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Progression of non-small cell lung cancer: Diagnostic and prognostic utility of matrix metalloproteinase-2, C-reactive protein and serum amyloid A

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Abstract. Matrix metalloproteinase-2 (MMP-2) is known to degrade type IV collagen, which is a major component of the cellular basement membrane, and to be involved in the invasion and metastasis of cancer cells. On the other hand, C-reactive protein (CRP) and serum amyloid A (SAA) are acute inflammatory biomarkers that increase in various conditions including infection, inflammation, malignancy and tissue disturbance. In the present study, we examined the serum levels of MMP-2, CRP and SAA in patients with localized and metastatic non-small cell lung cancer (NSCLC) to establish the clinical significance and changes in these biomarkers during NSCLC progression. In this study, 24 NSCLC patients were diagnosed at the Kitasato University Hospital and compared with 13 healthy controls. Measurement of MMP-2 levels in serum was determined by measuring pro-MMP-2 using a one-step sandwich enzyme immunoassay. CRP and SAA levels in the serum were measured by latex nephelometry. The serum levels of MMP-2, CRP and SAA in metastatic NSCLC patients were significantly higher than in localized NSCLC patients ($p<0.01$). There was a significant positive correlation between serum MMP-2 and CRP levels as well as SAA levels in metastatic NSCLC patients ($p<0.01$). Therefore, quantitation of MMP-2, CRP and SAA in NSCLC patients may be an auxiliary indicator to monitor tumor progression and poor prognosis of NSCLC disease.

Introduction

Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases that are known to degrade extracellular matrix (ECM). MMPs are secreted as inactive zymogens from cancer cells or macrophages and activated by proteases such as plasmin or trypsin (1,2). Biological activities of MMPs are downregulated by α2-macroglobulin (α2M) or tissue inhibitors of metalloproteinases (TIMPs) produced by macrophages, fibroblasts or other types of cells (3-5). Therefore, a quantitative imbalance between MMPs and their inhibitors such as α2M and TIMPs is thought to be a causative factor in invasion and metastasis. It has also been shown that MMP-2 degrades the ECM of atherosclerotic plaques and has an important role in plaque vulnerability at atherosclerotic regions (6,7). We previously reported that the quantitative imbalance between proteases, such as prostate-specific antigen (PSA) and MMP-2, and their inhibitors, including α2M and α2-plasmin inhibitor, is a causative factor in invasion and metastasis of prostate cancer (PCa) (8,9).

C-reactive protein (CRP) and serum amyloid A (SAA) are widely used as acute inflammatory biomarkers in various conditions such as infection, inflammation, malignancy and tissue disturbance (10-12). CRP is most widely used as a sensitive inflammatory biomarker in routine clinical examination. In recent years, the determination of high sensitivity CRP (hs-CRP) has been possible due to the wide use of low concentration range measurements in routine clinical examination. It has been demonstrated that hs-CRP reflects the degree of localized vascular inflammation and is a useful prognostic marker of cardiovascular events (13,14). On the other hand, serum SAA level is generally increased in patients with viral infection or in corticosteroid-treated patients in contrast to CRP (15,16). The production of CRP and SAA in liver cells is regulated by interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) secreted from macrophages in various conditions (17,18). α2M is the most abundant proteinase inhibitor in the blood, and it is also involved in the inflammatory reaction through its function as a carrier protein of IL-6 (19). We previously demonstrated that the serum levels of IL-6, CRP and SAA were affected by serum α2M concentration in PCa patients with or without α2M deficiency, and these markers are considered α2M-dependent acute inflammatory biomarkers (20,21).

However, the relationship between the serum levels of MMP-2 and acute inflammatory biomarkers in patients with NSCLC progression has yet to be demonstrated. Therefore, we quantified serum levels of MMP-2, CRP and SAA in localized

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Key words: matrix metalloproteinase-2, C-reactive protein, serum amyloid A, non-small cell lung cancer

Table I. Concentration (median value, range) of MMP-2, CRP and SAA in healthy controls and NSCLC patients.

