Downregulation of the CCK-B receptor in pancreatic cancer cells blocks proliferation and promotes apoptosis

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Fino KK, Matters GL, McGovern CO, Gilius EL, Smith JP. Downregulation of the CCK-B receptor in pancreatic cancer cells blocks proliferation and promotes apoptosis. Am J Physiol Gastrointest Liver Physiol 302: G1244–G1252, 2012. First published March 22, 2012; doi:10.1152/ajpgi.00460.2011.—Gastrin stimulates the growth of pancreatic cancer cells through the activation of the cholecystokinin-B receptor (CCK-BR), which has been found to be overexpressed in pancreatic cancer. In this study, we proposed that the CCK-BR drives growth of pancreatic cancer; hence, interruption of CCK-BR activity could potentially be an ideal target for cancer therapeutics. The effect of CCK-BR downregulation in the human pancreatic adenocarcinoma cells was examined by utilizing specific CCK-BR-targeted RNA interference reagents. The CCK-BR receptor expression was both transiently and stably downregulated by transfection with selective CCK-BR small-interfering RNA or short-hairpin RNA, respectively, and the effects on cell growth and apoptosis were assessed. CCK-BR downregulation resulted in reduced cancer cell proliferation, decreased DNA synthesis, and cell cycle arrest as demonstrated by an inhibition of G1 to S phase progression. Furthermore, CCK-BR downregulation increased caspase-3 activity, TUNEL-positive cells, and decreased X-linked inhibitor of apoptosis protein expression, suggesting apoptotic activity. Pancreatic cancer cell mobility was decreased when the CCK-BR was downregulated, as assessed by a migration assay. These results show the importance of the CCK-BR in regulation of growth and apoptosis in pancreatic cancer. Strategies to decrease the CCK-BR expression and activity may be beneficial for the development of new methods to improve the treatment for patients with pancreatic cancer.

gastrin; G protein-coupled receptor; ribonucleic acid interference

Pancreatic cancer ranks as the fourth most common cause of cancer-related mortality with a five-year survival rate of <1% and a median survival of 3–6 mo. Although numerous chemotherapeutic drugs have been tested in this malignancy, survival of advanced pancreatic cancer has not improved over the past several decades. Because of the lack of effective treatment options available for this disease, identification of novel targets and approaches is desperately needed. G protein-coupled receptors have been shown to play an important role in mediating tumor growth and metastasis (17). Through its interaction with gastrin, the G protein-coupled receptor, cholecystokinin-B receptor (CCK-BR), mediates the growth of several gastrointestinal cancers such as colon (29, 39, 46, 50), stomach (45, 48), and pancreas (40, 42, 43, 49).

The human CCK-BR has been shown to be upregulated in pancreatic cancer compared with normal tissues (41). The human pancreas produces gastrin during fetal development, after which gastrin expression is downregulated, and no gastrin can be detected in the healthy adult pancreas. However, gastrin is reexpressed in pancreatic tumors where it enhances proliferation through an autocrine mechanism (44). Evidence that both the receptor and ligand become upregulated in pancreatic cancer suggest it may be an important pathway involved in pancreatic cancer development and growth. Previous work has shown that stable, long-term reduction in gastrin expression through RNA interference (RNAi) techniques significantly reduces pancreatic tumor growth and metastasis in vivo (27). CCK is a ligand for both the CCK-BR and CCK-A receptor (AR), and is also reexpressed by pancreatic tumors. However, RNAi downregulation of endogenous CCK had virtually no effect on human pancreatic tumor growth (28), suggesting that the CCK-BR interaction with gastrin is the important mediator of growth in gastrointestinal malignancies.

The CCK-BR has also been reported to play an important role in carcinogenesis. Ectopic expression of the CCK-BR in a nontumorigenic, gastrin-producing colon epithelial cell line was sufficient to transform these cells into tumorigenic cells (9). Additional evidence of the CCK-BR’s contribution to carcinogenesis is supported by studies involving transgenic mice (12, 21, 38). Transgenic expression of the CCK-BR in murine pancreatic acinar cells results in increased pancreas growth, transdifferentiation of acinar cells into ductal structures, and development of pancreatic cancer (12). Although overexpression of progastrin in mice induces hyperproliferation of the colon and promotes colorectal cancer (38), mice carrying CCK-BR knockout alleles have reduced gastrin-dependent colonic proliferation, fewer aberrant crypt foci, and decreased tumor size (21). Furthermore, while progastrin may play an important role in proliferation and colon carcinogenesis through its actions at the annexin A2 receptor (36), gastrin-17 is the major form of gastrin involved in pancreatic cancer growth (28, 44).

