CHEMISTRY OF MATERIALS

pubs.acs.org/cm

Article

Targeted Delivery of siRNA-Gemcitabine Oligonucleotide Chimeras for High-Efficacy Synergistic Treatment of Pancreatic Cancer

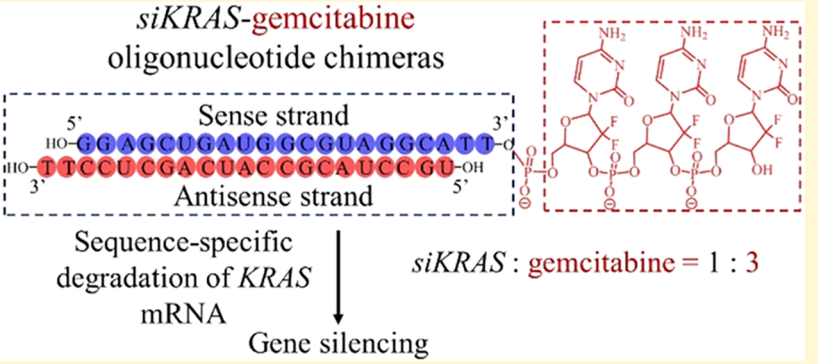
Published as part of the Chemistry of Materials special issue "Polymer-Drug Conjugate Materials".

Ri Huang, Hong Du, Liang Cheng, Ning Zhao, Peizhuo Zhang, Fenghua Meng,* and Zhiyuan Zhong*

Cite This: *Chem. Mater.* 2024, 36, 11881−11891

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Pancreatic cancer (PC) stands for the most intractable malignancy. Gemcitabine (GEM) is one of the few approved first-line treatments for PC patients. The fast clearance, demanded high dosage, and existence of drug resistance have, nevertheless, posed not only a significant limitation to its clinical efficacy but also serious toxicity concerns. $KRAS^{G12D}$ mutation is identified as a key driver in many PC patients, and its expression level shows a correlation with drug resistance and mortality. Here, we explored $KRAS^{G12D}$ siRNA-gemcitabine oligonucleotide chimeras (*siKRAS*-G_n) as a dual prodrug that was designed to specifically silence $KRAS^{G12D}$ gene and sensitize PC cells to GEM for the synergistic treatment of PC. *siKRAS*-G_n conjugates with 1, 2, 3, 4, or 5 units of GEM were synthesized and delivered using cRGD-decorated polymersomes. Interestingly, the proapoptotic activity of *siKRAS*-G_n was shown to highly depend on the number of GEM, in which three GEM units (*siKRAS*-G_3) were found to be optimal and induced strong apoptosis of PANC-1 cells (apoptosis rate: 64.2%). In contrast, minimal cell apoptosis was discerned for *siKRAS*, *siKRAS*-G_1, *siKRAS*-G_2, and free GEM (9-fold of that in *siKRAS*-G_3). *siKRAS*-G_3, while showing similar *KRAS* mRNA silencing ability to *siKRAS*, markedly enhanced the downregulation of *KRAS* protein *in vitro* and *in vivo*. Accordingly, *siKRAS*-G_3 significantly outperformed *siKRAS* and *siScramble*-G_3 in both tumor inhibition and survival benefits. The targeted delivery of the *siKRAS*-gemcitabine prodrug conjugate has emerged as an appealing treatment for pancreatic cancer.

1. INTRODUCTION

Pancreatic cancer (PC) is known as the most intractable malignancy.[1] Unlike other cancers, there is little success in the development of molecular targeted drugs against PC to date.[2] *KRAS* mutations, particularly $KRAS^{G12D}$, are identified as a main driver of PC in patients.[3−5] $KRAS^{G12D}$ mutation not only promotes the development and progression of PC but also leads to other problems such as oxidative phosphorylation dysfunction and drug resistance.[6,7] $KRAS^{G12D}$ was long regarded as an "undruggable" target[4] until a small-molecule inhibitor called MRTX1133 was recently developed.[8] Targeting $KRAS^{G12D}$ with siRNA drugs provides an emerging and valuable strategy for treating PC.[9,10] For instance, CD47-mediated iExosomes with siRNA targeting $KRAS^{G12D}$ have been shown to increase survival in PC mouse models,[11] and such a therapy is now in a phase I clinical trial (NCT03608631).[12] Poly(lactic-*co*-glycolic acid) polymeric implant $KRAS^{G12D}$-LODER is in phase II clinical trials (NCT01676259).[13]

Gemcitabine (GEM), a nucleoside derivative of cytosine, is one of the few first-line treatments for PC patients.[14] GEM can inhibit the tumor cell growth by replacing the nucleoside cytosine in the DNA replication process.[15,16] The fast clearance, demanded high dosage, and existence of drug resistance have, however, posed not only significant limitation

Received: August 19, 2024
Revised: November 21, 2024
Accepted: November 21, 2024
Published: December 5, 2024

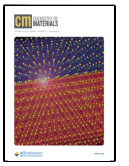


Table 1. *siKRAS*, *siScramble*, *siKRAS*-G_n (n = 1−5), and *siScramble*-G_3 Used in This Study

Entry	Name	Strand	Sequence (5' – 3')
1	*siKRAS*	sense antisense	GGAGCUGAUGGCGUAGGCATT UGCCUACGCCAUCAGCUCCTT
2	*siKRAS*-G_n	sense antisense	GGAGCUGAUGGCGUAGGCATT-O UGCCUACGCCAUCAGCUCCTT
3	*siScramble*	sense antisense	GGTGCTGGCGAGCTGAAGATT UCUUCAGCUCGCCAGCACCTT
4	*siScramble*-G_3	sense antisense	GGAGCUGAUGGCGUAGGCATT-O UGCCUACGCCAUCAGCUCCTT
5	Gemcitabine 3'-CE Phosphoramidite	-	

to its clinical efficacy but also serious toxicity concerns.[17,18] In recent years, combination therapies[19,20] and targeted delivery strategies[21−23] have been explored to enhance the antitumor efficacy of GEM. For example, the combination therapy of GEM with nab-paclitaxel was able to extend the overall survival of patients.[24,25] The combination of GEM with RNAi therapy is a promising treatment strategy for PC.[9,26−28] GEM in combination with *KRAS* siRNA (*siKRAS*) brought about better antitumor effects and survival than GEM alone.[9,29] Nevertheless, conventional combination therapies are mostly suboptimal due to the inherent differences in the physicochemical properties and pharmacokinetic behavior of different drugs.[30−32] The attempts to using mesoporous silica nanoparticles and micelles for the targeted delivery of GEM showed modest improvement.[33,34]

Here, we explored $KRAS^{G12D}$ siRNA-gemcitabine oligonucleotide chimeras (*siKRAS*-G_n) as a dual prodrug that specifically silences $KRAS^{G12D}$ gene expression and sensitizes PC cells to GEM for the synergistic treatment of PC (Scheme 1). *siKRAS*-G_n is unique as it enables simultaneous intracellular codelivery of *siKRAS* and GEM at prescribed ratios. cRGD-installed bioresponsive chimeric polymersomes (cRGD-BCP), prepared from coself-assembly of the poly(ethylene glycol)-*b*-poly(trimethylene carbonate-*co*-dithiolane trimethylene carbonate)-*b*-polyethylenimine (PEG-P(TMC-DTC)-PEI) triblock copolymer and cRGD-functionalized PEG-P(TMC-DTC), were used as the delivery vehicle.[35] PC cells have been reported to overexpress the $\alpha_v\beta_3$ integrin.[36] Strikingly, our results show that the number of GEM plays a critical role and *siKRAS*-G_3 induces a strong synergistic pro-apoptosis effect to PC cells *in vitro* and to PC tumor *in vivo*. The *siKRAS*-gemcitabine prodrug conjugate provides an emerging treatment strategy for PC.

