Advances in Brief

γ-Aminobutyric Acid as a Promoting Factor of Cancer Metastasis; Induction of Matrix Metalloproteinase Production Is Potentially Its Underlying Mechanism

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Abstract

We investigated expression of γ-aminobutyric acid (GABA), glutamate decarboxylase, and matrix metalloproteinase (MMP) in the prostates of patients with cancer or benign prostatic hypertrophy by immunohistochemical study. Marked expression of GABA, glutamate decarboxylase 67, and MMPs was observed in the prostates of cancer patients with metastasis (n = 72) and lymph node metastasis, although only sparse expression was noted in those of cancer patients without metastasis (n = 76) or patients with benign prostatic hypertrophy (n = 152). We then investigated the influence of GABA stimulation on in vitro MMP production and the invasive ability of cancer cells using human prostate cancer cell line C4-2. The production of MMPs increased significantly in cancer cells after a 24-h incubation with GABA. Cell invasion assay using a BioCoat Matrigel Invasion Chamber kit revealed that GABA stimulation significantly promoted the invasive ability of cancer cells and that addition of MMP inhibitor GM6001 significantly decreased GABA-induced migration. This may indicate the involvement of MMP activity in GABA-induced cancer cell invasion. We further analyzed the transmission pathway by performing GABA receptor modulation. The GABAB receptor agonist baclofen significantly increased MMP production as well as invasive ability. Moreover, blockade of the GABAB receptor pathway using GABAB receptor antagonist CGP 35348 significantly inhibited GABA-induced MMP production and invasive ability in cancer cells, whereas GABA receptor modulation did not influence MMP production or the invasive ability of cancer cells. Thus, increased expression of GABA may be implicated in cancer metastasis by promoting MMP production in cancer cells, and the GABAB receptor pathway may be involved in the process.

Introduction

Carcinoma of the prostate now constitutes a major and escalating international health problem. The current lifetime risk for men developing microscopic prostate cancer is roughly 30% (1, 2). Unlike most other forms of cancer, however, the probability of dying from the disorder is around 3%; not every prostate tumor is life-threatening (1, 2). Survival of a patient with prostatic carcinoma is closely related to the extent of the tumor. When the cancer is confined to the prostate gland, median survival in excess of 5 years can be anticipated. Patients with locally advanced cancer are not usually curable; median survival may be as long as 5 years. If the cancer has metastasized, however, current therapy will not cure it. The majority of patients with metastatic prostate cancer will die of the disease within 5 years (1–3). Considering the evidence, it would be highly beneficial to establish an efficient clinical marker, which may hold the key to distinguish dangerous tumors at an earlier stage. GABA was originally identified as a principal inhibitory neurotransmitter in the adult mammalian brain (4, 5). It has, however, become clear that GABA and GABA receptors exist in many non-neuronal peripheral tissues (4, 5). In the developing rat embryo, GABA has been found to play an important role in the morphogenesis and maturation of many tissues outside the nervous system (4, 6). Studies have also revealed that GABA participates in regulating cell division and affects the differentiation and maturation of cells in humans as well as in rodents (6, 7). Moreover, several reports recently showed that expression of GABA and its synthetic enzyme, GAD, significantly increased in neoplastic tissues, such as colorectal carcinoma, breast cancer, and gastric cancer, as compared with normal tissues (4, 8–10). We therefore examined the expression of GABA and GAD in prostate cancer and found that expression of both increased significantly in the prostates of cancer patients with metastasis, but not in those without metastasis or in patients with BPH. We then looked at the expression of MMPs, which have been known to promote cancer progression and metastasis (11–13), and found a marked expression of MMPs in the prostates of patients with metastasis along with GABA. We therefore investigated the mechanisms of GABA function on the metastasis, focusing on MMP activity using human prostate cancer cell line C4-2, which originated from LN metastasis. Our results may indicate that GABA is a promoting factor of prostate cancer metastasis and is a potential candidate for a new clinical marker, which should be of great value clinically.

Materials and Methods

Tumor Specimens. Formaldehyde-fixed, paraffin-embedded human prostate and LN specimens obtained from prostate cancer patients with metastasis (n = 72) or without metastasis (n = 76) or patients with BPH (n = 152) were studied. The ages of patients ranged from 54 to 88 years, with a mean age of 73 years.