Binding of gastrin to CCK-BR activates a diverse group of intracellular signaling pathways (1, 18). In the rat exocrine pancreatic cell line AR42J, gastrin stimulation activates protein kinase B (Akt), which promotes survival by phosphorylating the proapoptotic protein BAD (33, 47). Similarly, Akt is phosphorylated in response to gastrin stimulation in human esophageal cancer cells (20). Stimulation of gastric epithelial cells with gastrin upregulates expression of the anti-apoptotic proteins Bcl-2 and survivin (22). The mitogen-activated
protein kinase and Akt pathways also are involved in gastrin-induced cyclooxygenase-2 expression in colon cancer cells (13). In another gastrointestinal malignancy, gastric cancer, downregulation of the CCK-BR in cell lines increased caspase-3 expression and apoptosis (52). It was hypothesized that, since growth of pancreatic cancer cells is regulated by the interaction of gastrin with the CCK-BR, downregulation of the CCK-BR will inhibit pancreatic cancer growth and promote apoptosis. In this study, we examined the effects of downregulation of the CCK-BR on proliferation and apoptosis in human pancreatic cancer cells.

MATERIALS AND METHODS

Cell lines. Human pancreatic cancer cell lines AsPC-1, MIA PaCa-2, and Panc-1 were purchased from the ATCC (Rockville, MD) and were maintained in appropriate media with 10% FBS (Invitrogen, Carlsbad, CA). In a previous study, a panel of human pancreatic cancer cell lines was evaluated for CCK-BR mRNA expression by real-time RT-PCR (28), and Panc-1 cancer cells were found to express the most CCK-BR mRNA. Therefore, further studies establishing stable clones with short-hairpin RNA (shRNA) transfection were done using Panc-1 cancer cells.

Inhibition of CCK-BR with RNAi. Two independent CCK-BR-specific stealth small-interfering RNAs (siRNAs) [HS189635, si849 (CCGUACUGCCUGCUCCGUAGU) and HSS141489, si564 (CCGUACUGCCGAGGGGUUCCUA) and control siRNA (Medium GC Duplex no. 2, no. 12935–112) were obtained from Invitrogen. In addition, two independent shRNAs that targeted different regions of CCK-BR mRNA were selected based upon RNA secondary structure. The shRNA duplexes started at the following base locations in the CCK-BR RNA: GenBank no. AF441129, sites 52GACCGTGGTCTCCTAC and sh1413 (GAAACCTTGGCTCGTGC) (Oligoengine, Seattle, WA). A nonspecific shRNA control (NSC) containing a shRNA oligonucleotide duplex with no known homology to any mammalian gene sequence was used as a negative control. Each shRNA duplex was cloned into the pSuper.hygro vector, and shRNA-containing plasmids were verified by DNA sequence analysis.

For the RNAi experiments, AsPC-1, MIA PaCa-2, or Panc-1 cells were transfected with CCK-BR siRNA with Lipofectamine 2000 (Invitrogen). RNA was isolated after 48 h to evaluate mRNA downregulation. The cell pellet was resuspended in 50 mM Tris·HCl, pH 7.4, with 0.1 g/ml of proteinase K and 1 tablet of Complete, mini, EDTA-free Protease Inhibitor Cocktail protease inhibitor cocktail/50 ml buffer (Roche, Indianapolis, IN). Cells were homogenized with a Brinkman Polytron and then centrifuged at 48,000 × g for 30 min at 4°C to pellet cell membranes, and the membrane fraction was resuspended in an incubation buffer previously described (42) containing 0.1% BSA. Homogenates (total protein 200–400 μg/ml) were reacted with 125I-labeled gastrin (Perkin Elmer, Billerica, MA) for 60 min at 4°C, and the reaction was terminated by rapid filtration through Whatman GF/B filters with a Brandel harvester. Nonspecific binding was evaluated using 1 μM of unlabeled gastrin (Peninsula Laboratories, Carlsbad, CA). Radioactivity was assessed on a gamma scintillation counter with 80% efficiency, and receptor binding capacity (Bmax) was determined by using Prism Software (Graphpad). Each assay was performed in duplicate, and each sample was done in duplicate or triplicate.