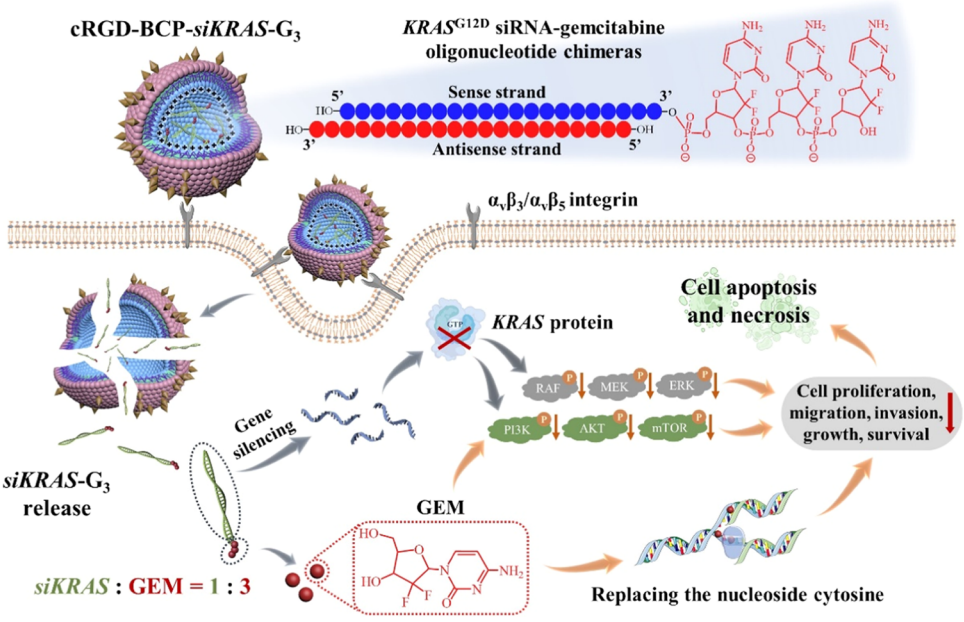
2. EXPERIMENTAL SECTION

2.1. Fabrication of siKRAS-G_n and cRGD-BCP-siKRAS-G_n. $KRAS^{G12D}$ siRNA-gemcitabine conjugates (*siKRAS*-G_n) with varying numbers of gemcitabine (n = 1−5) were synthesized with high yields and purity using standard procedures on a solid-phase oligonucleotide synthesizer. cRGD-BCP-*siKRAS*-G_n was fabricated by adding a dimethylformamide (DMF) solution of PEG-P(TMC-DTC)-PEI and cRGD-PEG-P(TMC-DTC) (molar ratio: 84.3:15.7) into *siKRAS*-G_n in phosphate buffer followed by dialysis, as previously reported.[35] The drug loading content, size, and stability of cRGD-BCP-*siKRAS*-G_n were studied.

2.2. *In Vitro* Transfection of siKRAS-G_n. PANC-1 cells were seeded in 12-well plates (200,000/well) for 24 h, treated with *siKRAS*-G_n, *siScramble*-G_n, or *siKRAS* encapsulated in cRGD-BCP (final siRNA concentration = 50, 100, or 200 nM, n = 3) for 6 h, and then further cultured in drug-free medium for 1, 2, or 3 days. *KRAS* mRNA in the cells was quantified using a quantitative real-time polymerase

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891

Scheme 1. $KRAS^{G12D}$ siRNA-Gemcitabine Oligonucleotide Chimeras (*siKRAS*-G_n) as a Dual Prodrug that Specifically Silences $KRAS^{G12D}$ Gene Expression and Sensitizes PC Cells to GEM for the Synergistic Treatment of PC[a]



[a]cRGD-functionalized bioresponsive chimeric polymersomes (cRGD-BCP) are used as targeted delivery vehicle. cRGD-BCP efficiently loads and delivers *siKRAS*-G_3 to PC cells, leading to simultaneous intracellular release of *siKRAS* and GEM. *siKRAS* and GEM cooperatively promote cell apoptosis and necrosis by affecting the RAF/MEK/ERK and PI3K/AKT/mTOR pathways.

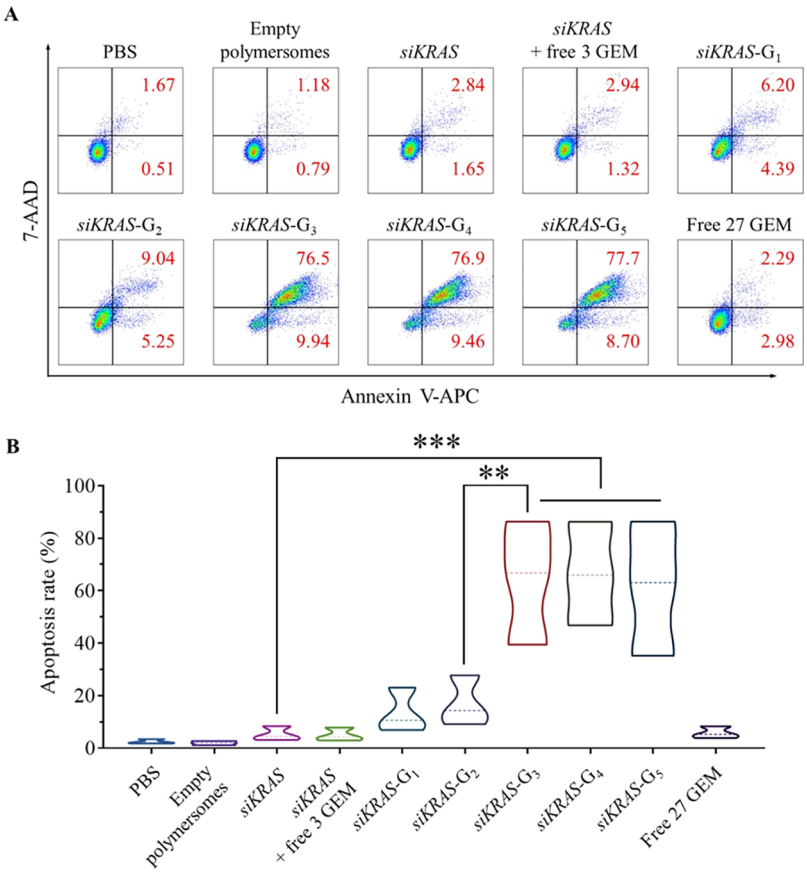


Figure 1. GEM unit number of *siKRAS*-G_n significantly affects the proapoptotic activity *in vitro*. Representative flow cytometry histograms of annexin V-APC/7-ADD staining (A) and relative apoptosis rate (B) of PANC-1 cells treated with PBS, empty polymersomes, *siKRAS* (200 nM), GEM alone (5.4 μM), and a mixture of *siKRAS* (200 nM) and GEM (600 nM); *siKRAS*-G_n (n = 1−5) (final siRNA conc.: 200 nM, final GEM conc. = 200−1000 nM) encapsulated in cRGD-BCP for 48 h. ** $p < 0.01$ and *** $p < 0.001$.

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891

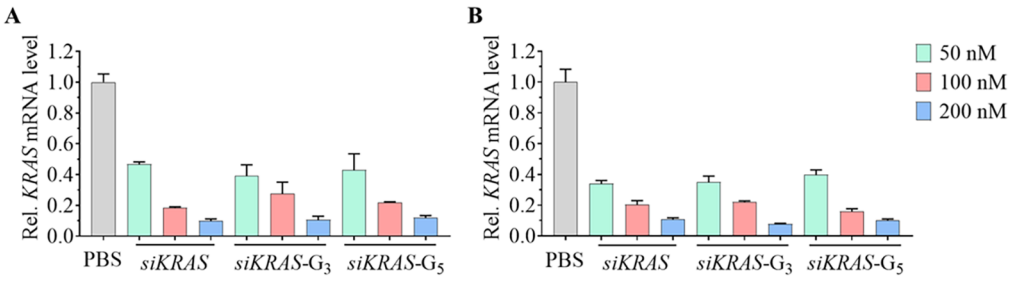


Figure 2. *In vitro* of *KRAS* mRNA by *siKRAS*-G_n encapsulated in cRGD-BCP. Relative *KRAS* mRNA expression was measured by RT-qPCR in PANC 1 cells after 48 h (A) and 72 h (B) of treatment (n = 3). Final expression was normalized to GAPDH and presented relative to PBS.

