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Different roles of GPR120 and GPR40 in the acquisition of malignant properties in pancreatic cancer cells

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ABSTRACT

Free fatty acids (FFAs) act as extracellular signaling molecules through binding to G-protein-coupled FFA receptors (FFARs). GPR120 and GPR40 are identified as FFARs for medium- and long-chain fatty acids. In the present study, we investigated roles of GPR120 and GPR40 in cellular functions of pancreatic cancer PANC-1 cells, using GPR120 and GPR40 knockdown cells (PANC-sh120 and PANC-sh40 cells respectively). In cell motility assay, PANC-sh120 cells showed the low cell motility, compared with control cells. In contrast, the cell motility of PANC-sh40 cells was significantly higher than that of control cells. Activity levels of matrix metalloproteinases (MMPs) were measured by gelatin zymography. While PANC-sh120 cells indicated the reduced MMP-2 activity, MMP-2 activity in PANC-sh40 cells was significantly higher than that in control cells. On the other hand, no activation of MMP-9 was detected in all cells. In colony assay, the large sized colonies were markedly formed in PANC-sh40 cells. No colony formation was observed in PANC-sh120 cells as well as control cells. These results suggest that distinct effects of GPR120 and GPR40 are involved in the acquisition of malignant property in pancreatic cancer cells.

1. Introduction

Free fatty acids (FFAs) are dietary nutrients and essential energy sources. Fatty acids are classified by the length of carbon chains. Short-chain fatty acids have fewer than 6 carbons, medium-chain fatty acids have 6 to 12 carbons and long-chain fatty acids contain more than 12 carbons [1–3]. FFAs act as extracellular signaling molecules through binding to FFA receptors (FFARs), which belong to a family of G-protein-coupled transmembrane receptors [3–5].

Among FFARs, GPR120 and GPR40 are functionally activated by long- and medium-chain FFAs [3–5]. However, the distribution and biological response of GPR120 and GPR40 are not uniform. High expression of GPR120 is found in gastrointestinal tract, lung, adipocytes and macrophages, suggesting that GPR120 is involved in the regulation of metabolism and immune response [4,6,7]. On the other hand, pancreatic beta cells highly expressed GPR40 in pancreatic tissues, suggesting that GPR40 stimulates insulin secretion [8]. Moreover, GPR40 expression was highly detected in insulinoma cells, but not in glucagonoma cells [9].

In colorectal carcinoma cells, activated GPR120 increased cell motile activity and angiogenesis process [10]. Recently, we showed that GPR120 enhanced and GPR40 suppressed cell motile activity of liver epithelial cells treated with chemical agents [11,12]. In the present study, we investigated the roles of GPR120 and GPR40 in cellular functions of pancreatic cancer cells. HPD1NR and HPD2NR cells used in this study were established from hamster pancreatic duct adenocarcinomas [13]. In addition, GPR120 and GPR40 knockdown cells were generated from human pancreatic PANC-1 cells.

2. Materials and methods

2.1. Cell culture

Three pancreatic cancer (HPD1NR, HPD2NR and PANC-1) cells were maintained in Dulbecco's modified Eagle's medium (DMEM)

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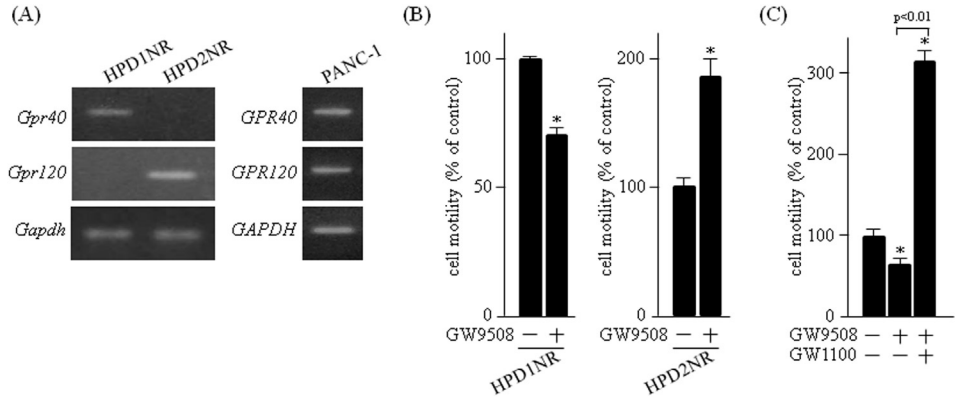

Fig. 1. Effects of GPR120 and GPR40 on cell motility of pancreatic cancer cells. (A) Expression patterns of GPR120 and GPR40 genes by semi-quantitative RT-PCR analysis. (B) Cell motility assay. Cells were pretreated with GW9508 (10 μM) for 60 min and seeded on the filters at 5×10^4 cells and incubated for 16 h. Columns indicate the mean of three studies; bars indicate SD. *; $p < 0.01$ vs. untreated cells. (C) Effects of GW1100 on cell motility. Before cell motility assay, cells were treated with GW9508 (10 μM) and GW1100 (1 μM) for 60 min. Columns indicate the mean of three studies; bars indicate SD. *; $p < 0.01$ vs. untreated cells.

