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Opposite effects of GPR120 and GPR40 on cell motile activity induced by ethionine in liver epithelial cells

Shuhei Ishii [a], Miku Hirane [a], Sayumi Kato [a], Nobuyuki Fukushima [b], Toshifumi Tsujiuchi [a,*]

[a] Division of Cancer Biology and Bioinformatics, Department of Life Science, Faculty of Science and Engineering, Kinki University, 3-4-1, Kowakae, Higashiosaka, Osaka 577-8502, Japan
[b] Division of Molecular Neurobiology, Department of Life Science, Faculty of Science and Engineering, Kinki University, 3-4-1, Kowakae, Higashiosaka, Osaka 577-8502, Japan

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ABSTRACT

Free fatty acids (FFAs) are dietary nutrients which act as ligands for FFAs receptors. G-protein-coupled receptor 120 (GPR120) and GPR40 are activated by long and medium chain FFAs. In the present study, we investigated the role of the GPR120 and GPR40 in cell motile activity stimulated by ethionine in rat liver epithelial WB-F344 cells. Cells were treated with ethionine at a concentration of 10 μM every 24 h for 2 days. The expression levels of the *Gpr120* and *Gpr40* genes in WB-F344 cells treated ethionine were significantly higher than those in untreated cells. In cell motility assay, the cell motile activity of WB-F344 cells was markedly elevated by ethionine, compared with untreated cells. To evaluate the effects of GPR120 on the cell motile activity by ethionine, we established GPR120 knockdown cells from WB-F344 cells. The cell motile activity stimulated by ethionine was significantly suppressed by GPR120 knockdown. In addition, a potent GPR40 antagonist GW1100 enhanced the cell motile activity by ethionine. These results suggest that opposite effects of GPR120 and GPR40 may be involved in the cell motile activity stimulated by ethionine in WB-F344 cells.

1. Introduction

G-protein-coupled receptors (GPCRs) comprise a family of seven transmembrane domain receptors that mediate a variety of cellular functions [1–3]. Among GPCRs, free fatty acids (FFAs) receptors have been identified, including GPCR 120 (GPR120) and GPR40. GPR120 and GPR40 are activated by the binding of long and medium chain FFAs [4,5]. GPR120 regulates the secretion of glucagon-like peptide-1 and indicates insulin-sensitizing and anti-inflammatory effects [6,7]. On the other hand, GPR40 promotes the insulin secretion stimulated by glucose in pancreatic islet cells [8]. Therefore, GPR120 and GPR40 are considered as target molecules for obesity, diabetes and inflammatory disorders [9].

Ethionine is an ethyl analog of methionine and has a variety of biological actions through an antagonistic effect to methionine in animals, such as growth inhibition, fatty liver and pancreatic acinar necrosis [10]. In addition, ethionine has potent liver carcinogenic potency in rats. The prolonged ethionine feeding at 0.25% level for 8 months induced liver tumors in rats [11]. Ethionine interferes with the methionine metabolism, leading to depletion of the primary methyl donor, S-adenosylmethionine. The hepatocarcinogenic effects of ethionine were prevented by the extra supplemented administration of methionine in rats [10].

In our recent study, we indicated that ethionine regulated cell motile activity through lysophosphatidic acid (LPA) signaling in rat liver epithelial WB-F344 cells [12]. LPA is a physiological lipid mediator which exhibits a wide range of cellular responses through G-protein-coupled LPA receptors [13,14]. In the present study, to assess the roles of GPR120 and GPR40 in cell motile activity induced by ethionine, we measured the expression levels of the *Gpr120* and *Gpr40* genes and the cell motile activity in WB-F344 cells treated with ethionine.

2. Materials and methods

2.1. Cell culture and treatment

WB-F344 cells used in this study were cultured in DMEM (Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 10% fetal bovine serum (FBS) in 5% CO_2 atmosphere at 37 °C. Cells were treated with ethionine (Sigma, St. Louis, MO, USA) at a

Abbreviations: GPR120, G-protein-coupled receptor 120; GPCR, G-protein-coupled receptor; RT, reverse transcription; PCR, polymerase chain reaction.

* Corresponding author. Fax: +81 6 6721 2721.
E-mail address: ttujiuch@life.kindai.ac.jp (T. Tsujiuchi).