Cell cycle analysis. For cell cycle analysis, Panc-1 cells were transfected with CCK-BR siRNA or control siRNA and, after 72 h, cells were fixed in 75% ethanol. Cells were then stained with 50 μg/ml of propidium iodide and treated with 1 μg/ml of RNase A. Control siRNA and CCK-BR siRNA samples were run on the FACSCalibur flow cytometer (BD Biosciences, San Diego, CA), and data were analyzed with CellQuest (Verity Software, Topsham, ME).

Histone H3 acetylation. Cells were transfected with CCK-BR siRNA and control siRNA, and, after 72 h, apoptosis assays. Panc-1 cells were transfected with CCK-BR siRNA or control siRNA, and, after 72 h, cells were fixed in 75% ethanol. Cells were then stained with 50 μg/ml of propidium iodide and treated with 1 μg/ml of RNase A. Control siRNA and CCK-BR siRNA were then labeled with BrdU for the last 16 h of the 72-h treatment with siRNA, and cell cycle analysis was performed as described in the manufacturer’s protocol.

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Protein extraction and western blot analysis. Protein for Western blots was harvested 72 h after transfection with siRNA. Total protein was determined using a MicroBCA assay (Pierce/Thermo Fisher Scientific, Rockford, IL). Whole cell lysates (50 μg of protein/well) were mixed with Laemmli’s reducing sample buffer, resolved by SDS-PAGE, and electrophoretically transferred to 0.2-membranes, and the membrane fraction was resuspended in an incubation buffer previously described (42) containing 0.1% BSA. Homogenates (total protein 200–400 μg/ml) were reacted with 125I-labeled gastrin (Perkin Elmer, Billerica, MA) for 60 min at 4°C, and the reaction was terminated by rapid filtration through Whatman GF/B filters with a Brandel harvester. Nonspecific binding was evaluated using 1 μM of unlabeled gastrin (Peninsula Laboratories, Carlsbad, CA). Radioactivity was assessed on a gamma scintillation counter with 80% efficiency, and receptor binding capacity (Bmax) was determined by using Prism Software (Graphpad). Each assay was performed in duplicate, and each sample was done in duplicate or triplicate.

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ersham, Piscataway, NJ), and the signal was detected using an enhanced chemiluminescence substrate (Pierce/Thermo Fisher Scientific).

Migration assay. Pancreatic cancer cells (wild-type, nonspecific control clones, and CCK-BR shRNA clones) were seeded on 12-well plates and allowed to grow to confluency. A scratch in the monolayer was created with a 200-μl pipet tip. Phase-contrast (CKX41; Olympus, Tokyo, Japan) photographs were taken immediately following the scratch (0 h) and 24 h after the scratch. Scratch width was measured using Axiolab Vision (Carl Zeiss MicroImaging) software, and the percentage of the scratch area closed was calculated as (width at 0 h − width at 24 h)/width at 0 h. Results were standardized to wild type.

Statistics. Results are expressed as means ± SE. Statistical comparisons were made using the two-tailed Student’s t-test. A P value of <0.05 was considered to be statistically significant, and a modified Bonferroni method was used to correct for multiple comparisons. For the real-time data, pairwise Student’s t-tests were performed on the normalized mean ΔΔCT values for each group.

RESULTS

CCK-BR RNAi effectively decreases receptor expression. Two CCK-BR-specific siRNAs (targeted to different regions of CCK-BR mRNA) were tested for their ability to downregulate the receptor in PANC-1 cells. Both siRNAs, si564 and si849, were effective in decreasing CCK-BR mRNA as demonstrated by real-time RT-PCR (Fig. 1A). Because CCK-BR si849 was highly effective in downregulating CCK-BR expression in PANC-1 cells >90%, si849 was used to downregulate CCK-BR in all subsequent siRNA experiments. The CCK-BR siRNA (si849)-transfected cells did not have altered expression of the CCK-AR (Fig. 1B), indicating the specificity of this siRNA for the CCK-BR. Similarly, CCK-BR mRNA expression was decreased in PANC-1 cells stably transfected with the sh377 or sh1413 construct (Fig. 1C). Although si849, sh377, and sh1413 each target separate regions of the CCK-BR mRNA, all were highly effective in knocking down CCK-BR mRNA. The CCK-BR mRNA was also decreased in siRNA-treated AsPC-1 and MIA PaCa-2 cancer cells (data not shown).

