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Research article

Diverse effects of G-protein-coupled free fatty acid receptors on the regulation of cellular functions in lung cancer cells

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ABSTRACT

Free fatty acids (FFAs) are dietary nutrients which mediate a variety of biological effects through binding to G-protein-coupled FFA receptors (FFARs). G-protein-coupled receptor 120 (GPR120) and GPR40 are identified as FFARs for long- and medium-chain fatty acids. Here we investigated whether GPR120 and GPR40 are involved in the acquisition of malignant properties in lung cancer cells. Three lung cancer RLCNR, LL/2 and A549 cells used in this study expressed *GPR120* and *GPR40* genes. The cell motile activities of all cells were significantly suppressed by a GPR40 antagonist GW1100. In addition, GPR40 knockdown inhibited the cell motile activity of A549 cells. In gelatin zymography, matrix metalloproteinase-2 (MMP-2) activity in GPR40 knockdown was significantly lower than that in control cells. Next, to evaluate effects of GPR120 and GPR40 on cellular functions induced by anti-cancer drug, the long-term cisplatin (CDDP) treated (A549-CDDP) cells were generated. The expression levels of *GPR120* and *GPR40* were significantly decreased in A549-CDDP cells. While A549-CDDP cells showed the high cell motile activity, GW1100 suppressed the cell motile activity of A549-CDDP cells. These results demonstrate that GPR120 negatively and GPR40 positively regulate cellular functions during tumor progression in lung cancer cells.

1. Introduction

G-protein-coupled receptors (GPCRs) are seven-transmembrane receptors which mediate several cellular functions, including cell growth, motility, differentiation and morphogenesis [1–3]. Free fatty acid (FFA) receptors belong to a member of GPCRs and exhibit a variety of biological responses via binding of FFAs [4–6]. GPCR 120 (GPR120) and GPR40 are identified as GPCRs for FFAs. FFAs are essential dietary nutrients and classified by the length of carbon chains. GPR120 and GPR40 are activated by long- and medium-chain FFAs [7,8]. The distribution of GPR120 and GPR40 expressions is closely related to tissues for metabolic controls. Moreover, GPR120 and GPR40 are involved in the regulation of hormone secretion from pancreatic islet cells and digestive tract [9–11]. Therefore, GPR120 and GPR40 are considered as potent target molecules for the treatment of metabolic disorders, such as diabetes and obesity [12–14].

Recently, it has been reported that GPR120 and GPR40 play important roles in the pathogenesis of cancer cells. The aberrant expressions of GPR120 were detected in colorectal carcinomas, compared with adjacent noncancerous tissues. The activation of GPR120 enhanced cell motile activity and angiogenic potency of colon cancer cells [15]. In contrast, GPR40 inhibited cell motile and invasive activities of fibrosarcoma cells [16]. In addition, GPR120 increased and GPR40 decreased cell motile and invasive activities of pancreatic cancer cells [17]. Therefore, these findings suggest that opposite effects of GPR120 and GPR40 regulate cellular functions of cancer cells.

Lung cancer is one of the most common human malignancies [18], but the rate-limiting molecular events during the development of lung cancer remain to be clarified. In the present study, to assess whether intracellular signaling via GPR120 and GPR40 may be a candidate molecule for chemotherapeutic treatment of lung cancers, we investigated the effects of GPR120 and GPR40 on cellular functions in three lung cancer cells.

2. Materials and methods

2.1. Cell culture and treatment

Rat RLCNR, mouse LL/2 and human A549 cells used in this study were cultured in Dulbecco's modified Eagle's medium

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(DMEM) (Wako Pure Chemical Industries Ltd., Osaka, Japan) containing 10% fetal bovine serum (FBS) in a 5% CO_2 atmosphere at 37 °C. To generate the long-term anti-cancer drug treated cells, A549 cells were treated by the stepwise treatment of cisplatin (CDDP) (Sigma, St. Louis, MO, USA) at a range of 0.01–1.0 μM for at least 6 months [16].

2.2. Cell proliferation assay

Cells were seeded at 4000 cells/well in 96-well plates and maintained in DMEM containing 10% FBS. The cell proliferation rate was measured with the Cell Counting Kit-8 (CCK-8) (Dojin Chemistry, Kumamoto, Japan). A solution from CCK-8 was added to each plate at 0, 1 or 3 days. To examine effects of GPR120 and GPR40 on cell proliferation, cells were cultured in DMEM containing 5% charcoal stripped FBS (Sigma) and treated with GW9508 (Sigma) and GW1100 (Sigma) at concentrations of 1–10 μM every 24 h for 3 days [16,17].

2.3. Cell motility assay

Cells were pretreated with GW9508 (10 μM) or GW1100 (1 μM) for 30 min and seeded into a Cell Culture Insert (BD Falcon, NJ, USA) with 8 μm pore size at 1×10^5 cells in 200 μl of serum-free DMEM (upper chamber). The filters were placed in 24-well plates (lower chamber) containing 800 μl of DMEM supplemented with 5% charcoal stripped FBS with GW9508 (10 μM) and cultured for 20 h. The percentage of cells moved to the lower side of the filter was counted after Giemsa staining [16,17].

