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原　　著

Serum Cathepsin B Levels, Urinary Excretion of Cathepsin B and Tissue Cathepsin B Content in the Patients with Gastric Cancer

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Abstract

Serum cathepsin B levels and urinary excretion of cathepsin B in the patients with gastric cancer were significantly higher than those in the control non-cancer patients. Moreover, cancer tissue cathepsin B content was significantly higher than that in the normal tissue. After radical curative operations for gastric cancers, both serum cathepsin B levels and urinary excretion of cathepsin B were restored to the control values. These results suggest a possible role of lysosomal enzyme, cathepsin B in the pathogenesis of tumor growth, and also suggest that these parameters might be possible indicators for tumor malignancy.

Introduction

Tumor growth is a complex sequence of events, that begins with the release of tumor cells from the primary tumor[1], and in this process of tumor invasion, it is well known that proteolytic enzymes can cause detachment of cells from each other.

Increases in lysosomal enzyme activities and in lysosomal enzyme release have been reported to correlate with the ability of primary tumor to invade normal tissue as well as with the occurence of metastatic tumors[2-4]. These reports suggest an important role of lysosomal enzymes in the pathogenesis of tumor growth and invasion.

In this study, we measured the serum cathepsin B levels, urinary excretion of cathepsin B and tissue cathepsin B content in the cancer patients, particularly with gastric cancer, since lysosomal enzyme, cathepsin B seems to play an crucial role in the process of tumor growth and invasion[5].

Key words: Lysosomal enzyme, Cathepsin B, Gastric cancer.
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Materials and Methods

Patients

Twenty one patients, who had been admitted to our department and department-related hospitals from January to July, 1992, were divided into the following two groups.

(a) Gastric cancer patients without remote metastasis (Can) (n=9) (age: 62±4 y.o., female : male=5 : 4). For all the patients, radical curative operations including regional lymphadenectomy were performed, and there were no remote metastasis identified both from preoperative examinations (chest X-rays, abdominal and chest computerized tomography and abdominal ultrasonography) and intraoperative findings. The sizes of all the tumors were above 2.0 cm in diameter, and all the tumors had already invaded submucosal layer or muscular layer of the stomach wall.

(b) Control non-cancer patients (Cont) (n=12) (age: 58±4 y.o., female : male=6 : 6). There were 6 internal hemorrhoids and 6 external inguinal hernias, and hemorrhoidectomy or herniorrhaphy was performed in all the patients.

Measurement of Serum Cathepsin B Levels and Urinary Excretion of Cathepsin B

In these two groups, before operations after a 12-hour fast, blood samplings were performed for ordinary preoperative hematology and biochemical study. By using a part of these bloods, serum cathepsin B levels were determined. Their urines were collected and stored in the refrigerator at 4°C. Urinary cathepsin B levels were determined within the same day, and urinary excretion of cathepsin B was calculated as U/kg.day.

In Can group, at 2 and 4 weeks after radical operations, the same blood samplings and urinary collection were performed, and both serum cathepsin B and urinary excretion of cathepsin B were determined.

Tissue Cathepsin B Content

For all the resected specimen, 500 mg of tumor tissues and normal tissues were homogenized in 5 ml of phosphate-buffered (pH 7.4) saline containing 0.5% Triton X-100 (Sigma Chemical Co., St. Louis, MO, U.S.A.). After a low speed centrifugation (150×g at 4°C for 15 min), for the resulting supernatant cathepsin B activity was measured, and cancer tissue cathepsin B content was expressed as a percentage of that in the normal tissue values (% of the normal tissue content).

Assay

Cathepsin B activity was measured spectrofluorometrically with carbobenzyloxy-arginyl-arginine-β-naphthylamide (Bachem Feinchemikalien AG, Budendorf, Switzerland) as the substrate by the method of McDonald and Ellis[6].

Statistics

Data were presented as the means±S.E.M. for n determinations. Differences between groups were evaluated by Student's t-test, and $p<0.05$ was considered to be significant.

