Noninvasive Detection of Lentiviral-Mediated Choline Kinase Targeting in a Human Breast Cancer Xenograft

Balaji Krishnamachary, Kristine Glunde, Flonne Wildes, Noriko Mori, Tomoyo Takagi, Venu Raman, and Zaver M. Bhujwalla

Introduction

Increased phosphocholine (PC) is one of the hallmarks of cancer, and several studies have established a strong correlation between increased PC and malignant progression (1, 2). One of the major causes of high PC in tumors is the increase in expression and activity of choline kinase (chk), the rate-limiting enzyme that phosphorylates and converts choline to PC (1–3). chk has been previously targeted with novel pharmacologic inhibitors (4–6) and chemical inhibitors are the long-term effect and specificity, and the ability to efficiently regulate various isoforms of target genes (8). Some of the problems that currently face conventional gene therapy are the lack of an efficient and reliable delivery system, stability of vectors (both viral and nonviral), the associated immunogenic response, problems associated with integration, and the inability to noninvasively detect the outcome of integration (9). Of the delivery mechanisms, viral vectors, and particularly lentivirus vectors, have potential for use in gene therapy (10).

Here, we have used a lentivirus vector to deliver chk-shRNA in established tumors and showed, for the first time, the therapeutic potential of chk down-regulation in vivo in a human breast cancer xenograft model. We cloned the shRNA against chk into a lentivirus vector with enhanced green fluorescent protein (EGFP) as a reporter gene and transduced MDA-MB-231 breast cancer cells in culture to test the efficacy of the knockdown. We subsequently transduced the virus in vivo in an established breast cancer xenograft model derived from MDA-MB-231 breast cancer cells in culture to test the efficacy of the knockdown. We subsequently transduced the virus in vivo in an established breast cancer xenograft model derived from MDA-MB-231 breast cancer cells by systemically injecting concentrated virus through the tail vein. Transduction of chk-shRNA in vivo reduced tumor growth due to decreased proliferation, as detected by Ki-67 staining (11). The decrease in chk mRNA and protein levels and the down-regulation of chk in tumors were detected noninvasively by a reduction of PC and phosphocholine (PC) and total choline (tCho) levels (7). Of the delivery mechanisms, viral vectors, and particularly lentivirus vectors, have potential for use in gene therapy (10).

Materials and Methods

Cells and cell culture conditions. The MDA-MB-231 breast cancer and the human embryonic kidney 293T cell lines were purchased from the American Type Culture Collection. Cells were cultured in RPMI 1640 and DMEM (Mediatech), respectively, and supplemented with 10% fetal bovine serum (FBS; Sigma), 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen Corp.). Cells were maintained in a humidified atmosphere of 5% CO2 in air at 37°C.

Cloning, virus preparation, and titration. shRNA against chk in a PCR3.1 basic vector (7) was digested and cloned between XbaI and PstI (New England Biolabs) into a human U6 promoter-driven pBRL vector (Irvin S.Y. Chen, University of California at Los Angeles, Los Angeles, CA) containing a reporter gene driven by a phosphoglycerate kinase promoter (Fig. L4). A vector with shRNA against luciferase (luc-shRNA) was used as control (12). Infectious viral supernatants (DMEM with 1% FBS) were derived by transient cotransfection of 293T (6 x 106 in 100 mm3 Petri dishes) with the U6 promoter-driven luciferase reporter vector (pGL3) and pMD18-T (Takara Shuzo Co., Ltd.) as the helper plasmid. Two days later, the supernatant was frozen and thawed three times to release the virus. The virus was quantified in 96-well plates by transducing cells with increasing concentrations of virus. The titration was performed in triplicate.

Requests for reprints: Zaver M. Bhujwalla, Department of Radiology, The Johns Hopkins University School of Medicine, Room 208C, Traylor Building, 720 Rutland Avenue, Baltimore, MD 21205. Phone: 410-955-9698; Fax: 410-614-1948; E-mail: zaver@mri.jhu.edu.

©2009 American Association for Cancer Research. doi:10.1158/0008-5472.CAN-08-4120
plates) cells using Lipofectamine 2000. A total of 19.5 μg of plasmid in the proportion of 12 μg of lentiviral vector carrying shRNA, 6 μg of packaging plasmid pCMVΔR8.2 DVPR (VPR deleted; ref. 13), and 1.5 μg of pCMV-VSVG were used, and viral supernatant was collected at 48, 72, and 96 h after transfection. Pooled supernatants were concentrated using an Amicon Ultra-15 100K cutoff filter device (Millipore). The viral titer of the transfection. Pooled supernatants were concentrated using an Amicon Ultra-15 100K cutoff filter device (Millipore). The viral titer of the supernatant was determined by performing a p24 ELISA kit (Cell Biolabs, Inc.) to detect the HIV-p24 core protein of the vector.