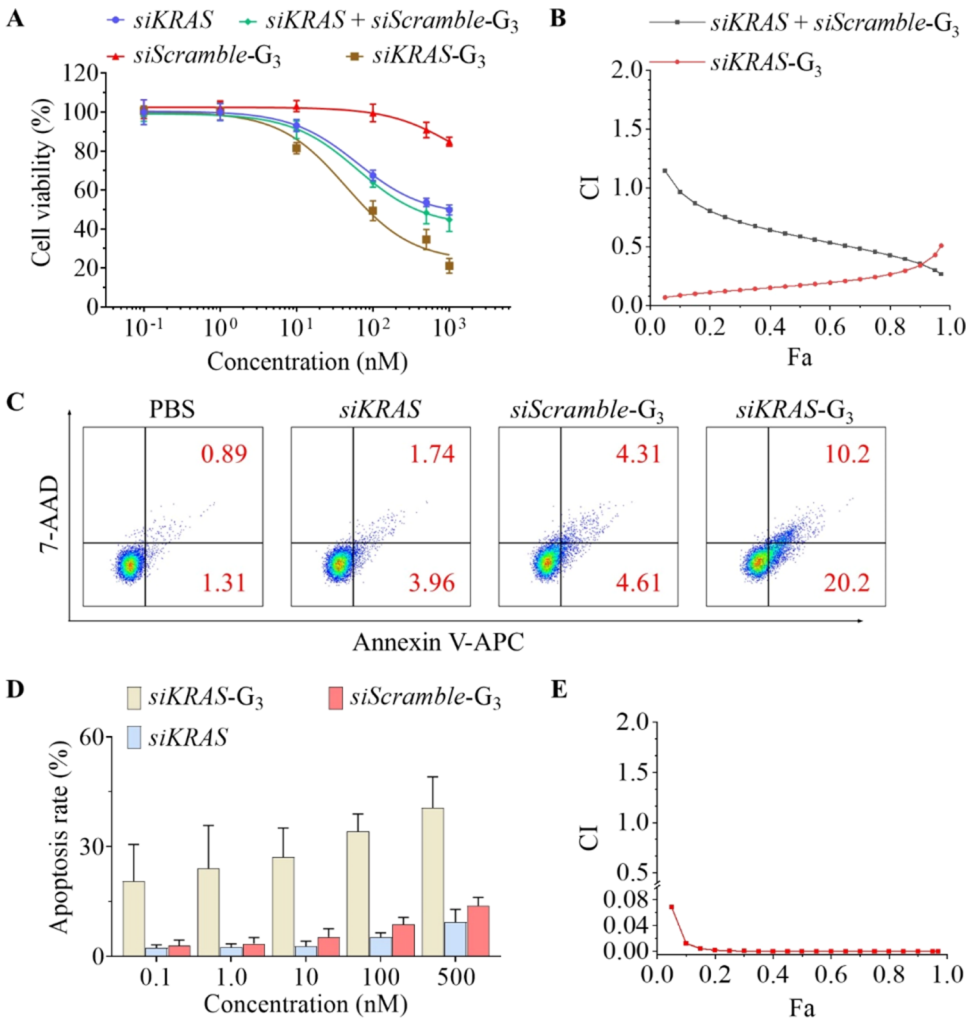


Figure 3. *siKRAS*-G_3 induces a strong synergistic effect to PANC-1 cells. (A) Cell viability (n = 5) and (B) CI plot of PANC-1 cells after 48 h of treatment with *siKRAS*, *siScramble*-G_3, *siKRAS*-G_3, and *siScramble*-G_3 + *siKRAS* encapsulated in cRGD-BCP. (C) Representative flow cytometry histograms of annexin V-APC/7-ADD (siRNA dosage: 100 nM), (D) apoptosis rate (n = 3), and (E) CI plot of PANC-1 cells treated with *siKRAS*, *siScramble*-G_3, and *siKRAS*-G_3 encapsulated in cRGD-BCP at different final siRNA concentrations for 24 h.

chain reaction (RT−qPCR) and normalized to the phosphate-buffered saline (PBS) group.

KRAS protein expression in PANC-1 cells after the above treatment (siRNA concentration: 200 nM) for 72 h was measured by Western blot (WB) using GAPDH as a reference (30 μg, n = 3) and normalized to the PBS group. Detailed procedures for RT−qPCR and WB are provided in the Supporting Information.

2.3. Apoptosis Studies. PANC-1 cells were seeded in 12-well plates (200,000/well). After 24 h, the culture medium was gently removed, and *siKRAS*-G_n, *siScramble*-G_n, or *siKRAS* encapsulated in cRGD-BCP (final siRNA concentration = 200 nM, n = 3) in fresh culture medium was added and incubated at 37 °C and 5% CO_2 for 48 h. The cells were then treated with trypsin and collected for treatment with Annexin V-APC/7-AAD staining at room temperature in the dark for 15 min. Measurements were performed on a BD FACS Calibur flow cytometer.

2.4. Synergistic Effect Studies. PANC-1 cells (10,000/well) were seeded in 96-well plates. After 24 h, *siKRAS*-G_n, *siScramble*-G_n, or *siKRAS* encapsulated in cRGD-BCP (final siRNA concentration siRNA: 0.1−1000 nM, n = 3) in fresh culture medium was added and incubated at 37 °C and 5% CO_2 for 48 h. The cells were washed with PBS three times. Cell counting kit-8 solution (10% in DMEM) was

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891

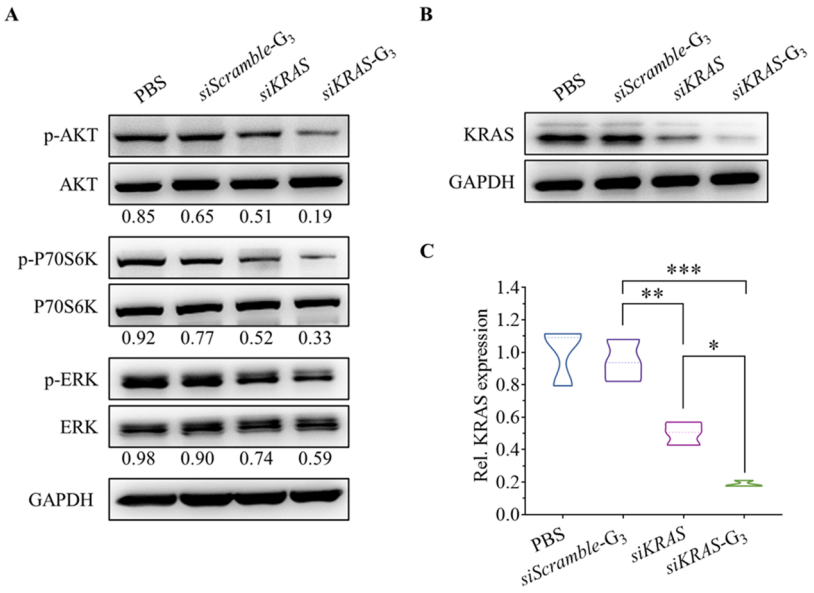


Figure 4. *siKRAS*-G_3 affects RAF/MEK/ERK and PI3K/AKT/mTOR pathway signaling *in vitro*. (A, B) Western blot analysis of cell lysates and (C) semiquantitative analysis of *KRAS* protein from PANC-1 cells after 72 h of treatment with *siKRAS*, *siScramble*-G_3, and *siKRAS*-G_3 encapsulated in cRGD-BCP (final siRNA conc.: 200 nM). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

added, and the mixture was incubated for another 1 h. The absorbance at 450 nm was measured using a microplate reader. The percentage of cell viability was calculated by comparing the absorbance of samples to the PBS group. The Chou−Talalay method was applied to study the synergistic effect by calculating the combination index (CI) and the fraction affected (Fa) through CompuSyn software. Fa-CI curves were constructed by using CompuSyn software, and the range of Fa was set from 0.10 to 0.97. The value of CI at Fa = 0.5 was used to determine the synergistic effect: CI < 1.0 was regarded as a synergistic effect, while CI < 0.5 was regarded as a strong synergistic effect.

2.5. Intracellular Signaling Study. PANC-1 cells (200,000/well) were seeded in 12-well plates. After 24 h, *siKRAS*-G_3, *siScramble*-G_3, or *siKRAS* encapsulated in cRGD-BCP (final siRNA concentration = 200 nM, $n = 3$) in fresh culture medium was added and incubated at 37 °C and 5% CO_2 for 72 h. The epidermal growth factor (50 ng/mL) was added to the mixture to incubate for 10 min. Cells were lysed in the lysis buffer with protease and phosphatase inhibitors, and the protein was quantified using a BCA protein assay kit. The cell lysates containing the same amount of proteins were further subjected to Western blotting measurements using 10% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) to determine p-AKT, p-ERK, and p-P70S6K (GAPDH as an internal reference). Detailed procedures are provided in the Supporting Information.

2.6. RNA Sequencing. PANC-1 cells (400,000/well) were seeded in 6-well plates. After 24 h, *siKRAS*-G_3, *siScramble*-G_3, or *siKRAS* encapsulated in cRGD-BCP (final siRNA concentration = 100 nM, $n = 3$) in fresh culture medium was added and incubated at 37 °C and 5% CO_2 for 48 h. The cells were lysed on ice with TRIzol, and an RNA-seq study was performed by Novogene Co., Ltd. (Beijing, P. R. China). Difference and enrichment analyses were completed using NovoMagic.

2.7. Therapeutic Efficacy of siKRAS-G_3 in PANC-1 Tumor-Bearing Mice. All mouse experiments were conducted under the approval of the Institutional Animal Care and Use Committee (IACUC) of GenePharma (Suzhou, P. R. China). PANC-1 cells (5×10^6) were mixed with Matrigel and implanted into the right dorsal abdomen. Mice bearing subcutaneous tumors received *siKRAS*-G_3, *siScramble*-G_3, or *siKRAS* encapsulated in cRGD-BCP (final siRNA dose: 1 or 3 mg/kg) or PBS via intravenous administration on days 0, 4, 8, and 12 following the size of tumor reaching 150 mm^3 ($n = 8$). All mice were weighed, and the volume of the tumor was measured with calipers every 4 days. On day 13, three mice from each group were sacrificed, and tumors and other major organs were collected and used for histological staining, immunoblot, and qRT−PCR studies. Tumors were photographed and weighed, and the tumor inhibition rate (TIR) was calculated using the following equation: TIR (%) = (1−tumor weight of the treated group/tumor weight of the PBS group) ×100. The survival rates were recorded ($n = 5$), and mice were considered dead when mice were dead, loss in mouse body weight ≥15%, or tumor volume >2000 mm^3.