(Wako Pure Chemical Industries Ltd., Osaka, Japan) containing 10% fetal bovine serum (FBS) in a 5% CO_2 atmosphere at 37 °C.

2.2. Establishment of GPR40 and GPR120 knockdown cells

To generate GPR40 and GPR120 knockdown (PANC-sh40 and PANC-sh120, respectively) cells, a HuSH short hairpin RNA plasmid (29-mer) against GPR120 or GPR40 (Origene, Rockville, MD) was used. Briefly, each plasmid was transfected into PANC-1 cells using X-tremeGENE HP Transfection Reagent (Roche Diagnostics GmbH, Mannheim, Germany). To obtain a stable clone, cells were selected by puromycin treatment for at least 3 weeks. PANC-RFP cells were established as control cells using a control vector plasmid without a target sequence [11,12].

2.3. Cell proliferation assay

Cells were seeded at 3000 cells/well in 96-well plates and cultured in DMEM containing 10% FBS. Cell growth rate was determined using the Cell Counting Kit-8 (CCK-8) (Dojin Chemistry, Kumamoto, Japan). CCK-8 reagent was added to each plate at 0, 1 or 3 days. To examine effects of GW9508 on cell growth, some cells were maintained in DMEM containing 5% charcoal stripped FBS (Sigma, St. Louis, MO, USA) and treated with GW9508 (Sigma) at concentrations of 1 and10 μM every 24 h. After 3 days, the CCK-8 was added to each well. These assays were performed in triplicate [11,12].

2.4. Cell motility assay

In cell motility assay, cells were pretreated with GW9508 (10 μM) for 60 min and seeded into a Cell Culture Insert (8 μm pore size) (BD Falcon, Franklin Lakes, NJ) at 5×10^4 cells in 200 μl serum-free DMEM (upper chamber) and placed in 24-well plates (lower chamber) containing 800 μl DMEM supplemented with 5% charcoal stripped FBS. After incubation for 16 h, the numbers of cells that had moved to the lower side of the filters were counted after Giemsa staining [11,12].

2.5. Scrape assay

Cells were seeded in 6-well plates and cultured in DMEM containing 10% FBS. After confluence, scrape lines were produced by 1000 μl tips. Cells were gently washed with PBS and maintained in serum-free DMEM with GW9508 (10 μM). The photographs were taken at 20 h after the scrape [14].

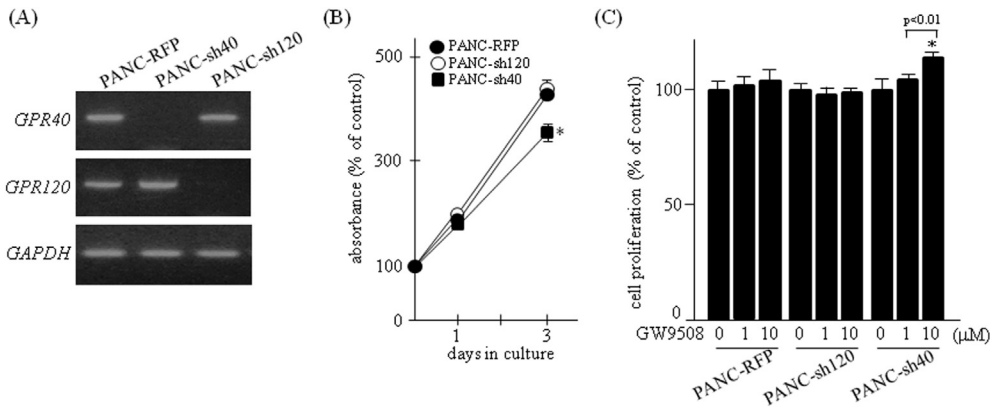

Fig. 2. Effects of GPR120 and GPR40 knockdown on cell proliferation. (A) Expression patterns of GPR120 and GPR40 genes by semi-quantitative RT-PCR analysis. PANC-RFP; control (vector) cells. PANC-sh40; GPR40 knockdown cells. PANC-sh120; GPR120 knockdown cells. (B) Cell proliferation rate of GPR120 and GPR40 knockdown cells. Cells were cultured in DMEM containing 10% FBS. Cell proliferation rate was measured using the CCK-8. Data are expressed as the percentage of cell number on day 0. *; $p < 0.01$ vs. PANC-RFP cells. (C) Effects of GW9508 on cell growth of GPR120 and GPR40 knockdown cells. Cells were cultured with or without GW9508 (1 or 10 μM) and cell growth rates were measured using the CCK-8. Columns indicate the mean of three studies; bars indicate SD. *; $p < 0.01$ vs. GW9508 untreated cells.

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