Elevated Serum Levels of Type I Collagen Degradation Marker ICTP and Tissue Inhibitor of Metalloproteinase (TIMP) 1 Are Associated with Poor Prognosis in Lung Cancer

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ABSTRACT

Purpose: **The aim of this study was to evaluate the correlation between type I collagen degradation marker ICTP, MMP (matrix metalloproteinase)-9, and tissue inhibitor of metalloproteinase (TIMP)-1 and to compare their value as prognostic factors in lung cancer.**

Experimental Design: **From the sera of 141 lung cancer patients, we assessed markers of type I collagen synthesis (PINP and PICP) and degradation (ICTP) by radioimmunoassays, and we assessed MMP-9 and its tissue inhibitor TIMP-1 by ELISA. There were 62 squamous cell carcinomas, 42 adenocarcinomas, 14 small cell carcinomas, and 23 cases with other histology. Seventeen of these patients had advanced disease. Sixty-seven patients had been operated on, 33 had received radiation therapy, 7 had received chemotherapy, and the rest had received other treatment combinations.**

Results: **We examined the relationship between these markers and found a correlation between ICTP and MMP-9 ($r = 0.201$; $P = 0.01$) or TIMP-1 ($r = 0.415$; $P = 0.00$). Elevated serum concentrations of ICTP (>5 μg/liter) and/or TIMP-1 (>300 ng/ml) correlated with poor prognosis. In univariate regression analysis, ICTP had prognostic value (odds ratio, 1.6462; $P < 0.03$): the patients with elevated serum concentrations of ICTP (>5 μg/liter) had a 64% higher risk of dying from lung cancer than did patients with opposite values.**

Conclusions: **Our results indicated that ICTP and TIMP-1 are good prognostic markers in lung cancer. The association between serum MMP-9 and ICTP suggests that MMP-9 could play a role in the degradation of the extracellular matrix producing ICTP in this pathological situation.**

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INTRODUCTION

Type I collagen is the major extracellular matrix component in skin, soft tissues, and bone, where it forms about 90% of the organic matrix. The NH_2-terminal (PINP) and the COOH-terminal (PICP) propeptides are cleaved off before fibril formation; their concentrations in blood directly indicate ongoing type I collagen synthesis. The COOH-terminal telopeptide of ICTP is released into the circulation when collagen fibers are degraded. Serum markers of type I collagen metabolism have previously been investigated as indicators of bone metastases in breast (1), prostate (2), and lung cancer (3) and as a factor for poor prognosis in ovarian (4) and lung cancer (5).

Tumor invasion is associated with degradation of the extracellular matrix and basement membranes, in a process in which MMPs[2] and TIMPs are strongly involved (6). Both MMPs and TIMPs can be overexpressed in malignant cells (7). In previous studies, MMP-9 has been associated with poor prognosis in lung cancer (8–10). TIMP-1 also correlates with poor prognosis in resected non-small cell lung carcinoma (11).

Expression of MMP-9 has been found in osteoclasts, and it has been suggested that MMP-9 may play a role in normal bone remodeling and especially in pathological bone resorption in human diseases (12). In periodontitis, an inhibitor of MMP reduces the concentration of ICTP and the activity of MMP-8 and MMP-13 simultaneously (13). The aim of our study was to examine, whether there was any correlation between type I collagen degradation, MMP-9, and TIMP-1 in lung cancer and to compare their value as prognostic factors.

PATIENTS AND METHODS

Patients. Serum samples were taken from 141 lung cancer patients treated from 1990–1991 and from 1996–1998 in Oulu University Hospital (Oulu, Finland). The samples were taken during the first visit before the treatment. The samples were stored at −70°C until use. The basic clinical data are shown in Table 1.

Immunoassays. The PICP, intact PINP, and ICTP concentrations were measured by competitive equilibrium radioimmunoassays with reagents supplied by Orion Diagnostica (Oulunsalo, Finland). The assays are based on human antigens, and the polyclonal antibodies have been raised in rabbits. Serum duplicate (100 μl) was used for the assay of PICP, 50 μl of serum duplicate were used for the assay of intact PINP, and 100 μl of serum duplicate were used for the ICTP assays. The intra- and interassay coefficients of variation were around 5% for all these assays at the concentrations obtained here.

