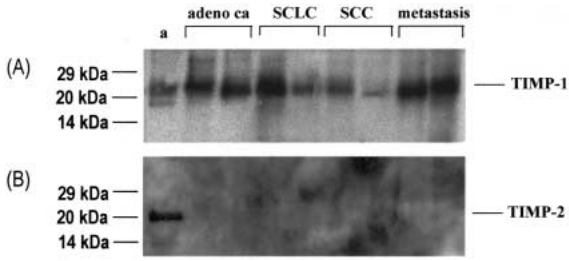


Figure 4

Western blot analysis for expression of TIMP-1 (panel A) and TIMP-2 (panel B) in bronchial washings obtained from the same samples analysed for MMP activity as presented in panel 1 A. Recombinant human TIMP-1 or TIMP-2 are presented in lane "a".

tive precursor of MMP-2. The finding of the specific expression of MMP-2 in bronchial washings obtained from patients with pulmonary adenocarcinoma or lung metastases could be confirmed at the protein level by Western blotting (figure 1 C).

MMP-1 expression was determined by casein zymography (data not shown) and by Western blotting (figure 1 D). Similarly to MMP-9, MMP-1 was detected in all samples investigated, with no obvious differences of expression when comparing the different tumour types and also in controls (figure 1 D). Neither of the other casein-digesting MMPs, MMP-3 and MMP-8, was detected either by zymography or by Western blotting.

To assess whether the expression of MMP correlates with the location of the tumour, bronchial washings were performed on both lung sites. To rule out false positive results, the disease-free lung was washed first. Comparing the enzymatic activity of MMP-9 in bronchial fluid obtained from the tumour site of the lung ("T") with that of the tumour-free lung site ("N") in the same patient, to our surprise we observed no specific expression pattern in the tumour-affected lung site, either for MMP-2, MMP-1 or MMP-9. In figure 3 we present typical gelatine zymographies for MMP-2 and MMP-9 for bronchial washings obtained from four adenocarcinomas (figure 3 A), four small-cell lung carcinomas (figure 3 B) and four patients with squamous-cell carcinoma (figure 3 C). MMP-2 bands are visible only in the patients with adenocarcinoma (figure 3 A) and in patients with metastasis of extrapulmonary tumours (data not shown), but are missing in the other two groups (figure 3 B, 3 C). However, we did not detect overexpression of MMP-2 in the tumour-affected lung site (figure 3). Both forms of MMP-9 were expressed in all 116 samples and no specific expression pattern could be observed comparing the tumour-free lung with the tumour-affected lung site (figure 3).

To assess a possible link between TIMP expression and tumour type or tumour stage, and in view of their inhibitory role in tumour genesis, we analysed all bronchial washings for the presence of TIMP-1 and TIMP-2 by Western-blot analysis and by enzyme-linked immunosorbent assay. TIMP-1 was expressed in all 116 samples (fig. 4 A). However, the expression pattern of TIMP-1 could be correlated neither to tumour type nor tumour staging, nor could it be related to tumour location. Comparison of the secreted amounts of TIMP-1 protein in the bronchial washings with the protein amount of its corresponding targets, MMP-1 or MMP-9 showed no correlation to tumour type, tumour location or tumour staging. In contrast, TIMP-2 protein could not be detected in any of the 116 bronchial washings (fig. 4 B). The data obtained by Western blotting was confirmed by the respective enzyme-linked immunosorbent assays for the presence of TIMP-1 or TIMP-2 (data not shown).

Discussion

Investigation of the expression pattern of MMP and TIMP in bronchial washings of patients with pulmonary malignancies revealed high levels of endobronchial inactive MMP-2 precursor and the abundance of its respective inhibitor, TIMP-2, exclusively in adenocarcinoma and patients with lung metastases of extrapulmonary tumours. In contrast, MMP-1, MMP-9 and TIMP-1 were constitutively expressed irrespective of tumour type and in controls. MMP-3 and MMP-8 were not detected in bronchial washings. A similar expression of all detectable MMP and TIMP was observed in the tumour-affected and the tumour-free lung site.