Establishing breast cancer xenografts. Approximately 2 × 10^6 MDA-MB-231 cells in 50 μL HBSS (Mediatech) were inoculated in the upper-right thoracic mammary fat pad of age-matched female severe combined immunodeficient mice. All surgical procedures and animal handling, including viral vector delivery, were performed in accordance with protocols approved by the Johns Hopkins University Institutional Animal Care and Use Committee, and conformed to the Guide for the Care and Use of Laboratory Animals published by the NIH. For histochemical analysis, frozen tissues were embedded in OCT for cryosectioning, and fresh tissues were fixed in 10% formaldehyde.

Noninvasive in vivo 31P MRS studies. In vivo single-voxel 31P MRS was performed on a 4.7-T Bruker Biospec spectrometer to dynamically monitor tumoral PME and PC levels through the time course of chk-targeted lentiviral gene therapy. Mice were anesthetized with an i.p. injection of ketamine (25 mg/kg; Phoenix Scientific, Inc.) and acepromazine (2.5 mg/kg; Aveco, Phoenix Scientific) diluted in saline. Body temperature was maintained during the experiment by using a blanket circulating with warm water as previously described (14). Single-voxel 31P MR spectra were acquired with a homebuilt surface coil using a single-pulse sequence and the following parameters: pulse width of 45 degrees, sweep width of 5,000 Hz, data size of 2,048 points, repetition time of 1 s, and 1,024 scans. Spectra were processed and analyzed with an in-house IDL program (Dr. D.C. Shungu, Hatch MR Research Center, Columbia University, New York, NY) using Gaussian and exponential multiplication (exponential line broadening = 0.15 Hz, Gaussian broadening = 0.03 Hz) to achieve the spectral resolution necessary to analyze the PC signal, or exponential multiplication (exponential line broadening = 20 Hz) for analysis of PME, which is relevant for spectra obtained at the lower field strengths used clinically, and a combination of linear and nonlinear least-square fitting in the time domain as previously described (4). The α-nucleoside triphosphate (NTP) signal was set to −10 ppm, and the signals were normalized and scaled to the β-NTP signal at −18.6 ppm, which remained constant during the course of the experiment.

RNA isolation, cDNA synthesis, and quantitative reverse transcription-PCR. Total RNA was isolated from freeze-clamped tumor tissue and frozen MDA-MB-231 breast cancer cells were transduced with lentivirus using QIAsherdder and RNeasy Mini kits (Qiagen). Finely powdered tumor tissues were further homogenized in RLT buffer (Qiagen Mini kit) before passing through the shredder. cDNA was prepared using the iScript cDNA synthesis kit (Bio-Rad). cDNA samples were diluted 1:10 and real-time PCR was performed using iQ SYBR Green Supermix and gene-specific primers in the iCycler real-time PCR detection system (Bio-Rad). All primers were designed using Beacon Designer software 5.1 (Premier Biosoft International). The expression of target RNA relative to the housekeeping gene hypoxanthine phosphoribosyltransferase 1 (HPRT1) was calculated based on
the threshold cycle \(C_i\) as \(R = 2^{-\Delta \Delta C_i}\), where \(\Delta C_i = C_i - C_{i \text{Hprt}} - \Delta C_i = \Delta C - \Delta C_{\text{chk-shRNA transduced}} - \Delta C_{\text{luc-shRNA}}\).

**Protein isolation and Western blots.** Total protein from tumor tissue or transduced cells was extracted using 1X cracking buffer (100 mmol/L Tris (pH 6.7), 2% glycerol) containing a protease inhibitor (Sigma) at 1:200 dilution, resolved on 10% SDS-PAGE, and incubated using a custom-made polyclonal antibody against chk at 1:100 dilution in 5% nonfat dry milk overnight at 4°C. A mouse monoclonal antibody against β-actin (Sigma) at 1:100,000 was used as a control. Suitable horseradish peroxidase-conjugated secondary antibody, either anti-mouse or anti-rabbit (Amersham Biosciences), was used at 1:2,500 dilution in milk. Immunoblots were developed using SuperSignal West Pico chemiluminescent substrate kit (Pierce Biotechnology, Inc.).