[2] The abbreviations used are: MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

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Table 1 The basic features of the lung carcinoma patients ($n = 141$)

Age (yrs)	65 (range, 38–85)
Sex	13 women and 128 men
Follow-up (mo)	22 (range, 0.5–90)
Histology	64 squamous cell carcinomas
	39 adenocarcinomas
	11 small cell carcinomas
	23 with other histology
	4 had missing information
Status of the disease	
	37 stage I
	15 stage II
	41 stage III
	34 stage IV (5 had only bone metastases, 19 had only soft tissue metastases, 8 both bone and soft tissue metastases, and 2 had missing information)
	14 had missing information
Treatments	
	46 treated with surgery (31 lobectomy/bilobectomy and 15 pneumonectomy)
	28 treated with surgery and radiation therapy
	43 treated with radiation therapy
	3 treated with cytostatic treatment
	5 treated with radiation therapy and cytostatic treatment
	9 treated with other treatment modalities
	7 had missing information

ELISA Quantitation of MMP-9 and TIMP-1. ELISAs were performed using standard protocols, as described previously (10). For the detection of MMP-9 and TIMP-1, the total protein was measured, *i.e.*, the protocol to quantitate the free and complex forms was used simultaneously. The monoclonal antibody against MMP-9 (code GE-213) recognizes both the free enzyme and that bound to its inhibitor, TIMP-1 (14). The monoclonal antibody against TIMP-1 (code DB-102D1) recognizes both the free TIMP-1 and that in complex with MMP-9. It does not cross-react with TIMP-2 (data not shown).

Statistical Analyses. Student's *t* test or the Mann-Whitney *U* test was used to compare the means and distributions. The overall survival rates were determined by the Kaplan-Meier method, and the differences between groups were analyzed by using the log-rank, Breslow, or Tarone-Ware analysis. Cox regression analysis was used to find significant predictors of survival. The correlations were calculated and analyzed by the Spearman method. The results are expressed as either mean ± SD or median (95% confidence interval).

RESULTS

There were no differences in the serum levels of these markers in different stages of lung cancer. The mean levels in the whole material ($n = 141$) were as follows: (*a*) ICTP, 4.6 ± 3.1 μg/liter; (*b*) PINP, 39 ± 28 μg/liter; (*c*) PICP, 103.8 ± 38.2 μg/liter; (*d*) MMP-9, 128 ± 103 ng/ml; and (*e*) TIMP-1, 354 ± 192 ng/ml. The serum level of MMP-9 was lower in small cell lung cancer patients (62 ± 42 ng/ml) than in patients in the other histological groups (squamous cell carcinoma, 141 ± 101 ng/ml, $P < 0.01$; adenocarcinoma, 123 ± 116 ng/ml, $P = 0.063$).