	Healthy controls (n=13)	Localized NSCLC (n=12)	Metastatic NSCLC (n=12)	p-value of healthy controls vs. localized NSCLC	p-value of healthy controls vs. metastatic NSCLC	p-value of localized vs. metastatic NSCLC
MMP-2 (ng/ml)	690 (624-782)	620 (347-844)	886 (685-1,040)	NS	<0.01	<0.01
CRP (μg/dl)	27.5 (8-401)	207.6 (18-594.5)	4,488 (224-9,940)	NS	<0.01	<0.01
SAA (μg/ml)	8.2 (7.2-18.6)	6.85 (2-337)	158 (5-1,474)	NS	<0.01	<0.01

MMP-2, matrix metalloproteinase-2; CRP, C-reactive protein; SAA, serum amyloid A; NSCLC, non-small cell lung cancer; NS, no stastically significant difference.

and metastatic NSCLC patients to establish the clinical significance and changes of these biomarkers during NSCLC disease progression. Although this study includes only a limited number of NSCLC patients, it is the first report to investigate the clinical significance and changes of MMP-2, CRP and SAA in patients with NSCLC in relation to disease progression.

Materials and methods

Patients. Thirty-seven untreated adult men participated in this study, of whom 13 were healthy controls (mean age 62.6 years, range 53-72) and 24 were diagnosed with non-small cell lung cancer (NSCLC) at the Kitasato University Hospital. The 24 cases included 12 localized NSCLC (6 adenocarcinomas and 6 squamous cell carcinomas) (mean age 64.8 years, range 52-78) and 12 metastatic NSCLC (6 adenocarcinomas and 6 squamous cell carcinomas) (mean age 67.6 years, range 54-79). NSCLC was clinically staged according to the TNM classification (22). Serum α2M levels in the 24 NSCLC patients and the 13 healthy controls were within reference range. Serum samples were obtained from these patients and stored at -80˚C until use. Informed consent was obtained from all subjects in this study.

Acute inflammatory biomarkers. The measurement of MMP-2 levels in serum was determined by measuring pro-MMP-2 using a one-step sandwich enzyme immunoassay (Fuji Chemical Industries, Toyama, Japan) (23). CRP and SAA levels in serum were measured by latex nephelometry using the LX-M (Eiken Chemical Co., Tokyo, Japan).

Statistical analysis. The Wilcoxon signed-rank test and the Mann-Whitney U test were used for statistical analyses, and $p<0.05$ was considered to indicate statistically significant differences.

Ethics approval. This study was conducted in accordance with the Declaration of Helsinki. This study had no impact on the management of patients, and informed consent was obtained from all subjects.

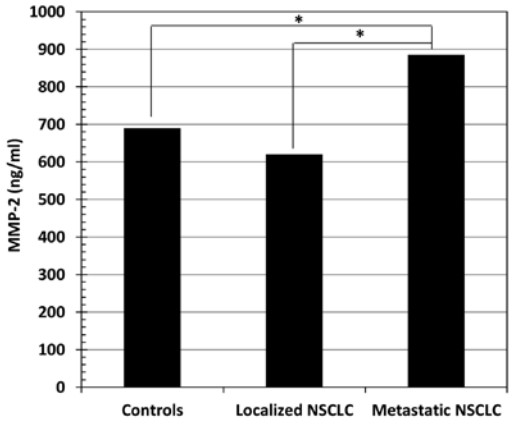

Figure 1. Serum MMP-2 levels in healthy controls, and localized and metastatic NSCLC patients. Serum MMP-2 levels were significantly increased in metastatic NSCLC patients as compared to healthy controls and localized NSCLC patients. NSCLC, non-small cell lung cancer; *p<0.01.

