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Research article

Gastrin regulates ABCG2 to promote the migration, invasion and side populations in pancreatic cancer cells via activation of NF-κB signaling

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ABSTRACT

Gastrin is absent in most normal adult pancreatic tissues but is highly expressed in pancreatic cancer tissues. Although Gastrin expression was reported to be associated with tumor proliferation in human pancreatic cancer, studies on the relationship between Gastrin and tumor metastasis in pancreatic cancer are rare. In this study, we performed an analysis to determine the effects of Gastrin on modulating the side populations, cell proportion and tumor cell metastatic potential and invasion activity and explored its mechanisms in pancreatic cancer. We indicated that Gastrin and ABCG2 were widely expressed in pancreatic cancer cell lines and overexpressed in cancer tissues. Gastrin induced ABCG2 expression, and this effect was mediated by NF-κB activation. Gastrin regulated the SP proportion of BxPC-3 cells via modulating ABCG2 expression. Through the regulation of the functions of NF-κB/ABCG2, Gastrin functionally promoted the migration and invasion in pancreatic cancer cell. The present study indicated that Gastrin induced ABCG2 expression by activating NF-κB and thereby modulated the SP proportion, tumor cell metastatic potential and invasion activity in pancreatic cancer. Gastrin could serve as an effective therapeutic target for the metastasis of pancreatic cancer.

1. Introduction

Pancreatic cancer has the lowest patient survival rate of any solid cancer [1]. One of the main reasons for its mortality is that pancreatic cancer cells have a high potential for invasion and metastasis [2,3]. Therefore, a better understanding of the molecular mechanisms underlying metastasis and invasion is urgently needed to find effective biomarkers and therapeutic targets for this type of tumor.

Cancer metastases represent a multistep biological process that is driven by the acquisition of genetic and/or epigenetic alterations within tumor cells. The cellular and molecular changes have implications for the cancer invasion-metastasis process and can serve as therapeutic targets.

ABCG2 was reported to be overexpressed in pancreatic cancer [4,5] and alters cellular epigenetic programming to promote cell survival. Moreover, ABCG2 expression is high in the side population(SP) cells of pancreatic cell lines [6,7], where it is likely to be important for xenobiotic protection and act as an SP cell marker. The recent hypothesis of the existence of SP tumor cells with inherent stem cell characteristics has been used to explain some phenomena observed in cancer biology, such as the initiation of tumor growth, tissue invasion, and formation of metastases [8]. Therefore, ABCG2 expression might play a vital role in cancer development, and additional investigations into the mechanisms underlying the expression of ABCG2 in pancreatic cancer are needed.

Some cytokines and growth factors have been proposed to be involved in ABCG2 regulation, such as transforming growth factor-β, interleukin 1-β and insulin-like growth factor 2 [9,10], however, they are not specific for tumor diagnosis and therapy. Gastrin peptides are normally produced at high levels by endocrine (G) cells located in the gastric antrum and proximal duodenal mucosa. Gastrin in various forms including amidated gastrin, progastrin, or glycine-extended gastrin, has been shown to induce proliferation in several types of cancers [11,12]. Moreover, as an autocrine factor, it is absent in most normal adult pancreatic tissues but are expressed in pancreatic cancer and related to tumor development [13–16], which might suggest their determinant value for cancer progression.

A number of studies have suggested that Gastrin (amidated gastrin-17)/CCK-B/gastrin receptor (CCKBR) can activate several signaling pathways linked to proliferation, cell adhesion, and anti-

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apoptotic effects, such as the JNK, JAK2, STAT3, and ERKs signaling pathways [11,17,18]. Amidated gastrin-17 was reported to be secreted by pancreatic cancer [15]. Gastrin has also been demonstrated to be capable of inducing NF-κB activation [17,19]. Inappropriate activation of the NF-κB protein has been shown to be linked to autoimmunity, chronic inflammation, and various cancers. NF-κB uses both Rel homology region domains to encircle the target DNA. The canonical NF-κB p50 · p65 heterodimer recognizes its target sequence via p50 binding to a 5′GGPyN half site and p65 binding to another 5′GGPyN site centered around an A:T base pair [20]. ABCG2 possess an NF-κB consensus binding site in its promoter region. It has been reported that NF-κB molecules bound directly to this region and regulated ABCG2 expression in breast cancer cell lines [21,22]. Consequently, Gastrin/NF-κB might be involved in modulating the ABCG2 function and expression and promoting cancer development. However, the regulatory role of Gastrin/NF-κB/ABCG2 molecule in pancreatic cancer progression is unknown.

In the present study, we hypothesized that Gastrin modulated ABCG2 expression and the proportion of SP cells, thereby affecting the tumor cell metastatic potential and invasion activity of pancreatic cancer. We detected the expressions of Gastrin and ABCG2 in human pancreatic cancer. We revealed the mechanisms by which Gastrin promoted the migration of human pancreatic cancer cells through the activation of the NF-κB/ABCG2 signal cascade. Finally, we showed that Gastrin/NF-κB signaling modulated the SP cell proportion through the regulation of ABCG2 expression and subcellular localization.

Our study demonstrated that Gastrin/NF-κB signaling initiated the migration of pancreatic cancer cells, which suggested that Gastrin might be an effective and potent therapeutic target for pancreatic cancer metastasis.