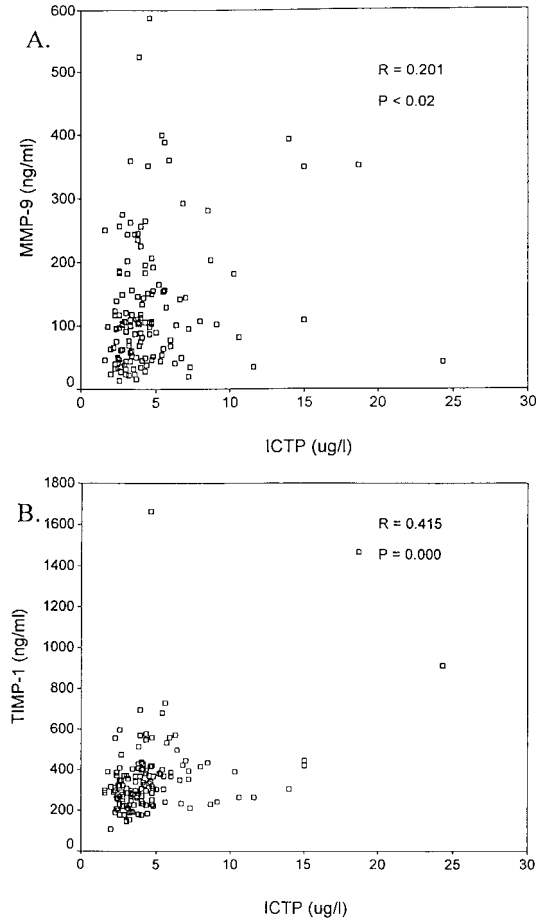

Fig. 1 The correlation between the serum values of ICTP and MMP-9 ($r = 0.201$; $P < 0.02$; *A*) and ICTP and TIMP-1 ($r = 0.415$; $P = 0.000$; *B*) in lung cancer patients ($n = 141$).

The serum levels of TIMP-1, ICTP, PINP, and PICP did not vary in different histopathological groups.

Significant correlations were found between the serum levels of MMP-9 and ICTP ($r = 0.201$; $P = 0.017$; Fig. 1*A*; Table 2) and between the serum levels of TIMP-1 and ICTP ($r = 0.415$; $P = 0.000$; Fig. 1*B*; Table 2). ICTP, PICP, and PINP correlated significantly between each other as well as MMP-9 and TIMP-1 (Table 2).

The patients with serum levels of TIMP-1 of >300 ng/ml had unfavorable prognosis when compared with the patients with serum levels of TIMP-1 of ≤300 ng/ml (1-year survival, 48% *versus* 67%; 5-year survival, 32% *versus* 40%; $P < 0.02$; Fig. 2*A*). The survival difference in Kaplan-Meier analysis by this cutoff value was seen in all histopathological subgroups, although it was significant only in the adenocarcinoma group. However, the reduction of the number of patients in subgroups prevented the statistical analyses (Table 3). A difference was also seen in different stages of the disease, mainly in stage III disease (Table 3). There were only two patients with stage IV disease, one had serum TIMP-1 ≤ 300 ng/ml, and the other had serum TIMP-1 > 300 ng/ml. They both died within a year.

The patients with serum ICTP > 5 μg/liter had an unfa-

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Table 2 The correlations between the serum values of MMP-9, TIMP-1, ICTP, PINP, and PICP ($n = 141$)

Variable	MMP-9	TIMP-1	ICTP	PINP	PICP
MMP-9					
rS[a]	1.000	0.299	0.201	−0.057	−0.152
P		0.000	0.017	NS[b]	0.078
TIMP-1					
rS		1.000	0.415	0.045	0.017
P			0.000	NS[b]	NS
ICTP					
rS			1.000	0.469	0.247
P				0.000	0.004
PINP					
rS				1.000	0.637
P					0.000
PICP					
rS					1.000
P					

[a] rS, correlation coefficient.
[b] NS, nonsignificant.

vorable prognosis when compared with the patients with ICTP ≤ 5 μg/liter (1-year survival, 42% *versus* 64%; 5-year survival, 37% *versus* 39%; $P = 0.0587$). A difference in survival was seen in different histopathological groups, although it did not reach statistical significance (Table 3). The difference in survival was seen in some stages of the disease and was significant in stage IIIB disease (Table 3). Only two patients had stage IV disease, and they both had serum ICTP > 5 μg/liter. They both died within a year.

The patients with serum TIMP-1 > 300 ng/ml and ICTP > 5 μg/liter had a 1-year survival rate of 38% and a 5-year survival rate of 33%, whereas the patients with serum TIMP-1 ≤ 300 ng/ml and ICTP ≤ 5 μg/liter had a 1-year survival rate of 69% and a 5-year survival rate of 40% (Fig. 2*C*). The difference was statistically significant ($P < 0.02$). This difference was also seen in different histopathological groups and stages and was significant in squamous cell carcinoma and adenocarcinoma (Table 3). MMP-9, PINP, and PICP did not have any effect on survival in this material.