17 human MMP have been identified by cloning and sequencing. MMP are proteinases characterised by a common core domain structure which interact with specific proteinase inhibitors, TIMP. Four different TIMP have been described. TIMP inhibit MMP in a ratio of 1:1 by noncovalent binding capping the active site of the MMP [5, 7, 9]. A shift in the MMP:TIMP ratio towards MMP expression facilitates cell invasion through the basement membrane. This mechanism is used by both normal cells and tumour cells to infiltrate tissue [17, 18]. Specifically, MMP-2 appears to be linked to an invasive tumour phenotype [18 - 24], and activation of MMP-2 is a key event in the acquisition of malignant potential [25]. Analysing the expression and activity of MMP and TIMP in pleural effusions, we reported earlier that the MMP-9: MMP-2 ratio in paraneoplastic pleural

effusions was greater than all others. We therefore concluded that the relative activity of MMP-2 is essential for its action [15]. This observation and the data presented in this study support the hypothesis of compartment-specific expression of MMP and TIMP as an important factor in tumour progression. In general, an altered MMP:TIMP balance plays a central role in tumour progression.

This hypothesis was underlined by recent data showing the combination of an overexpression of MMP-2 and low levels of its inhibitor TIMP-2 in various tumour types. This specific pattern of high MMP-2 and low TIMP-2 has been demonstrated in tumours of varying origin: breast [9], bladder [19], cervix [20], liver [21], brain [22, 23], oropharynx [24], and pancreas [18, 21]. High MMP-2 and low TIMP-2 were also associated with low patient survival. In accordance with our findings, the presence of MMP-9 was not correlated with tumour stage [22]. MMP-2, rather than MMP-9, may therefore facilitate malignant cell invasion and metastases.

Our data for adenocarcinoma- and lung metastases-specific expression of the MMP-2 precursor, and no expression of TIMP-2, suggest that these patients have more aggressive tumour progression and a lower survival rate and are in agreement with the current literature. However, the question how the MMP-2 precursor may be activated *in vivo* remains unanswered. Few studies have investigated the role of MMP and TIMP in lung carcinoma. Elevated mRNA levels of MMP-2 and collagen IV have been described in tissue samples of patients with squamous-cell carcinoma associated with disruption of basement membranes around the tumour. *In situ* hybridisation localised MMP-2, MMP-9 and TIMP-1 expression in tumour cells, stromal fibroblasts and endothelial cells [26]. These findings suggest that the production of MMP is not restricted to the tumour itself. In a histopathological study investigating adenocarcinomas of the lung, 41% of tissue sections stained positive for MMP-2 in the tumour cells, especially in poorly differentiated tumours. Patients with MMP-2 immunoreactivities showed poorer survival that those with an MMP-2 negative tumour [27]. An inverse correlation of patient survival with high MMP-2 expression has also been described in patients with melanoma [28] and neuroblastoma [22, 23]. MMP-2 was expressed in stromal and tumour tissue and associated with advanced clinical stages. It should be noted that immunohistochemical studies provide insight into the localisation of a protein only, but cannot differentiate between active and inactive or complexed MMP-2.

To identify the lung site of MMP and TIMP synthesis, we compared the expression of MMP in both the tumour-affected and the healthy lung site in each patient. The expression and activity of MMP-2 was similar at both lung sites, as confirmed by two independent methods, zymography and Western blotting. There are various possible explanations for this finding: (i) contamination of samples during bronchoscopy, (ii) exchange of bronchial secretions between the two lung sites, (iii) stimulation of MMP-2 by adenocarcinomas and metastases in all parts of the lung, and (iv) a pre-existing imbalance of the MMP-2:TIMP-2 ratio in patients which predisposes them to development of adenocarcinoma or metastases. Contamination during the bronchoscopic procedure is unlikely. Bronchial washings were always performed on the healthy lung site first and afterwards on the tumour-affected site. Furthermore, MMP-2 was documented in both lung sites of all patients with adenocarcinoma or metastases, and contamination would be expected to occur only occasionally. Based on cytology, malignant cells are rarely found in the tumour-free lung. Hence a massive exchange of epithelial lining fluid including MMP-2 is also unlikely. Adenocarcinoma and lung metastases may produce a stimulator for MMP-2 synthesis, resulting in a general increase of MMP-2 expression in the lung. This hypothesis is supported by the observation of MMP-2 expression in non-tumour cells surrounding the tumour [30-32]. In colorectal cancer, for example, increased levels of pro-enzyme and active enzyme forms of MMP-2 have been localised in regions of tumour invasion only [30]. Similar findings have been described in metastatic breast cancer expressing high MMP-2 mRNA levels in stromal fibroblasts [31]. There is further evidence of a tumour-associated general up-regulation of MMP-2. Elevated serum MMP-2 levels have been reported in patients with lung cancer metastases [1], gastric cancer [33], and prostate cancer [34]. Furthermore, a persistent imbalance of MMP-2 and TIMP-2 in the serum of patients suffering from urothelial cancer has been found to be correlated with advanced and recurrent disease [35]. In summary, there is evidence of a tumour-dependent enhanced systemic MMP-2:TIMP-2 ratio in several tumour types, rather than genetic pre-existence of the MMP-2:TIMP-2 imbalance observed.