2.8. Statistical Analysis. Data are presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied to determine the statistically significant differences among groups. The Kaplan−Meier survival rate and median survival time were analyzed by using GraphPad Prism 7. *($p < 0.05$) was regarded as statistically significant, while **($p < 0.01$) and ***($p < 0.001$) were considered highly significant.

3. RESULTS AND DISCUSSION

3.1. Design and Synthesis of siRNA-G_n Chimeras. The combination of chemotherapy with RNAi therapy has emerged as a potentially powerful strategy to manage malignant tumors.[37] The synergy of this combination is, however, most likely not optimal because chemotherapeutics and siRNA have completely different pharmacokinetics, and it is challenging to deliver them to the target at a constant ratio. In this study, we designed $KRAS^{G12D}$ siRNA-gemcitabine oligonucleotide chimeras (*siKRAS*-G_n) as a dual prodrug and systemically investigated the ratio of two drugs on the treatment of prostate cancer (PC). We have previously screened a highly specific siRNA sequence against $KRAS^{G12D}$.[35] Here, we synthesized five *siKRAS*-G_n chimeras with varying GEM numbers from 1 to 5 and *siScramble*-G_3 as a control. The structures of *siKRAS, siScramble, siKRAS*-G_n, and *siScramble*-G_3 are given in Table 1. All of the compounds were prepared with high yields using the standard procedures on a solid-phase oligonucleotide synthesizer and purified by preparative HPLC. HPLC showed a good purity of more than 90%. The mass spectrum and polyacrylamide gel electrophoresis (PAGE) confirmed their successful synthesis.

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891

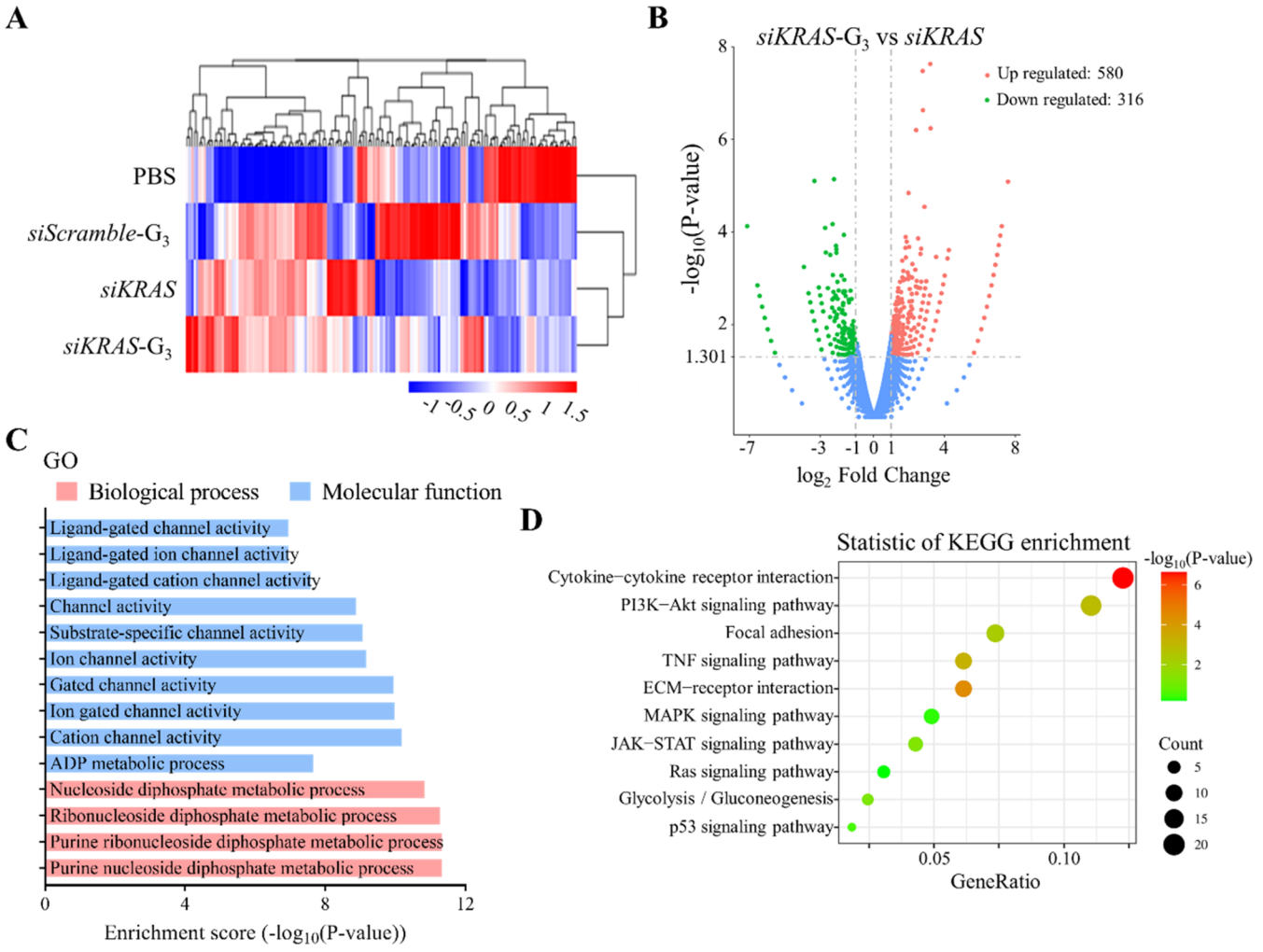


Figure 5. RNA-seq analysis of PANC-1 cells treated with *siKRAS*, *siKRAS*-G_3, and *siScramble*-G_3 encapsulated in cRGD-BCP for 48 h. (A) Heatmap of hierarchical clustering with DEGs. Red and blue indicate high and low gene expressions, respectively. (B) DEGs in a volcano plot (fold change >2 and *P* value <0.05) showing 580 upregulated genes (red) and 316 downregulated genes (green). (C) GO enrichment analysis and (D) KEGG pathway analysis of DEGs of *siKRAS*-G_3 relative to those of *siKRAS*.

3.2. Effect of GEM on the Proapoptotic Activity and Gene Silencing Efficiency of siKRAS-G_n. We have shown previously that cRGD-decorated chimeric polymersomes (cRGD-BCP) mediated the targeted delivery of *siKRAS* to PC *in vivo*.[35] *siKRAS*-G_n could be stably encapsulated into cRGD-BCP to yield cRGD-BCP-*siKRAS*-G_n with a small size of *ca.* 68 nm (Table S1) and quantitative loading of *siKRAS*-G_n at 5−15 wt % (Table S1 and Figure S1). Interestingly, the apoptosis assays showed that the GEM number in *siKRAS*-G_n played a crucial role in its proapoptotic activity to PANC-1 cells (Figure 1A). While *siKRAS*-G_1 and *siKRAS*-G_2 brought about only a moderate increase of apoptosis rate compared to *siKRAS* (13.6 and 17.1% versus 5.4%, respectively), *siKRAS*-G_3 resulted in massive apoptosis with an apoptosis rate of 64.2% (Figure 1B). No more increase of apoptosis rate was observed upon further increasing the GEM number to 4 and 5. These similar apoptotic activities of *siKRAS*-G_3, *siKRAS*-G_4, and *siKRAS*-G_5 indicate that after the silencing of the *KRAS* gene, a minimal amount of GEM is sufficient to inhibit DNA synthesis and induce apoptosis through caspase signaling. In contrast, cRGD-BCP-*siKRAS* plus 3 equiv. free GEM induced essentially the same apoptosis as cRGD-BCP-*siKRAS*, signifying the importance of GEM conjugation to *siKRAS*. Notably, free GEM even at 27 equiv. (*i.e.*, 9-fold that in *siKRAS*-G_3) caused little cell apoptosis. The high apoptotic activity of *siKRAS*-G_3 is likely due to its effective silencing of $KRAS^{G12D}$ gene expression that sensitizes PC cells to the GEM. $KRAS^{G12D}$ overexpression was reported to be a reason for drug resistance in PC, and silencing of $KRAS^{G12D}$ could effectively enhance chemotherapy.[6,9] It is remarkable that *siKRAS*-G_n shows such a high apoptotic activity at only 3 units of GEM (equiv to 600 nM free GEM).

The chemical modification of the siRNA sequence might alter its gene silencing activity.[38,39] Here, we studied the gene silencing ability of *siKRAS*-G_3 and *siKRAS*-G_5 in PANC-1 cells and compared them with *siKRAS*. The results showed that *siKRAS*-G_3, *siKRAS*-G_5, and *siKRAS* all could effectively silence $KRAS^{G12D}$ mRNA and GEM conjugation had not much influence on both the gene silencing efficacy and duration (Figure 2). *siKRAS*-G_3 achieved 92.3% knockdown of *KRAS* mRNA in PANC-1 cells at 72 h (siRNA concentration: 200 nM). Given its optimal *KRAS* silencing and strong apoptotic activity, *siKRAS*-G_3 was selected for further studies.

