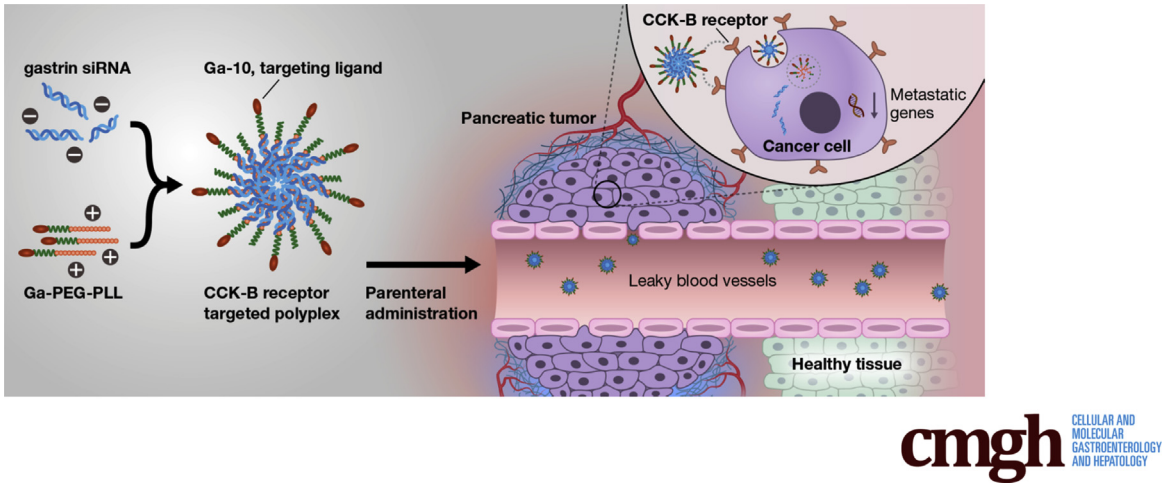
cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Cholecystokinin Receptor-Targeted Polyplex Nanoparticle Inhibits Growth and Metastasis of Pancreatic Cancer

Julian Burks,[1,*] Sandeep Nadella,[2,*] Abdullah Mahmud,[3,*] Charoen Mankongpaisarnrung,[2] Juan Wang,[2] Jong-In Hahm,[4] Robin D. Tucker,[5] Narayan Shivapurkar,[2] Stephan T. Stern,[3] and Jill P. Smith[2]

[1]Department of Oncology, [2]Department of Medicine, [4]Department of Chemistry, [5]Department of Comparative Medicine, Georgetown University, Washington, District of Columbia; [3]National Institutes of Health Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc, Frederick National Laboratory for Cancer Research, Frederick, Maryland



SUMMARY

Here, we report the development of a polyplex nanoparticle that selectively targets the cholecystokinin receptor on human pancreatic cancer and delivers small interfering RNAs specific to gastrin to block cancer cell growth in vitro and in vivo. One remarkable finding in our investigation was that this therapeutic approach completely prevented metastasis—the most common cause of death in this condition.

BACKGROUND & AIMS: Pancreatic ductal adenocarcinoma (PDAC) remains the most aggressive malignancy with the lowest 5-year survival rate of all cancers in part owing to the lack of tumor-specific therapy and the rapid metastatic nature of this cancer. The gastrointestinal peptide gastrin is a trophic peptide that stimulates growth of PDAC in an autocrine fashion by interaction with the cholecystokinin receptor that is overexpressed in this malignancy.

METHODS: We developed a therapeutic novel polyplex nanoparticle (NP) that selectively targets the cholecystokinin receptor on PDAC. The NP was characterized in vitro and stability testing was performed in human blood. The effects of the target-specific NP loaded with gastrin small interfering RNA (siRNA) was compared with an untargeted NP and with an NP loaded with a scrambled siRNA in vitro and in 2 orthotopic models of PDAC. A polymerase chain reaction metastasis array examined differentially expressed genes from control tumors compared with tumors of mice treated with the targeted polyplex NP.

RESULTS: The polyplex NP forms a micelle that safely delivers specific gastrin siRNA to the tumor without off-target toxicity. Consistent with these findings, cellular uptake was confirmed only with the targeted fluorescently labeled NP by confocal microscopy in vitro and by IVIS fluorescent based imaging in mice bearing orthotopic pancreatic cancers but not found with untargeted NPs. Tumor uptake and release of the gastrin siRNA NP was verified by decreased cellular gastrin gene expression by quantitative reverse-transcription polymerase chain reaction and peptide expression by immunohistochemistry. Growth of PDAC was inhibited in a dose-related fashion in cell culture and in vivo. The targeted NP therapy completely blocked tumor metastasis and altered tumor-specific genes.

CONCLUSIONS: Our polyplex nanoparticle platform establishes both a strong foundation for the development of receptor-targeted therapeutics and a unique approach for the delivery of siRNA in vivo, thus warranting further exploration of this

approach in other types of cancers. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.02.013)*

Keywords: CCK Receptor; Gene Therapy; Gastrin; Nanotechnology; Orthotopic.

