The Feasibility of Cathepsin B Level in Preoperatively Screening Patients with Thyroid Cancer and Nodular Hyperplasia

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To evaluate the feasibility of cathepsin-B levels in preoperatively screening patients with thyroid cancer, we assigned these patients to either the thyroid cancer group (n=32) or the nodular hyperplasia group (n=7). Five healthy volunteers served as controls (n=5). We quantified cathepsin-B expressions in cancerous lesions with follicular carcinoma and hyperplastic lesions with nodular hyperplasia, and compared the degrees to those of normal thyroid tissue, which was obtained from matched contralateral lobe. The activity of serum cathepsin B was significantly higher in patients with thyroid carcinoma (284.87±79.32, 10^5 μU) and those with nodular hyperplasia (255.45±95.68, 10^5 μU) than compared to normal control (168.94±15.10, 10^5 μU) (p<0.05). Based on the results of immunoassay, the concentrations of cathepsin B in the thyroid cancer group (15.50±7.86 ng/ml) and the nodular hyperplasia group (17.64±7.49 ng/ml) were higher than those of the control group (4.85±0.61 ng/ml). The degree of cathepsin-B mRNA expression was significantly higher in cancerous or hyperplastic lesions than normal thyroid tissues from matched contralateral lobe with follicular carcinoma or non-neoplastic thyroid disease. Our results indicate that the activity of serum cathepsin B is a useful indicator in screening patients with nodular hyperplasia or neoplastic thyroid disease and it may be involved in the abnormal proliferation of cells.

Key words : Cathepsin B, thyroid cancer, nodular hyperplasia, screening

Introduction

Extracellular matrix (ECM) determines the structural tissue organization. It also forms a complex network consisting of collagen, fibrillar glycoprotein and proteoglycan, thus determining the biological condition. Proteolysis of ECM is strongly associated with cancer progression. In other words, neoplastic cells must change their shape to penetrate into ECM, but this is not easy. However, ECM eventually allows neoplastic cells when it is degraded by proteolytic enzymes [1,25]. Thus, cancer invasion and metastasis are associated with the activity of various proteolytic enzymes in ECM.

Of these proteolytic enzymes, cathepsin is a lysosomal proteinase which is present in nearly all mammalian cells, whose variants include an aspartyl protease (cathepsin D) and a cysteine protease (cathepsin B, L, H, C, S, F, K, O, V, W and X) [29]. Cathepsin D-overexpression is commonly seen in patients with cervical cancer, ovarian cancer, lung cancer, G-I tract cancer, prostate cancer or breast cancer [2,19,21]. A poor prognosis is achieved particularly in patients with node-negative breast cancer in whom cathepsin-D overexpression is present. Cathepsin-D overexpression is therefore considered a prognostic indicator in these patients [5,8,14]. Cathepsin-B overexpression is also associated with brain tumor, colorectal cancer, hepatoma, melanoma or prostate cancer [15,17,31]. Furthermore, the degree of cathepsin-B overexpression is significantly higher in patients with differentiated thyroid cancer than normal healthy people or those with non-neoplastic thyroid disease [23,24]. Based on these reports, it can therefore be inferred that the concentration of serum cathepsin B is a useful indicator in preoperatively screening patients with thyroid cancer.

The aim of this study was to evaluate the feasibility of serum cathepsin-B level in preoperatively screening patients with thyroid cancer. To support this, we quantified cathepsin-B expression in cancerous or hyperplastic lesion and compared its degree to that of normal thyroid tissue from matched contralateral lobe.

Materials and Methods

Study patients

We examined 39 patients who underwent thyroidectomy at Kosin University Gospel Hospital between February 2005

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and June 2009. Thirty two patients of them had a diagnosis of differentiated thyroid carcinoma (31 cases of papillary cancer and one case of follicular one). The remaining seven patients had a non-neoplastic disease, nodular hyperplasia. We divided them into the thyroid cancer group (n=32) and the nodular hyperplasia group (n=7). Mean age was 50.8±12.3 years in the thyroid cancer group and 43.5±12.8 years in the nodular hyperplasia group. In addition, five healthy individuals voluntarily participated in the present study. These were served as control (n=5), with a mean age of 22.60±0.55 years (Table 1). We also performed a histopathologic analysis after we obtained tissue samples in three cases of thyroid carcinoma, one case of nodular hyperplasia and five normal thyroid tissues from matched contralateral lobes.