3.3. Synergistic Antitumor Effect of siKRAS-G_3. We further studied the synergistic antitumor effect of *siKRAS*-G_3 using *siKRAS* and *siScramble*-G_3 (sequences shown in Table 1)

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891

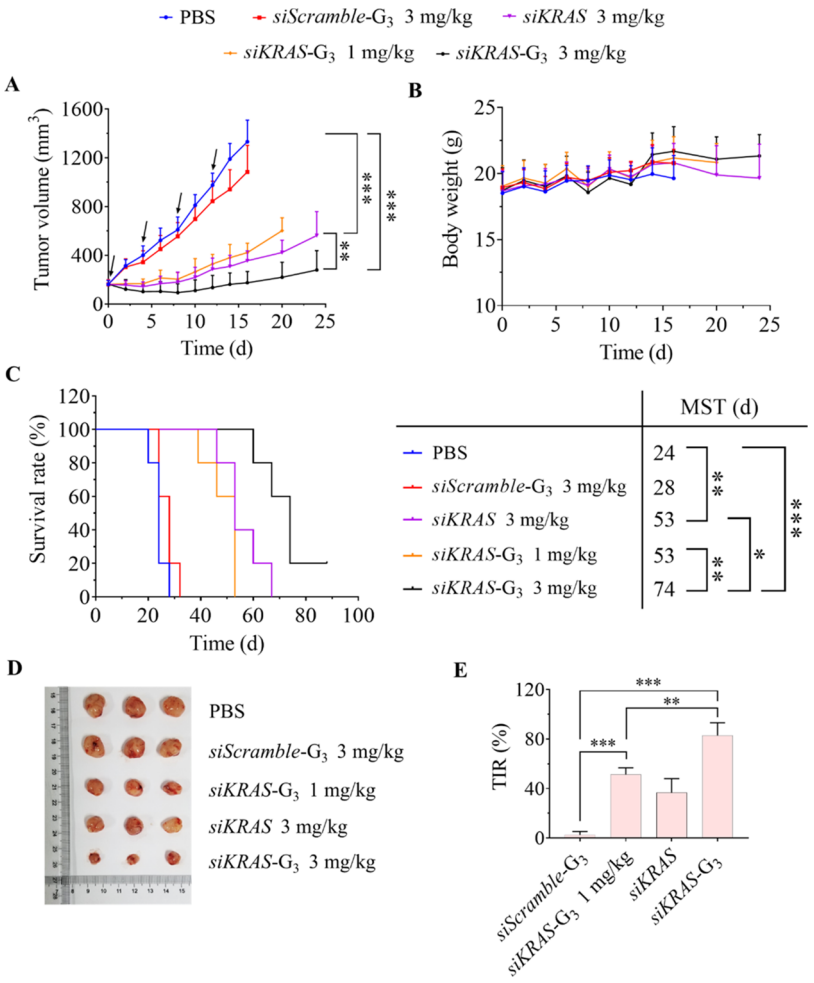


Figure 6. *siKRAS*-G_3 inhibits the tumor growth *in vivo*. Mice bearing PANC-1 tumors were treated with *siKRAS* (3 mg/kg), *siScramble*-G_3 (3 mg/kg), and *siKRAS*-G_3 (1 mg/kg and 3 mg/kg) encapsulated in cRGD-BCP and PBS on day 0, 4, 8, and 12 via *i.v.* injection. (A) Tumor growth curves; (B) body weight changes; and (C) Kaplan−Meier survival curves ($n = 5$). (D) Photo of excised tumors and (E) analysis of tumor inhibition rate (TIR) on day 13 ($n = 3$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

as controls. As shown above, free GEM induced little cell apoptosis at such a low dosage (600 nM), partly due to its inferior cell uptake. Figure 3A shows that the codelivery of *siKRAS* and *siScramble*-G_3 using cRGD-BCP led to a little increase in the inhibitory effect on PANC-1 cells compared to *siKRAS* alone, while *siKRAS*-G_3 exhibited clearly better inhibition of PANC-1 cells than *siKRAS* and *siScramble*-G_3 combination. The combination index (CI) calculated using the Chou−Talalay method indicated a strong synergistic inhibitory effect for *siKRAS*-G_3 (CI < 0.5, Figure 3B). This suggests that GEM and siRNA are more effective in tumor cell inhibition when delivered as a single drug. Moreover, Figure 3C,D shows that neither *siKRAS* nor *siScramble*-G_3 induced significant cell apoptosis at 24 h incubation, while *siKRAS*-G_3 caused significant early apoptosis. The CI analysis based on apoptosis at different concentrations confirmed a strong synergistic effect of *siKRAS*-G_3 (Figure 3E).

3.4. Mechanistic Analysis of the Synergistic Effect of siKRAS-G_3 at the Protein and Gene Levels. It was reported that *KRAS* protein inhibition and its protein phosphorylation inhibition pathway could improve the therapeutic efficacy of GEM formulations.[36,40] In *KRAS* mutant pancreatic cancer, the activation of the *KRAS* protein triggers downstream effector signaling pathways, such as the RAF/MEK/ERK and PI3K/AKT/mTOR pathways, to promote tumor cell proliferation and survival, and PI3K/AKT/mTOR pathway activation is associated with drug resistance to small-molecule antitumor drugs, such as GEM.[41,42]

To understand the mechanism of the synergistic effect of *siKRAS*-G_3, the effects of *siKRAS*, *siScramble*-G_3, and *siKRAS*-G_3 on the *KRAS* protein and the protein phosphorylation of its associated RAS downstream signaling pathway were investigated.

Western blotting confirmed that *siKRAS*-G_3 had a superior inhibitory effect on protein phosphorylation of both the RAF/MEK/ERK and PI3K/AKT/mTOR pathways (Figure 4). Specifically, p-AKT/AKT in the *siKRAS*-G_3 group was reduced by a factor of 3.4 and 2.7 compared with *siScramble*-G_3 and *siKRAS* groups, respectively, p-P70S6K/P70S6K was reduced by a factor of 2.3 and 1.6, and p-ERK/ERK was reduced by a factor of 1.5 and 1.3 (Figure 4A). Moreover, *siKRAS*-G_3 significantly downregulated the *KRAS* protein compared to *siScramble*-G_3 (***p) and *siKRAS* (*p) (Figure 4B,C).

RNA sequencing (RNA-seq) was used to investigate the genetic level differences in PANC-1 cells after cRGD-BCP transfection with *siKRAS*-G_3, *siKRAS*, or *siScramble*-G_3. As displayed in Figure 5A, all groups differed genetically from the

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891

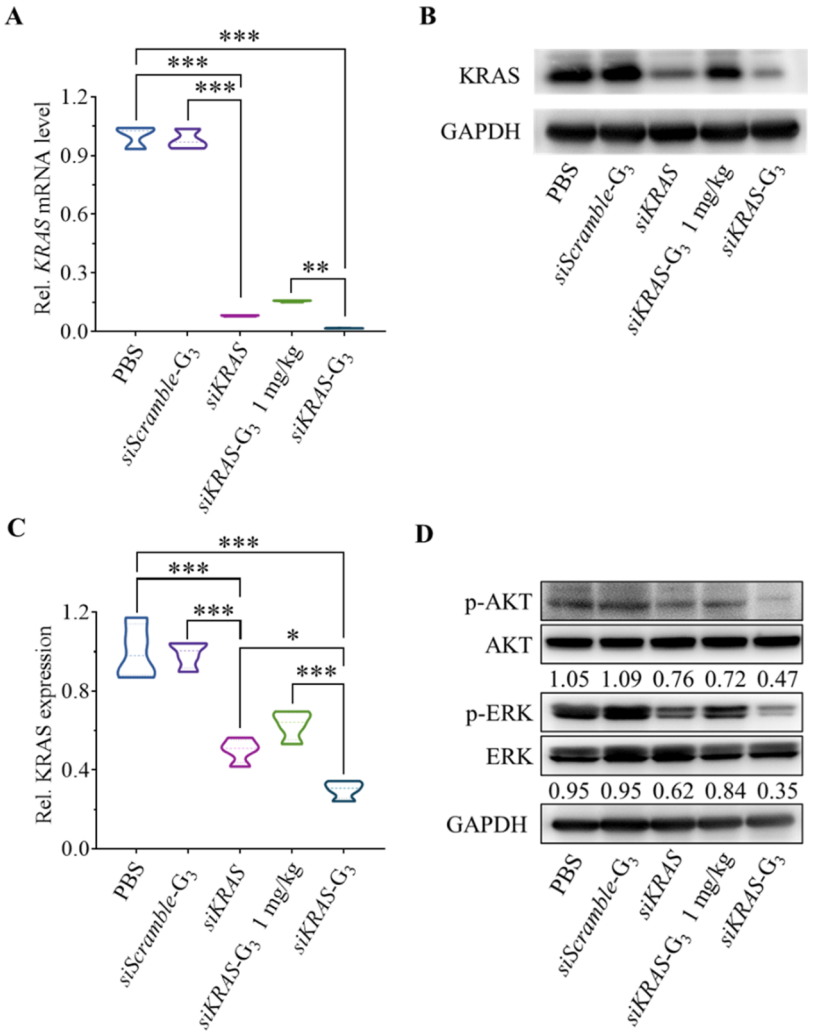


Figure 7. *siKRAS*-G_3 affects RAF/MEK/ERK and PI3K/AKT/mTOR pathway signaling *in vivo*. Mice bearing PANC-1 tumors were treated with *siKRAS* (3 mg/kg), *siScramble*-G_3 (3 mg/kg), *siKRAS*-G_3 (1 mg/kg and 3 mg/kg) encapsulated in cRGD-BCP, and PBS on day 0, 4, 8, and 12 via i.v. injection ($n = 5$). On day 13, three mice from each group were sacrificed. (A) Relative *KRAS* mRNA expression from tumor lysates measured by RT-qPCR; (B) Western blotting of *KRAS* protein and (C) semiquantitative analysis of *KRAS* protein from the tumor lysates; and (D) Western blotting of RAS downstream signaling pathway proteins. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