To confirm that the downregulation of the CCK-BR mRNA resulted in decreased receptor protein, receptor-binding assays were performed on the PANC-1 CCK-BR shRNA clones, NSCs, and wild-type cells with 125I-labeled gastrin-17. Receptor binding affinity was in the nanomolar range, comparable with the physiological range previously determined to stimulate growth (44). There was no difference in receptor number of the wild-type cells compared with the PANC-1 nonspecific control shRNA clones (Fig. 1D). The Bmax was significantly decreased by 73 and 71%, respectively, in sh377 and sh1413 clones compared with wild-type and nonspecific control clones.

Downregulation of the CCK-BR decreases proliferation. At 72 h after transfection, CCK-BR siRNA-treated PANC-1 cells displayed a significant decrease in cell number. Growth of CCK-BR siRNA-treated PANC-1 cells was reduced by 73% compared with cells treated with control siRNA and by 69% compared with vehicle-treated cells (P < 0.001, Fig. 2A). A decrease in cell viability was also seen in both sh377 and sh1413 stable CCK-BR shRNA clones compared with wild-type and nonspecific control shRNA cells (Fig. 2B) as measured by the MTS cell proliferation assay. The decrease in cell growth upon downregulation of the CCK-BR suggests that CCK-BR inactivation may block DNA synthesis, and this finding was confirmed by BrdU labeling (Fig. 2C) in three pancreatic cancer cell lines, AsPC-1, MIA PaCa-2, and PANC-1. All cancer cell lines treated with siRNA CCK-BR had decreased BrdU incorporation compared with control siRNA-treated cells and vehicle-treated cells. CCK-BR sh377 clones and sh1413 clones had significantly less BrdU incorporation compared with wild-type and nonspecific control clones.

Fig. 1. Downregulation of the cholecystokinin-B receptor (CCK-BR) in cells treated with small-interfering RNA (siRNA) and short-hairpin RNA (shRNA). A: real-time RT-PCR analysis of two independent CCK-BR-targeted siRNA (si564 and si849) were examined for their ability to downregulate the CCK-BR mRNA. siRNA-treated cells were standardized to the control siRNA (Ctrl siRNA), n = 6. B: expression of CCK-A receptor (CCK-AR) mRNA in cells treated with si849 is unchanged compared with control siRNA. C: two independent shRNA CCK-BR-targeted sequences (sh377 and sh1413) were examined by real-time RT-PCR for their ability to downregulate CCK-BR mRNA. CCK-BR shRNA cells were standardized to nonspecific shRNA control (NSC) cells, n = 4. For all real-time RT-PCR data, columns represent the fold change in mRNA levels calculated from the mean relative quantity (ΔΔCT), and bars represent a 95% confidence interval [CI, ΔΔCT = 95% CI]. D: receptor binding assay for CCK-BR shRNA clones. Columns represent median receptor binding capacity (Bmax) in fmol/mg protein of various shRNA clones compared with the wild-type (WT) controls and the clones transfected with NSC, n = 3. *P ≤ 0.05 and **P < 0.01.
Expression of XIAP is known to be upregulated in pancreatic cancer (25, 37, 37). XIAP binds to active caspase-3 (15, 16) and blocks the increased caspase-3 activity induced during apoptosis. XIAP expression in CCK-BR siRNA-treated cells and CCK-BR shRNA clones was examined. After downregulation of the CCK-BR with siRNA in AsPC-1, MIA PaCa-2, and PANC-1 cells, XIAP expression was significantly lower than in control siRNA- and vehicle-treated cells (P < 0.05, Fig. 5A). The reduction in XIAP expression also occurred in the CCK-BR shRNA clones. XIAP levels were reduced in both shRNA 377 and shRNA 1413 clones compared with wild-type and NSC clones (P < 0.05, Fig. 5B).