In conclusion, in bronchial washings of patients with lung adenocarcinoma and pulmonary metastases MMP-2 is enhanced, while TIMP-2 is absent. The MMP-2:TIMP-2 imbalance may thus be used as a marker for differential diagnosis and prognosis in lung malignancies. In regard to therapy, the data support the idea of using local administration of TIMP-2 or synthetic MMP-2 inhibitors as a new therapeutic strategy in adenocarcinoma.

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References

1 Garbisa S, Scagliotti G, Masiero L, et al. Correlation of serum metalloproteinase levels with lung cancer metastasis and response to therapy. Cancer Res 1992;52:4548–9.
2 Crawford HC, Matrisian LM. Tumour and stromal expression of matrix metalloproteinases and their role in tumour progression. Invasion Metastasis 1994;14:234–45.
3 Himelstein BOP, Canete-Soler R, Bernhard EJ, et al. Metalloproteinases in tumour progression: The contribution of MMP-9. Invasion Metastasis 1994;14:246–58.
4 Rouyer N, Wolf C, Chenard MP, et al. Stromelysin-3 gene expression in human cancer: An overview. Invasion Metastasis 1994;14:269–75.
5 Finlay G, K. Russell KJ, McMahon K, et al. Elevated levels of matrix metalloproteinases in bronchoalveolar lavage fluid of emphysematous patients. Thorax 1997;57:502–6.
6 Kleiner DE, and Stetler-Stevenson WG. Matrix metalloproteinases and metastasis. Cancer Chemothe. Pharmacol 1999;43 (Suppl.):S42–S51.
7 Ray JM, and Stetler-Stevenson WG. The role of matrix metalloproteinases and their inhibitors in tumour invasion, metastasis and angiogenesis. Eur Respir J 1994;7:2062–72.
8 Murphy G, and Knäuper V. Relating the matrix metalloproteinase structure to function: Why the "hemopexin" domain? Matrix Biology 1997;15:511–8.
9 Onisto M, Riccio MP, Scannapieco P, et al. Gelatinase A/TIMP-2 imbalance in lymph-node-positive breast carcinomas, as measured by RT-PCR. Int J Cancer 1995;63:621–6.
10 Benbow U, and Brinckerhoff CE. The AP-1 site and MMP gene regulation: What is all the fuss about? Matrix Biology 1997;15: 519–26.
11 Dunsmore SE and Rannels DE. Extracellular matrix biology in the lung. Am J Physiol 1996;270:L3–L27.
12 Laurent GF. Dynamic state of collagen: pathways of collagen degradation in vivo and their possible role in the regulation of collagen mass. Am J Physiol 1987;252:C1–C9.
13 O'Connor CM and FitzGerald MX. Matrix metalloproteinases and lung disaeses. Thorax 1994;49:602–9.
14 Woessner JF. Matrix metalloproteinases and their inhibitors in connective tissue remodelling. FASEB J 1991;5:2145–54.
15 Eickelberg O, Sommerfeld CO, Wyser C, et al. MMP and TIMP expression pattern in pleural effusion of different origins. Am J Respir Crit Care Med 1997;156:1987–92.
16 Eickelberg O, Koehler E, Reichenberger F, et al. Extracellular matrix deposition by primary human lung fibroblasts in response to TGF-β1 and TGF-β3. Am J Physiol 1999;276: L814–24.
17 Papakonstantinou E, Karakiuloakis G, Eickelberg O, et al. A 340 kDa hyaluronic acid secreted by human vascular smooth muscle cells regulates their proliferation and migration. Glycobiology 1998;8:821–30.
18 Koshiba T, Hosotani R, Wada M, et al. Involvement of matrix metalloproteinase-2 activity in invasion and metastasis of pancreatic carcinoma. Cancer 1998;82:642–50.
19 Gohji K, Fujimoto N, Fujii A, et al. Prognostic significance of circulating matrix metalloproteinase-2 to tissue inhibitor of metalloproteinases-2 ratio in recurrence of urothelial cancer after complete resection. Cancer Res 1996;56:3196–8.