PBS group and from each other. The *siKRAS*-G_3 group expressed obviously high levels of tumor suppressor-related genes (*LATS2*, *LZTS1*, *TP63*, *TP53I3*, etc.), in addition to invasion suppressor-related genes (*siKRAS*) and proliferative suppressor-related genes (GEM). Additionally, a volcano plot illustrated that *siKRAS*-G_3 differed from *siKRAS* in 580 upregulated genes and 316 downregulated genes (Figure 5B). The GO functional enrichment analysis revealed that differentially expressed genes (DEGs) were enriched for both diphosphate metabolic processes and ion channel activity functions in the *siKRAS*-G_3 group compared to *siKRAS* (Figure 5C). Metabolic processes, ion channel activity, and glycolytic pathways were known to associate with tumor progression and drug resistance.[43−45] Further KEGG pathway analysis showed that the *siKRAS*-G_3 group had enrichment of genes for glycolysis, receptor interactions, and tumor-related signaling pathways relative to the *siKRAS* group (Figure 5D). Moreover, cytokine−cytokine receptor interactions and the PI3K−AKT signaling pathway were significantly enriched in the *siKRAS*-G_3 group, revealing that synergistic effects may be associated with phosphorylation and resistance processes (Figure 5D). Therefore, the potent synergistic antitumor effect of *siKRAS*-G_3 is a result of the significant inhibition of PI3K/AKT/mTOR and RAF/MEK/ERK signaling pathways as well as the regulation of metabolic processes, ion channel activity, and glycolytic pathways.

3.5. Therapeutic Activity of cRGD-BCP-siKRAS-G_3 toward PC Tumor-Bearing Mice. We next evaluated the antitumor activity of *siKRAS*-G_n chimeras in nude mice bearing subcutaneous PANC-1 tumors. Mice were intravenously injected (i.v.) with PBS, *siScramble*-G_3 (3 mg/kg), *siKRAS* (3 mg/kg), or *siKRAS*-G_3 (1 mg/kg, 3 mg/kg) on days 0, 4, 8, and 12. All oligonucleotides were delivered using cRGD-BCP. The results showed that the three *siKRAS* and *siKRAS*-G_3 treatments significantly delayed tumor growth without obvious weight loss (Figure 6A,B), while *siScramble*-G_3 did not have advantages over the PBS group. Among them, *siKRAS*-G_3 (3 mg/kg) exhibited the highest antitumor activity, demonstrating a dose dependency and synergistic effects. Both *siKRAS*-G_3 and *siKRAS* treatments significantly extended the mouse survival time compared to PBS and *siScramble*-G_3. The median survival time (MST) of *siKRAS*-G_3 at 3 mg/kg group reached 74 d, in

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891

contrast to the 53 and 28 days for *siKRAS* and *siScramble*-G_3, respectively (Figure 6C). Notably, the *siKRAS*-G_3 group at 1 mg/kg achieved the same MST as *siKRAS* at 3 mg/kg. The photos of tumors excised on day 13 confirmed the best inhibitory effect of *siKRAS*-G_3 (Figure 6D). The tumor inhibition rate (TIR) of the *siKRAS*-G_3 (3 mg/kg) group was 82.8%, significantly higher than that of the *siKRAS* (**p) and *siScramble*-G_3 (***p) groups (Figure 6E). These results verify the synergistic effect of *siKRAS*-G_3.

The H&E and TUNEL staining of tumor slices showed that three *siKRAS* and *siKRAS*-G_3 groups caused significant shrinkage, nuclear chromatin condensation, apoptosis, and necrosis of tumor cells compared with the PBS and *siScramble*-G_3 groups, and *siKRAS*-G_3 (3 mg/kg) induced the most apoptotic and necrotic cells (Figure S2). In addition, *siKRAS*-G_3 (3 mg/kg) led to a reduction in the proliferating cell nuclear antigen (PCNA) and an escalation in cleaved caspase-3 in tumor slices.

KRAS gene silencing and pathway protein phosphorylation inhibition in the tumors were further examined. The WB results showed that *siKRAS* (3 mg/kg), *siKRAS*-G_3 (1 mg/kg), and *siKRAS*-G_3 (3 mg/kg) all significantly downregulated *KRAS* mRNA (***p), with silencing efficiencies of 91.9, 84.5, and 98.2%, respectively (Figure 7A). Immunoblotting showed that *siKRAS* (3 mg/kg) could achieve 50.1% protein downregulation, while *siKRAS*-G_3 (3 mg/kg) achieved a greater downregulation of *KRAS* protein (*p) up to 69.9% (Figure 7B,C). It is of interest to note that *siKRAS*-G_3, despite affording nearly complete silencing of *KRAS* mRNA, induces only partial downregulation of the *KRAS* protein. This discrepancy originates from the fact that *siKRAS*-G_3 can only prevent the production of more *KRAS* protein but cannot remove the *KRAS* protein already existing in the tumor. The combination of the *siKRAS* and *KRAS* inhibitor might further improve the therapeutic efficacy. The phosphorylation levels of PI3K/AKT/mTOR and RAF/MEK/ERK pathway proteins showed inhibitory effects similar to those *in vitro* (Figure 7D). The above results confirm that *siKRAS*-G_3 is a highly potent dual prodrug that achieves synergistic treatment of PC when delivered by cRGD-decorated chimeric polymersomes.

4. CONCLUSIONS

We have demonstrated that a new dual prodrug, *KRAS* siRNA-GEM chimeras with minimal three units of GEM (*siKRAS*-G_3), achieves strong synergy in the inhibition of pancreatic cancer, when delivered by cRGD peptide-modified bioresponsive chimeric polymersomes (cRGD-BCP). *siKRAS*-G_3 has shown over one magnitude higher proapoptotic activity compared to *siKRAS* plus free GEM control. Notably, the *in vivo* studies in the PANC-1 tumor model display that *siKRAS*-G_3 achieves nearly complete silencing of $KRAS^{G12D}$ mRNA in the tumor and significantly better downregulation of $KRAS^{G12D}$ protein over the *siKRAS* counterpart, leading to superior tumor inhibition and survival benefits to all controls. The therapeutic effect of *siKRAS*-G_3 might further be improved by proper chemical modification of *siKRAS* sequences, which can augment its stability and gene silencing duration. The *siKRAS*-G_3 prodrug with a precise molecular structure, easy synthesis, and excellent anticancer performance has a high potential for clinical translation. This dual prodrug strategy that elegantly combines siRNA and chemodrugs provides a new treatment for different malignancies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemmater.4c02335.

Materials, cells, animals, characterization, qRT-PCR and Western blot measurements, histological analysis, characterizations of cRGD-BCP-*siKRAS*-G_n, representative H&E, TUNEL, and PCNA, cleaved caspase-3 staining images of dissected tumor slices, and synthesis of *siKRAS*-G_n (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Fenghua Meng − *Biomedical Polymers Laboratory, College of Chemistry, Chemical Engineering and Materials Science, and State Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou 215123, P. R. China;* orcid.org/0000-0002-8608-7738; Phone: +86-512-65880098; Email: fhmeng@suda.edu.cn

Zhiyuan Zhong − *Biomedical Polymers Laboratory, College of Chemistry, Chemical Engineering and Materials Science, and State Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou 215123, P. R. China; College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, P. R. China;* orcid.org/0000-0003-4175-4741; Email: zyzhong@suda.edu.cn

Authors

Ri Huang − *Biomedical Polymers Laboratory, College of Chemistry, Chemical Engineering and Materials Science, and State Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou 215123, P. R. China; College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, P. R. China*

Hong Du − *Suzhou GenePharma Co., Ltd., Suzhou 215123, P. R. China*

Liang Cheng − *Biomedical Polymers Laboratory, College of Chemistry, Chemical Engineering and Materials Science, and State Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou 215123, P. R. China; College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, P. R. China*

Ning Zhao − *College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, P. R. China; Suzhou GenePharma Co., Ltd., Suzhou 215123, P. R. China;* orcid.org/0000-0003-3699-3369

Peizhuo Zhang − *Suzhou GenePharma Co., Ltd., Suzhou 215123, P. R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chemmater.4c02335

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by research grants from the National Natural Science Foundation of China (NSFC 52033006, 52233007).