**Downregulation of the CCK-BR decreases Akt phosphorylation and migration.** The prosurvival signaling protein Akt is known to be phosphorylated and activated as a result of normal interaction of gastrin with the CCK-BR (47). Akt phosphorylation leads to increased XIAP levels by blocking ubiquitination and proteasomal degradation of XIAP (14). Because downregulation of the CCK-BR drastically reduces XIAP levels, we would predict that downregulation of CCK-BR would also cause a reduction in Akt phosphorylation. Although total Akt levels were unchanged in CCK-BR siRNA-treated cells, Akt phosphorylation at Ser473, which is important for the activation of Akt, was significantly decreased by CCK-BR siRNA treatment in AsPC-1, MIA PaCa-2, and PANC-1 cells (P < 0.05, Fig. 6). Because the phosphatidylinositol 3-kinase (PI3K) pathway and Akt phosphorylation are known to mediate cell migration (4), scratch-wound assays were performed to assess cell migration in response to downregulation of the CCK-BR. Compared with untransfected wild-type cells or NSC clones, both CCK-BR shRNA clones demonstrated a decrease in migration (P < 0.005, Fig. 7).

**DISCUSSION**

The present study demonstrates that the CCK-BR is a potential target for developing novel strategies for the treat-
ment of pancreatic cancer and further defines pathways affected by CCK-BR signaling. This study establishes that downregulation of the CCK-BR increases apoptotic activity, and decreases proliferation, XIAP expression, Akt activation, and migration, suggesting that the CCK-BR is an important receptor regulating growth of pancreatic cancer. Although AsPC-1, MIA PaCa-2, and PANC-1 cells have different levels of CCK-BR mRNA expression (28), with expression highest in PANC-1 cells, downregulation of the receptor decreased BrdU incorporation, XIAP expression, and Akt phosphorylation to a similar level in all three cell lines regardless of CCK-BR expression or degree of differentiation.

In addition to decreasing cellular proliferation, G1/S progression is inhibited with downregulation of the CCK-BR, suggesting that downregulating this receptor induces cell cycle arrest. Cyclins that control G1/S transition have been found to be influenced by gastrin. Gastrin increases transcription of cyclin D1, D3, and E in a human gastric adenocarcinoma cell line and Swiss 3T3 cells that express the CCK-BR (31, 53). Therefore, it is possible that downregulation of the CCK-BR

![Graph showing cell cycle analysis](image)

Fig. 3. CCK-BR downregulation decreases G1/S phase progression. Cell cycle analysis by propidium iodide (PI) staining and flow cytometry in cells with CCK-BR knockdown compared with control siRNA. A: percentage of cells in G1/G0, G2, and S phase, n = 8. B: change in cell cycle phase of CCK-BR siRNA-transfected cells relative to control siRNA. C: representative cell cycle histograms demonstrating the change in cell cycle.

![Graph showing apoptotic activity](image)

Fig. 4. Apoptotic activity increases with downregulation of the CCK-BR. A: caspase-3 activity increases in cells transfected with CCK-BR siRNA. Results are standardized to vehicle, n = 7. B: caspase-3 activity is increased in CCK-BR shRNA clones. Results are standardized to WT, n = 5. C: percentage of TUNEL-positive cells measured by flow cytometry is increased in cells transfected with CCK-BR siRNA, n = 5. ***P < 0.005 and ‡P < 0.001.
may reduce cyclin D transcription and result in cell cycle dysfunction.

The increase in caspase-3 activity in PANC-1 cells treated with CCK-BR siRNA or in cells with stable shRNA-mediated downregulation of CCK-BR is consistent with other studies (27, 52). Herein, our results demonstrated for the first time that XIAP expression is decreased when the CCK-BR is downregulated. Many pancreatic cancer cell lines exhibit constitutive activation of Akt mediated through dysregulated phosphatase and tensin homolog (PTEN) expression (2, 3). Gastrin stimulation activates Akt phosphorylation through the CCK-BR (47), and this study demonstrates that downregulation of the CCK-BR in PANC-1 cells inhibits phosphorylation of Akt. Activated Akt is known to regulate XIAP by phosphorylation at Ser87. Phosphorylation of XIAP by Akt protects XIAP against ubiquitination and proteasomal degradation (14). The decrease in XIAP expression observed upon CCK-BR downregulation is consistent with the decrease in activated Akt brought on by the CCK-BR siRNA treatment.

Akt, an integral part of the PI3K pathway, is involved in mediation of cell migration. We demonstrated that CCK-BR downregulation inhibits Akt phosphorylation and cell migration. Others have demonstrated that signaling pathways activated by the CCK-BR can regulate cellular adhesion by gastrin-induced modifications of p120, α- and β-catenins, and E-cadherin in intestinal epithelial cells (19). More recently, in pancreatic cancer cells, the CCK-BR has been shown to regulate β1- and αv-integrins, which are involved in modulation of cell adhesion via the PI3K pathway (7, 8).

