

Materializing the Potential of Small Interfering RNA via a Tumor-Targeting Nanodelivery System

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Abstract

The field of small interfering RNA (siRNA) as potent sequence-selective inhibitors of transcription is rapidly developing. However, until now, low transfection efficiency, poor tissue penetration, and nonspecific immune stimulation by *in vivo* administered siRNAs have delayed their therapeutic application. Their potential as anticancer therapeutics hinges on the availability of a vehicle that can be systemically administered, safely and repeatedly, and will deliver the siRNA specifically and efficiently to the tumor, both primary tumors and metastases. We have developed a nanosized immunoliposome-based delivery complex (scL) that, when systemically administered, will preferentially target and deliver molecules useful in gene medicine, including plasmid DNA and antisense oligonucleotides, to tumor cells wherever they occur in the body. This tumor-targeting nanoparticle delivery vehicle can also deliver siRNA to both primary and metastatic disease. We have also enhanced the efficiency of this complex by the inclusion of a pH-sensitive histidine-lysine peptide in the complex (scL-HoKC) and by delivery of a modified hybrid (DNA-RNA) anti-HER-2 siRNA molecule. Scanning probe microscopy confirms that this modified complex maintains its nanoscale size. More importantly, we show that this nanoimmunoliposome anti-HER-2 siRNA complex can sensitize human tumor cells to chemotherapeutics, silence the target gene and affect its downstream pathway components *in vivo*, and significantly inhibit tumor growth in a pancreatic cancer model. Thus, this complex has the potential to help translate the potent effects of siRNA into a clinically viable anticancer therapeutic. [Cancer Res 2007;67(7):2938–43]

Introduction

Realization of the potential of small interfering RNA (siRNA) as anticancer therapeutics requires development of systemically administered, stable, efficient, tumor-specific nanodelivery vehicles. Although advances are being made (1–3), currently, only a few approaches potentially feasible in patients have been described (4–8). Our laboratory has developed an anti-transferrin receptor (TfR) single-chain antibody fragment-directed nanoimmunoliposome (scL), which self-assembles into ~100-nm size particles. This *in vivo* administered complex takes advantage of elevated TfR levels on tumor cells to deliver its payload specifically and efficiently to

primary/metastatic tumors (9–11) and is entering phase I clinical trials for gene therapy with p53. Inclusion of a histidine-lysine peptide (HoKC) in the complex (scL-HoKC) enhances endosomal release resulting in increased effect (12–14). Our prior work has shown that the presence of the targeting moiety on the surface of the liposome is crucial for tumor targeting and efficient uptake of these complexes (9–12, 14). We have previously used scL to systemically deliver high levels of siRNA specifically to tumors (15). Here, we show the increased potency of the scL-HoKC complex and the ability of these nanoimmunoliposomes carrying anti-HER-2 siRNA to target and sensitize tumor cells to chemotherapeutics, silence the target gene, affect its downstream pathway, and significantly inhibit tumor growth *in vivo*.

Materials and Methods

Cell lines. Human pancreatic (PANC-1) and breast (MDA-MB-435) cancer cell lines were obtained from Lombardi Comprehensive Cancer Center Tissue Culture Shared Resource (Washington, DC). MDA 435/LCC6 (a gift from R. Clarke, Lombardi Comprehensive Cancer Center) is a metastatic cell line derived from MDA-MB-435 (16). These cell lines were maintained in Improved MEM (Biosource Biofluids, Rockville, MD) supplemented with 2 mmol/L L-glutamine, 50 µg/mL each of penicillin, streptomycin, and neomycin, and 10% (PANC-1 and MDA-MB-435) or 5% (MDA 435/LCC6) heat-inactivated fetal bovine serum (FBS). H500, a normal human fibroblast cell line, was maintained in Eagle's MEM as described previously (17). Human lung (H157) and colon carcinoma (H630) cell lines were obtained from Dr. Daniel Longley (Queen's University, Belfast, United Kingdom). They were maintained in RPMI 1640 and DMEM plus 1 mmol/L sodium pyruvate, respectively, plus antibiotics, glutamine as above, and 10% FBS.

Scanning probe microscopy. Sample solutions of (a) TfRscFv/LipA/siRNA (scL/siRNA) and (b) TfRscFv/LipA-HoKC/siRNA (scL-HoKC/siRNA) were freshly prepared at Georgetown University (Washington, DC), immediately delivered to National Institute of Standards and Technology (Gaithersburg, MD), and imaged. For scanning probe microscopy (SPM), sample (a) was diluted 1:3 with deionized water and 5 µL micropipetted onto an ultrasonically cleaned silicon substrate with native oxide coating. For sample (b), 5 µL of undiluted solution were micropipetted onto an ultrasonically cleaned silicon substrate. SPM imaging was done using a Veeco MultiMode SPM with a Nanoscope IV controller. Topographical images were obtained in tapping mode using uncoated silicon high-resonant frequency Veeco RTESP cantilevers (resonant frequency of ~320–360 kHz and spring constant of ~20–60 N/m) and NTMDT NSC12 type C cantilevers (120–190 kHz and 3.5–12 N/m).