20 Davidson B, Goldberg L, Kopolovic J, et al. MMP-2 and TIMP-2 expression correlates with poor prognosis in cervical carcinoma – a clinicopathologic study using immunohistochemistry and mRNA in situ hybridization. Gynecol Oncol 1999;73:372–82.
21 Musso O, Théret N, Campion JP, et al. In situ detection of matrix metalloproteinase-2 (MMP2) and the metalloproteinase inhibitor TIMP2 transcripts in human primary hepatocellular carcinoma and in liver metastasis. J Hepatol 1997;26:593–605.
22 Ara T, Fukuzawa M, Kusafuka T, et al. Immunohistochemical expression of MMP-2, MMP-9, and TIMP-2 in neuroblastoma: association with tumor progression and clinical outcome. J Pediatr Surg 1998;33:L1272–8.
23 Sugiura Y, Shimada H, Seeger RC, et al. Matrix metalloproteinases-2 and -9 are expressed in human neuroblastoma: contribution of stromal cells to their production and correlation with metastasis. Cancer Res 1998;58:2209–16.
24 Sutinen M., Kainulainen T, Hurskainen T, et al. Expression of matrix metalloproteinases (MMP-1 and -2) and their inhibitors (TIMP-1, -2 and -3) in oral lichen planus, dysplasia, squamous cell carcinoma and lymph node metastasis. Br J Cancer 1998;77: 2239–45.
25 Stetler-Stevenson WG. Progelatinase A activation during tumor cell invasion. Invasion Metastasis 1994;14:259–68.
26 Nakagawa H, and Yagihashi S. Expression of type IV collagen and its degradating enzymes in squamous cell carcinoma of lung. Jpn J Cancer Res 1994;85:934–8.
27 Kodate K, Kasai T, Hashimoto H, et al. Expression of matrix metalloproteinase (gelatinase) in T1 adenocarcinoma of the lung. Pathol Int 1997;47:461–9.
28 Väisänen A, Kallioinen M, Taskinen PJ, and Turpeenniemi-Hujanen T. Prognostic value of MMP-2 immunoreactive protein (72 kD type IV collagenase) in primary skin melanoma. J Pathol 1998;186:51–8.
29 Prontera C, Mariani B, Rossi C, et al. Inhibition of gelatinase A (MMP-2) by batimastat and captopril reduces tumor growth and lung metastases in mice bearing Lewis lung carcinoma. Int J Cancer 1999;81:761–6.
30 Emmert-Buck MR, Roth MJ, Zhang Z, et al. Increased gelatinase A (MMP-2) and cathepsin B activity in invasive tumor regions of human colon cancer samples. Am J Pathol 1994; 145:1285–90.
31 Gilles C, Polette M, Seiki M,1 et al. Implication of collagen type I-induced membrane-type 1-matrix metalloproteinase expression and matrix metalloproteinase-2 activation in the metastatic progression of breast carcinoma. Lab Invest 1997;76:651–60.
32 Boyd RS, and Balkwili FR. MMP-2 release and activation in ovarian carcinoma: the role of fibroblasts. Br J Cancer 1999;80:315–21.
33 Endo K, Maehara Y, Baba H, et al. Elevated levels of serum and plasma metalloproteinases in patients with gastric cancer. Anticancer Res 1997;17:2253–8.
34 Gohji K, Fukjimoto N, Hara I, et al. Serum matrix metalloproteinase-2 and its density in men with prostate cancer as a new predictor of disease extension. Int J Cancer Res 1998;79: 96–101.
35 Gohji K, Fujimoto N, Ohkawa J, et al. Imbalance between serum matrix metalloproteinase-2 and its inhibitor as a predictor of recurrence of urothelial cancer. Br J Cancer Res 1998; 77:650–5.

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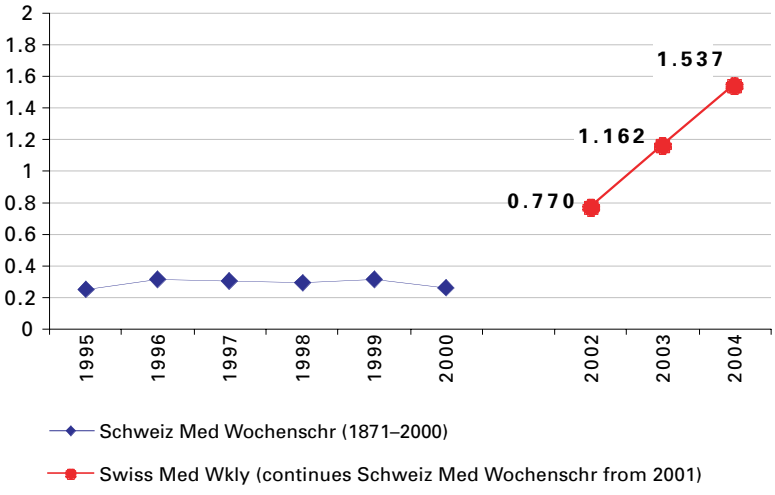

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