In univariate Cox regression analysis models, only ICTP had prognostic value. When the serum value of ICTP was >5 μg/liter, the risk of dying was 64% higher than it was when the serum value of ICTP was ≤5 μg/liter (odds ratio, 1.65; 95% confidence interval, 1.08–2.52; $P = 0.0215$). When ICTP was analyzed together with stage (odds ratio, 2.02; 95% confidence interval, 1.53–2.70; $P = 0.0000$), this marker lost its significance. In univariate regression analysis, no significant correlation with survival was found when MMP-9, TIMP-1, PINP, PICP, or TIMP-1 was analyzed. Also, when ICTP and TIMP-1 were grouped together (group 1, ICTP ≤ 5 μg/liter and TIMP-1 ≤ 300 ng/ml; group 2, ICTP > 5 μg/liter and TIMP-1 > 300 ng/ml), there was no prognostic effect on survival in univariate regression analysis. It was not possible to analyze them together in the same multivariate regression analysis model because of the high correlation between them.

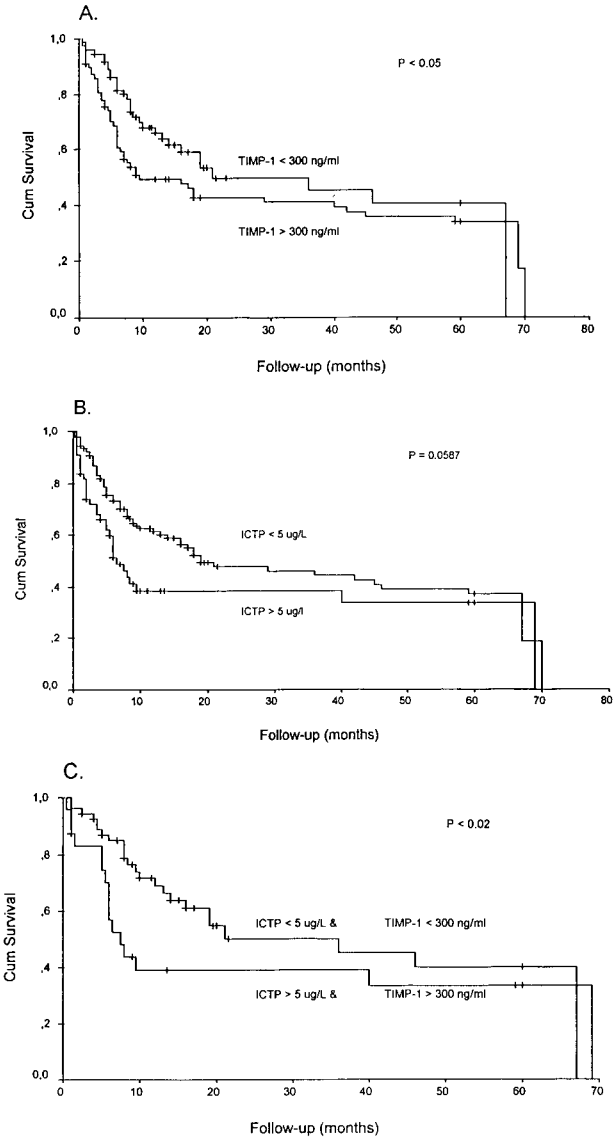

Fig. 2 Kaplan-Meier survival curves for lung cancer patients ($n = 141$) according to concentrations of serum TIMP-1 (*A*), ICTP (*B*), and both TIMP-1 and ICTP (*C*).

DISCUSSION

In our study, MMP-9 and TIMP-1 correlated significantly with ICTP, the degradation marker of type I collagen. It is likely that MMP-9 is functional in the degradation of type I collagen in lung cancer patients. MMP-9 has been found to degrade type I, IV, V, VII, and X collagen (15). Expression of MMP-9 has also been detected in osteoclasts, suggesting that MMP-9 plays a role in bone resorption and is also involved in tissue destruction in various pathophysiological conditions (16). Type I collagen in bone is also degraded by cathepsin K during physiological bone resorption, and this end product cannot be recognized by the ICTP assay (16).