**Blood sampling and tissue preparation**

Prior to surgery, a 10 cc whole-blood sampling was done in normal controls and patients. The whole-blood sample was treated with heparin 1 cc, and was preserved at -70°C for further laboratory procedure. Cancer tissue and its adjacent normal tissue were intraoperatively extracted, which were also preserved in a deep-freezer at -70°C until use.

**Isolation of crude extract**

A whole-blood sample was centrifuged at 200× g at 4°C for ten min, and thus supernatant was obtained. Using a Polytron apparatus, supernatant and WBC layer were homogenized five times for 15 sec. This was followed by a 30,000× g, 60-minute centrifugation. The resultant supernatant was preserved at -70°C. The remaining precipitates were treated with Tris-Cl pH 7.3, and were subject to a 30,000× g, 60-minute centrifugation. The resultant supernatant was harvested and then preserved at -70°C.

**Cathepsin-B activity**

Gelatinase assay

According to a modification of the method described by Keene et al., the gelatinase activity of cathepsin B was quantified with the sample 10 μl diluted at a ratio of 1/10 [9].

The electrophoresis was performed using a 12% Tris-running gel containing Gelatin 0.1% at 4°C. The running gel was placed in a 2.5% Triton-X 100 solution and then shaken for two hr. The sample was treated with 5 mM dithiothreitol (DTT), 0.1 M sodium acetate pH 4.5 at 37°C for 18 hr. 0.1% Coomassie Brilliant Blue R solution was applied to develop the color of cathepsin-B band. To confirm whether cathepsin-B activity was effectively blocked, the sample was treated with leupeptin. Thus, the final concentration of leupeptin was set at 100 μM.

**Peptidolytic assay**

The sample 10 μl was treated with a 20 mM Tris-buffer solution 80 μl pH 5.5 for 37°C for two hr. Then, it was reacted with a synthetic fluorogenic substrate, z-arginyl-arginyl-7-amido-4-methylcoumarin hydrochloride (z-Arg-Arg-AMC), thus making a final concentration of 100 μM. The reaction was performed at 37°C for 15 min. Then, fluorescence and observance were measured with a Microplate Fluorometer (Packard Co., USA) at wavelengths of 380 nm and 460 nm. A 1-unit (U) cathepsin-B activity was defined as the amount of 7-amido-4-methylcoumarin (AMC) released at 37°C for a minute.

**Inhibition of cathepsin-B activity**

The sample 10 μl was placed in a 20 mM Tris-buffer solution 70 μl pH 5.5, and it was treated with EDTA, PMSF, pepstatin A and leupeptin. Thus, the final concentration of sample was set at 10-100 μM. The reaction was performed at 37°C for two hr. The sample was reacted with a synthetic fluorogenic substrate, z-arginyl-arginyl-7-amido-4-methylcoumarin hydrochloride (z-Arg-Arg-AMC), thus making a final concentration of 100 μM. The reaction was performed at 37°C for 15 min. Then, fluorescence and observance were measured with a Microplate Fluorometer (Packard Co., USA) at wavelengths of 380 nm and 460 nm.

**Western Blot**

The electrophoresis was performed according to a modification of the method described by Laemmli [13]. To denature the sample diluted at a ratio of 1/10 and human lysosomal cathepsin B diluted at a ratio of 1/20, pH was adjusted to 6.8. The sample was heated in a 60 mM Tris-buffer solution containing 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol and 0.1% bromophenol blue for two min. The electrophoresis was performed using a 12% SDS-running gel and the transblotting system (Bio-rad) was used to transblot

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Age (mean±SD)</th>
<th>Male-to-female ratio</th>
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</thead>
<tbody>
<tr>
<td>Thyroid cancer group (n=32)</td>
<td>50.8±12.3 years</td>
<td>1:7</td>
</tr>
<tr>
<td>Nodular hyperplasia group (n=7)</td>
<td>43.5±12.8 years</td>
<td>0:7</td>
</tr>
<tr>
<td>Control group (n=5)</td>
<td>22.60±0.55 years</td>
<td>5:0</td>
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protein on a nitrocellulose sheet [28]. The nitrocellulose sheet was placed in a PBS solution containing a 5% skim milk solution and then shaken at room temperature for an hour. After three-time washing with a PBS solution containing a 0.1% Tween-20, the nitrocellulose sheet was reacted with anti-human cathepsin-B antibody (1:1,000, R&D systems) in a PBS solution containing a 0.1% Tween-20 at 4°C for 18 hr. After three-time washing with a PBS solution containing a 0.1% Tween-20, the nitrocellulose sheet was reacted with the secondary antibody, alkaline-phosphatase (AP)-conjugated rabbit anti-goat IgG (1:1,000, Calbiochem) in a PBS solution containing a 0.1% Tween-20 for two hours. The band was detected using an AP-Conjugate Substrate Kit (Bio-rad).

