Phosphorus MRS effectively monitors lentiviral-mediated gene therapeutic silencing of choline kinase in a human breast cancer xenograft

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Introduction

Choline kinase (Chk), an enzyme that converts free choline to phosphocholine (PC), is overexpressed in several cancers, including breast cancer [1-2]. Chk contributes to the increased phosphocholine (PC) and total choline (tCho) levels in breast tumors, which can be detected by ^{1}H and ^{31}P magnetic resonance spectroscopy (MRS) [1-2]. Because Chk is associated with tumor aggressiveness, it can be used as a target for anticancer therapies using gene silencing by RNA interference [1]. We are currently developing systemic RNA interference-based anticancer therapies that target Chk for breast cancer treatment [3]. Because lentiviral vectors have emerged as vectors of choice for long-term, stable *in vitro* and *in vivo* gene transfer, we have generated an HIV-based lentivirus to target Chk *in vitro* and *in vivo* in MDA-MB-231 breast cancer cells and xenografts [3]. This lentivirus produces double-stranded short hairpin RNA (shRNA) specific to Chk (shRNA-chk), and efficiently transduces MDA-MB-231 breast tumor xenografts as previously shown [3]. Here we have utilized *in vivo* single-voxel ^{31}P MRS to monitor the functional efficacy of lentiviral-mediated Chk silencing following intravenous injection of this lentivirus in mice bearing MDA-MB-231 breast tumor xenografts. We optimized the treatment protocol with this lentiviral vector, which was monitored by quantitation of the ratios of the signal integrals from PC/β-nucleoside triphosphate (NTP) and phosphomonoester (PME)/ β-NTP. Treatment efficacy was monitored by measuring tumor volumes.

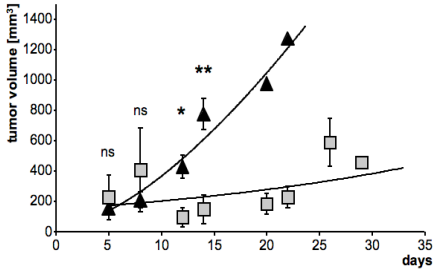

Figure 1: Tumor growth of human MDA-MB-231 breast cancer xenografts in SCID mice systemically treated with shRNA-chk to target Chk (gray squares) and treated with shRNA-luc (black triangles) as controls.
ns: not significant; *, p<0.05; **, p<0.01. Values are mean ± standard error (n=4).

Methods

Lentiviral particles contained a construct encoding for shRNA-chk expression in the pRRL-pGK-EGFP lentiviral vector, which was produced as previously described [3]. Controls were transduced with virus expressing shRNA against Luciferase (shRNA-luc) [3]. Human MDA-MB-231 breast cancer cells were orthotopically inoculated into the mammary fat pad of severe combined immunodeficient (SCID) mice. An average of 2.7×10^7 lentiviral particles in 200 µl phosphate-buffered saline per mouse was injected into the tail vein of MDA-MB-231 breast tumor-bearing SCID mice. *In vivo* single-voxel ^{31}P MRS was performed on a 4.7T Bruker Biospec spectrometer to dynamically monitor tumoral PME and PC levels throughout the time course of Chk-targeted lentiviral gene therapy. Phosphorus MR spectra were processed and analyzed with an in-house IDL program (Dr. D. C. Shungu), using gaussian multiplication and a combination of linear and nonlinear least-square fitting [4]. Statistical analysis was performed using a t-test assuming unequal variances in the software package JMP (www.jmpin.com). Because the lentiviral vector also transduced enhanced green fluorescent protein (EGFP) expression [3], efficiency of viral transduction was evaluated by fluorescence microscopy of EGFP expression in freshly cut 2-mm thick tumor slices once tumors reached volumes of approximately 800 mm^3. Tumors were also sectioned and stained with hematoxylin and eosin (H&E) to assess necrosis, and with the proliferation marker Ki67 to assess proliferation.