2. Materials and methods

2.1. Reagents, antibodies and cell culture

recombinant human adminated Gastrin-17 (rhGastrin) was purchased from the Bachem Group (H-3085, Bachem, Bubendorf, Switzerland). The working concentration of rhGastrin was 10^{-8} M. The NF-κB inhibitor Bay 11-7082 (B5556), Hoechst 33342 (B2261) and L-365,260 (L4795) were purchased from Sigma (Sigma, St. Louis, MO, USA). The working concentration of Bay 11-7082 was 20 μM. Matrigel was purchased from Corning (356234, Corning, New York, NY, USA). Antibodies for the ChIP assays (rabbit anti-NF-κB/p50 antibody (sc-7178) and rabbit anti-NF-κB/p65 antibody (sc-372)) were obtained from Santa Cruz Biotechnology (Santa Cruz Biotech, Santa Cruz, CA, USA). Antibodies used for western blotting were as follows: rabbit anti-ABCG2 (sc-25821, Santa Cruz Biotech, Santa Cruz, CA, USA), mouse anti-histone H3.1 (B1201, Sungene Biotech, Tianjin, China), and mouse anti-β-actin (C1213, Sungene Biotech, Tianjin, China); the rabbit anti-NF-κB/p50 antibody and rabbit anti-NF-κB/p65 antibody were the same antibodies used in the ChIP assays. Antibodies for flow cytometry analysis (PE mouse anti-human CD338) were obtained from BD Biosciences (34372, BD, San Jose, CA, USA). Antibodies for confocal microscopy included the mouse monoclonal anti-ABCG2 antibody (AB3380, Abcam, Cambridge, UK). Antibodies for immunohistochemistry included mouse monoclonal anti-ABCG2 antibody (AB3380, Abcam, Cambridge, UK). The pancreatic cancer cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). The BxPC-3, Capan-2, SW-1990 and AsPC-1 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum(Biological Industries, Kibbutz Beit-Haemek, Israel). PANC-1 and MIA PaCa2 were cultured in DMEM medium supplemented with 10% fetal bovine serum. All cell lines were grown at 37 °C in 5% CO_2.

2.2. Patients and sample preparation

Fresh pancreatic adenocarcinoma and paracarcinoma tissues were obtained from patients who received a radical pancreatectomy for pancreatic adenocarcinoma without radiation or chemotherapy prior to the operation. The diagnosis was confirmed according to the standards of the American Joint Committee on Cancer (AJCC, 2010). Histological slides were reviewed by two experienced pathologists blinded to the clinical data. The study was approved by the Human Research Committee of Nankai University and China Anti-Cancer Association (CACA) and had been performed in accordance with the *Helsinki Declaration*. Each pancreatic cancer surgical specimen was cut into pieces 1 cm^3 in size and fixed with 100 g/L formaldehyde solution as soon as they were picked up, then embedded in paraffin for further study.

2.3. Immunohistochemistry

A 3-step immunoperoxidase technique was employed to test the expression of ABCG2 with 1:100 diluted anti-ABCG2. Sections were deparaffinized, hydrated, immersed in citrate buffer and autoclaved, followed by incubation with rabbit serum for half an hour. Incubation with primary antibodies was performed for 24 h at room temperature. The slides were washed using phosphate buffered saline (PBS) and incubated with the secondary antibody. Chromogen 3, 3′-diaminobenzidine tetrachloride (Serva, Heidelberg, Germany) was applied as a substrate. The cell nucleus was stained with Harris hematoxylin. The expression level of ABCG2 were scored according to the extent and intensity of the staining. The extent of positive staining was scored by the percentage of the positively stained area. Stained areas in each region of interest were scored as follows: 0 for a percentage $< 5\%$, 1 for 5–25%, 2 for 25–50%, 3 for 50–75%, and 4 for $\geq 75\%$. The intensity of staining was scored as 0, 1, 2 and 3 for the representation of negative (no staining), mild (weak but detectable above control), moderate (distinct) and intense (strong). The percentage of positively stained areas and the intensity of staining were multiplied to produce a weighted score. The scoring was performed by two independent evaluators without knowledge of the patients' pathological and clinical characteristics.

2.4. Cell transfection and RNA interference

The small interfering RNAs (siRNAs) targeting the human Gastrin gene were purchased from Santa Cruz Biotechnology (sc-37103, Santa Cruz Biotech, Santa Cruz, CA, USA). The siRNAs targeting the human ABCG2 and NF-κB/p65 genes were designed and synthesized by GenePharma (Shanghai, China): si-ABCG2 (5′-GAAGAAGAUCACAGUCUUCTT-3′), its control sequence (5′-GAAGACUGUGAUCUUCUUCTT-3′); si-NF-κB/p65, sense (5′-G CCCUAUCCCUUUACGUC-3′), antisense (5′-GACGUAAAGGGAUAGGGC-3′); and its negative control, sense (5′-UUCUCCGAACGUGUCACGUTT-3′), antisense (5′-ACGUGACACGUUCGGAGAATT-3′). The cells were transfected with the siRNAs and their controls using the INTERFER transfection reagent (Polyplus, Bioparc, France).

2.5. Western blotting

Tissues and whole cell extracts were lysed in RIPA III lysis buffer (Sangon Biotech, Shanghai, China). Additionally, nuclear proteins were prepared using the Nucleoprotein Extraction Kit (Keygen Biotech, Jiangsu, China). Then, the protein concentration was determined using the bicinchoninic acid protein assay kit (Thermo Scientific, Rockford, IL, USA). Quantified cell lysates (20 μg protein loaded for each lane) were separated by sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (SDS-PAGE) and transferred to