Immunohistochemical Analysis of GABA Content, GAD Activity, and MMP Expression in the Prostates and LNs. We performed immunohistochemical staining of GABA, GAD67, and MMPs (MMP-2, -3, -7, and -9) using the following primary antibodies: rabbit polyclonal antibodies against GABA (1:300 dilution; Sigma); goat polyclonal antibodies against GAD67 (1:500 dilution; Chemicon, Temecula, CA); or MMP-2, -3, -7, or -9 (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). Paraffin sections of each sample were pretreated with goat or donkey serum (1:10 dilution; Jackson Laboratories, West Grove, PA) and incubated with each primary antibody overnight at 4°C. Sections were then incubated with a proper secondary antibody (Alexa Fluor 488 goat antirabbit IgG or Alexa Fluor 488 donkey antigoat IgG; 1:250 dilution; Molecular Probes, Eugene, OR) for 60 min in

8 The abbreviations used are: GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; MMP, matrix metalloproteinase; BPH, benign prostatic hypertrophy; BMI, body mass index; FV, field of view; LN, lymph node.
darkness at room temperature. Sections incubated without primary antibody were used as negative controls. Immunoactivity of each sample was evaluated using a confocal laser scanning microscope system (Axioplan2; Carl Zeiss GmbH, Jena, Germany; Radiance 2000; Bio-Rad, Hercules, CA) equipped with fluorescence intensity measurement software (Laser Sharp 2000; Bio-Rad) and the MCID program (Imaging Research Inc., Ontario, Canada). The result was expressed as a ratio of fluorescence intensity in each sample to that of a standard sample.

**Influence of GABA Stimulation on MMP Production in Cancer Cells in Vitro as Analyzed by Flow Cytometry and Immunohistochemistry.** We investigated the causal relationship between GABA and MMP production using human prostate cancer cell line C4-2, which originated from LN metastasis. Aliquots of cancer cells (2 × 10^6) were cultured in RPMI 1640 containing 5% FCS in the presence or absence of GABA at a concentration of 100 μM for 24 h. The cells were harvested after treatment with 0.2% EDTA. After fixation with 4% paraformaldehyde solution, the cells were incubated with a 10-fold dilution of goat antihuman polyclonal antibodies against MMP-2, -3, -7, or -9 (Santa Cruz Biotechnology) at 4°C overnight. The cells were then incubated with an 80-fold dilution of FITC-conjugated donkey antigoat IgG (Santa Cruz Biotechnology) at 4°C for 60 min. After each incubation, the cells were washed three times with PBS and analyzed with an EPICS ELITE flow cytometer (Coulter, Hialeah, FL).

For immunohistochemistry, C4-2 cancer cells were incubated with GABA at various concentrations (0, 100, or 1000 μM) for 24 h. After washing, cells were fixed with 4% paraformaldehyde. The expression of MMP-2, -3, -7, and -9 was assessed by the methods described above.

**Detection of the GABA Receptor Pathway by GABA Receptor Modulation Using GABA Receptor Agonist and Antagonist.** The C4-2 cancer cells were incubated with GABA receptor agonist muscimol (Sigma) or GABA receptor antagonist baclofen (Sigma) at 100 μM for 24 h. The production of MMP-3 was assessed by flow cytometric and immunohistochemical analysis. We then performed receptor blocking using GABA A receptor antagonist BMI (Sigma) or GABA B receptor antagonist CGP 35348 at various concentrations (0, 100, or 1000 μM) for 24 h. The production of MMP-3 was assessed by flow cytometry and immunohistochemistry.