Results

The optimal treatment protocol was achieved by two intravenous injections of lentiviral particles encoding for shRNA-chk per week (on days 4 and 7), based on the evaluation of the ^{31}P MR spectra and tumor growth curves. Phosphorus MRS was performed twice a week on each day after injecting the lentiviral particles. Tumor growth was significantly attenuated in the treatment group treated with lentiviral particles expressing shRNA-chk as compared to controls (Fig. 1). *In vivo* ^{31}P MRS demonstrated that the PME/β-NTP (Fig. 2) and PC/β-NTP (Fig. 3) ratios significantly decreased in shRNA-chk lentivirus treated tumors compared to shRNA-luc treated controls. All tumors displayed significant transduction as measured by EGFP expression.

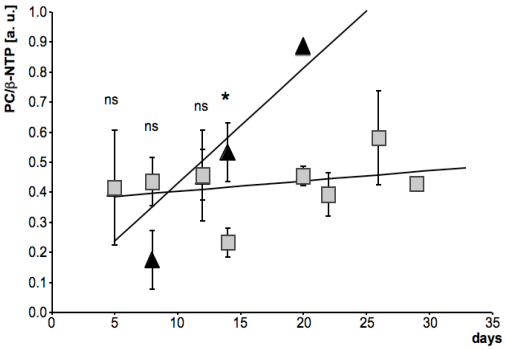

Figure 3: PC/β-NTP ratios quantified from *in vivo* ^{31}P MR spectra of MDA-MB-231 tumors throughout the course of treatment with shRNA-chk (gray sqares) and shRNA-luc controls (black triangles) in MDA-MB-231 tumor-bearing SCID mice.
ns: not significant; *, p<0.05. NTP, nucleoside triphosphate; PC, phosphocholine. Values are mean ± standard error (n=4).

Tumors from mice treated with shRNA-chk lentiviral particles contained large necrotic regions in the H&E stained sections, and decreased numbers of Ki67-positive-staining cells as compared to shRNA-luc treated tumors, indicative of decreased proliferation.

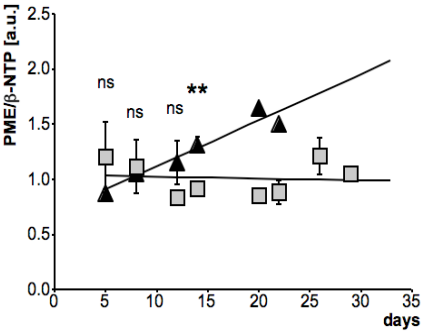

Figure 2: PME/β-NTP ratios quantified from *in vivo* ^{31}P MR spectra of MDA-MB-231 tumors throughout the course of treatment with shRNA-chk (gray sqares) and shRNA-luc controls (black triangles) in MDA-MB-231 tumor-bearing SCID mice.
ns: not significant; **, p<0.01. NTP, nucleoside triphosphate; PME, phosphomonoesters. Values are mean ± standard error (n=4).

Discussion

Here we have demonstrated that tumoral Chk silencing can be achieved by bi-weekly intravenous administration of lentiviral particles expressing shRNA-chk into SCID mice bearing human MDA-MB-231 breast tumor xenografts. This lentiviral particle-mediated Chk-targeted therapy significantly reduced tumor growth, and decreased proliferation *in vivo*, which is in good agreement with our previous *in vitro* studies [1]. Systemic lentiviral shRNA-chk delivery *in vivo* decreased the tumoral PC and PME levels as monitored by PC/β-NTP and PME/β-NTP ratios from *in vivo* ^{31}P MR spectra. Decreased PC and PME levels following shRNA-chk delivery are in good agreement with our previous studies [1]. Systemic viral particle-mediated Chk-targeted gene therapy may be feasible for clinical translation, and can be monitored by single-voxel ^{31}P MRS *in vivo*.

References: [1] Glunde K et al, *Cancer Res* 65, 11034 (2005) [2] de Molina AR et al, *Oncogene* 21, 4317 (2002) [3] Glunde et al, ISMRM 2007, abstract #465 (2007) [4] Glunde K et al, *Magn Reson Med* 48, 819 (2002). This work was supported by NIH 1R01 CA82337 and P50 CA103175 (JHU ICMIC Program).