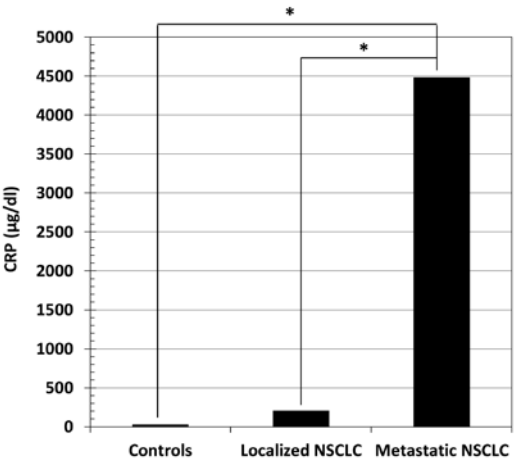

Figure 2. Serum CRP levels in healthy controls, and localized and metastatic NSCLC patients. Serum CRP levels were markedly increased in metastatic NSCLC patients as compared to healthy controls and localized NSCLC patients. NSCLC, non-small cell lung cancer; *p<0.01.

Results

Concentrations of MMP-2, CRP and SAA in serum. Table I shows the concentrations (median value, range) of MMP-2, CRP and SAA in the sera of healthy controls and NSCLC patients. The serum levels of MMP-2 (Fig. 1), CRP (Fig. 2) and SAA (Fig. 3) in metastatic NSCLC patients were significantly

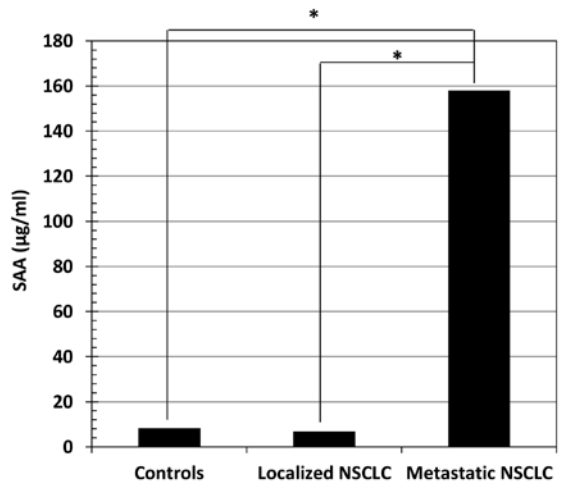

Figure 3. Serum SAA levels in healthy controls, and localized and metastatic NSCLC patients. Serum SAA levels were markedly increased in metastatic NSCLC patients as compared to healthy controls and localized NSCLC patients. NSCLC, non-small cell lung cancer; *p<0.01.

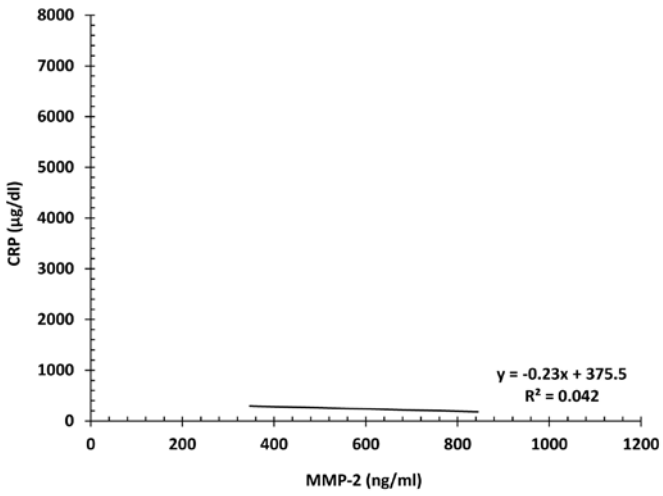

Figure 4. Correlation between serum MMP-2 and CRP levels in localized NSCLC patients. There was a weak but significant negative correlation between serum MMP-2 and CRP levels in localized NSCLC patients. NSCLC, non-small cell lung cancer.

higher than in healthy controls (p<0.01 for all three markers) and localized NSCLC patients (p<0.01 for all three markers).