**Influence of GABA Stimulation on the Invasive Ability of Cancer Cells as Analyzed by in Vitro Invasion Assay.** We investigated the influence of GABA stimulation on the invasive ability of cancer cells by *in vitro* invasion assay using a BioCoat Matrigel Invasion Chamber kit (Becton Dickinson Bioscience, Bedford, MA) according to the manufacturer’s instructions. We prepared cell suspensions of C4-2 cells (5 × 10^5 cells/ml) with or without GABA incubation at 100 μM for 24 h in serum-free RPMI 1640. We seeded the cells (2.5 × 10^5 cells in 500 μl) into the transwell insert chamber with a filter coated with Matrigel and placed the inserts in the lower chambers filled with 750 μl of RPMI 1640 containing 5% FCS. Chambers were incubated at 37°C under 5% CO2 atmosphere for 24 h. Thereafter, we removed the inserts and scraped off the noninvading cancer cells remaining on the upper side of the filter. The cells that had invaded to the lower side of the filter were viewed under a Nikon phase-contrast microscope and counted in >10 fields of view at ×200 magnification. The invasive ability of cancer cells was expressed as the mean number of cells that had invaded to the lower side of the filter, and results were represented as mean ± SD of cells/FV. The assay was done in triplicate, and two independent experiments were performed.

**Influence of MMP Blocking on GABA-Induced Cancer Cell Invasion.** We investigated whether GABA-induced cancer cell invasion is attributable to an increase in MMP activity; we performed blocking analysis using MMP inhibitor GM6001 (Calbiochem, San Diego, CA) in an *in vitro* invasion assay. Cancer cells were incubated with GM6001 at a concentration of 27 nm followed by GABA stimulation. Cells incubated with GM6001-negative, which is the specific product of negative control for GM6001, were used for negative controls. The invasive ability of cancer cells in each condition was examined using the BioCoat Matrigel Invasion Chamber kit as described above.

**Involvement of the GABA Receptor Pathway in the Invasive Ability of Cancer Cells.** To investigate and/or confirm the involvement of the GABA receptor pathway in the invasive ability of cancer cells, we performed an invasion assay with GABA receptor modulation using the GABA receptor agonists and antagonists as described above.

**Statistical Analysis.** The data on the expression of GABA, GAD, and MMPs in immunohistochemical study were subjected to an unpaired Student’s *t* test. The correlation between expression of GAD67 and Gleason score was examined using Fisher’s exact test. A binomial logistic regression analysis was performed to investigate which factor indicates a higher risk of cancer metastasis, the expression of GAD67 or a higher Gleason score. An unpaired Student’s *t* test was performed for the data from the *in vitro* invasion assay and flow cytometry.

**Results**

**Marked Expression of GABA, GAD67, and MMPs in the Prostate Sections of Cancer Patients with Metastasis and LN Metastasis, but not in Those of Prostate Cancer Patients without Metastasis or Patients with BPH.** We performed immunohistochemical staining of GABA and GAD67 in the prostates of cancer patients or patients with BPH. We observed marked expression of GABA and GAD67 in the prostate sections of cancer patients with metastasis (*n* = 72), whereas only sparse expression was noted in those of cancer patients without metastasis (*n* = 76) or patients with BPH (*n* = 152; Table 1; Fig. 1). The mean values of fluorescence intensity evaluated by confocal laser scanning microscope were significantly higher in the prostate sections of cancer patients with metastasis than in those without metastasis or in patients with BPH (Table 1). We then looked into staining of GABA and GAD67 in the LN metastases and compared them with that in normal LNs. We observed dense expression of GABA and GAD67 in the prostate sections of cancer patients with metastasis (*n* = 72), whereas only faint staining was seen in normal LNs.

We next examined the expression of MMP-2, -3, -7, and -9, which are believed to be important factors for cancer metastasis. The expression of these MMPs, especially MMP-3, was significantly higher in the prostate sections of cancer patients with metastasis than in those

