siRNA-mediated silencing of the glycerophosphocholine phosphodiesterase GDPD5 in breast cancer cells

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Introduction

We recently demonstrated that the glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) gene expresses a glycerophosphocholine phosphodiesterase (E.C. 3.1.4.2; GPC-PDE) enzyme that is at least partially responsible for the relatively low glycerophosphocholine (GPC) levels in human breast cancer cells and human breast tumors [1]. Human breast cancers with malignant choline metabolite profiles consisting of low GPC and high phosphocholine (PC) levels highly co-expressed GDPD5, choline kinase alpha (CHKA), and phosphatidylcholine-specific phospholipase D1 (PLD1), while cancers containing high GPC and relatively low PC levels displayed low co-expression of GDPD5, CHKA, and PLD1 [1]. These data suggest that GDPD5 is associated with breast cancer malignancy, and that GDPD5 catalyzes the degradation of GPC to free choline (Cho) and glycerol-3-phosphate [1]. In the present study, we transiently silenced GDPD5 using short interfering RNA (siRNA) against GDPD5 in human breast cancer cells, which was studied by quantitative high-resolution (HR) ^{1}H magnetic resonance spectroscopy (MRS).

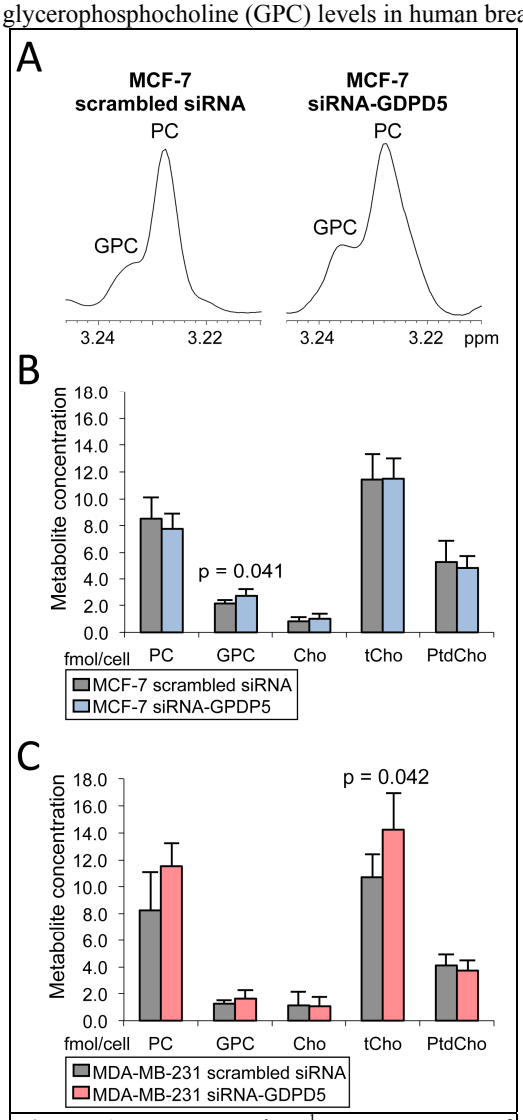

Figure A: Representative ^{1}H MR spectra of control MCF-7 (left) cells treated with scrambled siRNA and MCF-7 cells transiently silenced with siRNA-GDPD5 (right). **B:** Quantification of metabolite levels from ^{1}H MR spectra of scrambled siRNA treated control (gray bars, n=3) *versus* siRNA-GDPD5 treated MCF-7 cells (blue bars, n=8). Values are mean ± standard deviation. **C:** Quantification of metabolite levels from ^{1}H MR spectra of scrambled siRNA treated control (gray bars, n=3) *versus* siRNA-GDPD5 treated MDA-MB-231 cells (red bars, n=9). Values are mean ± standard deviation.

Methods

We tested three different siRNA targeting sequences for silencing GDPD5 (siRNA-GDPD5) in weakly malignant estrogen receptor positive (ER^+) human MCF-7 breast cancer cells and highly malignant estrogen receptor negative (ER^-) human MDA-MB-231 breast cancer cells. The siRNA-GDPD5 treated cells were compared with respective controls that were treated with scrambled siRNA. The knockdown efficiency of GDPD5 was assessed by quantitative RT-PCR (qRT-PCR) using iCycler (Bio-Rad) and iQ SYBR Green (Quanta BioSciences). For MRS, dual phase extraction of cells treated with scrambled siRNA or siRNA-GDPD5 for 48 hours was performed as previously described [2, 3, 4]. Quantitative, fully relaxed HR ^{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 as previously described [3, 4].

Results

GDPD5 silencing with the three tested siRNA-GDPD5 targeting sequences resulted in a significant reduction of GDPD5 mRNA in MCF-7 as well as MDA-MB-231 cells to about 10% of the respective scrambled siRNA controls as detected by qRT-PCR (data not shown). Since all three sequences displayed comparable knockdown efficiency, we pooled the ^{1}H MRS data obtained with these three siRNA-GDPD5 sequences. Cellular GPC levels increased following transient siRNA-GDPD5 silencing (Figure A), which was significant (p=0.041) in MCF-7 cells (Figure B). PC decreased, and free choline (Cho), total choline (tCho: sum of PC, GPC, and Cho), and membrane phosphatidylcholine (PtdCho) remained unchanged in MCF-7 cells treated with siRNA-GDPD5. In MDA-MB-231 breast cancer cells, GPC, PC, and tCho (p=0.042) increased following siRNA-GDPD5 treatment as compared to cells treated with scrambled siRNA (Figure C).

Discussion and Conclusion

Our data suggest that GDPD5 catalyzes the degradation of GPC to Cho and glycerol-3-phosphate in human breast cancer cells, which can be concluded from the GPC increase upon GDPD5 silencing with siRNA-GDPD5. However, the observed increases in GPC are relatively small, although significant in MCF-7 cells, indicating that additional enzymes with GPC-PDE activity may be actively participating in choline phospholipid metabolism of breast cancer cells. tCho and PC levels increased in highly malignant ER^- MDA-MB-231 cells, but not in weakly malignant ER^+ MCF-7 cells upon GDPD5 silencing, suggesting different roles for GDPD5 in these two cell lines. Further investigation of the molecular and metabolic effects of GDPD5 silencing in breast cancer cells and tumor models is necessary to uncover the role of GDPD5 in choline metabolism and malignancy of breast cancer. We will also explore the role of other candidate genes with GPC-PDE activity in choline metabolism of breast cancer.

References

[1] Cao M.D. et al, NMR in Biomed in press, 2011. [2] Tyagi et al, MRM 35, 194-200, 1996. [3] Glunde et al, Cancer Res 64, 4270-6, 2004. [4] Glunde et al, Cancer Res 65, 11034-43, 2005. **Acknowledgements:** This work was supported by NIH R01 CA134695.