**Dual-phase extraction and high-resolution MRS studies.** Dual-phase extraction and MR studies were carried out with tumor tissue (<0.3g) or ~2 x 10⁷ cells from water-soluble fractions using methanol/chloroform/water (1:1:1, v/v/v) as previously described (7). Briefly, tumor samples were freeze clamped, pulverized with liquid N₂, and homogenized in 4 mL of ice-cold methanol. Pelleted cells were mixed with 4 mL of ice-cold methanol and vigorously vortexed. After keeping tumor and cell samples on ice for 10 min, 4 mL of chloroform were added, vortexed vigorously, and incubated on ice for additional 10 min. Finally, 4 mL of water were added and the samples were vigorously shaken. All procedures were performed on ice, and samples were stored at 4°C overnight for phase separation and later centrifuged at 15,000 x g at 4°C for 30 min. The water/methanol phase containing the water-soluble cellular metabolites such as choline, PC, and glycerophosphocholine (GPC) was treated with ~100 mg of Chelex beads (Sigma-Aldrich) to remove any divalent cations. After removing the beads, methanol was evaporated using a rotary evaporator. The remaining water phases were lyophilized. Cell extracts were resuspended in 0.6 mL deuterated water (D₂O) for MRS analysis. Five microliters of 0.75% (w/w) 3-(trimethylsilyl)-propionic 2,2,3,3-d₄ acid sodium salt (TMSP) in D₂O were used as an internal standard. Fully relaxed 1H MR spectra of the water-soluble extracts were acquired on a Bruker Avance 500 spectrometer (Bruker BioSpin Corp.) as previously described (7). Signal integrals of metabolites such as choline, PC, and tCho (PC + GPC + choline) were compared with that of the internal standard TMSP according to the following equations:

\[
\text{[metabolite] = } \frac{I_{\text{metabolite}}}{T_{\text{MRS}}N_{\text{cell}}} \quad (A)
\]

\[
\text{[metabolite]_{tumor} = } \frac{I_{\text{metabolite}}}{T_{\text{MRS}}W_{\text{tumor}}} \quad (B)
\]

In the equations, [metabolite] is the molar concentration per cell of the metabolite expressed as mol/cell, and [metabolite]ₜₘᵢₜᵢₜ is the molar concentration in tumor samples. \(T_{\text{MRS}}\) is the number of moles of TMSP in the sample, \(N_{\text{cell}}\) is the cell number, and \(W_{\text{tumor}}\) is tumor volume (based on 1 g = 0.001 L). Because the number of protons contributing to the signal of all the choline metabolites at 3.20 to 3.24 ppm and to the TMSP peak at 0 ppm is the same, correction for differences in the number of protons was not required. Cell culture data are the mean of three independent transduction experiments. Ex vivo tumor extract data are from an average of four animals with tumor weight <300g.

**Immunohistochemistry.** Formalin-fixed, 5-μm-thick sections were stained for H&E using a standard protocol. OCT-embedded frozen tissue samples were cut at 5 μm thickness on a cryomicrotome and stained for Ki-67 using DAKO Rapid EnVison system as published elsewhere (15). Cut sections were fixed in acetone for 1 min and incubated with primary antibody (Ki-67 rabbit polyclonal antibody, 1:20 dilution; Novus Biologicals) for 3 min. Following this treatment, sections were incubated with the EnVision complex at 37°C for an additional 3 min before incubation with substrate solution (3,3'-diaminobenzidine). After each incubation, sections were washed three times with TBS for 10 s each. Before counterstaining with a hematoxylin quick staining kit (Vector Laboratories), sections were washed with tap water and mounted on DAKO Paramount aqueous mounting solution. Photomicrographs were taken on a Nikon microscope equipped with a CCD camera. Using ImageJ analysis software, photomicrographs of H&E sections, captured at ×4 magnification, were quantified for percentage necrotic area as a ratio of the pixels in the necrotic region divided by the total pixel in the tumor section. Ki-67 quantification was achieved by measuring the ratio of the number of cells positive for brown staining to the total cells in a given field of view at ×40 magnification. Five fields of view were analyzed per section.

**Statistical analyses.** Data were analyzed using the software package JMP³ or Microsoft Excel software² using a two-sided t test, assuming unequal variances. Values of \(P < 0.05\) were considered significant.

