Effects of targeting the glycerophosphocholine phosphodiesterase GDPD5 in breast cancer models

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Introduction: Glycerophosphocholine phosphodiesterase (E.C. 3.1.4.2; GPC-PDE) is an enzyme in choline phospholipid metabolism that catalyzes the degradation of glycerophosphocholine (GPC) to free choline (Cho) and glycerol-3-phosphate. We recently demonstrated that the glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) gene expresses a GPC-PDE enzyme that is at least in part responsible for the low GPC concentration in breast cancer cells [1]. In the present study, we stably silenced GDPD5 using short hairpin RNA (shRNA) against GDPD5 and investigated the effects of this stable GDPD5 downregulation in breast cancer cells and tumor xenograft models.

Methods: We stably downregulated GDPD5 using shRNA against GDPD5 (GDPD5-shRNA) delivered by lentiviral transduction in MCF7 breast cancer cells (MCF7-GDPD5-shRNA). Fully relaxed high-resolution ^{1}H MR spectra of the water-soluble and lipid cell extracts were measured on a Bruker Avance 500 MR Spectrometer, and analyzed using the MestReC 4.9.9.6 software. Quantitative RT-PCR (qRT-PCR) was performed to detect GDPD5 mRNA levels in MCF7 cells using iCycler (Bio-Rad) and iQ SYBR Green (Quanta BioSciences). To further evaluate the effects of GDPD5 silencing on tumor growth, we orthotopically inoculated 8 athymic nude mice each with parental MCF7 and MCF7-GDPD5-shRNA cells (2 million cells in 50 µl Hank's buffer). We monitored tumor growth using standard calipers. 3D water-suppressed magnetic resonance spectroscopic imaging (MRSI) was performed on a Bruker 9.4T small animal imaging system using a standard 3D chemical shift imaging sequence. Water-unsuppressed MRSI was performed on each tumor as internal reference. The total choline (tCho) concentration of tumors (n=9) was calculated by normalizing to the unsuppressed water signal [2].

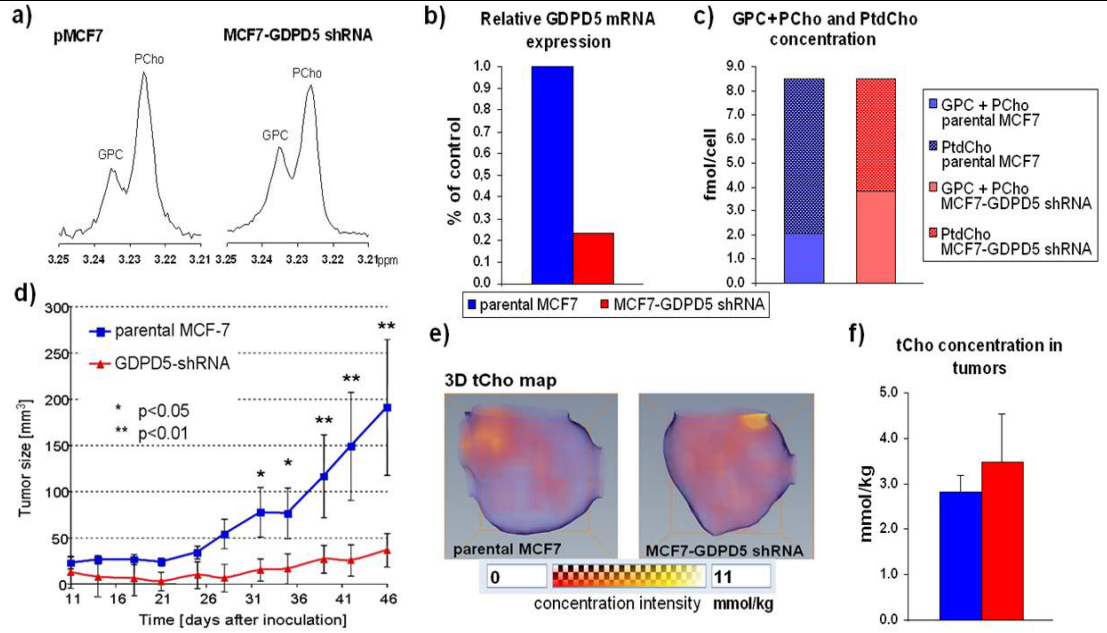

Figure a) Representative ^{1}H MR spectra of parental MCF7 (left) and MCF7-GDPD5-shRNA cells (right). **b)** Relative GDPD5 mRNA expression of parental MCF7 (blue) and MCF7-GDPD5-shRNA cells (red). **c)** GPC+PCho and PtdCho concentrations of parental MCF7 and MCF7-GDPD5-shRNA cell extracts. **d)** Growth curves of solid tumors (n=8 each) in mice inoculated with parental MCF7 (blue) and MCF7-GDPD5-shRNA cells (red). **e)** 3D total choline (tCho) map of representative parental MCF7 and MCF7-GDPD5-shRNA tumors. **e)** tCho concentration in parental MCF7 (blue) and MCF7-GDPD5-shRNA tumors (red). Values are mean ± standard deviation (n=9).

Results: A decreased phosphocholine (PCho)/GPC ratio was detected in MCF7-GDPD5 shRNA cells (1.52) compared to parental MCF7 cells (1.97) as shown in Figure a). Figure b) shows the relative GDPD5 mRNA expression level in MCF7-GDPD5-shRNA cells, which was decreased by 77% as compared to the parental MCF7 control cell line. The sum of GPC+PCho was higher in MCF7-GDPD5-shRNA cells (3.8 fmol/cell) than in parental MCF7 cells (2.1 fmol/cell), while the phosphatidylcholine (PtdCho) level was lower in MCF7-GDPD5-shRNA cells compared to parental MCF7 cells, 4.6 *vs.* 6.4 fmol/cell, respectively (Figure c). Figure d) demonstrates that tumors that stably expressed GDPD5-shRNA grew significantly more slowly than parental MCF7 tumors. Figure e) and f) show representative 3D distributions of the tCho concentrations in MCF7-GDPD5-shRNA tumors (3.49±1.11 mmol/kg), which was higher than in parental MCF7 tumors (2.81±0.14 mmol/kg), and consistent with the cell culture data shown in Figure c).

Discussion and Conclusion: Here we demonstrated for the first time that silencing the specific GPC-PDE enzyme GDPD5 increased the tCho levels in breast cancer cells and tumor models. Silencing GDPD5 resulted in an accumulation of GPC, which may disturb choline phospholipid metabolism through inhibition of membrane breakdown and turnover. The choline metabolite profile in the GDPD5-downregulated cells was at the same time changed toward a less malignant profile, characterized by a decreased PCho/GPC ratio. The tumor growth rate of the GDPD5-silenced breast tumor xenografts was significantly reduced. These findings emphasize that increased tCho levels can be associated with decreased tumor growth in this particular case. Careful analysis of MRSI data is therefore required to understand the effects of high tCho concentrations in terms of breast tumor growth and aggressiveness, as the elevated tCho signal may be due to increased GPC levels associated with decreased tumor growth. GDPD5 may have a potential role as an anticancer target in regulating choline phospholipid metabolism in breast cancer. We are currently further investigating the molecular and metabolic effects of GDPD5 silencing in breast cancer cells and tumor models.

References: [1] Doepkens M. et al, ISMRM 2010; [2] Bolan P.J. et al, Magn Reson Med 50, 1134-43, 2003. **Acknowledgements:** This work was supported by NIH R01 CA134695. We thank Tiffany R. Blackwell for technical laboratory assistance.