Gemcitabine, the current standard of care treatment for pancreatic cancer, offers a low response rate, and only increases survival time by a mean of six months. Given alone, most therapeutic agents that target cancer cell-signaling pathways, particularly tyrosine kinase receptor-signaling pathways, are not effective in decreasing pancreatic tumor growth and metastasis (32). Even small molecule inhibitors of the PI3K/Akt pathway have failed to demonstrate efficacy in clinical trials with pancreatic cancer patients (34). Unfortunately, the approach of “one size fits all” has not been effective in treating pancreatic cancer. Recently, a set of genes identified as “driver genes” have been described in pancreatic cancer (23). Among these, Carter et al. (6) describe a PI3K suppressor gene SNP (PIK3CG/R839C), which has the second highest scoring predicted driver mutation among these genes in pancreatic cancer. Because PI3Kγ transmits signals downstream from G protein-coupled receptors, Korc (23) suggested that there may be a connection between mutations in PIK3CG and activation of the CCK-BR. Knowing this is a key pathway in pancreatic cancer growth, interruption of the CCK-BR signaling pathway may lead to advances in the treatment of pancreatic cancer. Therapies directed toward Kras, another driver mutation, which is
mutated early on in the development of pancreatic cancer in the precursor pancreatic intraepithelial neoplasms lesions, have not improved survival (26), suggesting that Kras alone is not controlling growth of pancreatic cancer. Moreover, recent studies suggest that G protein-coupled receptor signaling systems play a critical role in mitogenic signaling and are implicated in the growth of multiple solid tumors, including the pancreas (35, 51). Because the CCK-BR promotes growth primarily in gastrointestinal cancers like pancreatic cancer and downregulation of the CCK-BR blocks pancreatic cancer cell growth, targeting the CCK-BR as a possible pancreatic cancer therapy is promising.

Although many CCK-BR antagonists have been identified, there are no drugs targeting the CCK-BR in clinical use because of their poor biodistribution and pharmacokinetics (5). Two pharmacological agents that target the CCK-BR have been evaluated in clinical trials for treatment of pancreatic cancer. The first compound, gastrazole, is a high-affinity CCK-BR antagonist. When compared with placebo, pancreatic cancer patients treated with gastrazole showed a slight increase in survival (10). Unfortunately, the very low oral bioavailability of gastrazole required that the drug be administered intravenously. Z-360, a more recently developed orally active CCK-BR antagonist, was evaluated in phase Ib/IIa clinical trials in combination with gemcitabine (30). Although a trend toward reduced pain and increased survival were observed, these differences were not statistically significant. However, in both trials that compared CCK-BR antagonists with conventional therapeutics, Z-360 and gastrazole had low toxicity and high tolerability. The results of tolerability of these drugs agree with previous work where the CCK-BR was knocked out in mice.

Although the potential for CCK-BR-targeted therapies is evident, the current pharmacological antagonists for the CCK-BR are inadequate. The development of an RNAi-based approach to selectively downregulate CCK-BR expression holds promise for a more efficient therapy. Previous work examining CCK-BR knockout mice demonstrated that these animals had few phenotypic differences from wild-type mice. CCK-BR-deficient mice had increased gastric pH, more G cells, and a decrease in the thickness of the oxyntic mucosa of the stomach (11, 24). Evidence from the CCK-BR knockout mouse model and the CCK-BR antagonist clinical trials supports the idea that targeting the CCK-BR as a therapeutic strategy can be effective and well tolerated. Our current studies indicate that the development of targeted therapeutics against

Fig. 6. Downregulation of the CCK-BR inhibits protein kinase B (Akt) phosphorylation. A: representative Western blot of Akt phosphorylation in CCK-BR siRNA-transfected AsPC-1, MIA PaCa-2, and PANC-1 cells. B: quantification of phosphorylated (p) Akt by densitometry. The ratio of pAkt to each corresponding β-actin band was calculated. Results are expressed as a percentage and standardized to vehicle, n = 3. *P < 0.05.

Fig. 7. Downregulation of the CCK-BR decreases cell migration by scratch-wound assay. Migration in CCK-BR shRNA clones is expressed as a percentage of wound repair and standardized to WT, n = 6. ***P < 0.005.
the CCK-BR, including RNAi-based therapies, for pancreatic cancers and for other gastrin-dependent malignancies will be an important new direction in treating these diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES