Silencing GDPD5, a novel anticancer target, increases glycerophosphocholine in human breast cancer cells

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Introduction: Phosphocholine (PC) and total choline-containing compounds (tCho = glycerophosphocholine (GPC) + PC + free choline (Cho)) are elevated in human breast cancers, as demonstrated by numerous ^{1}H magnetic resonance (MR) spectroscopy (MRS) studies [1, 2]. A switch from high GPC and low PC to low GPC and high PC characterizes the choline metabolite profile of breast [3] and ovarian [4] cancers. Choline phospholipid metabolism consists of a complex network of biosynthetic and catabolic pathways, with one or more enzymes acting per pathway [5]. Some of the enzymes in choline phospholipid metabolism may be potential targets for anticancer therapy. Previous studies on choline kinase downregulation by RNA interference (RNAi) reduced proliferation and increased differentiation in breast cancer cells [6]. Glycerophosphocholine phosphodiesterase (E.C. 3.1.4.2; GPC-PDE) is an enzyme in choline phospholipid metabolism that catalyzes the degradation of GPC to Cho and glycerol-3-phosphate. To our knowledge, the gene(s) for the GPC-PDE responsible for the low GPC concentration in breast cancer cells have not yet been identified. Glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) is a GPC-PDE that is rapidly inhibited by NaCl and urea in renal cells [7], and may be a candidate gene for GPC-PDE in breast cancer cells. In our current study, we inhibited for the first time GPC-PDE chemically by high concentrations of NaCl and urea, and downregulated GDPD5 by RNAi using short hairpin RNA (shRNA) against GDPD5.

Methods: We incubated MCF-12A, a nonmalignant human mammary epithelial cell (HMEC) line, and MCF-7 and MDA-MB-231, two human breast cancer cell lines for 24 h with 100 mM urea and 100 mM NaCl in culture medium (NaCl/urea). Fully relaxed high-resolution ^{1}H MR spectra of the water-soluble cell extracts were measured on a Bruker Avance 500 MR Spectrometer, and analyzed using the MestReC 4.9.9.6 software. Cell viability/proliferation was measured with the WST-1 proliferation assay (Roche Diagnostics). Quantitative RT-PCR (qRT-PCR) was performed to detect GDPD1, 2, 3, 4, and 5 mRNA levels in MCF-12A, MCF-7, and MDA-MB-231 cells using iCycler (Bio-Rad) and iQ SYBR Green (Quanta BioSciences). DNA encoding short-hairpin RNA (shRNA) against GDPD5 was cloned between the *Nde1* and *Pst1* restriction sites of a human U6 promoter-driven pRRL vector containing enhanced green fluorescent protein (EGFP) as a reporter gene driven by a phosphoglycerate kinase promoter. Infectious viral supernatants were derived by transient co-transfection of HEK 293T cells with the following. A total of 19.5 µg of plasmid in the proportion of 12 µg lentiviral vector carrying shRNA, 6 µg of packaging plasmid pCMVΔR8.2 DVPR, and 1.5 µg of pCMV-VSVG were used. Viral supernatant was collected at 48, 72, and 96 hours after transfection. Viral supernatant with 1 mg/mL polybrene was added to MCF-7 cells for 4 to 5 h. This procedure was repeated for 3 days to obtain stable GDPD5 knockdown clones. Transduction efficiency was reported by EGFP expression. Water-soluble metabolites were extracted from the cells, and fully relaxed high-resolution ^{1}H MR spectra were measured and analyzed as described above.

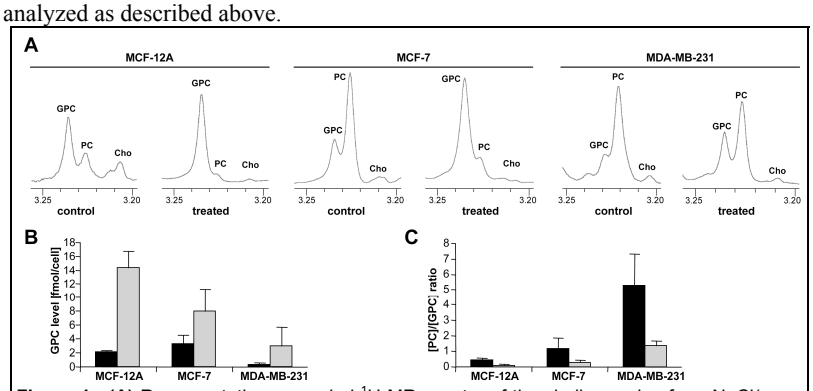

Figure 1. (A) Representative expanded ^{1}H MR spectra of the choline region from NaCl/urea-treated, GDPD5-inhibited (treated) *versus* control MCF-12A, MCF-7, and MDA-MB-231 cells. **(B)** GPC levels in treated (gray bars) *versus* control (black bars) quantified from the spectra shown in A (n=3). **(C)** [PC]/[GPC] ratios in treated (gray bars) *versus* control (black bars) quantified from the spectra shown in A (n=3). Values are mean ± standard deviation.

