Systemic Administration and Targeted Radiosensitization via Chemically Synthetic Aptamer–siRNA Chimeras in Human Tumor Xenografts

Xiaohua Ni, Yonggang Zhang, Kenji Zennami, Mark Castanares, Amarnath Mukherjee, Raju R. Raval, Haoming Zhou, Theodore L. DeWeese, and Shawn E. Lupold

Abstract

Radiation therapy is a highly effective tool for treating all stages of prostate cancer, from curative approaches in localized disease to palliative care and enhanced survival for patients with distant bone metastases. The therapeutic index of these approaches may be enhanced with targeted radiation-sensitizing agents. Aptamers are promising nucleic acid delivery agents for short interfering RNAs (siRNA) and short hairpin RNAs (shRNA). We have previously developed a radiation-sensitizing RNA aptamer–shRNA chimera that selectively delivers DNA-PK knockdown shRNAs to prostate-specific membrane antigen (PSMA) positive cells in the absence of transfection reagents. Although these chimeras are effective, their synthesis requires in vitro transcription and their evaluation was limited to intratumoral administration. Here, we have developed a second-generation aptamer–siRNA chimera that can be assembled through the annealing of three separate chemically synthesized components. The resulting chimera knocked down DNA-PK in PSMA-positive prostate cancer cells, without the need of additional transfection reagents, and enhanced the efficacy of radiation-mediated cell death. Following intravenous injection, the chimera effectively knocked down DNA-PK in established subcutaneous PSMA-positive tumors. Systemic treatment with these radiation-sensitizing agents selectively enhanced the potency of external beam radiation therapy for established PSMA-positive tumors. Mol Cancer Ther; 14(12); 1–8. ©2015 AACR.

Introduction

Ionizing radiation (IR) is a widely utilized tool in the management of prostate cancer (1, 2). External beam radiation therapy (EBRT) is applied to treat low-risk primary prostate cancer as well as intermediate-to-high-risk primary prostate cancer, in combination with androgen deprivation therapy or as an adjuvant after surgery. EBRT or systemic radionuclides, such as strontium-89 or samarium-153, can further be utilized for the management of pain associated with prostate cancer bone metastases. More recently, the bone seeking alpha-emitting particle, radium 223 dichloride, was shown to enhance the overall survival of men with castration-resistant metastatic prostate cancer to bone (3). Although IR therapy is commonly utilized and effective, the long-term benefits can be limited for some patients. For example, the estimated 10-year disease-free survival rate for locally advanced prostate cancer following IR therapy is less than 50% (4, 5). The survival benefits of radium are also limited, providing only 3 months of additional survival in men who previously received standard chemotherapy (3). Thus, there are several opportunities to improve IR therapies.

Improvements in IR therapy can be achieved through new mechanisms in delivery or dosing, or through IR-sensitizing strategies. Radiation sensitization is an attractive approach because it has the potential to either improve the potency of existing therapies, or to reduce the amount of IR required. Importantly, to improve the therapeutic index tumor-targeted or tumor-selective IR sensitization is required, to spare healthy tissues from similar IR sensitization (6).

Radiation therapy primarily causes DNA double-strand breaks (DSB), which are considered to be the most lethal lesion (7–9). A critical pathway of DSB repair is nonhomologous end joining, which involves signaling through the DNA protein kinase (DNA-PK) complex (10). The catalytic subunit of DNA-PK was recently identified as one of the most potent targets for prostate cancer IR sensitization in a high throughput siRNA library screen (11). In light of this, we developed a DNA-PK-targeted radiation-sensitizing agent. Prostate-selective targeting was achieved through a previously developed PSMA-targeting RNA aptamer, A10-3 (12). A short hairpin RNA (shRNA), targeting DNA-PK, was then affixed to the end of this aptamer, generating an internalizing...
aptamer–shRNA chimera (11, 13). When these chimera were intratumorally administered into established PSMA-positive human xenograft tumors, the therapeutic effect of IR was significantly enhanced (11). Although these results are promising, the clinical translation of this strategy may be challenging.

The chemical synthesis of long, 2'-modified RNA aptamers can be costly and are generally limited to a maximum product length of 50–60 nucleotides (14). Consequently, aptamer-targeted therapeutics above this size have been generated by in vitro transcription (11,13). Recently, a truncated form of the A10-3 aptamer was identified and shown to be sufficiently small for chemical synthesis and targeted siRNA knockdown of Polo-like kinase 1 (15). Intrapерitoneal injection of these aptamer–siRNA chimera caused a pronounced regression of established PSMA-expressing tumors, revealing the potential for systemically administered PSMA aptamer–targeted siRNAs. In light of these results, and other successful systemic applications of aptamer–siRNAs (16,17), we sought to develop a chemically synthesized aptamer–siRNA chimera for systemic prostate cancer radiation sensitization. Here we describe the development and characterization of this chimera in a human prostate cancer xenograft model of EBRT.

