

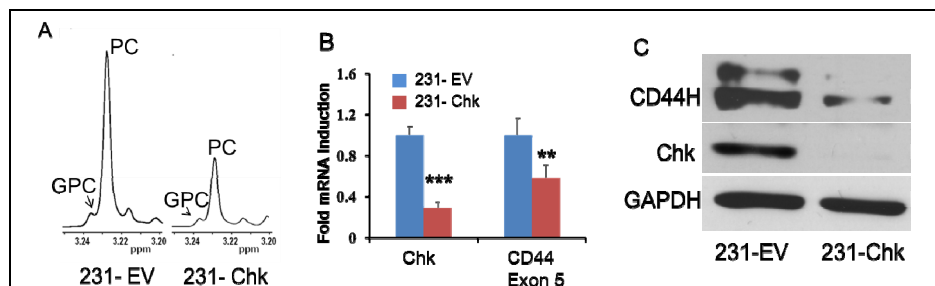
# Lentiviral choline kinase shRNA transduction of cells and tumors *in vivo* regulates breast cancer stem cell markers CD44 and ABCG2

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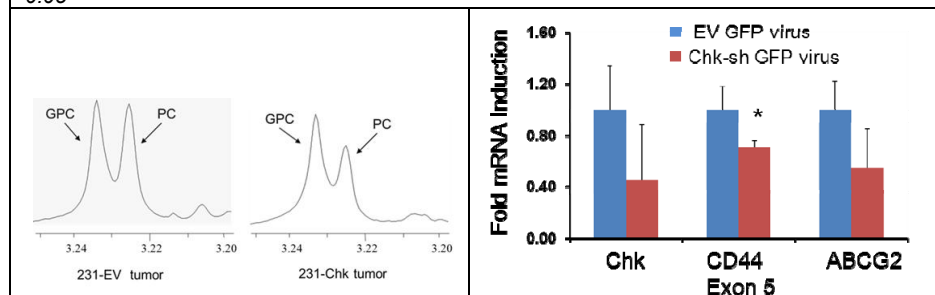
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**Introduction:** The discovery of subpopulations of breast cancer cells with stem-like characteristics (stem-like breast cancer cells or SBCCs) is offering new paradigms for understanding and treating tumor recurrence and metastasis [1]. Magnetic resonance spectroscopy (MRS) studies have shown that an increase of phosphocholine (PC) is one of the signatures of cancer, and this elevation is closely related to malignant transformation, invasion, and metastasis. Increased PC is primarily due to increased choline kinase alpha (Chk $\alpha$ ) expression and activity following malignant transformation [2, 3]. We recently demonstrated that down-regulating Chk in an invasive and metastatic breast cancer cell line reduced proliferation, induced differentiation, decreased metastasis, and sensitized cells to conventional chemotherapy such as treatment with 5-fluorouracil (5-FU) [4, 5]. We were able to successfully transduce established tumors with short hairpin RNA (shRNA) targeting Chk using lentiviral vector delivery, and demonstrated a reduction of growth and proliferation, as well as down-regulation of Chk $\alpha$ , and a reduction of PC in the MR spectra. We found that overexpressing Chk $\alpha$  in the less invasive MCF-7 human breast cancer cell line resulted in cells becoming resistant to treatment with 5-FU, and in greater exclusion of rhodamine-123, one of the characteristics of SBCCs [6]. Here we have determined the relationship between Chk downregulation and two markers of SBCCs, CD44 and ABCG2, in lentivirally transduced MDA-MB-231 cells *in vitro* and in transduced tumors *in vivo* with shRNA that targets Chk.

**Methods:** Studies were performed with MDA-MB-231 human breast cancer cells and its variants. *In vitro* studies were performed with MDA-MB-231 cells that were transduced with lentivirus that expressed shRNA against Chk (231-Chk) and that stably silence Chk expression or an empty vector (EV). Tumors were grown orthotopically in female severe combined immunodeficient (SCID) mice. We used an HIV-based lentivirus to target Chk in MDA-MB-231 breast cancer cells and *in vivo* in MDA-MB-231 breast cancer xenografts. We constructed a lentivirus containing a pol III promoter, which enables efficient gene delivery for stable integration, producing double-stranded shRNA specific to Chk (shRNA-Chk). The design, production, concentration, and delivery of lentivirus expressing shRNA-Chk or empty vector (EV) and gene expression analysis were performed following our previously established protocol [7]. Spectral changes following lentiviral transduction of tumors with Chk-targeting shRNA are from our previous study [7]. Here we have additionally characterized CD44 and ABCG2 in these tumors, as well as performed additional transduction studies with MDA-MB-231 tumor variants.



**Figure 1. (A)** High-resolution <sup>1</sup>H MR spectra of water-soluble metabolites of MDA-MB-231 cells stably expressing either EV or – shRNA-Chk showing PC levels. **(B)** Quantitative real-time PCR (qRT-PCR) showing Chk $\alpha$  and CD44 mRNA expression in MDA-MB-231 stably expressing shRNA against Chk. **(C)** Western blot showing Chk and CD44 protein expression. GAPDH was used as loading control. Values are mean  $\pm$  S.E obtained from triplicates. \*  $p \leq 0.05$



**Figure 2.** Representative expanded regions of <sup>1</sup>H MR spectra from tumors transduced with **(A)** shRNA-luc and **(B)** shRNA-Chk. Abbreviations: GPC, glycerophosphocholine; PC, phosphocholine; tCho, total choline-containing compounds [7].

**Figure 3.** qRT-PCR data following systemic intravenous injection of lentivirus expressing shRNA-Chk *in vivo*. Decreased expression of CD44, Chk $\alpha$  and ABCG2 mRNA was observed in tumors. Values are mean  $\pm$  S.E obtained from 5 animals. \*  $p \leq 0.05$

**Results and Discussion:** Lentiviral transduction of MDA-MB-231 cells with shRNA-Chk resulted in a significant decrease of PC (**Figure 1A**), of Chk $\alpha$  and CD44 mRNA (**Figure 1B**), and protein expression of CD44 and Chk $\alpha$  (**Figure 1C**). *In vivo*, lentivirally transduced tumors also showed a similar decrease of PC (**Figure 2**) and of Chk $\alpha$ , CD44 and ABCG2 mRNA expression levels (**Figure 3**), confirming the effects observed in cells in culture. Here we have shown that Chk downregulation induces decreases in the expression of two SBCC markers. These data highlight the potential importance of targeting Chk to minimize the burden of cells with stem-like characteristics in tumors.

**References:** 1. Al-Hajj, M *et al.*, Proc Natl Acad Sci U S A, 2003; 2. Ramirez de Molina, AR *et al.*, Oncogene, 2002; 3. Ramirez de Molina, AA. *et al.*, Biochem Biophys Res Commun, 2002; 4. Mori, N *et al.*, Cancer Res., 2007. 5. Glunde, K *et al.*, Cancer Res, 2005. 6. Shah, T *et al.*, NMR in Biomed., 2010. 7. Krishnamachary, B *et al.*, Cancer Research, 2009.

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