■ REFERENCES

(1) Siegel, R. L.; Miller, K. D.; Fuchs, H. E.; Jemal, A. Cancer statistics, 2022. *Ca-Cancer J. Clin.* **2022**, *72*, 7−33.

(2) Zhang, Z.; Song, J.; Xie, C.; Pan, J.; Lu, W.; Liu, M. Pancreatic cancer: recent progress of drugs in clinical trials. *AAPS J.* **2021**, *23*, No. 29.
(3) Liu, P.; Wang, Y.; Li, X. Targeting the untargetable KRAS in cancer therapy. *Acta Pharm. Sin. B* **2019**, *9*, 871−879.
(4) Huang, L.; Guo, Z.; Wang, F.; Fu, L. KRAS mutation: from undruggable to druggable in cancer. *Signal Transduction Targeted Ther.* **2021**, *6*, No. 386.
(5) Jiang, W.; Li, H.; Liu, X.; Zhang, J.; Zhang, W.; Li, T.; Liu, L.; Yu, X. Precise and efficient silencing of mutant Kras(G12D) by CRISPR-CasRx controls pancreatic cancer progression. *Theranostics* **2020**, *10*, 11507−11519.
(6) Zhao, H.; Wu, S.; Li, H.; Duan, Q.; Zhang, Z.; Shen, Q.; Wang, C.; Yin, T. ROS/KRAS/AMPK signaling contributes to gemcitabine-induced stem-like cell properties in pancreatic cancer. *Mol. Ther.–Oncolytics* **2019**, *14*, 299−312.
(7) Won, E. J.; Park, H.; Chang, S. H.; Kim, J. H.; Kwon, H.; Cho, Y. S.; Yoon, T. J. One-shot dual gene editing for drug-resistant pancreatic cancer therapy. *Biomaterials* **2021**, *279*, No. 121252.
(8) Hallin, J.; Bowcut, V.; Calinisan, A.; Briere, D. M.; Hargis, L.; Engstrom, L. D.; Laguer, J.; Medwid, J.; Vanderpool, D.; Lifset, E.; Trinh, D.; Hoffman, N.; Wang, X.; Lawson, J. D.; Gunn, R. J.; Smith, C. R.; Thomas, N. C.; Martinson, M.; Bergstrom, A.; Sullivan, F.; Bouhana, K.; Winski, S.; He, L.; Fernandez-Banet, J.; Pavlicek, A.; Haling, J. R.; Rahbaek, L.; Marx, M. A.; Olson, P.; Christensen, J. G. Anti-tumor efficacy of a potent and selective non-covalent KRAS-(G12D) inhibitor. *Nat. Med.* **2022**, *28*, 2171−2182.
(9) Anthiya, S.; Ozturk, S. C.; Yanik, H.; Tavukcuoglu, E.; Sahin, A.; Datta, D.; Charisse, K.; Alvarez, D. M.; Loza, M. I.; Calvo, A.; Sulheim, E.; Loevenich, S.; Klinkenberg, G.; Schmid, R.; Manoharan, M.; Esendagli, G.; Alonso, M. J. Targeted siRNA lipid nanoparticles for the treatment of KRAS-mutant tumors. *J. Controlled Release* **2023**, *357*, 67−83.
(10) Luo, D.; Xu, X.; Iqbal, M. Z.; Zhao, Q.; Zhao, R.; Farheen, J.; Zhang, Q.; Zhang, P.; Kong, X. siRNA-loaded hydroxyapatite nanoparticles for KRAS gene silencing in anti-pancreatic cancer therapy. *Pharmaceutics* **2021**, *13*, No. 1428.
(11) Kamerkar, S.; LeBleu, V. S.; Sugimoto, H.; Yang, S.; Ruivo, C. F.; Melo, S. A.; Lee, J. J.; Kalluri, R. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* **2017**, *546*, 498−503.
(12) Aghamiri, S.; Raee, P.; Talaei, S.; Mohammadi-Yeganeh, S.; Bayat, S.; Rezaee, D.; Ghavidel, A. A.; Teymouri, A.; Roshanzamiri, S.; Farhadi, S.; Ghanbarian, H. Nonviral siRNA delivery systems for pancreatic cancer therapy. *Biotechnol. Bioeng.* **2021**, *118*, 3669−3690.
(13) Taniguchi, H.; Suzuki, Y.; Imai, K.; Adachi, Y. Antitumoral RNA-targeted oligonucleotide therapeutics: The third pillar after small molecule inhibitors and antibodies. *Cancer Sci.* **2022**, *113*, 2952−2961.
(14) Inkoom, A.; Ndemazie, N. B.; Smith, T.; Frimpong, E.; Bulusu, R.; Poku, R.; Zhu, X.; Han, B.; Trevino, J.; Agyare, E. Biological evaluation of novel gemcitabine analog in patient-derived xenograft models of pancreatic cancer. *BMC Cancer* **2023**, *23*, No. 435.
(15) Muggia, F.; Diaz, I.; Peters, G. J. Nucleoside and nucleobase analogs in cancer treatment: not only sapacitabine, but also gemcitabine. *Expert Opin. Investig. Drugs* **2012**, *21*, 403−408.
(16) Balboni, B.; El Hassouni, B.; Honeywell, R. J.; Sarkisjan, D.; Giovannetti, E.; Poore, J.; Heaton, C.; Peterson, C.; Benaim, E.; Lee, Y. B.; Kim, D. J.; Peters, G. J. RX-3117 (fluorocyclopentenyl cytosine): a novel specific antimetabolite for selective cancer treatment. *Expert Opin. Invest.. Drugs* **2019**, *28*, 311−322.
(17) Thummuri, D.; Khan, S.; Underwood, P. W.; Zhang, P.; Wiegand, J.; Zhang, X.; Budamagunta, V.; Sobh, A.; Tagmount, A.; Loguinov, A.; Riner, A. N.; Akki, A. S.; Williamson, E.; Hromas, R.; Vulpe, C. D.; Zheng, G.; Trevino, J. G.; Zhou, D. Overcoming gemcitabine resistance in pancreatic cancer using the BCL-X(L)-specific degrader DT2216. *Mol. Cancer Ther.* **2022**, *21*, 184−192.
(18) Thompson, B. R.; Shi, J.; Zhu, H. J.; Smith, D. E. Pharmacokinetics of gemcitabine and its amino acid ester prodrug following intravenous and oral administrations in mice. *Biochem. Pharmacol.* **2020**, *180*, No. 114127.
(19) Xia, Y.; Wei, J.; Zhao, S.; Guo, B.; Meng, F.; Klumperman, B.; Zhong, Z. Systemic administration of polymersomal oncolytic peptide LTX-315 combining with CpG adjuvant and anti-PD-1 antibody boosts immunotherapy of melanoma. *J. Controlled Release* **2021**, *336*, 262−273.
(20) Lai, X.; Liu, X. L.; Pan, H.; Zhu, M. H.; Long, M.; Yuan, Y.; Zhang, Z.; Dong, X.; Lu, Q.; Sun, P.; Lovell, J. F.; Chen, H. Z.; Fang, C. Light-triggered efficient sequential drug delivery of biomimetic nanosystem for multimodal chemo-, antiangiogenic, and anti-MDSC therapy in melanoma. *Adv. Mater.* **2022**, *34*, No. e2106682.
(21) Guo, B.; Wei, J.; Wang, J.; Sun, Y.; Yuan, J.; Zhong, Z.; Meng, F. CD44-targeting hydrophobic phosphorylated gemcitabine prodrug nanotherapeutics augment lung cancer therapy. *Acta Biomater.* **2022**, *145*, 200−209.
(22) Guo, B.; Qu, Y.; Sun, Y.; Zhao, S.; Yuan, J.; Zhang, P.; Zhong, Z.; Meng, F. Co-delivery of gemcitabine and paclitaxel plus NanoCpG empowers chemoimmunotherapy of postoperative "cold" triple-negative breast cancer. *Bioact. Mater.* **2023**, *25*, 61−72.
(23) Ying, N.; Lin, X.; Xie, M.; Zeng, D. Effect of surface ligand modification on the properties of anti-tumor nanocarrier. *Colloids Surf. B.* **2022**, *220*, No. 112944.
(24) Kunzmann, V.; Siveke, J. T.; Algul, H.; Goekkurt, E.; Siegler, G.; Martens, U.; Waldschmidt, D.; Pelzer, U.; Fuchs, M.; Kullmann, F.; Boeck, S.; Ettrich, T. J.; Held, S.; Keller, R.; Klein, I.; Germer, C. T.; Stein, H.; Friess, H.; Bahra, M.; Jakobs, R.; Hartlapp, I.; Heinemann, V.; On behalf of the German Pancreatic Cancer Working Group (AIO-PAK) and NEOLAP investigators; et al. Nab-paclitaxel plus gemcitabine versus nab-paclitaxel plus gemcitabine followed by FOLFIRINOX induction chemotherapy in locally advanced pancreatic cancer (NEOLAP-AIO-PAK-0113): a multicentre, randomised, phase 2 trial. *Lancet Gastroenterol. Hepatol.* **2021**, *6*, 128−138.
(25) Ozaka, M.; Nakachi, K.; Kobayashi, S.; Ohba, A.; Imaoka, H.; Terashima, T.; Ishii, H.; Mizusawa, J.; Katayama, H.; Kataoka, T.; Okusaka, T.; Ikeda, M.; Sasahira, N.; Miwa, H.; Mizukoshi, E.; Okano, N.; Mizuno, N.; Yamamoto, T.; Komatsu, Y.; Todaka, A.; Kamata, K.; Furukawa, M.; Fujimori, N.; Katanuma, A.; Takayama, Y.; Tsumura, H.; Fukuda, H.; Ueno, M.; Furuse, J.; Hepatobiliary, G. Pancreatic Oncology Group of Japan Clinical Oncology, A randomised phase II study of modified FOLFIRINOX versus gemcitabine plus nab-paclitaxel for locally advanced pancreatic cancer (JCOG1407). *Eur. J. Cancer* **2023**, *181*, 135−144.
(26) Tang, S.; Hang, Y.; Ding, L.; Tang, W.; Yu, A.; Zhang, C.; Sil, D.; Xie, Y.; Oupicky, D. Intraperitoneal siRNA nanoparticles for augmentation of gemcitabine efficacy in the treatment of pancreatic cancer. *Mol. Pharmaceutics* **2021**, *18*, 4448−4458.
(27) Rehman, U.; Abourehab, M. A. S.; Alexander, A.; Kesharwani, P. Polymeric micelles assisted combinatorial therapy: Is it new hope for pancreatic cancer? *Eur. Polym. J.* **2023**, *184*, No. 111784.
(28) Tang, H.; Xue, Y.; Li, B.; Xu, X.; Zhang, F.; Guo, J.; Li, Q.; Yuan, T.; Chen, Y.; Pan, Y.; Ping, Y.; Li, D. Membrane-camouflaged supramolecular nanoparticles for co-delivery of chemotherapeutic and molecular-targeted drugs with siRNA against patient-derived pancreatic carcinoma. *Acta Pharm. Sin. B* **2022**, *12*, 3410−3426.
(29) Réjiba, S.; Wack, S.; Aprahamian, M.; Hajri, A. K-ras oncogene silencing strategy reduces tumor growth and enhances gemcitabine chemotherapy efficacy for pancreatic cancer treatment. *Cancer Sci.* **2007**, *98*, 1128−1136.
(30) Nishimoto, A. Effective combinations of anti-cancer and targeted drugs for pancreatic cancer treatment. *World J. Gastroenterol.* **2022**, *28*, 3637−3643.
(31) Zhang, R. X.; Wong, H. L.; Xue, H. Y.; Eoh, J. Y.; Wu, X. Y. Nanomedicine of synergistic drug combinations for cancer therapy - strategies and perspectives. *J. Controlled Release* **2016**, *240*, 489−503.
(32) Zhang, M.; Liu, E.; Cui, Y.; Huang, Y. Nanotechnology-based combination therapy for overcoming multidrug-resistant cancer. *Cancer Biol. Med.* **2017**, *14*, 212−227.