Materials and Methods

Materials

Aptamers were purchased from IDT Technologies and RNA oligonucleotides from Sigma Aldrich. Anti-DNA-PK (AB2) and antihuman ACTB (AC-15) were purchased from Millipore and Sigma-Aldrich. Power vision poly-horseradish peroxidase (HRP) antimouse immunoglobulin G (IgG) was purchased from Leica Biosystems.

Cell culture

LNCaP and PC-3 cells were originally obtained from ATCC in 2006. Parental cells were authenticated and verified Mycoplasma free by DDC medical in November of 2014. LNCaP-MLuc cell derivation is previously described (18). All cells are verified mycoplasma free semiannually by MycoAlert Mycoplasma Detection Kit, last performed September of 2014. Cells were grown in RPMI 1640 supplemented with 10% FBS and maintained at 37°C and 5% CO2.

Aptamer–siRNA chimeras

Aptamers A10-3 (PSMA-targeting) or Neg (control) were chemically synthesized with 2'-Fluoro-pyrimidine, 5'-amine, and deoxy-T 3′-3′ cap modifications (12). DNAPK antisense (DNA-PK targeting) and Con antisense (control) siRNA were synthesized as nonmodified RNA. Bridge oligo DNAPK (sense)-link A10-3, Con siRNA (sense)-link A10-3, DNAPK (sense)-link Neg, and Con siRNA (sense)-link Neg were synthesized as nonmodified RNA for annealing to A10-3 and Neg aptamers, respectively. Aptamer–siRNA chimera were annealed with equimolar concentrations by brief denaturation at 95°C followed by gentle cooling to room temperature. Sequences are provided in Supplementary Table S1.

Dicer assay

One microgram of annealed chimera was incubated with recombinant Dicer following the manufacturer's recommendations (Recombinant Human Turbo Dicer Kit; ref. 11). Products were resolved by ethidium bromide stained agarose gel electrophoresis.

In vitro gene silencing and radiosensitization

A total of 2 × 105 cells (LNCaP, LNCaP-MLuc) were Hiperfect (Qiagen) transfected with 100 nmol/L siRNA in 6-well plates or treated with 400 nmol/L of aptamer–shRNA. After 48 hours, cells were either collected for RNA or protein extraction or seeded in 96-well plates (500–1,000 cells/well). For cell viability studies, 96-well plates were irradiated 24 hours later with 0 or 6 Gy using a GammaCell 40 (Nordion) 137Cs radiators (0.6 Gy/min). Viability was assessed after 7 days by MLuc assay (18) or Cell Titer-Blue assay (Promega) per manufacturer’s instructions. Gene silencing was assessed by quantitative RT-PCR as previously described (11). Briefly, 1 μg RNA was reverse-transcribed using Quantitect Reverse Transcription Kit (Qiagen) and SYBR Green (Bio-Rad) with a Bio-Rad iCycler. Standard curves were generated by serial dilution of each sample and the relative amount of target gene mRNA was normalized to GAPDH mRNA. Details of primers are provided in Supplementary Table S2.

Immunoblotting

Cells were lysed by Nupage LDS Sample Buffer (Life Technologies), heated at 70°C for 10 minutes and separated by 4–15% Criterion Tris-HCl Gel and transferred to Immunoblot PVDF (Bio-Rad). Membranes were probed with primary and secondary antibodies at optimized concentrations and protein visualized by ChemiDoc SRS+ and Image Lab software (Bio-Rad).

A10-3 siRNA stability assay

A10-3-DNAPK chimera (1 μmol/L final concentration) was incubated in 200 μL RPMI 1640 medium containing 50% or 5% mouse serum (Jackson ImmunoResearch Laboratories) or human serum (Sigma-Aldrich) at 37°C. Between 2 and 72 hours, 20 μL aliquots of the reaction were withdrawn and then stored at −80°C until all incubations were completed. Each mixture was then analyzed by nondenaturing polyacrylamide gel electrophoresis with ethidium bromide staining.