Small interfering RNA. The anti-HER-2 siRNAs were synthesized and purified by TriLink Biotechnologies, Inc. (San Diego, CA; ref. 18) based on a previously published siRNA sequence reported to have substantial anti-HER-2 activity (19). The hybrid siRNA is a 19-mer blunt-ended version of this published RNA/RNA duplex wherein the sense RNA strand was replaced by DNA with the following sequence (DNA is italicized): 5'-TCTCTCGGGTGGTGGCAT-3' (sense) and 3'-AGAGACGCCACCAACCGUA-5' (antisense). The control sequence for this hybrid siRNA is 5'-TTCTCCGAACGGTCACTG-3' (sense) and 5'-AAGAGGCUUGCACUGAGCA-3' (antisense). The second

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blunt-ended 19-mer siRNA is a modification of this hybrid sense DNA/antisense RNA molecule, where O-Me substituents have been incorporated in the central region of the sense strand with DNA flanks (modified hybrid). The sequence is 5'-TTTTTgcgggguuGICIT-3' (sense) and 3'-AGAGACGCCACCAACCGUA-5' [(RNA) antisense], where I = 2' deoxyinosine and lowercase letters indicate 2'-O-Me/RNA. The control sequence for this modified hybrid siRNA is 5'-TICICcgaaguguCICIT-3' (sense) and 3'-AAGAGGCUUGCACA-GUGCA-5' (antisense).

Nanoimmunoliposome complex formation. The scL and scL-HoKC complexes were formed as described previously (12, 15).

Confocal microscopy. 5' Fluorescein (6-FAM)-labeled (sense strand) control modified hybrid siRNA was synthesized and purified by PAGE electrophoresis by TriLink Biotechnologies. PANC-1 cells (5.5×10^4) were seeded on coverslips in a 24-well tissue culture plate and transfected (15) 24 h later with either scL or scL-HoKC carrying 0.2 $\mu\text{mol/L}$ of 6-FAM siRNA, or 0.2 $\mu\text{mol/L}$ of uncomplexed (free) 6-FAM siRNA, for 6 h in serum-free medium. The cells were washed and fixed with 3.7% paraformaldehyde and the slides were mounted using Prolong Antifade kit (Molecular Probes, Inc., Eugene, OR) and observed with an Olympus IX-70 Laser Confocal Scanning Microscope imaging system (Center Valley, PA) with an upright confocal microscope and an X60 oil immersion objective.

XTT assay. The XTT assays were done as described previously (20).

In vivo studies. The fluorescence tumor-targeting experiment was done as described previously (11), except that scL-HoKC complex was used.

To further show the tumor specificity over normal tissue, tumors were induced in female athymic nude (NCR *nu/nu*) mice by the s.c. inoculation of approximately 1×10^7 of H157 or H630 cells suspended in Matrigel collagen basement membrane (BD Biosciences, Bedford, MA). When the tumors were at least 350 mm^3 , the mice were i.v. injected once with scL encapsulated modified hybrid siRNA at 9 mg/kg. After 24 h, the animals were sacrificed and tumor, liver, lungs, and brain were removed. The siRNA was isolated as total RNA using Trizol reagent (Invitrogen, Carlsbad, CA). Each RNA (25 μg) was run on a 19% PAAG and 7 mol/L urea gel, transferred to nylon membrane, and hybridized with an oligonucleotide probe, labeled by the Gene Images AlkPhos Direct kit (GE Healthcare, Piscataway, NJ), specific for either the sense or antisense strand of modified hybrid siRNA.

To assess gene silencing *in vivo*, tumors were induced in female athymic nude mice by s.c. inoculation of 6×10^6 MDA-MB-435 cells suspended in Matrigel. Mice bearing tumors of at least 100 mm^3 were treated with scL-HoKC complexed hybrid or modified hybrid siRNA or their respective control molecules (3 mg/kg). The complex, prepared as described previously (15), was given by i.v. injection thrice over 24 h. Mice were sacrificed 46 h after the first injection. Total protein (40 μg) isolated from each tumor was electrophoretically fractionated using a Criterion Precast 4% to 20% gradient gel, transferred to nitrocellulose membrane, and probed

for expression of various genes. The signal was detected by enhanced chemiluminescence (GE Healthcare).

For the efficacy study, PANC-1 s.c. tumors were induced in female athymic nude (NCR *nu/nu*) mice by the serial passage of 1 mm^3 s.c. tumor sections. Mice bearing tumors of approximately 50 to 70 mm^3 were tail vein injected thrice weekly with the scL-HoKC complexed modified hybrid siRNA, alone or in combination with gemcitabine. The doses of siRNA were 3 mg/kg (days 0–15), 2 mg/kg (days 15–21), and 1.5 mg/kg (days 21–33). Gemcitabine was given i.p. twice weekly at 60 mg/kg. Tumor sizes were measured using calipers and the tumor volume in mm^3 ($L \times W \times H$) \pm SE (8–20 tumors/group) was plotted versus time.

Results and Discussion

The nanoimmunoliposome complexes are composed of siRNA encapsulated by a cationic liposome, the surface of which is decorated with an anti-TfR single-chain antibody fragment (TfRscFv; refs. 21, 22) that serves to target the complex to both primary and metastatic disease (9–11, 15). We assessed the size of the parent complex (scL) and that containing the pH-sensitive peptide (scL-HoKC) by SPM. SPM images surface topography in tapping mode by oscillating a cantilever with a sharp tip close to the cantilever resonance frequency. A feedback circuit maintains the oscillation of the cantilever at constant amplitude. Figure 1 shows SPM topographical images of isolated and aggregated scL/siRNA (Fig. 1A) and scL-HoKC/siRNA (Fig. 1B) particles. The aggregation is a result of the SPM preparation method. The sizes of both the scL and scL-HoKC particles are fairly uniform, averaging ~ 100 nm. These sizes are clearly in the nanoparticle range and agree with our previous findings with encapsulated plasmid DNA (9). The insets represent higher-magnification SPM phase images. Phase imaging indicates that inclusion of HoKC peptide results in a surface structure that is clearly different than that of scL, being less homogenous, with a more highly structured appearance under dry imaging conditions.

We have synthesized two 19-mer blunt-ended siRNA sense DNA/antisense RNA hybrid analogues (hybrid and modified hybrid) directed against HER-2 (19) that are hypothesized to be more stable and potent than the corresponding standard duplex siRNA molecule (18). We compared the level of cell killing by the hybrid form to that of the standard RNA duplex, both delivered by scL, in PANC-1 cells *in vitro* (Fig. 2A). Both siRNAs showed significant cell

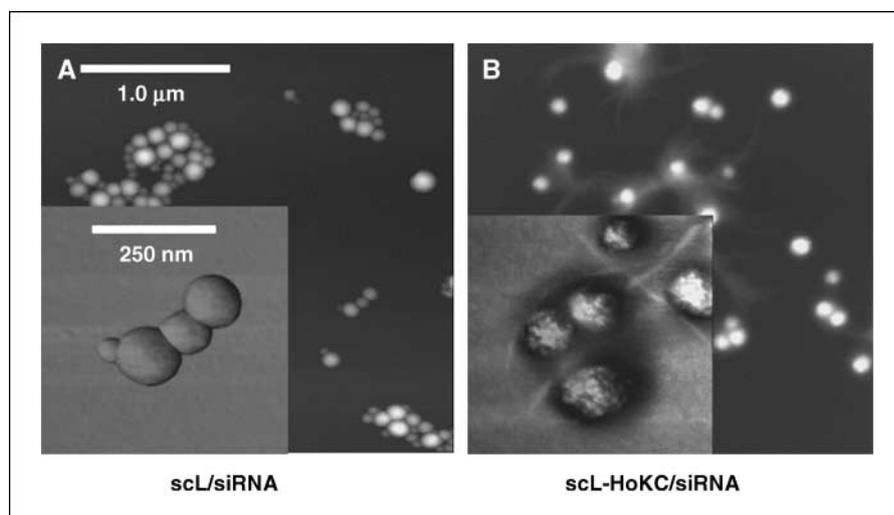


Figure 1. Topographical and phase SPM images of nanoimmunocomplexed anti-HER-2 siRNA. A and B, inset, magnified phase-contrast image. The scales are identical in (A) and (B). A, imaging of the scL/siRNA complex. B, imaging of the scL-HoKC/siRNA complex.

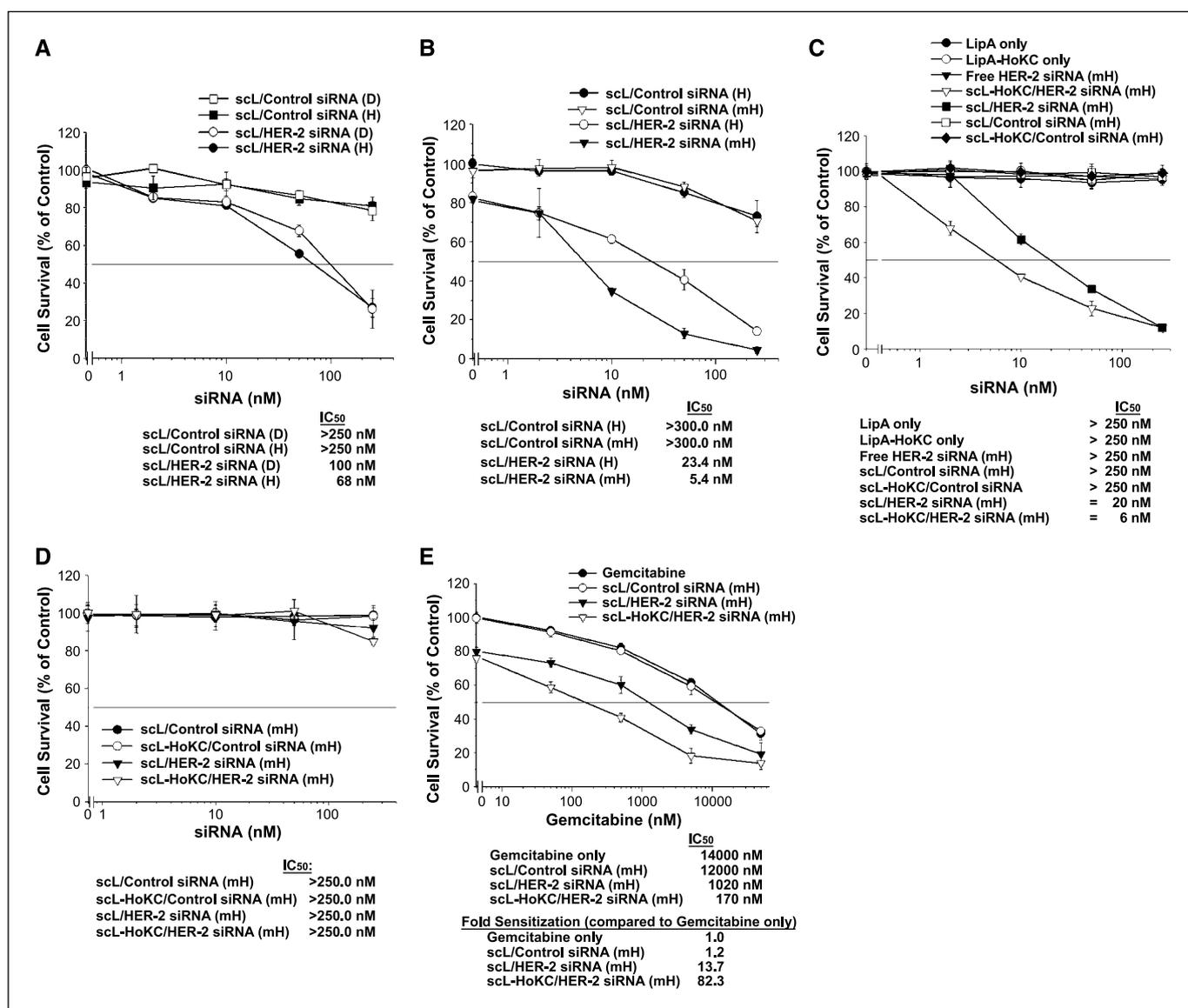


Figure 2. XTT cell survival assays. **A**, comparison of the effect of nanoimmunocomplex scL-delivered duplex and hybrid anti-HER-2 siRNAs on human pancreatic cancer cell line PANC-1 survival *in vitro*. Controls are the appropriate nonsequence-specific molecules. IC_{50} values are the concentration of siRNA (nmol/L) that kills 50% of the cells. **B**, similar experiment in PANC-1 cells as in (A) comparing hybrid and modified hybrid sequences. **C**, comparison of the effects of free (uncomplexed) and nanoimmunocomplexed modified hybrid anti-HER-2 siRNA on human breast cancer cell line MDA-MB-435 *in vitro*. The modified hybrid siRNA was delivered by both the scL and scL-HoKC complexes. To show that the cell killing observed is siRNA specific and not due to nonspecific cytotoxicity of oligonucleotides or of the liposomes, cells were also transfected with liposome or liposome-HoKC only as well as scL and scL-HoKC encapsulated control molecules. **D**, assessment of the effect of modified hybrid siRNA delivered by scL and scL-HoKC complexes on normal cells (human fibroblast cell line H500). **E**, comparison of the level of sensitization of PANC-1 cells to chemotherapeutic agent gemcitabine by scL-delivered and scL-HoKC-delivered modified hybrid anti-HER-2 siRNA (125 nmol/L). Fold sensitization is the ratio of the IC_{50} value of each treatment compared with gemcitabine only.

killing. However, the hybrid form was ~1.5-fold more potent compared with duplex based on IC_{50} values (concentration resulting in 50% cell killing). Neither the duplex nor the hybrid control molecules had significant effect. Thus, the results observed with the scL-delivered anti-HER-2 siRNA was not due to nonspecific cytotoxicity or to the scL vehicle. The difference in IC_{50} was even more dramatic when scL/siRNA (hybrid) was compared with scL/siRNA (modified hybrid; Fig. 2B). The modified hybrid form lowered the IC_{50} by >4-fold over hybrid. Here, also, the control molecules had no effect. Therefore, we focused our study on delivery of the modified hybrid analogue. The extent of cell killing between the complexed and free modified hybrid anti-HER-2

siRNA was assessed (Fig. 2C). The free siRNA, such as both of the unliganded liposomes as well as the scL and scL-HoKC complexed control molecules, had no effect on MDA-MB-435 cells (IC_{50} , >250 nmol/L). In contrast, both scL and scL-HoKC complexed modified hybrid siRNA resulted in significant cell death with IC_{50} values of 20 and 6 nmol/L, respectively. These results not only show that delivery via the ligand-liposome complex greatly enhances the effect of the siRNA but also indicate that inclusion of the pH-sensitive peptide increases this effect by >3-fold. To show tumor cell specificity, scL and scL-HoKC encapsulated anti-HER-2 modified hybrid siRNA and control molecules were assessed in normal human fibroblast cell line H500. There was no significant

effect of the siRNA on cell survival in these nontumor cells (IC_{50} , >250 nmol/L), confirming that the anti-HER-2 siRNA does not have an effect on normal cells (Fig. 2D).

Subsequently, we examined the ability of modified hybrid, when delivered by the nanoimmunoliposomes, to *in vitro* sensitize PANC-1 and MDA-MB-435 cancer cells to conventional chemotherapeutic agents gemcitabine and docetaxel, respectively. We also compared the effect of sCL-HoKC versus sCL as the delivery vehicle to confirm the previous results showing enhancement with HoKC. As shown in Fig. 2E with PANC-1, both nanoimmunoliposome delivery systems significantly increased the response of the cells to gemcitabine (13- to 82-fold). Similarly, an 11- to 23-fold increase in sensitization of MDA-MB-435 cells to docetaxel was observed. The IC_{50} values of docetaxel alone, sCL/HER-2 siRNA plus docetaxel, and sCL-HoKC/HER-2 siRNA plus docetaxel were 1.2, 0.11, and 0.05 nmol/L, respectively. In both cell lines, a greater level of sensitization was induced by the presence of the pH-sensitive peptide in the complex.

Due to the small size and ligand-directed tumor-targeting nature of these complexes, it is likely that the strong *in vitro* response seen is due to enhanced cellular uptake and cytoplasmic delivery. To confirm this, we examined PANC-1 cells transfected with free, sCL, and sCL-HoKC complexed 6-FAM-labeled control siRNA (0.2 nmol/L) by confocal microscopy. Both the sCL/siRNA-transfected and sCL-HoKC/siRNA-transfected cells showed intense cytoplasmically localized fluorescence signal in virtually 100% of the cells 6 h after transfection (Fig. 3A). In contrast, there was very little if any uptake of the free siRNA at this concentration and time.

We have previously shown improved tumor targeting and tumor-specific uptake of sCL-delivered 6-FAM siRNA *in vivo* over free siRNA after i.v. administration (15). Figure 3B shows a similar high level of tumor specificity and transfection in MDA-MB-435/LCC6 lung

metastases by the i.v. delivered sCL-HoKC/siRNA complex. The metastasis indicated by the arrow displays a high level of fluorescence with no significant signal in the adjacent normal lung tissue, confirming the tumor-targeting ability and the efficient delivery of the siRNA by the nanocomplex containing the pH-sensitive peptide.

Figure 3C further shows the specific delivery of intact siRNA to tumor versus normal tissues *in vivo* in mice bearing human lung (H157) and colon (H630) xenografts after i.v. administration of sCL/siRNA (modified hybrid). Both the intact sense (modified DNA/RNA; Figure 3C, a) and antisense (RNA; Figure 3C, b) strands are present in the tumors but are not detectable in the brain, liver, or lung of the mice.

The ultimate test of the therapeutic potential of this efficient systemic delivery approach for siRNA is its ability to effectively silence the target gene and modulate the downstream components of its signaling pathway after reaching the tumor, leading to tumor response. To assess the silencing of the target *HER-2* gene and affect downstream proteins by nanoimmunoliposome-delivered siRNA, mice bearing MDA-MB-435 s.c. tumors were i.v. injected with sCL-HoKC complexed hybrid and modified hybrid siRNA at 3 mg/kg. The sCL-HoKC-delivered hybrid form resulted in ~50% decrease in HER-2 expression in the tumor compared with untreated control, whereas treatment with the modified hybrid form virtually eliminated HER-2 expression (Fig. 4A). No effect was observed in the tumors from the mice that received sCL-HoKC complexed control molecules. Similar down-modulation of HER-2 levels by sCL-HoKC/siRNA (modified hybrid) was observed in PANC-1 cells (Fig. 4B).

Two of the signal transduction pathways influenced by HER-2 are the phosphatidylinositol 3-kinase/AKT and RAS/mitogen-activated protein kinase (MAPK) pathways, both of which play a crucial role

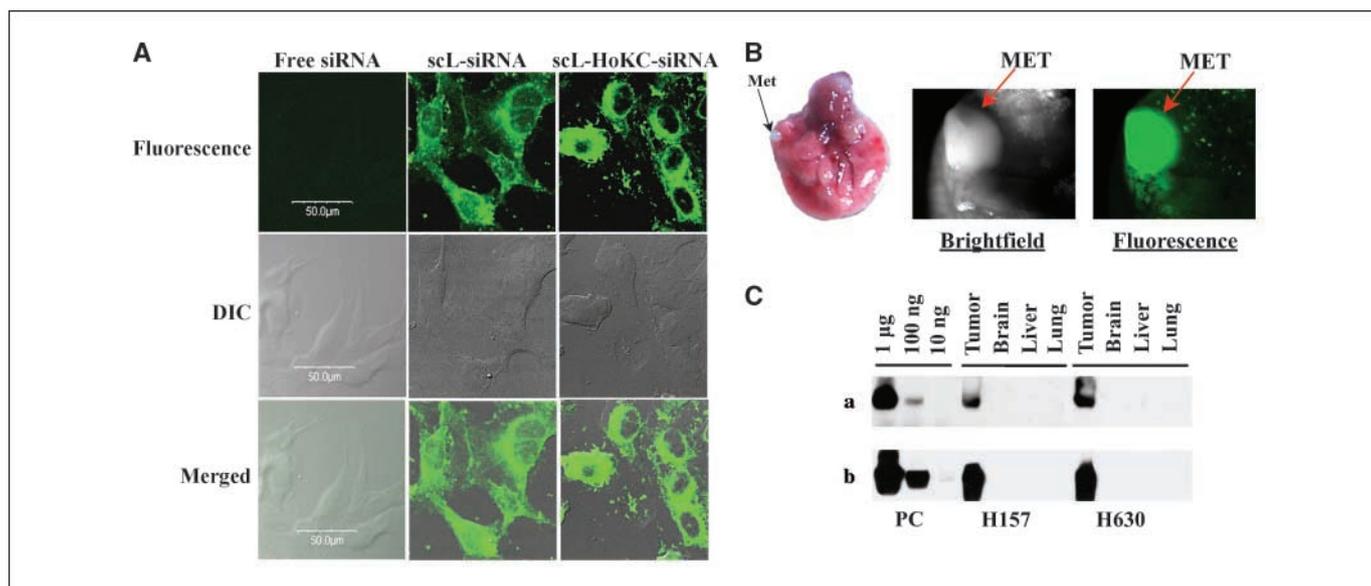


Figure 3. A, uptake of fluorescently labeled siRNA in PANC-1 cells *in vitro*. Confocal microscopy was used to compare the uptake and cellular localization of free, sCL, and sCL-HoKC complexed 6-FAM-labeled modified hybrid siRNA (0.2 μ mol/L) 6 h after transfection into PANC-1 cells. All images are at the identical magnification. DIC, differential interference contrast microscopy. B, *in vivo* tumor-specific fluorescence targeting in a metastatic mouse model. MDA 435/LCC6 lung metastases were induced in female athymic nude mice as described previously (11). Three hours after i.v. tail vein injection of sCL-HoKC carrying the modified hybrid 6-FAM siRNA, the animal was euthanized and tissues were imaged. The identical field is shown in bright-field and fluorescence views with arrow indicating metastases. C, *in vivo* delivery of siRNA to tumor versus normal tissues. Human lung (H157) and colon (H630) carcinoma xenograft tumors were induced in nude mice as described in Materials and Methods. Twenty-four hours after i.v. tail vein injection of sCL/siRNA (modified hybrid; 9 mg/kg), the tumor and normal tissues were harvested and total RNA was isolated, electrophoretically separated, transferred to nylon membrane, and probed with oligonucleotides specific for each strand of the siRNA. a, membrane hybridized with an oligonucleotide probe complementary to the sense (modified DNA/RNA) strand. b, membrane hybridized with an oligonucleotide probe complementary to the antisense (RNA) strand. PC, intact modified hybrid siRNA as a positive control loaded on the gel in the amounts indicated.

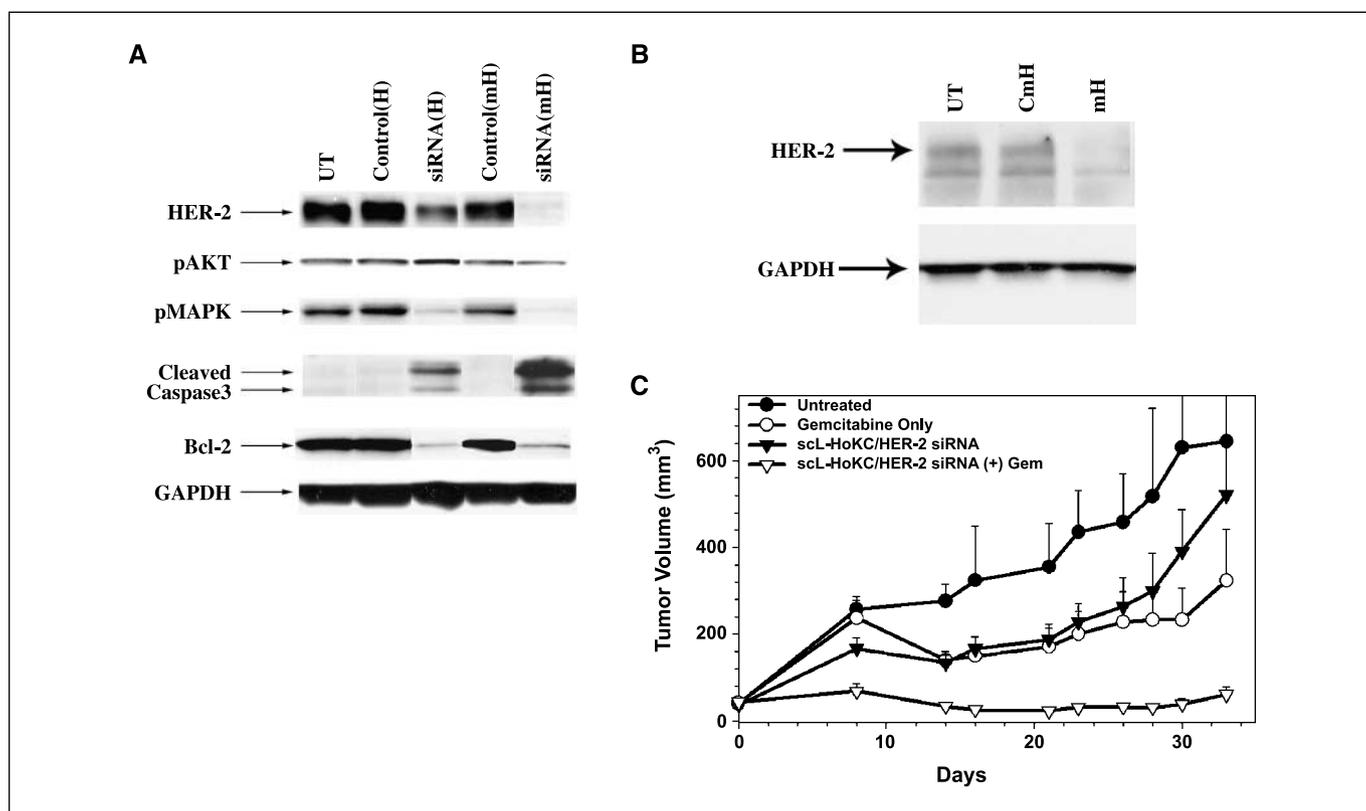


Figure 4. *In vivo* tumor response. **A**, Western blot analysis of expression of HER-2, signaling pathway components, and apoptotic indicators *in vivo* in MDA-MB-435 xenograft tumors. Down-modulation of HER-2 levels *in vivo* by scL-HoKC-delivered hybrid (H) and modified hybrid (MH) anti-HER-2 siRNA was assessed by Western blot analysis of s.c. MDA-MB-435 xenograft mouse tumors induced as described in Materials and Methods. The complexes carrying 3 mg/kg siRNA, or the respective controls, were i.v. given via the tail vein. The membrane was probed with anti-HER-2 rabbit polyclonal antibody C-18 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). In addition to HER-2, the expression levels of pAKT and pMAPK (using mouse monoclonal antibody Ser⁴⁷³ and mouse monoclonal antibody, Thr²⁰²/Tyr²⁰⁴, E10; Cell Signaling Technology, Beverly, MA) were determined. The induction of apoptosis after treatment was assessed by changes in levels of the 17-kDa band of cleaved caspase-3 (up-regulated; rabbit polyclonal antibody Asp¹⁷⁵; Cell Signaling Technology) and the antiapoptotic protein Bcl-2 (down-modulated; rabbit polyclonal antibody N-19; Santa Cruz Biotechnology). Equal tumor protein loading was determined using an antibody to the housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase* (GAPDH; rabbit polyclonal antibody; Trevigen, Inc., Gaithersburg, MD). UT, tumor from an untreated mouse. **B**, down-modulation of HER-2 levels in PANC-1 cells after transfection with scL-HoKC carrying the modified hybrid form of the siRNA or its control molecule. **C**, antitumor efficacy in a PANC-1 xenograft tumor model. S.c. tumors were induced in female athymic nude mice as described in Materials and Methods. Tumor-bearing animals were i.v. tail vein injected with scL-HoKC complexed modified hybrid anti-HER-2 siRNA alone or in combination with gemcitabine (Gem). Points, mean of 8 to 20 tumors per group; bars, SE.

in cell survival and proliferation (23–27). Thus, we assessed the effect of scL-HoKC/siRNA down-modulation of HER-2 on the phosphorylated, active forms of AKT (pAKT) and MAPK (pMAPK; Fig. 4A). Nanoimmunocomplex-mediated systemic delivery of both the hybrid and modified hybrid anti-HER-2 siRNA resulted in significant knockdown of pMAPK in the tumors, with almost total inhibition of expression by modified hybrid as expected based on the strong effect of modified hybrid on HER-2 expression. The hybrid form of the scL-HoKC-delivered siRNA had virtually no effect on pAKT levels. However, delivery of the modified hybrid form resulted in an ~20% decrease in pAKT expression. Thus, the relative efficacy of the hybrid and modified hybrid siRNAs on HER-2 expression levels is also translated to these downstream proteins, indicating that this response is mediated through the HER-2 effect. This is supported by the fact that no down-modulation of HER-2, pAKT, or pMAPK was observed in the tumors from the mice receiving the complexes carrying the control hybrid or control modified hybrid molecules. It also seems that the down-modulation of HER-2 via the siRNA may be having a greater influence on signaling through the RAS/MAPK pathway.