The major enzymes responsible for the collagen turnover in soft tissue are the various MMPs (6). The degradation products

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Table 3 Effect of serum TIMP and ICTP on 1-year (1-y) and 5-year (5-y) survival (%) in lung cancer patients ($n = 141$) according to different histopathological diagnoses or stages

	TIMP1 ≤ 300 ng/ml	TIMP-1 > 300 ng/ml	ICTP ≤ 5 μg/liter	ICTP > 5 μg/liter	ICTP ≤ 5 μg/liter and TIMP1 ≤ 300 ng/ml	ICTP > 5 μg/liter and TIMP-1 > 300 ng/ml
Squamous cell carcinoma						
n	26	33	47	12	24	10
1-y	79	61	67	54	81	64[a]
5-y	47	45	42	45	44	51
Adenocarcinoma						
n	18	20	29	9	16	7
1-y	79	44[a]	63	22	65	14[a]
5-y	27	31	39	22	28	14
Small cell carcinoma						
n	5	6	10	1	5	1
1-y	53	44	54	0	53	0
5-y	53	44	54	0		
Stage I						
n	19	15	33	7	13	4
1-y	83	83	84	80	82	75
5-y	58	53	80	52	51	75
Stage II						
n	11	5	11	9	4	6
1-y	90	75	89	83	75	83
5-y	75	56	63	62	75	62
Stage IIIA						
n	20	14	34	10	16	4
1-y	73	30[a]	60	42	73	0
5-y	17	20	42	26	17	0
Stage IIIB						
n	11	19	38	22	10	7
1-y	38	8	28	0[a]	42	0
5-y	25	8	28	0	28	0
All						
n	71	76	102	31	54	24
1-y	67	48	64	42[a]	69	38
5-y	40	32	39	37	40	33

[a] $P < 0.05$.

formed in this process are detectable in the ICTP assay (16). Thus, ICTP also measures the degradation of mature soft tissue type I collagen, although the concentration of ICTP also increases in pathological bone degradation (1–3). The human ICTP assay detects only relatively large degradation products of type I collagen that contain two copies of a phenylalanine-rich region located between the triple helical domain and the lysine-derived cross-link (16). This determinant is specific for the mature, trivalently cross-linked collagen, and its content is low in malignant tissue (17, 18). In our study, the excess ICTP in the serum of patients was a partial cause of bone metastases in patients with advanced disease, but it must have originated mainly from enhanced breakdown of type I collagen in the extracellular matrix of soft tissue around the tumor. These kinds of results have been described previously in ovarian cancer (19, 20).

ICTP has previously been investigated as a factor for poor prognosis in ovarian and lung cancer (4, 10, 18) and as a factor for advanced disease in breast cancer (21). In ovarian cancer, serum ICTP remained the only significant prognostic indicator of overall survival together with stage (18). The invasive growth and prognosis in ovarian cancer have been linked to MMPs (22, 23).

TIMP-1, a natural inhibitor of MMP enzymes, inhibits *in vitro* and *in vivo* tumor cell invasion (24). TIMP-1 is capable of binding to a number of members of the metalloproteinase family. TIMP-1 has also been shown to act as a metastasis suppressor gene (25). However, the expression of TIMPs is coordinated with that of MMPs. Thus, the activated MMPs are locally inhibited rapidly by TIMPs, and the degradation of collagens is tightly regulated (26). Thus, the correlation between a degradation marker (*e.g.*, ICTP) and TIMP should be positive, such as that seen between MMP-9 and TIMP-1. If the correlation had been negative, it would have indicated that increased degradation is a failure of inhibition, which is not likely. Also, TIMP-1 RNA expression or serum TIMP-1 has been shown to be of prognostic value in lung cancer (11).

In our present study, elevated serum TIMP-1 and ICTP concentrations were associated with shorter survival, and of the two, ICTP was the stronger prognostic factor. This information could serve as an extra tool for clinical decision-making when selecting patients for adjuvant or neoadjuvant treatments for lung cancer. In stage IIIA disease, patients who had elevated values of these markers had a shorter survival than did the patients with low values of these markers. This indicates that these markers could be a possible object for studies on combin-

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ing cytostatic treatment or radiation therapy with operation. Our material was too small in these subgroups to allow a more detailed analysis; thus, this area should be further investigated with larger numbers of patients.

Our results clearly indicate that ICTP and TIMP-1 are valuable markers for predicting poor prognosis and survival in lung cancer patients. MMP-9 is likely to be associated with the degradation of type I collagen in the extracellular matrix producing ICTP fragments.

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