Animal model studies

Studies were performed according to the protocols approved by the Animal Care and Use Committee at Johns Hopkins University. Eight-week-old athymic nude mice (nu/nu; Harlan Laboratories) were obtained and housed at the Animal Center Isolation Facility at Johns Hopkins University. Mice were inoculated with 5 × 106 (50% Matrigel) cells subcutaneously, and tumors grown to at least 0.8 cm in diameter. At least four animals were included in each study group. Animals were injected via tail vein with 1 nmol aptamer–chimeras on days −3 and −2. On day 0, the tumor was harvested and partitioned for RNA extraction or formalin fixation. Chimera treatment doses were based on preliminary studies (200 pmol−1 nmole) to identify functional doses and existing literature (15). For radiosensitization, animals with established tumors were randomized and intravenously treated with aptamer–siRNA chimeras as described above on days −3 and −2. On day 0, animals were mock irradiated or treated 6 Gy local IR (5.8 Gy/min) to the tumor-bearing leg from a J.L. Shepherd Mark 137Cs irradiator, with the remainder of the body shielded. Tumors were measured every 2 days to calculate tumor volume: (w × l × h) × 0.52. Tumor response was determined as reaching four times its volume at the start of treatment.
Immunohistochemistry

Slides were deparaffinized, rehydrated, and stained for DNA-PK as previously described (11). Stains were developed with diaminobenzidine (DAB Kit, Vector Laboratories) and counterstained with Haemotoxylin Mayers. Images were captured by Nikon 50i microscopy with Nikon NIS-Elements software and charge-coupled device digital camera. For quantification of DNA-PK, whole DAB staining slides were scanned via ScanScope CS system at the Tissue Micro Array Core of Johns Hopkins University School of Medicine, and total DNA-PK expression per cell nucleus was measured from multiple randomized areas of each tissue specimen using Framework for Image Dataset Analysis (FrIDA) software as previously described (19).

5' RACE assay

5' RACE was performed as previously described (11). Briefly, miRNA (5 μg) from treated cells or tumor was ligated to GeneRacer adaptor (Invitrogen). Ligated RNA was reverse transcribed with GSP[DNAPK] reverse-1 and PCR amplified with GR 5' primer and GSP[DNAPK] reverse-2 (Supplementary Table S2). Products were resolved by ethidium bromide stained polyacrylamide gels.

Statistical analysis

Tumor size was evaluated by two-way ANOVA. P value of 0.05 or less was considered significant. Extensions to tumor quadrupling: events (tumor volume <4-fold from injection) were plotted by Kaplan–Meier curve and analyzed by log-rank (Mantel–Cox) test. Paired samples were evaluated by Student t-test.

Results

Chemically synthesized aptamer–siRNA chimera

The A10-3-DNAPK-siRNA chimera was generated through a three-part chemical synthesis and annealing process (Fig. 1A). Previous truncations of the A10-3 aptamer had identified the 5’-terminal 39 nucleotides (nt) as the minimally functional region (15). We therefore hypothesized that a portion of the 3’-terminus of A10-3 was unnecessary and could be used as a “bridge” to anneal radiation-sensitizing siRNAs. A similar approach has been successful with other aptamer–siRNA chimera (15, 20). A complementary “bridge” RNA oligonucleotide was designed by fusing the sense strand of the DNA-PK-targeting siRNA to a 13 nt extension with complementarity to the 3’-terminus of the aptamer (Fig. 1A, S-siRNA). A dinucleotide uridine spacer was placed between the bridge and sense siRNA portions to mimic a 3’-overhang. A complementary antisense DNA-PK siRNA was then applied and also designed to contain a 3’-overhang (Fig. 1A, AS-siRNA). Control chimera containing non-targeting aptamers (Neg-DNAPK) or nonspecific siRNA (A10-3-Con) were also generated using the same “bridge” strategy as above (Supplementary Table S1). The efficiency of generating the annealed aptamer–siRNA chimera is high, with over 90% efficiency (Fig. 1B). The chimera are functional substrates of Dicer, resulting in the desired siRNA product (Fig. 1C).