Results: Proton MR spectra of water-soluble cell extracts obtained from nonmalignant MCF-12A cells revealed a GPC increase and a PC decrease after treatment with NaCl/urea (Figure 1A). Malignant MCF-7 and MDA-MB-231 cells exhibited a switch from low GPC and high PC to high GPC and low PC following NaCl/urea treatment (Figure 1A). Metabolite quantification (n=3) demonstrated a significant increase in GPC levels (Figure 1B) and a decrease of the PC/GPC ratio (Figure 1C) for cells treated with NaCl/urea in all tested cell lines, compared to their respective controls. GDPD5 inhibition by NaCl/urea significantly decreased cell proliferation/viability in MCF-12A, MCF-7, and MDA-MB-231 cells (data not shown). qRT-PCR detected significantly higher GDPD5 mRNA levels compared to the mRNA levels of GDPD1, 2, 3, and 4 in the respective cell line for MCF-12A, MCF-7, and MDA-MB-231 cells. GDPD5 levels were significantly higher in MDA-MB-231 compared to MCF-7 and MCF-12A cells (data not shown). In MCF-7 cells transduced with GDPD5-shRNA, we also observed a switch from low GPC and high PC to high GPC and low PC (Figure 2A). The PC/GPC ratio decreased in MCF-7 cells with stably down-regulated GDPD5 as compared to wild type MCF-7 cells (Figure 2B, n=2).

Discussion: Exposure of MCF-12A, MCF-7, and MDA-MB-231 cells to high NaCl and urea, as well as transduction with GDPD5-shRNA in MCF-7 cells, increased GPC and decreased PC, resulting in a decreased [PC]/[GPC] ratio. An increased [PC]/[GPC] ratio is associated with increased malignancy in breast cancer cell lines [3]. Our current study demonstrated for the first time that inhibiting or down-regulating GDPD5, a specific gene encoding a GPC-PDE, altered the choline phospholipid metabolite profile of breast cancer cells toward a less malignant metabolic profile. GDPD5 is at least partially responsible for the decreased GPC levels in breast cancer cells, as indicated by high GDPD5 mRNA and low GPC metabolite levels in MDA-MB-231 cells. Decreased proliferation detected upon GDPD5 inhibition with NaCl/urea further corroborated the importance of GPDP5 in breast cancer. These results indicate that GDPD5 may provide a future target for anticancer therapy. MRS could be used to monitor the GPC increase following downregulation of GDPD5 with shRNA or small interfering RNA (siRNA) in such future therapies.

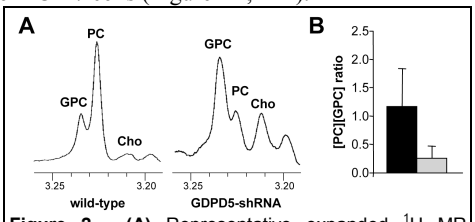

Figure 2. (A) Representative expanded ^{1}H MR spectra of the choline region from GDPD5-shRNA *versus* wild-type MCF-7 cells. **(B)** [PC]/[GPC] ratios in MCF-7 wild-type (black bar) and GDPD5-shRNA (gray bar) cells quantified from the spectra shown in A (n=2). Values are mean ± standard deviation.

References: [1] Podo F. *NMR Biomed* 12(7), 413-439 (1999). [2] Ackerstaff E *et al*. *J Cell Biochem* 90(3), 525-533 (2003). [3] Aboagye EO *et al*. *Cancer Res* 59(1), 80-84 (1999). [4] Iorio E *et al*. Cancer Res 65(20), 9369-76 (2005). [5] Glunde K *et al*. *Expert Rev Mol Diagn* 6(6), 821-829 (2006). [6] Glunde K *et al*. *Cancer Res* 65(23), 11034-11043 (2005). [7] Gallazzini M *et al*. *Proc Nati Acad Sci* 105(31), 11026-11031 (2008).
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