Pancreatic ductal adenocarcinoma (PDAC) has a dismal prognosis,[1,2] and the current chemotherapeutic regimens provide a stagnant 5-year survival rate of only approximately 7%.[3] With the recent increase in the incidence of pancreatic cancer it is anticipated that this malignancy will surpass colon and breast cancer in the next decade to become the second leading cause of cancer-related deaths in the United States.[4] In this new era of precision medicine and genomic profiling, targeted therapies directed at cancer-specific receptors have improved the outcome of many recalcitrant cancers.[5,6] Reasons for the poor outcome in pancreatic cancer includes its propensity to metastasize rapidly[1] and the lack of available targeted therapies.[6]

We previously showed that pancreatic cancer overexpresses the cholecystokinin-B (CCK-B) receptor.[7] Although CCK-B receptors are present at a very low density in normal pancreas tissue,[8] their expression increases in early precancerous pancreatic intraepithelial neoplasia (PanIN) lesions of the pancreas[9] and becomes markedly overexpressed in cancer.[7,8] Down-regulation of the CCK-B receptor in pancreatic cancer cells has been shown to reduce cancer cell proliferation, decrease DNA synthesis, induce cell-cycle arrest, increase apoptosis, and decrease cell migration.[10]

CCK receptors also are expressed on pancreatic stellate cells,[11,12] the cell responsible for the dense fibrosis in the pancreatic tumor microenvironment,[13,14] and blockade of the CCK-B receptor in (Pdx1 [pancreatic and duodenal homeobox 1] promoter; Cre recombinase; Lox-Stop-Lox; G12D mutation results in an amino acid substitution at position 12 in KRAS [Kirsten rat sarcoma virus], from a glycine (G) to an aspartic acid [D]) Pdx1-Cre/LSL-Kras^{G12D} transgenic mice halts progression of the precancerous PanIN lesions and also reverses the fibrosis.[9] Gastrin is the major ligand for the CCK-B receptor,[15] and gastrin stimulates the growth of pancreatic cancer in an autocrine fashion.[16] Although gastrin is expressed in the fetal pancreas,[17–19] its expression is turned off at week 14 of gestation and gastrin is not found in the normal adult human pancreas.[20] However, the gastrin peptide becomes re-expressed in precancerous pancreatic PanIN lesions,[21] and is expressed markedly in pancreatic cancer.[20] Down-regulation of gastrin messenger RNA (mRNA) by RNA interference techniques inhibits growth and metastasis of human pancreatic cancer.[22,23] These properties make gastrin mRNA and its receptor, the CCK-B receptor, ideal targets for cancer therapeutics. RNA interference is an effective tool for studying gene expression in vitro; however, applying this technique in the clinic has been challenging. Various vehicles have been attempted to transport small interfering RNA (siRNA) to tissues in vivo, although safe and effective delivery methods remain problematic. Furthermore, drugs or molecules that target selective cancer cell membrane–associated receptors significantly improve efficacy and limit off-site toxicity.

Because the CCK-B receptor is overexpressed on PDAC, researchers have been trying to develop imaging strategies for CCK-B–receptor–positive cancers using ^{111}In-labeled-CCK[24] and ^{68}Ga-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-minigastrin.[25] Currently, radiopeptide imaging with ^{111}In-minigastrin labeling to detect CCK-B receptors is established and in clinical use for medullary cancer (another cancer that expresses CCK-B receptors).[26] By using a similar maleimide coupling technique to target the CCK-B receptor as previously described,[27] we developed a polyplex nanoparticle (NP) that selectively targets the CCK-B receptor and serves as a target-specific vehicle to deliver gene therapy to inhibit growth and metastasis of PDAC. Biodegradable nontoxic nanoparticles that serve as vehicles to carry siRNA without off-target toxicity, such as the polyplex NP described in this work, have the potential to revolutionize cancer therapeutics.

Materials and Methods

Synthesis of the CCK-B–Receptor–Targeted Polyplex

The targeted polyplex was synthesized from gastrin-10 peptide conjugated poly (ethylene glycol)-*block*-poly (L-lysine) (Ga–polyethylene glycol [PEG]5k-PLL27) (5k indicates PEG molecular weight [MW] and 27 is the PLL degree of polymerization; termed *Ga-PEG-PLL*). Ga-PEG-PLL was synthesized from thiol functionalized PEG5K-PLL27 (sulfhydryl [SH]-PEG-PLL). Briefly, SH-PEG-PLL was synthesized from trityl-S-poly(ethylene glycol)-*block*-poly (L-lysine) (Tr-S-PEG-PLL) (average MW, 9700 g/mol; PEG MW, 5000 g/mol; Tr-S-PEG-PLL: custom synthesized; Alamanda Polymers, Huntsville, AL) by reducing with trifluoroacetic acid and triethylsilane (98:2 vol/vol). Maleimide functionalized gastrin 10 peptide (3-maleimido-propionyl-Glu-Glu-Glu-Ala-Tyr-Gly-Trip-Met-Asp-Phe-NH_2; MW, 1426.48 g/mole) (Ga-10) was conjugated to the resulting SH-PEG-PLL via Michael addition reaction at pH 7 in deoxygenated HEPES buffer (100 mmol/L) under an inert atmosphere. Next, the reaction mixture was dialyzed (Spectrapor [Rancho Dominguez, CA] RC membrane; MW cut off, 8–10

***Authors share co-first authorship.**

Abbreviations used in this paper: **CCK, cholecystokinin; Ex/Em, maximal excitation and emission wavelengths; Ga-10, gastrin 10 peptide; mRNA, messenger RNA; MW, molecular weight; N/P, ratio of "amines" of poly (L-lysine) unit and "phosphates" of siRNA complexed in the polyplex; NMR, nuclear magnetic resonance; NP, nanoparticle; PanIN, pancreatic intraepithelial neoplasia; PBS, phosphate-buffered saline; PDAC, pancreatic ductal adenocarcinoma; PEG, polyethylene glycol; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; siRNA, small interfering RNA.**

2352-345X
https://doi.org/10.1016/j.jcmgh.2018.02.013