**Cathepsin-B immunoassay**

To measure the concentration of serum cathepsin B in both patients and normal controls, Quantikine kit (Human pro-cathepsin B immunoassay, R&D systems) was used. Based on the method described earlier, the sample and standard solution (0.313 - 100 μg/ml) were diluted at a ratio of 1/5. A 50-μl aliquot of each dilution was placed in a microplate well. The reaction was performed at 4°C for two hr. A washing solution 250 μl was added to a microplate well and the microplate was shaken to gently mixing and then discarded all solutions. A 200 μl aliquot of conjugate solution was placed in a microplate well. The reaction was performed at 4°C for two hr. All solutions were discarded. A 200 μl aliquot of substrate solution was placed in a microplate well. The reaction was performed at room temperature for 30 min after illumination was blocked. To terminate the reaction, a 50 μl aliquot of stop solution was placed in a microplate well. The absorbance was measured at wavelengths of 450 nm and 540 nm.

**Immunohistochemical staining**

Tissue sample was fixed in a 10% neutral formalin solution, and was embedded in a paraffin block. Paraffin-embedded tissue sample was sectioned at a thickness of 4 μm, and it was mounted on a glass slide. Based on the conventional avidin-biotin-complex (ABC) method, an immunohistochemistry was performed. Paraffin was removed with xylene for ten minutes. Tissue sample was hydrated with alcohol, and it was rinsed with a Tris-buffer solution (10× Immunoassay buffer: Biomed, CA, USA) for ten minutes. Using a dilution solution (research Genetics, USA), cathepsin-B primary antibody (monoclonal, Abcam, Cambridge, UK) was diluted at a ratio of 1: 30. The reaction was performed at room temperature for an hour. Tissue sample was rinsed with a Tris-buffer solution, and was reacted with biotin-conjugated secondary antibody for 15 min. Again, tissue sample was rinsed with a Tris-buffer solution and then reacted with avidin-conjugated peroxidase for 15 mins. Then, it was rinsed with a Tris-buffer solution. The color of tissue sample was developed with 3-amino-9-ethyl carbazole (AEC). Tissue sample was counter-stained with a Meyer’s hematoxylin. For light microscopy, it was mounted with a crystal mount. Skin tissue was served as a positive control. Not using the primary antibody, a PBS solution was served as a negative control. Positive immunohistochemical finding was defined as the appearance of brownish granule in the cytoplasm of cancer cells.

**RNA isolation and cDNA synthesis**

With the use of TRIZOL (Invitrogen, USA), RNA was isolated from tissue sample. To synthesize the first-strand cDNA, the isolated RNA 2 μg was treated with 10× reaction buffer (100 mM Tris, 500 mM KCl, pH 8.3) 2 μL, 25 mM MgCl2 4 μl, 10 mM dNTP mixture 3.2 μl oligo (dT) 18 primer 0.5 μl, RNase inhibitor 1 μl, 0.1 M dithiothreitol 2 μl and AMV reverse transcriptase 1.0 μl. A 20 μl solution was prepared using a DEPC-treated distilled water and then placed in a thermal cycler at 25°C for ten min. The reaction was performed at 42°C for 60 min. To block the activity of reverse transcriptase, a 20 μl solution was maintained at 99°C for five min and then preserved at -20°C until use. To quantify the purified cDNA, absorbance was measured at wavelengths of 260 nm and 280 nm.

**Real-time PCR**

Quantitative real-time PCR was performed using ABI 7900 HT (Applied Biosystems, Foster city CA, USA). PCR solution was 2× SYBR Green PCR Master Mix (Applied Biosystems, Foster city CA, USA). To prepare PCR solution, cDNA 0.5 μl (10 ng/μl) each, primer 0.8 μl each and 2× SYBR

<table>
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<th>Table 2. The primer for cathepsin B</th>
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<tr>
<td><strong>Forward primer sequence</strong></td>
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<td>GGA GAA TGG CAC ACC CTA CT</td>
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1 mix 10 μl were placed in a test tube. A sterilized distilled water 8.7 μl was added to a test tube and then well mixed. Thus, PCR solution 20 μl was prepared. PCR solution was transferred to a glass capillary tube. Then, PCR was performed at 95°C (30 s), 60°C (30 s) and 72°C (30 s). Thus, the amplification of PCR was performed at a total of 40 cycles. Following the real-time PCR, the signal value was expressed as Rn by comparing the emission intensity of ROX dye (passive reference) with that of SYBR green, obtained during the PCR. At each cycle, the amplification was plotted using Rn value. Here, CT was defined as the Rn value obtained in the first cycle of PCR amplification. The expression of target gene was relatively quantified by comparing the CT of GAPDH between the samples. In each sample, the CTs were averaged after the real-time PCR was completed independently three times. Besides, dissociation curve (DC) analysis can be used to confirm the melting temperature of amplified products. Thus, the amplification of single product was confirmed in the PCR amplification of target gene and GAPDH.

Statistical analysis

Statistical analysis was done using SPSS® version 12.0 for windows (SPSS, Inc., Chicago, IL). ANOVA was used to analyze the differences in the activity and amount of cathepsin B between the three groups. A post-hoc analysis was performed using Turkey. Using Student's t test, we examined whether the activity and amount of cathepsin B is correlated with the clinical course of thyroid cancer. Statistical significance was set at p < 0.05.

Results

Cathepsin-B activity and its inhibition

Gelatinase activity

Cathepsin B showed a broad-spectrum of gelatinolytic activity. The active proteolytic activity of cathepsin B was associated with proteins of 31 kDa and those of 25-27 kDa, but it was not seen in proteins of 44 kDa, the inactivated precursor. Furthermore, the activity of cathepsin B was blocked by the addition of leupeptin 100 μM, a cysteine protease inhibitor (Fig. 1).

Peptidolytic Activity

The degree of cathepsin-B expression relative to protein 1 mg was 168.94±15.10 mU (<10², mU/mg protein) in the control group, 255.45±95.68 mU (<10³, mU/mg protein) in the nodular hyperplasia group and 284.87±79.32 mU (<10³, mU/mg protein) in the thyroid cancer group. These results showed a significant difference between the control group and the nodular hyperplasia group and that between the control group and the thyroid cancer group (p<0.05). However, this statistical significance was not observed between the nodular hyperplasia group and the thyroid cancer group (Table 3) (Fig. 2). The degree of cathepsin-B activity was the highest at pH 5.5 (Fig. 3).

Inhibition of cathepsin-B activity

To examine whether the serum cathepsin-B activity is specifically inhibited by a cysteine protease, the sample was treated with 100 μM Pepstatin A, 100 μM PMSF and 100 μM EDTA. These inhibitors did not block cathepsin-B activity, but 100 μM leupeptin did (Table 4).

Serum cathepsin-B expression

Western blot was performed to examine the pattern of cathepsin-B expression. This provided an indistinct band at 31 kDa in the control group and two distinct bands at 31

Table 3. Cathepsin-B activities

<table>
<thead>
<tr>
<th>Thyroid cancer group</th>
<th>Nodular hyperplasia group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>284.87±79.32 (×10², mU/ mg protein)</td>
<td>255.45±95.68 (×10³, mU/ mg protein)</td>
<td>168.94±15.10 (×10², mU/ mg protein)</td>
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</table>
Fig. 2. Cathepsin-B activities. Diluted blood samples were treated with 20 mM Tris-Cl containing 5 mM dithiothreitol (pH 5.5), and were incubated with 100 mM z-phe-arg-7-amido-4-methylcoumarin hydrochloride at 37°C for 15 min. The peptidolytic activities were measured by spectrophotometry. The release rate of 7-amido-4-methylcoumarin by peptidase was determined after the fluorescence increased at excitation and emission wave lengths of 380 and 460 nm, respectively.

Table 4. Inhibition of cathepsin B activity by specific protease inhibitors

<table>
<thead>
<tr>
<th>Subfamily of protease</th>
<th>Inhibitor</th>
<th>Cathepsin-B activity (100 μM) (%) of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic</td>
<td>Pepstatin A</td>
<td>127.88±14.69</td>
</tr>
<tr>
<td>Serine</td>
<td>PMSF</td>
<td>116.18±25.08</td>
</tr>
<tr>
<td>Metalloprotease</td>
<td>EDTA</td>
<td>103.57±8.14</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Leupeptin</td>
<td>26.94±5.30</td>
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Fig. 4. Western blotting of blood samples. SDS-PAGE and transferring were performed as described previously. Nitrocellulose membrane was incubated with anti-human cathepsin-B antibody (1:1,000, R&D systems) in PBS buffer containing 0.1% Tween-20 at 4°C for 18 hr, and it was also incubated with alkaline phosphatase labeled rabbit anti-goat IgG (1:1,000, Calbiochem) at room temperature for two hr. The protein band was expressed by an AP Conjugate Substrate Kit (Bio-rad) (5× thyroid carcinoma sample number).

Fig. 3. The correlation between pH and cathepsin-B activity. Protease activity was determined using a z-arg-arg-AMC substrate under the pH range of 3-7. The activities are shown relative to that under pH 6.0 being 100%. The results were averaged from four experiments, thus expressed as mean±SD (SD: standard deviation).

kDa and 27 kDa, respectively in the nodular hyperplasia group and the thyroid cancer group (Fig. 4). An immunoblot assay was performed to quantify cathepsin-B expression. This showed that the mean amount of cathepsin B detected in the control group, the nodular hyperplasia group and the thyroid cancer group was 4.85±0.61, 17.64±7.49 and 15.50±7.86 ng/ml, respectively. These results showed a significant difference between the control group and the nodular hyperplasia group and that between the control group and the thyroid cancer group (p<0.05). However, this statistical significance was not observed between the nodular hyperplasia group and the thyroid cancer group (Table 5).

Immunohistochemical findings
Light-brown granules appeared in the cytoplasm of papillary thyroid cancer, but were not present in inflammatory cells. Of particular note, tumor cells infiltrating between the normal ones had a strong immunohistochemical pattern. This was notable in the basement membrane at the periphery of thyroid tissue (Fig. 5).

mRNA expressions of the cancerous or hyperplastic lesions
The difference in mRNA expression was quantified between the cancerous or hyperplastic lesion and the normal tissue on the contralateral lobe in the thyroid cancer group (FC24 versus N24) and the nodular hyperplasia group (NH34 versus N34). This showed a significant difference in mRNA expression between the cancerous or hyperplastic lesion and the normal tissue on the contralateral lobe in both groups (Fig. 6).
Table 5. Cathepsin B immunoassay

<table>
<thead>
<tr>
<th>Thyroid cancer group (n=32)</th>
<th>Nodular hyperplasia group (n=7)</th>
<th>Control group (n=5)</th>
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<tbody>
<tr>
<td>15.50±7.86 ng/ml</td>
<td>17.64±7.49 ng/ml</td>
<td>4.85±0.61 ng/ml</td>
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</table>

Fig. 5. Cathepsin-B immunohistochemistry in papillary carcinoma. Papillary carcinoma cells show a weak cytoplasmic reactivity to cathepsin B on immunohistochemistry (<400). A neoplastic papillary structure in papillary carcinoma had an increased staining property for cathepsin-B activity along the basement membrane of neoplastic cells lining the papillary structure.

Fig. 6. The change of cathepsin-B mRNA expression. A real-time PCR analysis was performed for total RNAs extracted from all three groups (N: control group, FC: thyroid cancer group and NH: nodular hyperplasia group).

Discussion

It has been reported that such proteinases as matrix metalloproteinases (MMP) and serine protease uPAs (urokinase-type plasminogen activators) are involved in various stages of cancer progression, including the growth, invasion and metastasis [3,11,20]. Cathepsin B is matured from its precursor, procathepsin B, in which cathepsin D, elastase and cathepsin G are synergistically mediated. Cathepsin B mediates the activation of urokinase-type plasminogen, thus involved in converting plasminogen to plasmin. Cathepsin B also mediates the conversion of proMMPs to MMPs, thus involved in the degradation of ECM protein [4,22,30]. Besides, cathepsin K is involved in bone resorption mediated by osteoclasts, which degrades bone matrix protein and thereby causes osteoporosis [16]. Cathepsin L has a similar activity to cathepsin B. Cathepsin H and K are involved in carcinogenesis [10,12].

Cathepsin B and D are released by lysosomes, and are overexpressed in cancer tissue and cell lines of patients with hepatoma, breast cancer, melanoma, colorectal cancer or prostate cancer. Based on these findings, it was speculated that serum cathepsin B and D will be useful in screening patients with cancer and predicting their clinical course. In particular, Skrzydlewski et al. reported that the activities of cathepsin B and D were significantly correlated with tumor size and lymph node metastasis in patients with colorectal cancer [26]. Nishikawa et al. reported that there was no significant difference in the concentration of serum cathepsin B between patients with benign ovarian tumor and their malignant counterparts [18]. We used serum cathepsin B of patients with thyroid carcinoma, and showed that the activity of cathepsin B was significantly higher compared to control. In our series, however, there was no significant difference between the thyroid cancer group and the nodular hyperplasia group. According to Shuja et al., the activity of cathepsin B was 1.5 times higher in patients with papillary thyroid carcinoma compared to control [23,24]. These authors noted, however, that it was 1.3-3 times higher in patients with polynodular goiter (a non-neoplastic disease); 1.2 times higher in those with Hashimoto’s thyroiditis; and 7.6 times higher in those with papillary thyroid carcinoma compared to control [24].

In the present study, we evaluated the degree of mRNA expression in a case of nodular hyperplasia and another case of thyroid cancer in which cathepsin-B activity increased most markedly compared to control. This showed that the degree of mRNA expression was higher in the cancer lesion than the normal one although both tissue samples were ex-
tracted in the same patient.

In addition, cathepsin containing granules are located in the peri-nuclear region of cells with a normal differentiation. However, these granules migrate to the basal area in the protoplasm in cases of cancer progression [7,27]. The migration of cathepsin granules occurs synchronously with the destruction of basement membrane. This is particularly notable because cancer invasion locally occurs with the protein synthesis. Our immunohistochemical findings showed that the staining of cathepsin B was increased in the basement membrane of thyroid cancer. This supports the argument that cathepsin-B granules migrate to the basement membrane.

Gocheva et al. conducted an animal experiment using mice with a mutation in cysteine cathepsin gene. According to these authors, cancer growth was inhibited in cases in which cathepsin B, L and S were absent, but cathepsin C was not associated with it; cathepsin B and S were involved in angiogenesis; and cathepsin B and L were involved in cancer proliferation [6]. Our results showed that the degree of cathepsin-B expression was significantly higher in both serum and tissue of patients with thyroid carcinoma and those with nodular hyperplasia compared to control. These results indicate that cathepsin B is involved in the proliferation of cancer cells in patients with thyroid carcinoma or non-neoplastic thyroid disease.

To summarize, our results showed that the activity of serum cathepsin B was significantly higher in patients with thyroid carcinoma and those with nodular hyperplasia than compared to control. This indicates that the activity of serum cathepsin B is a useful indicator in screening patients with nodular hyperplasia or neoplastic thyroid disease. Our results also showed that the degree of cathepsin-B expression was significantly higher in hyperplastic or cancerous lesion than normal tissue of patients with non-neoplastic thyroid disease or thyroid carcinoma. This suggests that the activity of cathepsin B is involved in the abnormal proliferation of cells, and deserves further prospective large-scale studies.

References

17. Miyake, H., I. Hara, and H. Eto. 2004. Serum level of cathe-
초록: 감상선암 및 결절성 촉증증 환자의 수술전 스크라닝을 위한 cathepsin B의 발현 양상

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현장 cathepsin B 활성도 측정이 감상선암 및 결절성 촉증증의 수술 전 진단에 도움이 되는지를 알아보고자 분화감상선암 32례, 결절성 촉증증 7례, 대조군 5례의 현장 cathepsin B 발현양상을 대조군과 함께 관찰하였다. 수술시 제거된 악모양암의 암조직, 결절성 촉증증 조직의 cathepsin B 발현을 정상 조직과 비교하였다. 현장 cathepsin B 활성도는 정상 대조군에서 168.94±15.10 (×10^7) mU, 결절성 촉증증에서 255.45±95.68 (×10^7) mU, 악모양암에서 284.87±79.32 (×10^7) mU로 악모양암군과 결절성 촉증증군에서 cathepsin B 발현이 정상 대조군보다 비교적 높게 나타났다(p<0.05). 현장 cathepsin B의 정량적 비교를 위한 immunoassay결과에서도 결절성 촉증증군(17.64±7.09 ng/ml)이 악모양암군(15.50±7.86 ng/ml)에서 정상대조군(4.85±0.61 ng/ml)보다 높은 수치를 나타내었다(p<0.05). 결절성 촉증증 및 악모양암군의 조직 내 cathepsin B mRNA발현이 정상 조직에서보다 높게 나타났다. 따라서 현장 cathepsin B는 감상선세포의 비정상적인 촉증시에 증가를 알 수 있으며, 감상선암 혹은 결절성 촉증증을 스크라닝하는데 이용될 수 있을 것으로 사료된다.