Results

Serum cathepsin B levels in Can group (2.8±0.2 U/ml) were significantly higher than those in Cont group (1.8±0.2 U/ml). Urinary excretion of cathepsin B in Can group (47±4 U/kg・day) were significantly increased as compared with Cont group (21±3 U/kg・day) (Fig.

1a and b).

In Can group, for all the resected specimen, cancer tissue cathepsin B contents were significantly higher (188±8% of the normal tissue values) than those in the normal tissues (Fig. 2).

In Can group, at 2 weeks after radical operations, both serum cathepsin B levels (2.0±0.2 U/ml) and urinary excretion of cathepsin B (26±4 U/kg · day) were significantly decreased as compared with before operations. At 4 weeks after radical operations both serum cathepsin B levels (1.7±0.2 U/ml) and urinary excretion of cathepsin B (23±3 U/kg · day) were restored almost to the control values (Fig. 3a and b).

Discussion

Tumor growth and invasion are the result of an intricate sequence of events initiated by the invasion of cancer cells into normal tissue, detachment and dissemination of cancer cells to distant organs, and completed by establishment of secondary tumors. The malignancy of a tumor depends upon its ability to invade normal tissue at the primary and secondary sites of growth.

Increases in lysosomal enzyme activities and in lysosomal enzyme release in cancer tissue and from cancer tissue have been reported to be correlated with the ability of primary tumors to invade normal tissue as well as to make a remote metastasis[2-4]. Furthermore, there has been another report regarding a close relationship between serum lysosomal enzymes and tumor malignancy in vaginal clear-cell adenocarcinoma[7]. Thus these reports suggest an important

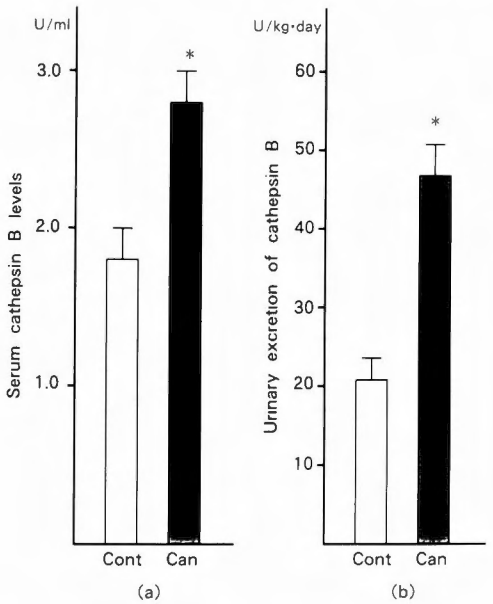

Fig. 1 Serum cathepsin B levels (a) and urinary excretion of cathepsin B (b) in the patients with gastric cancer. □: Control non-cancer patients (n=12), ■: Gastric cancer patients (n=9), As compared with Cont group, *p<0.02.

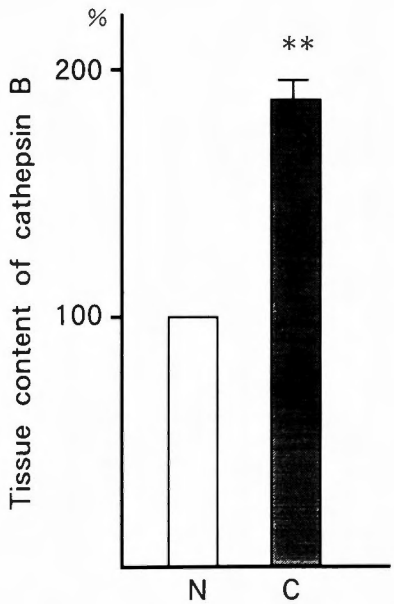

Fig. 2 Tissue cathepsin B content in the gastric cancer patients. N: normal tissue, C: cancer tissue, Cancer tissue content was expressed as a percentage of the normal tissue content. As compared with N, **p<0.01.