**Table 1 Expression of GABA, GAD67, MMP-2, -3, -7, and -9 in the prostates and lymph nodes**

<table>
<thead>
<tr>
<th></th>
<th>Prostates</th>
<th>LN (metastasis)</th>
<th>LN (nonmetastasis)</th>
</tr>
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<tbody>
<tr>
<td>GABA</td>
<td>65.28 ± 7.50</td>
<td>17.34 ± 3.73</td>
<td>13.36 ± 3.40</td>
</tr>
<tr>
<td>GAD67</td>
<td>81.84 ± 10.15</td>
<td>20.97 ± 4.68</td>
<td>7.35 ± 1.83</td>
</tr>
<tr>
<td>MMP-2</td>
<td>27.12 ± 5.46</td>
<td>2.91 ± 1.01</td>
<td>0.63 ± 0.18</td>
</tr>
<tr>
<td>MMP-3</td>
<td>46.21 ± 6.33</td>
<td>4.88 ± 1.35</td>
<td>1.28 ± 0.65</td>
</tr>
<tr>
<td>MMP-7</td>
<td>19.52 ± 4.99</td>
<td>1.89 ± 0.72</td>
<td>0.48 ± 0.13</td>
</tr>
<tr>
<td>MMP-9</td>
<td>23.31 ± 5.53</td>
<td>2.56 ± 0.98</td>
<td>0.53 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Cancer (meta +)</td>
<td>Cancer (meta -)</td>
<td>BPH (meta +)</td>
</tr>
<tr>
<td></td>
<td>34.21 ± 8.33</td>
<td>3.06 ± 1.31</td>
<td>40.25 ± 10.56</td>
</tr>
<tr>
<td></td>
<td>23.17 ± 5.61</td>
<td>0.62 ± 0.35</td>
<td>33.52 ± 9.12</td>
</tr>
<tr>
<td></td>
<td>20.94 ± 5.26</td>
<td>0.62 ± 0.22</td>
<td>22.46 ± 5.78</td>
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*a* *P < 0.01, Cancer (meta +) versus Cancer (meta -).*

*b* *P < 0.01, LN (meta +) versus LN (meta -).*

8091

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without metastasis or those with BPH (Table 1; Fig. 1) and appeared in an area similar to that where GABA and GAD67 were observed. Moreover, marked expression of MMPs was found in the LN metastases, similar to the manner in which GABA and GAD67 expression appeared, whereas no significant staining was observed in normal LNs (Table 1; Fig. 1).

Expression of GAD67 Indicates a Significantly Higher Risk of Metastasis than Higher Gleason Score. We performed histological examination in each sample according to Gleason’s grading system and investigated the correlation between expression of GAD67 and Gleason score using Fisher’s exact analysis (Table 2). We found a significantly higher frequency of GAD67 expression in the prostates with higher Gleason scores ($P < 0.001$) when we divided prostate cancer cases into two groups, those with a Gleason score of $\leq 7$ ($n = 52$) and those with a Gleason score of $> 7$ ($n = 96$). On the basis of the data, we performed a binomial logistic regression analysis to investigate which factor indicates a higher risk of cancer metastasis (expression of GAD67 or a higher Gleason score). We found that expression of GAD67 was associated with a significantly higher risk of metastasis (odds ratio, 46.861; 95% confidence interval, 16.01–137.21) than a higher Gleason score (odds ratio, 3.463; 95% confidence interval, 1.05–11.48).

Increased in Vitro MMP Production in Cancer Cells after GABA Stimulation. We investigated the influence of GABA stimulation on in vitro MMP production using the C4-2 prostate cancer cell line. Cancer cells were incubated with or without GABA at 100 $\mu$m for 24 h, and production of MMP-2, -3, -7, and -9 was assessed by flow cytometry. The result was expressed as a ratio of each MMP-positive cell to total cells (mean ± SD %).

Table 2. Expression of GAD67 and Gleason scores in the prostates of cancer patients with or without metastasis

<table>
<thead>
<tr>
<th></th>
<th>Gleason ≤7</th>
<th>Gleason &gt;7</th>
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<tbody>
<tr>
<td>GAD67-positive</td>
<td>8 (4)</td>
<td>66 (61)</td>
</tr>
<tr>
<td>GAD67-negative</td>
<td>44 (4N)</td>
<td>30 (3)</td>
</tr>
</tbody>
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Table 3. Expression of MMP-2, -3, -7, and -9 in cancer cells with or without GABA stimulation

<table>
<thead>
<tr>
<th></th>
<th>MMP-2</th>
<th>MMP-3</th>
<th>MMP-7</th>
<th>MMP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.18</td>
<td>2.48</td>
<td>2.26</td>
<td>23.08</td>
</tr>
<tr>
<td>GABA (+)</td>
<td>63.24</td>
<td>88.30</td>
<td>33.00</td>
<td>64.68</td>
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</table>

$^{a} P < 0.01$ versus control.