**Results.**

**Molecular and functional characterization of transduced MDA-MB-231 cells.** To determine the efficacy of viral vectors, viral supernatants prepared from either luc-shRNA (control) or chk-shRNA were added to MDA-MB-231 breast cancer cells. EGFP expression in photomicrographs of control and chk-shRNA–transduced cells shown in Fig. 1B confirmed ~90% transduction efficiency. To evaluate the silencing efficiency, transduced cells were characterized for endogenous chk mRNA by quantitative reverse transcription-PCR (qRT-PCR) using specific primers against endogenous chk and for protein expression in immunoblots obtained using a custom-made rabbit polyclonal antibody against chk (Fig. 1C and D). MDA-MB-231 cells transduced with chk-shRNA showed ~80% reduction in chk mRNA and protein relative to control cells. Wild-type and luciferase-transduced control cells had comparable expression levels of chk mRNA and protein.

To detect the efficiency of transduction by lentivirus expressing chk-shRNA in cells, ¹H MRS was performed on water-soluble extracts obtained from transduced cells. Representative fully relaxed ¹H MR spectra from chk-shRNA transduction showed that PC levels were significantly higher in control cells transduced with luc-shRNA compared with those transduced with chk-shRNA (compare Fig. 2A versus B). Levels of PC and, as a result, tCho-containing compounds were found to be significantly lower (\(P < 0.01\)) in chk-shRNA–transduced cells (Fig. 2C). GPC was not altered in control and chk-shRNA–transduced cells, but the decrease of PC resulted in a significantly lower ratio of PC/GPC following chk-shRNA transduction (Fig. 2D). These data represent an average of three separate experiments for each cell line.

**Evaluation of lentivirus-mediated transduction of tumors in vivo.** Data characterizing the chk-shRNA transduction of tumors are shown in Fig. 3. As shown in Fig. 3A, tumor growth in mice injected with chk-shRNA was reduced compared with control tumors. There was no evidence of weight loss or physical distress resulting from the treatment protocol. At the end of the i.v. injection protocol, the tumor and other organs, such as kidney, lung, and liver, were harvested. Representative photomicrographs of EGFP expression in 2-mm-thick sections of control and chk-shRNA–transduced tumor tissue are shown in Fig. 3B. EGFP was not observed in other organs (data not shown). A representative immunoblot shown in Fig. 3C (top) shows the maximum chk protein down-regulation that was achieved following treatment. Reduction of protein expression was primarily due to the efficient.

---

¹ http://www.jmpin.com
² http://www.microsoft.com

**Choline Kinase Targeting in a Breast Cancer Xenograft**
down-regulation of chk mRNA as shown in Fig. 3C (bottom). Compared with control, chk mRNA was down-regulated on average by ~35% in tumors transduced with chk-shRNA (Fig. 3C, bottom). Quantitative PCR of genomic DNA from tumor and organ tissue detected expression of EGFP as well as lentivirus vector markers by day 14 of the treatment protocol or after five i.v. chk-shRNA injections, tumoral PME/PC levels in tumors of animals injected with chk-shRNA were evident in the in vivo levels in tumors of animals injected with chk-shRNA compared with control, chk mRNA was down-regulated on average by ~35% in tumors transduced with chk-shRNA (Fig. 3C, bottom). Compared with control, chk mRNA was down-regulated on average by ~35% in tumors transduced with chk-shRNA (Fig. 3C, bottom).

**In vivo and ex vivo spectroscopic analyses of transduced tumor xenografts.** To validate the efficiency of virus-mediated gene targeting, noninvasive in vivo 31P MRS was performed to detect tumoral PME and PC levels. A decrease in both PC and PME levels in tumors of animals injected with chk-shRNA compared with those injected with luc-shRNA was evident in the in vivo single-voxel 31P MRS spectra (Fig. 4A). As shown in Fig. 4B and C, by day 14 of the treatment protocol or after five i.v. chk-shRNA injections, tumoral PME/PC levels were significantly (P < 0.01 and 0.05, respectively) decreased. This decrease in PME and PC levels was consistent with the observation of regions of EGFP expression in the tumor tissue. To further substantiate this finding, water-soluble extracts from tumor tissue were analyzed for PC, GPC, and tCho levels by 1H MR spectroscopy. Four tumors with matching volumes (tumor weight <300 mg) were selected from each group for this analysis. Representative fully relaxed 1H MR spectra from a luc-shRNA–transduced and chk-shRNA–transduced cells are shown in Fig. 5A and B. A decrease of the PC peak is evident in the chk-shRNA–transduced tumor. Quantitative data of PC, GPC, and tCho levels in luc-shRNA–transduced and chk-shRNA–transduced tumors are shown in Fig. 5C. Consistent with the reduction of PC in chk-shRNA–transduced cells, a significant decrease of PC and tCho was detected in the chk-shRNA–transduced tumors. However, the PC/GPC ratio was not significantly different (Fig. 5D) because of the slight decrease of GPC in the chk-shRNA–transduced tumors.