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concentration of 10 μM every 24 h for 2 days [12]. In addition, to assess the effects of long-term ethinonine treatment, cells were treated with increasing concentrations of ethionine at a range of 0.1–2 μM for approximately 6 months.

2.2. Reverse transcription (RT) – polymerase chain reaction (PCR) analysis for Gpr120 and Gpr40 expressions

Single-strand cDNAs were synthesized with Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Semi-quantitative RT-PCR analysis for the amplification of the *Gpr120* and *Gpr40* genes was performed. The PCR products were separated on 2% agarose gels containing 0.05 μg/ml ethidium bromide. For quantitative real-time RT-PCR analysis, SYBR *Premix Ex Taq* (Tli RNaseH Plus) (TaKaRa Bio, Inc., Shiga, Japan) and a Smart Cycler II System (TaKaRa Bio) were used as described previously [12]. The primer sets used in this assay were as follows: rat *Gpr120* (GenBank accession number; AB207868) F: 5′-CTGGCC GTCCCTTTTCTTCT-3′, R: 5′-TGTTCCTCCACTCGCTCCTG-3′; and rat *Gpr40* (GenBank accession number; AF539810) F: 5′-CCCACTTTGC GCCCCTCTAT-3′, R: 5′-GCCTCCGGATGGCTTGGTAT-3′. The expression levels of target genes were normalized to those of rat *Gapdh*.

2.3. Cell motility assay

For cell motility assay, cells were seeded on Cell Culture Inserts with 8 μm pore size (BD Falcon, Franklin Lakes, NJ) at 1×10^5 cells in 200 μl of serum-free DMEM (upper chamber). The filters were then placed in 24-well plates containing 800 μl of DMEM supplemented with 10% FBS (lower chamber), and incubated for 20 h. Some cells were pretreated with GW9508 (10 μM) (Sigma) and/or GW1100 (1 μM) (Sigma) for 30 min. The number of cells moved to the lower side of the filter was counted after Giemsa staining [12,15].

2.4. Establishment of GPR120 knockdown cells

To assess the effects of GPR120 on the cell motile activity induced by ethionine, we generated GPR120 knockdown (WB-sh120) cells form WB-F344 cells. Briefly, a HuSH short hairpin RNA plasmid (29-mer) against GPR120 (Origene, Rockville, MD) was transfected into WB-F344 cells using X-tremeGENE HP Transfection Reagent (Roche Diagnostics GmbH). The transfected cells were selected with puromycin for at least 4 weeks and a stable clone (WB-sh120) was obtained. As control cells, WB-cont cells were also generated using a control (vector) plasmid [12].

2.5. Cell proliferation assay

For cell proliferation assay, cells were plated at 3000 cells/well in 96-well plates and cultured in 100 μl of DMEM containing 10% FBS. To measure cell proliferation rate for 3 days, solution from Cell Counting Kit-8 (Dojin Chemistry, Kumamoto, Japan) was added to each well at 0, 1 or 3 days. After incubation for 1 h, the absorbance of the culture medium at 450 nm was determined [16].

2.6. Animal treatment

Male F344 rats, at 6 week of age (Japan SLC Inc., Shizuoka, Japan) received the diet containing 0.05% ethionine. Animals were killed at 1 and 3 weeks after the beginning of the experiment and the livers were immediately excised. All animals were housed in our facilities and cared in accordance with the institutional laboratory animal care guidelines.

2.7. Statistical analysis

Statistical analysis was performed using Student's *t*-test. The data were recognized to differ significantly for values of $p < 0.01$. The results are given as means ± SD.

3. Results

3.1. Gpr120 and Gpr40 gene expressions and cell motile activity induced by ethionine

To assess effects of ethionine on the *Gpr120* and *Gpr40* expressions and the cell motile activity, cells were treated with ethionine (10 μM) every 24 h for 2 days. The *Gpr120* gene was expressed in WB-F344 cells treated with or without ethionine. In contrast, while the *Gpr40* expression was undetectable in WB-F344 cells by semi-quantitative RT-PCR analysis, ethionine elevated the *Gpr40* expression (Fig. 1(A)). In addition, the expression levels of the *Gpr120* and *Gpr40* genes were confirmed by quantitative real time RT-PCR analysis. The *Gpr120* and *Gpr40* expressions were significantly elevated in WB-F344 cells treated with ethionine, compared with untreated cells (Fig. 1(B)). The cell motile activity of WB-F344 cells treated with ethionine was significantly higher than that of untreated cells. Moreover, some cells were pretreated with GW9508 (10 μM) which is an agonist of GPR120 and GPR40 [17]. The cell motile activity was significantly enhanced by GW9508 in WB-F344 cells treated with or without ethionine (Fig. 1(C)).