Aptamer–siRNAs knockdown DNA-PK and sensitize human prostate cancer cells to IR

Chemically synthesized and annealed aptamer–siRNA chimera were evaluated for the ability to knockdown DNA-PK and to sensitize PSMA expressing cells to IR. A previously developed
PSMA-positive reporter cell line, LNCaP-MLuc, was applied for these studies (18). Cells were treated with 400 nmol/L of A10-3-DNAPK or a negative control chimera with nonspecific siRNA (A10-3-Con), in the absence of transfection reagents, or transfected with 100 nmol/L siDNA PK. Relative DNA-PK expression levels were quantified by real-time RT-PCR. Within 48 hours of treatment, A10-3-DNAPK treated cells had ~40% less DNA-PK mRNA levels when compared with cells treated with A10-3-Con (Fig. 2A). siRNA transfection was utilized as a positive control for these studies (Fig. 2A, siDNAPK). Knockdown of DNA-PK protein was verified by immunoblotting (Fig. 2B). 5′-Rapid amplification of cDNA ends (5′-RACE) was then applied to verify that DNA-PK knockdown was mediated by RNAi-induced mRNA cleavage, as detected by the predicted cleavage product (Supplementary Fig. S1).

LNCaP-MLuc cells express a secreted Metridia Luciferase (MLuc), which is driven by a β-actin promoter and enhancer to provide a signal that is representative of viable cell numbers (18). These reporter cells were treated with 400 nmol/L of aptamer–siRNA chimera in the absence of transfection reagents, or transfected with the positive control DNA-PK siRNA. Two days after treatment, cells were irradiated with 2–6 Gy of IR. Cell viability and proliferation was then measured by secreted MLuc activity (Fig. 2C) and verified by the commercial Cell Titer-Blue assay (Fig. 2D). Both assays detected significantly enhanced IR-mediated cytotoxicity in A10-3-DNAPK treated and siDNA PK transfected cells, when compared with A10-3-Con control-treated cells. Thus, in an in vitro cell model, the chemically synthesized aptamer–siRNA chimeras were confirmed to function as radiation sensitizers.

Systemic knockdown of DNA-PK in PSMA-positive tumors

Sufficient nuclease stability is required for the systemic application of aptamer–siRNA chimera. Prior to in vivo application, the stability of A10-3-DNAPK chimera were therefore analyzed in media supplemented with mouse or human serum for several days. Results indicate that over half of the chimera remain stable for up to 12 hours at 37°C (Supplementary Fig. S2). Therefore, sufficient chimera should be available for tumor delivery.

The chemically synthesized and annealed chimera were then evaluated as systemic siRNA delivery agents in PSMA-positive and PSMA-negative mouse tumor xenograft models. On two consecutive days, 1 nmol of each chimera was administered intravenously into athymic nude mice hosting established subcutaneous PSMA-positive LNCaP or PSMA-negative PC-3 tumors. Tumors were then harvested for analysis 2 days postinjection. PSMA-positive LNCaP tumors from mice treated with A10-3-DNAPK had significantly reduced DNA-PK mRNA and protein levels, as quantified by qRT-PCR (Fig. 3A) and immunohistochemistry (Fig. 3B), when compared with A10-3-Con treated animals. Targeted DNA-PK mRNA and protein knockdown was further
confirmed in LNCaP-MLuc xenograft tumor models (Supplementary Fig. S3). Conversely, A10-3-DNAPK treatment did not reduce DNA-PK mRNA or protein levels in PSMA-negative PC-3 tumors (Fig. 3C and D). These results support PSMA-selective targeting by the A10-3-DNA-PK chimera.

**Systemic radiosensitization of PSMA-positive tumors**

Subcutaneous human PSMA-positive LNCaP or PSMA-negative PC-3 prostate tumor xenografts were again established in nude mice. Mice were then treated with 1 nmol of A10-3-DNAPK, A10-3-Con, NEG-DNAPK (a control chimera containing a non-targeted aptamer with DNA-PK targeting siRNA), or PBS by tail vein injection on 2 consecutive days. Two days after the last injection, half of each treatment group received direct tumor irradiation with a single nonablative dose of 6 Gy. Tumor growth was then measured for several weeks after treatment. The therapeutic effect of IR was significantly greater in A10-3-DNAPK treated PSMA-positive LNCaP tumors when compared with irradiated animals treated with control chimera (Fig. 4A). However, A10-3-DNAPK treatment did not enhance IR therapy in PSMA-negative PC-3 tumors (Fig. 4B). The tumor volumes of nonirradiated cohorts were similar, indicating that A10-3-DNAPK treatment alone has no significant therapeutic effect in the absence of irradiation for either LNCaP or PC-3 tumors. The combination of A10-3-DNAPK
and IR significantly extended the time for PSMA-positive tumor volume quadrupling by approximately 6 weeks, when compared with 3.5 weeks for animals with irradiated tumors that were treated with A10-3-Con control or Neg-DNAPK chimeras (Fig. 4C). This effect was not observed in PSMA-negative PC-3 tumors, indicating PSMA selective radiation sensitization (Fig. 4D).