As both of these pathways influence the apoptotic machinery (25–27), we examined the change in expression of two key

components in apoptosis: the antiapoptotic gene *Bcl-2* and the cleaved active form of caspase-3. Although both the scL-HoKC-delivered hybrid and modified hybrid siRNAs resulted in induction of cleaved caspase-3 (as indicated by the 17-kDa band), consistent with our other data, the modified hybrid effect was approximately 3- to 4-fold greater. The down-modulation of *Bcl-2* was similar with both forms of the siRNA. This suggests that other genes in addition to *Bcl-2* may also be involved here in the induction of apoptosis. Thus, as hypothesized, systemic delivery of modified hybrid siRNA by the nanoimmunoliposome complex can efficiently silence the *HER-2* gene and affect components in multiple signal transduction pathways leading to apoptosis in the tumor.

Tumor response was assessed using the PANC-1 s.c. xenograft mouse model. Tumor-bearing mice were i.v. injected with scL-HoKC/antiHER-2 siRNA (modified hybrid) either alone or in combination with gemcitabine, a currently used chemotherapeutic agent for pancreatic cancer. Mice receiving gemcitabine only served as controls. The initial dose of siRNA was 3 mg/kg. To ascertain how low a dose could be used to inhibit tumor growth, on day 15, the dose was decreased to 2 and to 1.5 mg/kg on day 21. Although there was some effect of single-agent treatment, the

combination of scL-HoKC/siRNA (modified hybrid) and drug resulted in statistically significant differences between this combination group and the untreated group ($P \leq 0.0033$), the gemcitabine only group ($P \leq 0.01$), and the scL-HoKC/siRNA only group ($P \leq 0.002$; Fig. 4C). These findings show that, even at low doses, the efficient and targeted delivery of siRNA to the tumors via the nanoimmunoliposome results in an increased response to conventional chemotherapy with dramatic tumor growth inhibition. Moreover, during this period of tumor growth inhibition, the animals that received the combination treatment remained healthy and continued to gain weight (data not shown). Thus, this treatment regimen may not result in systemic toxicity. Thus, use of this unique, nanosized, tumor-targeting systemic delivery system may be one means to overcome the current limitations to use of siRNA in the clinic.

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