**Tumor-associated phenotypic changes.** Both anatomic and morphologic differences were observed between the luc-shRNA–transduced and the chk-shRNA–transduced tumors. To further evaluate the effects of chk-shRNA transduction, formalin-fixed tumor samples were evaluated for necrosis, and immunohistochemistry of frozen OCT-embedded tissue was performed for the proliferation marker Ki-67. Representative photomicrographs of H&E-stained sections from luc-shRNA–transduced and chk-shRNA–transduced tumors are shown in Fig. 6A at ×4 (i and ii) and ×20 (iii and iv) magnification. There was a trend toward increased necrosis in the chk-shRNA–transduced tumor (compare i versus ii, and iii versus iv). On average, however, the increase in necrosis in chk-shRNA–transduced tumors was not statistically significant (Fig. 6B). Cells in the Ki-67–immunostained sections obtained from luc-shRNA–transduced control tumors showed intense brown staining in the nucleus compared with the chk-shRNA–transduced treated tumors due to the presence of the Ki-67 antigen in the granular compartment of the nucleolus region. Representative photomicrographs of Ki-67 antigen–stained sections from luc-shRNA–transduced and chk-shRNA–transduced tumors shown in Fig. 6C at ×20 (i and ii) and ×40 (iii and iv) magnification reveal a decrease of Ki-67 staining in chk-shRNA–transduced tumors compared with luc-shRNA–transduced control tumors. Summarized data are presented in Fig. 6D and show significantly lower Ki-67–positive cells in chk-shRNA–treated tumors compared with luc-shRNA control tumors (35% versus 64%, P ≤ 0.005).
Discussion

Here, we have shown the ability of a viral-based delivery system to down-regulate chk expression in established tumors. chk down-regulation was detected noninvasively by MRS and resulted in reduced tumor growth together with decreased proliferation.

Virus-based therapy, especially lentivirus-based therapy, has shown promising results in the treatment of both inherited and acquired diseases (16). The use of HIV-1–based lentivirus as a mode of gene delivery was envisaged almost a decade ago (17) and its transduction efficacy was shown in over 42 different cell lines representing 10 different human tumor types (18). A careful dissection of various elements in the virus and the elimination of sequences not required for transduction and integration has led to a safer self-inactivating vector system. The incorporation of regulatory elements to the vector backbone has resulted in better transduction efficiency and transgene expression (18). Such a modified virus has been previously used to successfully transduce dividing and nondividing cells in culture (17) as well as in vivo (20, 21). The pRRL vector used in our study incorporated the required safety elements and showed over 95% transduction efficiency on addition of the pseudotyped viral supernatant to a safer self-inactivating vector system. The incorporation of regulatory elements to the vector backbone has resulted in better transduction efficiency and transgene expression (18). The packaging vector used in this study (ΔR8.2 DVPR) has the accessory element VPR deleted (19) and thus incorporates safety into the system without any negative effects on yield or infection efficiency. We used the VSV-G protein to pseudotype the envelope of the virus to increase the transduction efficiency of the transgene. Such a modified virus has been previously used to successfully transduce dividing and nondividing cells in culture (17) as well as in vivo (20, 21). The pRRL vector used in our study incorporated the required safety elements and showed over 95% transduction efficiency on addition of the pseudotyped viral supernant to MDA-MB-231 cells.

The robust EGFP expression observed in tumors was consistent with the reduction of chk expression at both mRNA and protein levels and resulted in a significant decrease of PC. A similar reduction in mRNA and protein with a corresponding decrease of mRNA and protein with a corresponding decrease of PC has been previously reported in MDA-MB-231 (7) and MCF-7 cells transfected transiently with small interfering RNA (22) and in MDA-MB-231 cells stably expressing U6-driven chk-shRNA (7).