**Discussion**

Efficient delivery and cellular internalization has been a challenge for RNA interference (RNAi) therapeutics. Over the last decade, aptamers have evolved as promising RNAi delivery vehicles, capable of binding a variety of specific cell-surface ligands and shuttling associated RNAi agents into cells (21, 22). The use of modified nucleic acids has provided sufficient stability for RNA aptamer–siRNA chimera to perform well in multiple complex and challenging in vivo disease models. PSMA-targeting RNA aptamers have successfully delivered polo-like kinase 1 targeting siRNAs to established prostate tumors after intraperitoneal injection, resulting in marked tumor regression (15). In a different systemic delivery model, aptamers targeting the HIV-1 envelope protein gp120 delivered tat/rev-targeted siRNAs by intravenous injection, causing significant decreases in viral loads and resulting in the recovery of CD4+ T cells (17). In a model of HIV transmission, the intravaginal application of gel-formulated CD4-targeting aptamers delivered antiviral siRNAs and blocked the sexual transmission of virus (23). Here we have demonstrated in another challenging in vivo model that RNA aptamers are capable of targeting established PSMA-positive tumors, following intravenous injection, to cause sufficient siRNA-mediated DNA-PK knockdown and tumor radiation sensitization.

There are several strategies for generating aptamer–siRNA chimeras including the continuous in vitro transcription of aptamer–shRNAs, the solid-phase synthesis of aptamers and siRNAs followed by complementary annealing, or a combination thereof. The goal of this project was to develop radiation-sensitizing aptamer–siRNA chimera using fully synthesized and annealed components (Fig. 1). This has previously been accomplished through a similar annealing strategy, where the truncated PSMA aptamer A10-3.2 was extended to include the guide or passenger strand of the siRNA, and the complementary siRNA strand was then separately annealed (15). Similarly, the addition of complementary sticky bridge sequences to aptamers and siRNAs has resulted in successful assembly and fully functional chimera (20). Here we utilized a region of the 3'-terminus of the A10-3 aptamer as a bridging region for chimera assembly.

**Figure 4.**

Systemic and targeted radiosensitization in tumor models. Mice bearing established subcutaneous tumors were intravenously injected with aptamer–siRNA chimeras (1 nmol/injection) or PBS on days –3 and –2. On day 0, tumors were either untreated or irradiated (0 or 6 Gy IR). A, systemic radiosensitization of established PSMA-positive LNCaP tumors. Relative tumor volume over time (Vt/Vo). Radiation similarly affected growth in all treatment groups except irradiated A10-3-DNAPK. **, P < 0.05; ***, P < 0.001, A10-3-DNAPK + 6 Gy vs. A10-3-Con + 6 Gy and Neg-DNAPK + 6 Gy; 2-way ANOVA. Mean ± SEM. B, systemic radiosensitization of established PSMA-negative PC3 tumors. Relative tumor volume over time (Vt/Vo) of established PSMA-negative PC-3 tumors. Mean ± SEM. C, extension of tumor quadrupling for PSMA-positive LNCaP tumors. Events (animals whose tumor volume was not yet 4-fold the size at the time of injection) were plotted by Kaplan–Meier curve. **, P < 0.001, A10-3-DNAPK + 6 Gy vs. A10-3-Con + 6 Gy and A10-3-DNAPK + 6 Gy vs. Neg-DNAPK + 6 Gy; log-rank (Mantel-Cox) test. D, extension of tumor quadrupling for PSMA-negative PC3 tumors. Events (animals whose tumor volume was not yet 4-fold the size at the time of injection) were plotted by Kaplan–Meier curve.
sequences were incorporated into the sense siRNA to bridge and join the aptamer, sense and antisense siRNA (Fig. 1). In addition, we intentionally engineered dinucleotide overhangs onto the 3'-ends of both sense and antisense siRNAs as previous studies have reported enhanced activity in chimera containing dinucleotide overhangs (15). Notably, we utilized nonmodified RNA for the sense and antisense components. Nonetheless, the annealed chimera demonstrated significant stability in human and mouse serum supplemented media (Supplementary Fig. S2), suggesting the aptamer may provide some protection to the conjugated siRNA duplex.