Correlation between serum MMP-2 and CRP levels. There was a weak, but significant negative correlation between serum MMP-2 and CRP levels in localized NSCLC patients (r^2=0.042) (p<0.05) (Fig. 4). On the other hand, there was a weakly significant positive correlation between serum MMP-2 and CRP levels in metastatic NSCLC patients (r^2=0.051) (p<0.01) (Fig. 5).

Correlation between serum MMP-2 and SAA levels. There was a significant positive correlation between serum MMP-2 and SAA levels in localized (r^2=0.312) (p<0.01) (Fig. 6) and metastatic (r^2=0.231) (p<0.01) (Fig. 7) NSCLC patients.

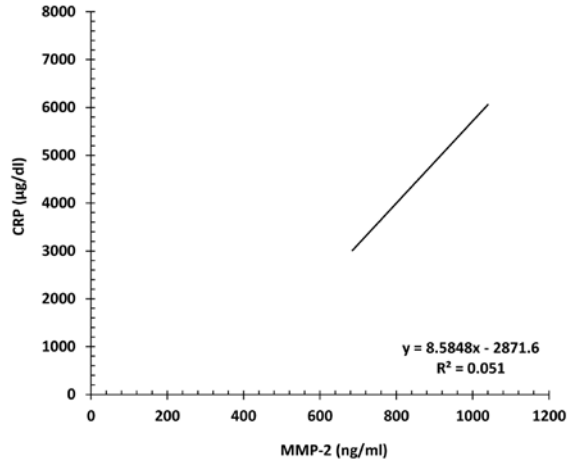

Figure 5. Correlation between serum MMP-2 and CRP levels in metastatic NSCLC patients. There was a weakly significant positive correlation between serum MMP-2 and CRP levels in metastatic NSCLC patients. NSCLC, non-small cell lung cancer.

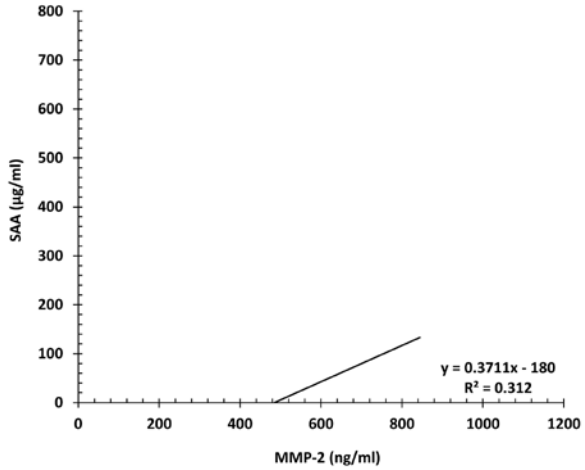

Figure 6. Correlation between serum MMP-2 and SAA levels in localized NSCLC patients. There was a significant positive correlation between serum MMP-2 and SAA levels in localized NSCLC patients. NSCLC, non-small cell lung cancer.

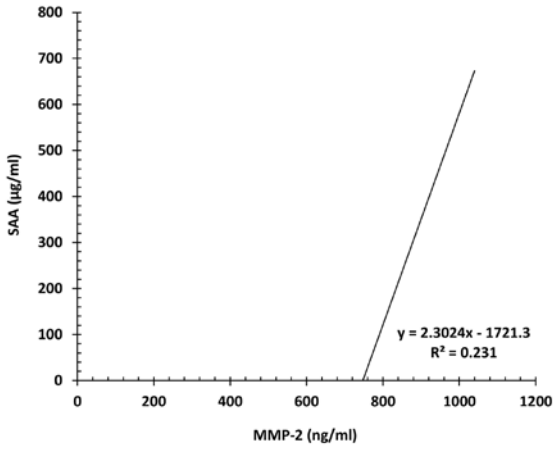

Figure 7. Correlation between serum MMP-2 and SAA levels in metastatic NSCLC patients. There was a significant positive correlation between serum MMP-2 and SAA levels in metastatic NSCLC patients. NSCLC, non-small cell lung cancer.