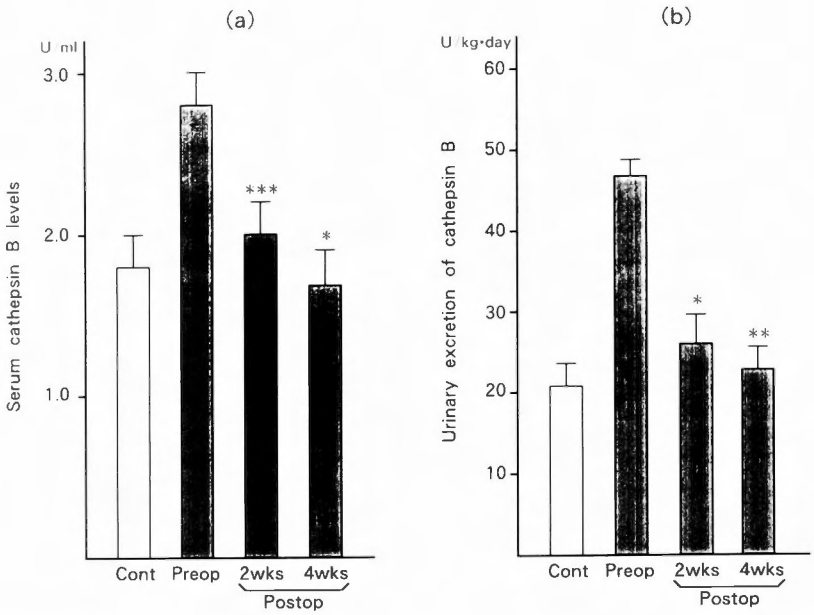

Fig. 3 Serum cathepsin B levels (a) and urinary excretion of cathepsin B (b) at 2 and 4 weeks after radical operations in the gastric cancer patients. □: Control non-cancer patients (n=12), ■: Gastric cancer patients (n=9), Preop: before operations, Postop: after operations, As comapred with Preop, $^{*}p<0.02$, $^{**}p<0.01$ and $^{***}p<0.05$.

role of lysosomal enzymes in thepathogenesis of tumor invasions and metastasis.

Lysosomal enzyme, cathepsin B is a cysteine proteinase with significant activity at neutral pH[8]. Since cathepsin B can degrade pericellular protein[9], cathepsin B released from tumor cells might contribute to detachment of the cells from the primary tumor and hence to invade into normal tissue. Two of the major constituents of the extracellular matrix, collagen and proteoglycan, might be degraded by cathepsin B[9]. Moreover, cathepsin B can degrade the major constituents of the venule wall, such as actin and myosin[10]. Thus cathepsin B might facilitate both growth and invasion of tumor cells.

The results of this study indicate that both serum cathepsin B levels and urinary excretion of cathepsin B in the patients with gastric cancers were significantly higher than those in the non-cancer patients, and also indicate that tumor tissue cathepsin B contents were significantly higher than those in the normal tissues, suggesting an important role of lysosomal enzyme, cathepsin B in the process of tumor growth.

Since in the gastric cancer patients without remote metastasis, after radical operations of tumors, serum cathepsin B levels and urinary excretion of cathepsin B were returned to the control values, these parameters might be possible indicators for tumor malignancy.

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和文抄録

胃癌患者における血中カテプシン B 値，尿中カテプシン B 排出量および組織カテプシン B 含有量について

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平野鉄也，吉岡秀憲

胃癌患者での血中カテプシン B 値および尿中カテプシン B 排出量はコントロールの非癌患者に比べ有意に高値を示した．さらに，切除標本での検討にて，胃癌組織中でのカテプシン B 含有量は正常組織と比べ有意に高値を示した．胃癌患者では，根治手術後には，血中カテプシン B 値も尿中カテプシン B 排出量もともにコントロール値に回復した．これらの結果は，ライソゾーム酵素であるカテプシン B の癌発育過程での何らかの役割を示唆させるとともに，これらのパラメーターが，悪性腫瘍の指標となりうることも示唆させた．