Involvement of the GABA B Receptor Pathway on MMP Production in Cancer Cells. We then performed GABA receptor modulation using GABA receptor agonist (GABA B, muscimol; GABA A, baclofen) or antagonist (GABA B, BMI; GABA A, CGP 35348). The C4-2 prostate cancer cells were incubated with 100 $\mu$m muscimol or baclofen. Flow cytometric analysis demonstrated that GABA stimulation significantly increased the expression of all MMPs we analyzed, especially that of MMP-3 (Table 3; Fig. 2, A and B-a). More than 80% of cells treated with GABA expressed MMP-3, although <5% (2.48 ± 0.53%) of cancer cells without GABA stimulation were MMP-3 positive. Immunohistochemical study corroborated the results of flow cytometry, showing marked expression of MMPs in cancer cells with GABA stimulation but not in those without GABA stimulation (Fig. 2B, a and b).

Fig. 1. Representative pictures of H&E-stained sections (a, d, and g) and expression of GAD67 (b, e, and h) and MMP-3 (c, f, and i) analyzed by immunohistochemistry in the prostates of cancer patients with metastasis and LN metastasis or those without metastasis. We observed marked expression of GAD67 and MMP-3 in the prostates of cancer patients with metastasis (b and c) and LN metastasis (e and f) but only sparse expression in those of cancer patients without metastasis (h and i). Original magnification, $\times$100 in a–c and g–i; $\times$200 in d–f; $\times$400 in boxed area.

Table 3. Expression of MMP-2, -3, -7, and -9 in cancer cells with or without GABA stimulation
tion. No significant influence was noted on GABA-induced MMP production by addition of GABA receptor antagonist BMI at a concentration of 100 μM or 1 mM. More than 80% of cells treated with BMI followed by GABA stimulation showed production of MMP-3 (Fig. 2, A and B-e). In contrast, adding GABA receptor agonist CGP 35348 at a concentration of 1 mM significantly inhibited MMP-3 expression caused by GABA stimulation (Fig. 2, B, e and f).

GABA Stimulation Increases the Invasive Ability of Cancer Cells. We examined the influence of GABA stimulation on the invasive ability of cancer cells by an in vitro invasion assay using a BioCoat Matrigel Invasion Chamber kit. A filter coated with Matrigel was used as a model of the basement membrane. Cells that invaded to lower compartment of chamber through the filter were counted after a 24-h incubation. The number of invasive cells with GABA stimulation was significantly higher than that in cells without GABA stimulation (Fig. 2B).

Fig. 2. Influence of GABA stimulation and GABA receptor modulation on in vitro MMP-3 production in C4-2 cancer cells analyzed by flow cytometry and immunohistochemistry. A, percentage of MMP-3-positive cells after GABA stimulation or GABA receptor modulation analyzed by flow cytometry. We performed GABA stimulation (100 μM, 24 h) or GABA receptor modulation using GABA receptor agonist (GABA_A, muscimol; GABA_B, baclofen) or antagonist (GABA_A, BMI; GABA_B, CGP 35348). GABA stimulation increased MMP-3 expression in cancer cells compared with control cells. The GABA_B receptor agonist baclofen increased MMP-3 expression in cancer cells, and addition of the GABA_A antagonist CGP 35348 inhibited GABA-induced MMP-3 expression. In contrast, no significant influence of GABA_A receptor modulation was noted. B, representative data of flow cytometry and immunohistochemistry. a, no production of MMP-3 in cancer cells without GABA stimulation. b, markedly increased production of MMP-3 in cancer cells after incubation with GABA at a concentration of 100 μM for 24 h. c, no production of MMP-3 in cancer cells incubated with the GABA_A receptor agonist muscimol (100 μM, 24 h). d, increased MMP-3 production in cancer cells after incubation with the GABA_B receptor agonist baclofen (100 μM, 24 h). e, no influence of GABA_A receptor antagonist BMI (1 mM, 24 h) on production of MMP-3 caused by GABA stimulation. f, significant inhibition of GABA-induced MMP-3 production attributable to pretreatment with the GABA_B receptor antagonist CGP 35348 (1 mM, 24 h).
Inhibitor. We next performed blocking analysis in an invasion assay using GM6001 to investigate whether GABA-induced cancer cell invasion is attributable to an increase in MMP activity. Cancer cells were incubated with GM6001 followed by GABA stimulation. Cells incubated with GM6001-negative followed by GABA stimulation were used as negative controls. The addition of GM6001 significantly decreased GABA-induced cancer cell invasion, although GM6001-negative did not influence the invasive ability (the number of invasive cells incubated with GM6001 versus GM6001-negative = 16.73 ± 3.22 versus 59.3 ± 5.61; P < 0.01; Fig. 3, A and B-c).

Involvement of the GABA<sub>B</sub> Receptor Pathway in Cancer Cell Invasion. We further investigated the involvement of the GABA receptor pathway on the invasive ability of cancer cells by GABA receptor modulation. We observed a marked increase in the number of invasive cells when cells were incubated with the GABA<sub>A</sub> receptor agonist baclofen, but not with the GABA<sub>A</sub> receptor agonist muscimol (36.16 ± 7.62 versus 8.96 ± 1.35 cells/FV; P < 0.01; Fig. 3, A, B-d, and B-f). Moreover, addition of the GABA<sub>B</sub> receptor antagonist CGP 35348 greatly inhibited the GABA-induced increase in the number of invasive cells, but addition of the GABA<sub>A</sub> receptor antagonist BMI had minimal influence (GABA + CGP 35348, 17.22 ± 3.96 cells/FV; GABA + BMI, 59.17 ± 5.71 cells/FV; P < 0.01; Fig. 3, A, B-g, and B-h).

Discussion

In the present study, we found that expression of GABA and its major synthetic enzyme in prostate tissues, GAD67, increased significantly in the prostates of cancer patients with metastasis and LN metastasis, as compared with cancer patients without metastasis or patients with BPH. The results suggest that these molecules are potential candidates for a new clinical marker to predict patient prognosis, and this should be of great value clinically because the prognosis of the prostate cancer patients is closely related to the stage of the disease (1–3). There are several factors to indicate patients who are at risk of metastasis and predict patient prognosis. The Gleason score (14), which is the most widely used histological grading system for prostate cancer, is an important factor in predicting their prognosis. A recent meta-analysis found that the annual rate of developing metastasis was 2.1% in patients with Gleason scores of <4, compared with 5.4% in patients with Gleason scores between 5 and 7 and 13.5% in patients with Gleason scores of >7 (1–3). In the present study, we performed histological examination of each sample according to Gleason’s grading system and investigated the correlation between expression of GAD67 and Gleason score using Fisher’s exact test. We found a significantly higher frequency of GAD67 expression in the prostates with higher Gleason scores (P < 0.001) when we divided prostate cancer cases into two groups, those with a Gleason score of ≤7 (n = 52) and those with a Gleason score of >7 (n = 96). Moreover, we performed a binomial logistic regression analysis to investigate which factor indicates a higher risk of metastasis, expression of GAD67 or a higher Gleason score. The results clearly revealed that expression of GAD67 was associated with a significantly higher risk of metastasis than a higher Gleason score. These results indicate that increased GABA content and GAD activity are potentially involved in cancer metastasis, and these molecules can be candidate markers for metastasis in patients with prostate cancer.