Discussion

MMPs are endopeptidases which degrade the ECM of the cellular basement membrane. Among members of the MMP family, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have been shown to degrade type IV collagen which is a major component of the cellular basement membrane. Therefore, it is thought that MMP-2 and MMP-9 are associated with invasion and metastasis of cancer cells. Noh *et al* showed that MMP-2 concentration in ascites might be a prognostic marker in advanced gastric cancer patients with disseminated metastasis (24). It has also been reported that the expression of MMP-2 in body fluid can be used as an additive diagnostic marker for metastatic breast cancer patients (25). Safranek *et al* demonstrated that mRNA expression of MMP-7 and MMP-9 in lung tissue of patients with NSCLC is higher than in the surrounding tissue and in benign lung disease tissue, and these findings support the important roles of these MMPs in the growth of lung cancer (26). Furthermore, it has also been shown that high expression of MMP-9 is associated with poor prognosis in patients with NSCLC (27). In the present study, we demonstrated that serum MMP-2 levels were markedly increased in metastatic NSCLC patients as compared to localized NSCLC. Therefore, it is hypothesized that MMP-2 is involved in the invasion and metastasis of NSCLC, as is the case with other types of cancer, and high serum MMP-2 levels in NSCLC patients can predict tumor progression.

CRP is a plasma protein produced by liver cells following cytokine stimulation, mainly IL-6, but also IL-1β and TNF-α (17,18). Serum CRP levels are increased in various conditions including infection, inflammation and tissue disturbance such as malignancy or myocardial infarction, but serum CRP levels are rarely increased in viral infection, multiple myeloma and non-active systemic lupus erythematosus (15,28,29). It has been shown that elevated serum CRP levels are associated with tumor progression and poor prognosis of esophageal cancer (12). Chua *et al* demonstrated that inflammatory and tumor markers in serum predict survival in patients with epithelial appendiceal neoplasms undergoing surgical cytoreduction and intra-peritoneal chemotherapy (30). It has also been reported that elevated preoperative serum CRP levels predict poor survival in patients undergoing resection for NSCLC (31).

SAA is a plasma protein produced by liver cells following cytokine stimulation, mainly IL-1β, but also IL-6 and TNF-α, and is generally increased in patients with viral infection and corticosteroid treatment, a characteristic that differs from CRP (17,18). The degree of change in serum SAA levels is believed to be larger compared to CRP in various conditions, and IL1-β stimulation of SAA production is hard to suppress by corticosteroid treatment (16). It has been reported that serum SAA levels are useful in predicting survival of patients with gastric cancer (32). Cocco *et al* demonstrated that SAA may be a novel biomarker to monitor disease recurrence and response to therapy in patients with uterine serous papillary cancer (33). It has also been shown that elevated serum SAA levels may be used as a potential biomarker for gastric cancer (34). In this study, serum CRP and SAA levels in metastatic NSCLC patients were significantly higher than in healthy controls and localized NSCLC patients, and there was a significant positive correlation between serum MMP-2 and CRP levels as well as SAA levels in metastatic NSCLC patients. Elevated serum levels of CRP and SAA in metastatic NSCLC patients are considered to reflect the tissue disturbance and inflammation that are associated with invasion and metastasis of NSCLC, and high serum CRP and SAA levels can predict tumor progression and poor prognosis of NSCLC.

In conclusion, the present study demonstrated that serum MMP-2 levels were notably increased in metastatic NSCLC patients. Furthermore, serum levels of CRP and SAA were also markedly increased with NSCLC disease progression, and there was a significant positive correlation between serum MMP-2 and these acute inflammatory biomarkers in metastatic NSCLC patients. Therefore, the measurement of MMP-2, CRP and SAA in NSCLC patients may be an auxiliary indicator to monitor tumor progression and poor prognosis of NSCLC.

Acknowledgements

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