3.2. Effects of GPR120 knockdown on cell motile activity induced by ethionine

To investigate the roles of GPR120 and GPR40 in the cell motile activity induced by ethionine, we established GPR120 knockdown (WB-sh120) cells from WB-F344 cells (Fig. 2(A)). While ethionine stimulated the cell motile activity of control WB-cont cells, the cell motile activity of WB-sh120 cells was significantly suppressed by ethionine. GW9508 enhanced the cell motile activity stimulated by ethionine in WB-cont cells. In contrast, the cell motile activity was markedly inhibited by GW9508 in WB-sh120 cells treated with or without ethionine (Fig. 2(B)).

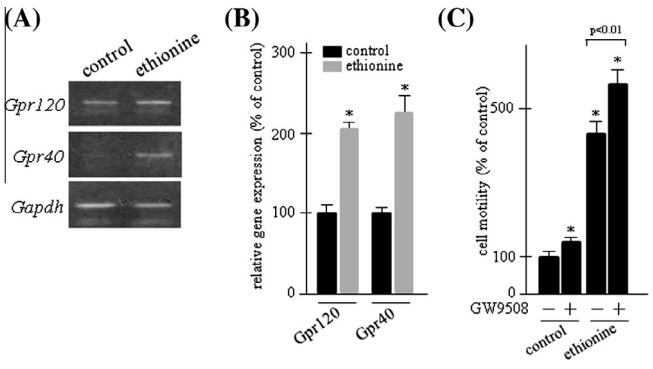

Fig. 1. GPR120 and GPR40 gene expressions and cell motile activity by ethionine. Cells were treated with ethionine at a concentration of 10 μM every 24 h for 3 days. (A) Expression patterns of *Gpr120* and *Gpr40* genes by semi-quantitative RT-PCR analysis. control; ethionine untreated cells. (B) Expression levels of *Gpr120* and *Gpr40* genes by quantitative real-time RT-PCR analysis. Columns indicate the mean of three studies; bars indicate SD. $^*p<0.01$ vs. untreated cells (control). (C) The cell motility assay with Cell Culture Insert. After pretreatment of GW9508 (10 μM) for 30 min, some cells were seeded on the filters at 1×10^5 cells and incubated for 20 h. Columns indicate the mean of three studies; bars indicate SD. $^*p < 0.01$ vs. untreated cells (control).

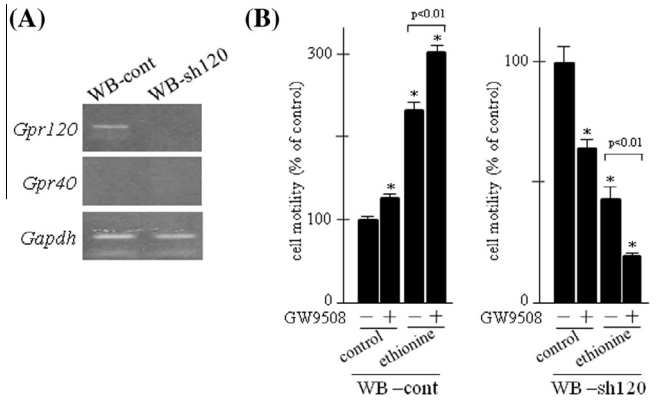

Fig. 2. Effects of GPR120 knockdown on cell motile activity. (A) Expression patterns of *Gpr120* and *Gpr40* genes in GPR120 knockdown (WB-sh120) and control (WB-cont) cells by semi-quantitative RT-PCR analysis. (B) Cell motile activity of WB-sh120 cells treated with ethionine. Cells were pretreated with or without ethionine at a concentration of 10 μM every 24 h for 2 days. Before cell motility assay, some cells were treated with GW9508 (10 μM) for 30 min. Data are indicated as the percentages of untreated cells. Columns indicate the mean of three studies; bars indicate SD. $^{*}p < 0.01$ vs. untreated cells (control).

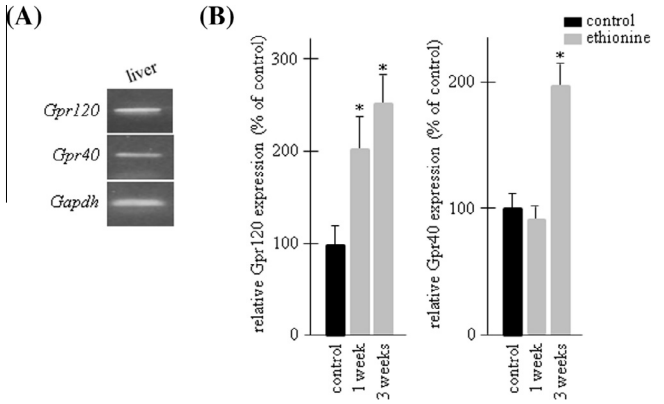

Fig. 3. *Gpr120* and *Gpr40* expressions in livers of rats fed ethionine-containing diet. (A) The expression patterns of the *Gpr120* and *Gpr40* genes in normal rat liver by semi-quantitative RT-PCR analysis. (B) Expression levels of *Gpr120* and *Gpr40* genes in livers of rats fed ethionine for 1 and 3 weeks by quantitative real-time RT-PCR analysis. Columns indicate the mean of three studies; bars indicate SD. $^{*}p < 0.01$ vs. normal liver tissues (control).

3.3. Gpr120 and Gpr40 expressions in livers of rats fed ethionine-containing diet

The expressions of the *Gpr120* and *Gpr40* genes were detected in normal liver tissue of rats (Fig. 3(A)). To examine whether ethionine increases the *Gpr120* and *Gpr40* expression levels in rat livers, rats received the diet containing 0.05% ethionine for 1 and 3 weeks. For quantitative real time RT-PCR analysis, total RNA was extracted from the pooled whole liver samples from 3 rats fed ethionine for 1 or 3 weeks. The *Gpr120* gene expressions were significantly elevated in the livers of rats fed ethionine for 1 and 3 weeks, compared with control livers. The increased *Gpr40* expressions were also found in the livers of rats fed ethionine for 3 weeks, but not 1 week (Fig. 3(B)).

3.4. Effects of long-term ethionine treatment on cell motile activity of WB-F344 cells

Finally, to investigate the effects of long-term ethionine treatment on cell motile activity, we established WB-et cells by continuous treatment of ethionine at a range of 0.1–2 μM for approximately 6 months (Fig. 4(A)). The cell growth rate of WB-et cells was significantly higher than that of WB-F344 cells (Fig. 4(B)). The expression levels of the *Gpr120* and *Gpr40* genes were elevated in WB-et cells in comparison with control cells (Fig. 4(C)). The cell motile activity of WB-et cells was significantly higher than that of control cells. GW9508 enhanced the cell motile activity of both cells. In addition, some cells were pretreated with a GPR40 antagonist GW1100 (1 μM) [17]. GW1100 markedly enhanced the cell motile activity increased by GW9508 in both cells (Fig. 4(D)).

4. Discussion

Recently we reported that the induction of LPA receptor-3 (LPA_3) was involved in the cell motile activity stimulated by ethionine in WB-F344 cells [12]. Moreover, LPA_3 enhanced the cell motile activities of WB-F344 cells treated with 12-O-tetradecanoylphorbol-13-acetate, 17β-estradiol and ethinyl estradiol [18,19]. In contrast, diethylstilbestrol suppressed the cell motile activity of WB-F344 cells, correlating with the induction of LPA_1 expression [19]. Since GPR120 and GPR40 belong to GPCRs as well as LPA receptors, we assessed the roles of GPR120 and GPR40 in cell motile activity induced by ethionine in WB-F344 cells. The present study indicated that ethionine increased the expression levels of the *Gpr120* and *Gpr40* genes in WB-F344 cells and simultaneously elevated the cell motile activity. The increased cell motile activity by ethionine was significantly suppressed by