2.4. Establishment of GPR40 knockdown cells from A549 cells

GPR40 knockdown (A549-40) cells were established using the X-tremeGENE HP Transfection Reagent (Roche Diagnostics Co. Ltd., Mannheim, Germany). Briefly, a HuSH short hairpin RNA plasmid (29-mer) against human GPR40 (Origene, Rockville, MD) was transfected into A549 cells. After 3 days, the transfected cells were treated with puromycin (Wako) for at least 2 weeks. Control (A549-R) cells were also generated using a vector plasmid without the target sequence [16,17].

2.5. Gelatin zymography

Cells were maintained in serum-free DMEM with or without GW9508 (10 μM) for 48 or 72 h. The supernatants obtained from the individual cells were loaded on a 10% SDS-PAGE containing 0.1% gelatin. After electrophoresis, the gels were washed twice with washing buffer (50 mM Tris–HCl (pH 7.5), 100 mM NaCl and 2.5% Triton X-100) for 30 min and incubated in reaction buffer (50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 10 mM $CaCl_2$ and 0.02% NaN_3) at 37 °C for 16 h. The gels were stained with 0.25% Coomassie Brilliant Blue R250 (Wako). The densities of bands were quantitatively determined with image analysis software (NIH Image, Bethesda, MD) [16,17].

2.6. Tube formation assay

To assess effects of GPR120 and GPR40 on tube formation of endothelial F-2 cells, cells were maintained in serum-free DMEM for 2 days. For tube formation assay, Matrigel (BD Falcon, NJ, USA) at 100 μl/well was plated in 96-well plates and incubated at 37 °C. After 30 min, F-2 cells were seeded onto Matrigel-coated plates at 4×10^4 cells in 100 μl of supernatants from the individual cells and cultured at 37 °C for 4 h. The length of total tube formation was measured from three representative 100 × fields/well [19].

2.7. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis

After RNA extraction using ISOGEN (Nippon Gene, Inc. Toyama, Japan), cDNA was synthesized with a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics). For quantitative real-time RT-PCR analysis, the target genes were amplified with SYBR *Premix Ex Taq* (Tli RNaseH Plus) (TaKaRa Bio Inc., Shiga, Japan) and the expression values were measured using a Smart Cycler II System (TaKaRa) [16,17].

2.8. Statistical analysis

Quantitative differences were statistically analyzed using ANOVA (analysis of variance) with a post-hoc test. The data were recognized to differ significantly for values of $p < 0.01$. The results were given as means ± SD.

3. Results

3.1. Roles of Gpr120 and Gpr40 on cell proliferation and motility in RLCNR and LL/2 cells

The expression patterns of *Gpr120* and *Gpr40* genes in RLCNR and LL/2 cells are shown in Fig. 1A. To assess effects of Gpr120 and Gpr40 on cell proliferation and motile activity, cells were treated with GW9508 which is an agonist of GPR120 and GPR40 [20,21]. In addition, GW1100 was used as a GPR40 antagonist [20–22]. In cell proliferation assay, no effect of GW9508 and GW1100 on cell growth rate was found in RLCNR and LL/2 cells (Fig. 1B). The cell motile activities of RLCNR and LL/2 cells were significantly stimulated by GW9508, compared with untreated cells. On the other hand, the cell motile activities of both cells stimulated by GW9508 were markedly suppressed by GW1100 (Fig. 1C).

3.2. Roles of GPR120 and GPR40 on cell proliferation and motility in A549 cells

The expressions of *GPR120* and *GPR40* genes were detected in A549 cells (Fig. 2A). While no change of cell proliferation rate was found in A549 cells treated with GW9508 alone, the treatment of GW9508 and GW1100 inhibited the cell proliferation rate of A549 cells (Fig. 2B). The cell motile activity of A549 cells treated with GW9508 was significantly lower than that of untreated cells. Moreover, the cell motile activity of A549 cells treated with GW9508 was markedly suppressed by GW1100 (Fig. 2C).

3.3. Effects of GPR40 knockdown on cellular functions of A549 cells

To confirm effects of GPR120 and GPR40 on cell proliferation and motile activity of A549 cells, GPR40 knockdown (A549-40) cells were generated from A549 cells (Fig. 3A). The cell proliferation rate of A549-40 cells was similar to that of control A549-R cells (Fig. 3B). The cell growth activity of A549-40 cells for 3 days was significantly inhibited by GW9508 at a concentration of 10 μM, but not A549-R cells (Fig. 3C). The cell motile activity of A549-40 cells was markedly lower than that of A549-R cells. In addition, GW9508 decreased the cell motile activities of both cells (Fig. 3D). In gelatin zymography, matrix metalloproteinase-2 (MMP-2) activity of A549-40 cells was significantly lower than that of A549-R cells. GW9508 reduced the MMP-2 activities of both cells. No activation of MMP-9 was found in all cells (Fig. 3 E and F).

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