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891

(33) Moharil, P.; Wan, Z.; Pardeshi, A.; Li, J.; Huang, H.; Luo, Z.; Rathod, S.; Zhang, Z.; Chen, Y.; Zhang, B.; Fernandez, C. A.; Sun, J.; Li, S. Engineering a folic acid-decorated ultrasmall gemcitabine nanocarrier for breast cancer therapy: dual targeting of tumor cells and tumor-associated macrophages. *Acta Pharm. Sin. B* **2022**, *12*, 1148−1162.
(34) Tarannum, M.; Holtzman, K.; Dreau, D.; Mukherjee, P.; Vivero-Escoto, J. L. Nanoparticle combination for precise stroma modulation and improved delivery for pancreatic cancer. *J. Controlled Release* **2022**, *347*, 425−434.
(35) Huang, R.; Du, H.; Cheng, L.; Zhang, P.; Meng, F.; Zhong, Z. Targeted nanodelivery of siRNA against KRAS G12D inhibits pancreatic cancer. *Acta Biomater.* **2023**, *168*, 529−539.
(36) Kang, Y. W.; Lee, J. E.; Jung, K. H.; Son, M. K.; Shin, S. M.; Kim, S. J.; Fang, Z.; Yan, H. H.; Park, J. H.; Han, B.; Cheon, M. J.; Woo, M. G.; Lim, J. H.; Kim, Y. S.; Hong, S. S. KRAS targeting antibody synergizes anti-cancer activity of gemcitabine against pancreatic cancer. *Cancer Lett.* **2018**, *438*, 174−186.
(37) Ma, M. J.; Shi, Y. H.; Liu, Z. D.; Zhu, Y. Q.; Zhao, G. Y.; Ye, J. Y.; Li, F. X.; Huang, X. T.; Wang, X. Y.; Wang, J. Q.; Xu, Q. C.; Yin, X. Y. N6-methyladenosine modified TGFB2 triggers lipid metabolism reprogramming to confer pancreatic ductal adenocarcinoma gemcitabine resistance. *Oncogene* **2024**, *43*, 2405−2420.
(38) Foster, D. J.; Brown, C. R.; Shaikh, S.; Trapp, C.; Schlegel, M. K.; Qian, K.; Sehgal, A.; Rajeev, K. G.; Jadhav, V.; Manoharan, M.; Kuchimanchi, S.; Maier, M. A.; Milstein, S. Advanced siRNA designs further improve in vivo performance of GalNAc-siRNA conjugates. *Mol. Med.* **2018**, *26*, 708−717.
(39) Hassler, M. R.; Turanov, A. A.; Alterman, J. F.; Haraszti, R. A.; Coles, A. H.; Osborn, M. F.; Echeverria, D.; Nikan, M.; Salomon, W. E.; Roux, L.; Godinho, B.; Davis, S. M.; Morrissey, D. V.; Zamore, P. D.; Karumanchi, S. A.; Moore, M. J.; Aronin, N.; Khvorova, A. Comparison of partially and fully chemically-modified siRNA in conjugate-mediated delivery in vivo. *Nucleic Acids Res.* **2018**, *46*, 2185−2196.
(40) Lee, J. E.; Kang, Y. W.; Jung, K. H.; Son, M. K.; Shin, S. M.; Kim, J.-S.; Kim, S. J.; Fang, Z.; Yan, H. H.; Park, J. H.; Yoon, Y. C.; Han, B.; Cheon, M. J.; Woo, M. G.; Seo, M. S.; Lim, J. H.; Kim, Y. S.; Hong, S. S. Intracellular KRAS-specific antibody enhances the antitumor efficacy of gemcitabine in pancreatic cancer by inducing endosomal escape. *Cancer Lett.* **2021**, *507*, 97−111.
(41) LoRusso, P. M. Inhibition of the PI3K/AKT/mTOR pathway in solid tumors. *J. Clin. Oncol.* **2016**, *34*, 3803−3815.
(42) Tian, Z.; Tan, Y.; Lin, X.; Su, M.; Pan, L.; Lin, L.; Ou, G.; Chen, Y. Arsenic trioxide sensitizes pancreatic cancer cells to gemcitabine through downregulation of the TIMP1/PI3K/AKT/mTOR axis. *Transl. Res.* **2023**, *255*, 66−76.
(43) Faubert, B.; Solmonson, A.; DeBerardinis, R. J. Metabolic reprogramming and cancer progression. *Science* **2020**, *368*, No. eaaw5473.
(44) Arcangeli, A.; Becchetti, A. Novel perspectives in cancer therapy: Targeting ion channels. *Drug Resistance Update* **2015**, *21*−*22*, 11−19.
(45) Gonçalves, A. C.; Richiardone, E.; Jorge, J.; Polonia, B.; Xavier, C. P. R.; Salaroglio, I. C.; Riganti, C.; Vasconcelos, M. H.; Corbet, C.; Sarmento-Ribeiro, A. B. Impact of cancer metabolism on therapy resistance - Clinical implications. *Drug Resistance Update* **2021**, *59*, No. 100797.

https://doi.org/10.1021/acs.chemmater.4c02335
Chem. Mater. 2024, 36, 11881−11891