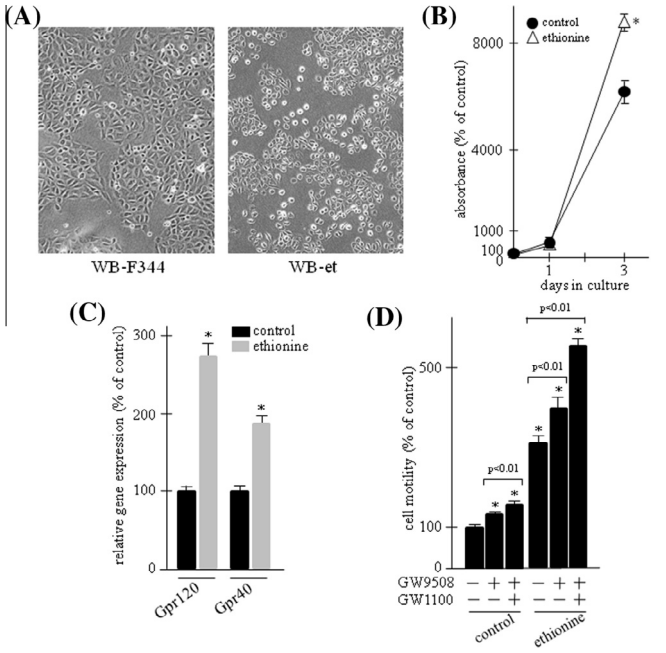

Fig. 4. Effects of long-term ethionine treatment on cell motile activity of WB-F344 cells. Cells were treated with increasing concentrations of ethionine at a range of 0.1–2 μM for approximately 6 months. (A) Morphology of WB-F344 and the long-term ethionine treated (WB-et) cells in a serum-containing medium. (B) Cell proliferation rate of control WB-F344 and WB-et cells. Cells were cultured in a serum-containing medium. Data are expressed as a percentage of cell number on day 0. Bars indicate SD. $^{*}p < 0.01$ vs. control cells. (C) Expression levels of *Gpr120* and *Gpr40* genes by quantitative real-time RT-PCR analysis. Columns indicate the mean of three studies; bars indicate SD. $^{*}p < 0.01$ vs. untreated cells. (D) Cell motile activity of WB-et cells treated with ethionine. Cells were pretreated with or without ethionine at a concentration of 10 μM every 24 h for 2 days. Before cell motility assay, some cells were treated with GW9508 (10 μM) and GW1100 (1 μM) for 30 min. Data are indicated as the percentages of untreated cells. Columns indicate the mean of three studies; bars indicate SD. $^{*}p < 0.01$ vs. control cells.

GPR120 knockdown. GW9508 is used as the agonist of GPR120 and GPR40 [17]. While the cell motile activity was enhanced by GW9508 in WB-F344 cells treated with ethionine, GW9508 inhibited the cell motile activity of GPR120 knockdown cells. In addition, the GPR40 antagonist GW1100 elevated the cell motile activity by ethionine [17]. Therefore, these results demonstrate that GPR120 enhanced and GPR40 suppressed the cell motile activity, suggesting that opposite effects of GPR120 and GPR40 are involved in the cell motile activity stimulated by ethionine in WB-F344 cells, similar to the case for LPA_1 and LPA_3 [12,18,19].

In normal tissues, the expression patterns of GPR120 and GPR40 are not uniform. GPR120 is highly expressed in adipocytes, lung and intestinal tract, while GPR40 is shown to be expressed in pancreatic islet cells and intestinal tract [7,20]. It is considered that the distribution of the GPR120 and GPR40 expressions may be closely related to energy homeostasis, such as metabolism and immune process [7,21]. In normal liver tissues, *Gpr120* and *Gpr40* genes were well expressed. In addition, the expression levels of the *Gpr120* and *Gpr40* genes were significantly elevated by the feeding of ethionine in rat livers. Based on these results, we generated the long-term ethionine treated cells and measured the expression levels of the *Gpr120* and *Gpr40* genes and the cell motile activity. The expression levels of the *Gpr120* and *Gpr40* genes were increased and the cell motile activity was enhanced by the long-term ethionine treatment, similar to the case for the short-term ethionine treamtment. Since ethionine is one of liver carcinogens, the roles of GPR120 and GPR40 in the development of liver tumors induced by ethionine in rats should be further clarified.

In conclusion, we demonstrated that the diverse roles of GPR120 and GPR40 are involved in the activation of cell motile activity induced by ethionine in WB-F344 cells. Recently, it has been reported that the high expression levels of GPR120 were found in colorectal cancers in comparison with adjacent noncancerous tissues. The cell motile activity and angiogenesis process were enhanced by the activation of GPR120 in colorectal carcinoma cells [22]. Taken together with these findings, it is possible that GPR120 and GPR40 may be novel chemopreventive targets for the establishment of anti-cancer therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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