An important aspect of gene therapy is the mode of injection (8). Viruses can be administered by an intratumoral injection of the viral supernatant, the intratumoral implantation of a polymeric device that can release viral vectors locally, or by an i.v. injection of the viral supernatant through the tail vein. The i.v. administration protocol used in our study resulted in robust EGFP expression in transduced tumors and an efficient knockdown of chk. The biodistribution and rate of accumulation of virus following an i.v. injection will depend on the virus type, total surface area of the microvessel, microvascular permeability, and rate of cellular uptake (8). Following i.v. administration, lentiviruses disperse within the vascular space and are filtered by the liver (23) where they are scavenged by Kupffer cells (8). Because these viral particles are of the order of 10 nm or larger, they will not leak out easily through normal tissue vasculature into normal tissues but will leak out into the tumor interstitium from the permeable vasculature found in tumors (24). In a previous study involving BALB/c mice, i.v. injection of lentivirus through the tail vein, generated using a first-generation packaging construct, resulted in GFP expression that was sustained for 40 days in bone marrow. Additional organs such as liver and spleen showed GFP expression to a lesser extent (23). However, after 40 days, GFP expression diminished in liver and spleen. Although in the present study we did not look for GFP expression in bone marrow, the lack of GFP detection in the lung, kidney, and liver in our study was most likely due to the inability of viral particles to penetrate through normal vasculature and due to their degradation in the liver. The expression of EGFP was more robust in chk-shRNA–transduced tumors compared with luc-shRNA–transduced control tumors. These differences may be attributed to the reduced proliferation and tumor growth in the chk-shRNA–transduced tumors.

Figure 3. In vivo transduction of lentivirus. A, tumor growth characteristics with lentivirus-mediated chk targeting. Tumor volume in mm³ of luc-shRNA–transduced (gray circles, n = 5) and chk-shRNA–transduced (black circles, n = 5) tumors. Points, mean; bars, SE. *, P < 0.05; †, P < 0.07. B, representative bright-field and fluorescence photomicrographs of a tumor section showing distribution of EGFP in a luc-shRNA–transduced and chk-shRNA–transduced tumor. C, top, representative immunoblot showing chk protein from tumor samples. Actin was used as loading control. Bottom, quantitative RT-PCR for chk mRNA expression from tumor samples (n = 5). D, quantitative PCR of genomic DNA from tumor tissue to detect viral vector genes to establish the integration of virus in both luc-shRNA–transduced (gray columns) and chk-shRNA–transduced (black columns) tumors.
One of the major challenges facing cancer gene therapy is the ability to detect the response of the tumor to the treatment. chk is an attractive target for gene therapy as its down-regulation has been shown to reduce proliferation (ref. 7 and this study) and increase differentiation as well as to increase cell kill in combination with conventional chemotherapy (22). A major advantage of chk targeting is the ability to detect viral transduction noninvasively with 1H or 31P MRS. Our data show that the reduction in tumoral chk protein levels through lentiviral-mediated chk-shRNA delivery resulted in decreased PC levels, which is the metabolic product of the chk enzyme reaction. The decrease in PC and, hence, PME levels could be dynamically monitored over a time period of 30 days in our preclinical studies. Residual PC observed in our in vivo 31P MRS studies may be attributed to the, possibly even compensatory, action of other PC-producing enzymes, such as phosphatidylycholine-specific phospholipase C. A decrease of tCho was also observed in ex vivo studies of tissue extracts. Future clinical assessment of successful chk-shRNA gene therapy could be...
performed with single-voxel $^{31}$P MRS, or with $^1$H MRS, which detects the tCho signal with high detection sensitivity. In summary, these preclinical data show that i.v. administered lentivirus expressing chk-shRNA can be used to target chk in established tumors. The down-regulation of chk, which can be detected noninvasively with $^{31}$P or $^1$H MRS, results in a reduction of cell proliferation and tumor growth. Our data suggest that chk down-regulation with this system resulted in a cytostatic rather than cytoreductive effect. Future studies using virus with increased plasma half-life can enhance the rate of accumulation and be more effective in target gene silencing in vivo. A comparable lentivirus dose used in the mouse studies may be possible to achieve in humans by injecting a larger volume and increasing the titer. In spite of safety concerns involving use of lentivirus as a mode of gene delivery, these studies are an important forerunner of future gene therapy trials targeting chk in tumors.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

Received 10/27/08; revised 12/30/08; accepted 1/30/09; published OnlineFirst 3/31/09.

Grant support: NIH grant P50 CA103175.

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.

We thank Gary Cromwell for maintaining the cell lines and inoculating the tumors.

**References**


Noninvasive Detection of Lentiviral-Mediated Choline Kinase Targeting in a Human Breast Cancer Xenograft

Balaji Krishnamachary, Kristine Glunde, Flonne Wildes, et al.

Cancer Res  Published OnlineFirst March 31, 2009.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-08-4120

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.