In summary, these data support that the pretreatment of animals bearing PSMA-positive tumors with chemically synthesized and systemically administered aptamer–siRNA chimeras (2 days prior to IR therapy) can significantly enhance tumor IR responses. The experimental design intentionally applied a single nonablative IR treatment, so that additional therapeutic efficacy from IR-sensitization could be observed. Nonetheless, we have observed one animal, treated with A10-3-DNAPK and 6 Gy IR, to have complete tumor ablation for more than 3 months after the termination of therapy. We anticipate that higher or longer IR doses would have resulted in more long-term tumor responses. Fractionated IR doses may also benefit if longer term gene knockdown is achieved, such as those reported in aptamer–siRNA chimera protective models of HIV transduction (23).

RNA aptamers are a unique class of targeting agents that have potential for clinical translation. There is currently one FDA-approved RNA aptamer therapeutic that targets VEGF for the treatment of macular degeneration. In addition, there are several aptamer-mediated therapeutic strategies in development for the treatment of cancer, and other diseases, where aptamers function as selective enzyme inhibitors or delivery vehicles for nanoparticles, drugs, and RNA interference agents (24). Here we sought to develop a first-in-class chemically synthesized IR-sensitizing aptamer–siRNA chimera. This design was pursued to overcome potential hurdles in GMP synthesis associated with plasmid purification, large-scale in vitro transcription and full-length product purification of longer aptamer–shRNA chimera (11). We felt that chemical synthesis may be more amendable to larger scale production in a GMP environment. If these chimeras can be safely administered in the clinic, they may enhance a variety of IR therapies for prostate cancer. For example, the chimera could be systemically administered prior to EBRT or brachytherapy for locally advanced prostate cancer. Similarly, pretreatment with the chimera may enhance the palliative or cytotoxic potential of systemic radionuclides in the treatment of bone metastatic prostate cancer. However, such studies would need to be mindful of PSMA expression in non-target tissues, such as in the kidney, to avoid potential IR sensitization of healthy tissues. Although these tissues would theoretically receive lower doses due to reduced PSMA expression, when compared with tumors, and due to the targeted nature of external beam radiation therapy and systemically targeted radiotherapeutics, such as the bone seeking radium 223 dichloride. In addition to targeting specificity, it is notable that larger double-stranded RNAs may nonspecifically induce inflammatory responses through Toll-like receptors. Previous studies of aptamer–siRNA chimera in human cells and in mouse models have not yet observed any significant inflammatory responses to date (11, 13, 15, 17, 20, 25, 26). Further, interferons are well-known enhancers of radiation therapy (27). However, there were no observed increases in the potency of radiation our studies of PSMA-negative A10-3-DNAPK treated tumors or in any cells or tumors treated with control aptamer–siRNA chimeras. Nonetheless, these preclinical results do not preclude the possibility of aptamer–siRNA chimera-mediated inflammatory reactions in humans. Therefore, additional studies will be needed to verify the safety and efficacy of these of these and other IR-sensitizing agents in phase I trials with safety-focused endpoints.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: X. Ni, K. Zennami, T.L. DeWeese, S.E. Lupold

Development of methodology: X. Ni, Y. Zhang, K. Zennami, T.L. DeWeese, S.E. Lupold

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Ni, Y. Zhang, K. Zennami, M. Castanares, A. Mukherjee, R.R. Raval, H. Zhou, T.L. DeWeese, S.E. Lupold

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Ni, K. Zennami, R.R. Raval, T.L. DeWeese, S.E. Lupold

Writing, review, and/or revision of the manuscript: X. Ni, K. Zennami, M. Castanares, A. Mukherjee, R.R. Raval, T.L. DeWeese, S.E. Lupold

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Ni, K. Zennami

Study supervision: X. Ni, K. Zennami, T.L. DeWeese, S.E. Lupold

Acknowledgments

The authors thank Alan Meeker and the IHC Core for assistance with analysis.

Grant Support

NIH grant 5P50CA058236-15 (to T.L. DeWeese and S.E. Lupold), the David H. Koch Charitable Foundation (to S.E. Lupold), the National Natural Science Foundation of China 81372768 (to X. Ni), and Shanghai Pujiang Program 13pj1407100 (to X. Ni).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 13, 2015; revised August 28, 2015; accepted September 20, 2015; published OnlineFirst October 5, 2015.

References


Molecular Cancer Therapeutics

Systemic Administration and Targeted Radiosensitization via Chemically Synthetic Aptamer–siRNA Chimeras in Human Tumor Xenografts

Xiaohua Ni, Yonggang Zhang, Kenji Zennami, et al.

Mol Cancer Ther Published OnlineFirst October 5, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-15-0291-T

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2015/10/02/1535-7163.MCT-15-0291-T.DC1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.