The development of cancer metastasis is a complex cascade of events involving tumor dissemination from the primary site to distant organs. The cancer cells must detach from the primary tumor, invade the stromal tissue, enter the circulation, extravasate, and invade the target organ, forming a metastatic colony. During this process, proteolytic degradation of the extracellular matrix is an indispensable step, and MMPs are shown to be crucial protein-
ases that enable tumor cells to permeate the basement membrane and invade the surrounding tissues (11–13). MMPs were originally identified as degenerative proteases that cleave the matrix components. However, recent studies revealed that MMPs may not only cleave matrix components but may also have functions to release bioactive peptides such as growth factors and growth factor-binding proteins at a site of extracellular matrix remodeling. These molecules potentially influence cancer cell behavior such as cell shape, movement, growth, differentiation, and survival by controlling cell adhesion and/or the cytoskeletal machinery (11–13). For instance, MMP-3 may cleave the matrix molecule decorin, thereby releasing the growth factor transforming growth factor-β, which modulates cancer cell behavior such as cell proliferation and angiogenesis (15). Moreover, cleavage products of laminin 5 mediated by MMP-2-induced proteolysis have been shown to promote migration of keratinocyte cells (16). In addition to remodeling of matrix itself, MMPs can cleave matrix receptors. One of these receptors is E-cadherin. Down-regulation of E-cadherin may promote cancer cell invasion (17). Overexpression of MMP-3 in breast tumorigenic cells leads to the breakdown of cell junctions, and this has been related to an enhanced proteolysis of E-cadherin (18). The expression of MMPs is regulated by exogenous signals, e.g., cytokines, growth factors, or altered cell matrix and cell-cell contacts (11–13). MMPs are not constitutively expressed on the cells in vivo but are induced at times of active tissue remodeling. Activation of one MMP may trigger the activity of another MMPs, initiating a cascade-like reaction. Stromelysin-1, MMP-3, is one of key enzymes of this cascade. It degrades various types of extracellular matrices and activates other MMPs, such as MMP-7 and -9. This self-augmenting cascade reaction may play important roles in cancer invasion and metastasis (11–13).

In the present study, we observed marked expression of MMP-3 as well as other MMPs in the prostates of cancer patients with metastasis and LN metastasis along with dense expression of GABA and GAD67, although the expression level of these molecules was significantly lower in the prostates of cancer patients without metastasis or of patients with BPH. We therefore investigated the causal relationship between GABA expression and MMP production using human prostate cancer cell line C4-2, which originated from LN metastasis. MMP production was significantly increased in cancer cells when cells were stimulated with GABA. Moreover, the GABAA receptor agonist baclofen significantly increased MMP production, and the GABAA receptor antagonist CGP 35348 significantly inhibited an increase in MMP production caused by GABA stimulation, although we observed no influence on MMP production by GABA receptor modulation. These results suggest that GABA is a positive stimulator for production of MMP and that the GABAA receptor-mediated pathway may be involved in its signal transmission.

We next examined the influence of GABA stimulation and involvement of MMP activity on cancer cell invasion using a BioCoat Matrigel Invasion Chamber kit. We found that GABA stimulation significantly promoted the invasive ability of cancer cells, and blockade of MMP activity using MMP inhibitor GM6001 significantly decreased GABA-induced cancer cell invasion. These findings may indicate that GABA stimulation promotes cancer cell invasion and that GABA-induced cancer cell invasion is attributable to an increase in MMP.

We then performed GABA receptor modulation in an invasion assay to investigate and confirm the involvement of the GABAB receptor pathway in the invasive ability of cancer cells. We found that the GABAA receptor agonist baclofen significantly increased cancer cell invasion and that GABA-induced invasive ability was greatly inhibited by the GABAA receptor antagonist CGP 35348, whereas GABAA receptor modulation did not influence the invasive ability of cancer cells. This may be strong evidence that GABA-induced cancer cell invasion is mediated through the GABAB receptor pathway. Recently, however, Joseph et al. (19) reported that in vitro GABA stimulation significantly reduced norepinephrine-induced migratory activity in the SW 480 colon carcinoma cell line, and its inhibitory effect was mediated by GABAB receptor. Different cell types and/or different extracellular conditions may explain the discrepancy between their results and ours in these two studies. SW 480 is a colon carcinoma cell line originating from primary cancer sites. In contrast, C4-2, which we used in this study, is a human prostate cancer cell line originating from LN metastasis. Although further investigation is necessary to clarify the exact mechanisms or functioning action of GABA on cancer metastasis, we propose that GABA influences the invasive ability of cancer cells.

In conclusion, our present results indicate that GABA promotes cancer cell invasion via the GABAB receptor pathway, and an increase in MMP activity is an underlying mechanism of functioning action. Several previous studies have demonstrated that inhibition of MMP activity may not satisfactorily prevent cancer growth or metastasis (20). Although further investigation is necessary, blockade of the GABA-mediated pathway, possibly combined with MMP inhibition, may have potential therapeutic value to prevent cancer progression or metastasis and may warrant further attention, especially for treatment of cancer patients with metastasis.

Acknowledgments

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References


8095
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