Targeting choline phospholipid metabolism: GDPD5 and GDPD6 silencing decreases breast cancer cell proliferation and invasion

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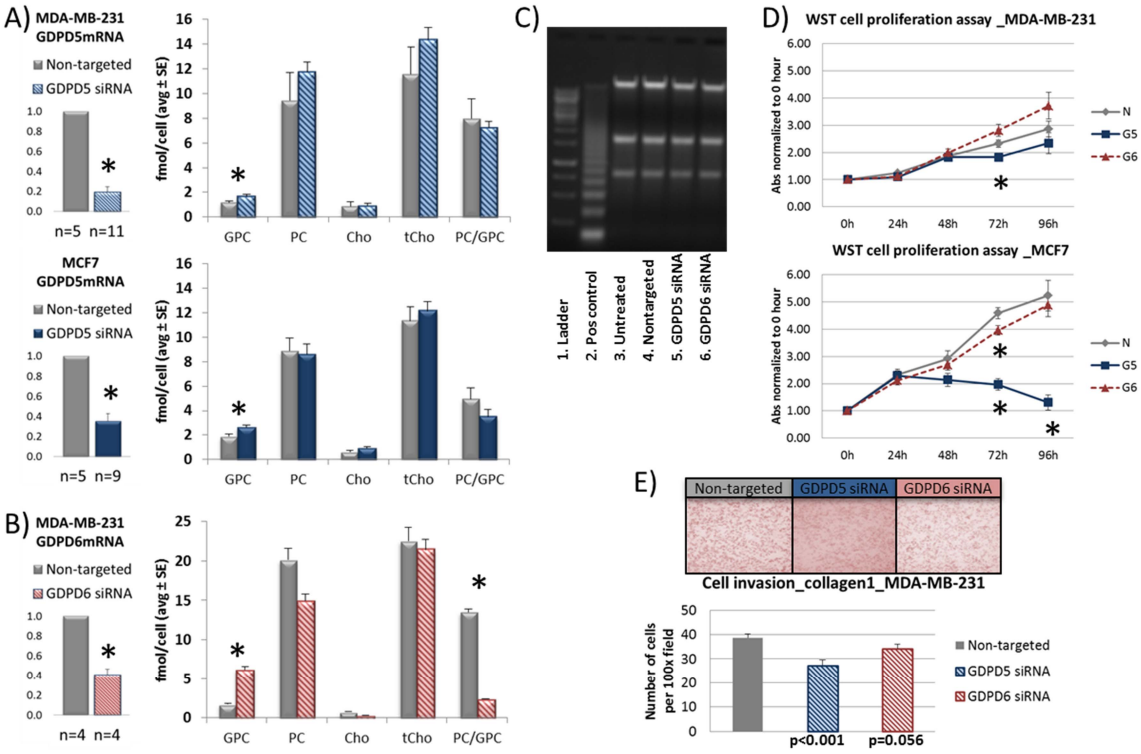
TARGET: Researchers interested in finding new targets for treatment of breast cancer and the biological effects of targeting cancer metabolism.

INTRODUCTION: As choline phospholipid metabolism has been shown to be associated with tumor malignancy and treatment response, the genes and enzymes regulating this pathway are interesting potential targets for treatment of breast cancer. We have demonstrated that the glycerophosphodiester phosphodiesterase GDPD5 is partially responsible for the relatively low glycerophosphocholine (GPC) levels in human breast cancer cells and human breast tumors [1]. In a recent study, GDPD6 has been shown to be involved in cell migration and metastasis [2]. Our purpose was to investigate the biological effects of targeting GDPD5 and GDPD6 for treatment of breast cancer.

METHODS: Down-regulation of GDPD5 and GDPD6 was performed by lipofectamine 2000 (Invitrogen) mediated transient transfection of small interfering RNA (siRNA) in weakly malignant human MCF-7 and highly malignant human MDA-MB-231 breast cancer cells. Non-targeted (scrambled) siRNA was used as a control. The knockdown efficiency of GDPD5 and GDPD6 was assessed by qRT-PCR using iCycler (Bio-Rad) and iQ SYBR Green (Quanta BioSciences). Quantitative, fully relaxed high resolution ^{1}H MR spectra of the water-soluble extracts were measured on a Bruker Avance 500 MR Spectrometer, and analyzed using the MestReC 4.9.9.6 software as previously described [3]. DNA laddering assay was performed using Apoptotic DNA Ladder Kit (Roche) and cell proliferation was monitored using Cell proliferation Reagent WST-1 (Roche). Cell invasion assay was performed using Transwell (CORNING) coated with 15ug/cm^2 Collagen1. Growth medium with 10% FBS was used as chemoattractant.



RESULTS:

A) GDPD5 siRNA qRT-PCR and ^{1}H MR. GDPD5 was successfully down-regulated by 80% and 65% in MDA-MB-231 and MCF7 cells, respectively. Significant increase in GPC (∗=p<0.05) was observed in GDPD5 siRNA treated cells compared to control.

B) GDPD6 siRNA qRT-PCR and ^{1}H MR. GDPD6 was successfully down-regulated by 60% for both MDA-MB-231 and MCF7 (data not shown) cells. GPC was increased in MDA-MB-231 cells compared to control. (MCF7 data in progress).

C) Apoptosis assay. DNA laddering was negative for both cell lines treated with GDPD5 and GDPD6 siRNA (n=2).

D) Cell proliferation. In GDPD5 siRNA treated cells, both cell lines experienced a decrease in cell proliferation compared to control at 72h. MCF7 cells treated with GDPD6 siRNA displayed a decrease in cell proliferation compared to control at 72h (n=6 per time point).

E) Cell invasion. GDPD5 and GDPD6 treated MDA-MB-231 showed a significant decrease in cell invasion compared to control. However, lower cell invasion was observed in GDPD5 compared to GDPD6 siRNA treated cells (p=0.024, n=3, MCF7 data in progress).

DISCUSSION and CONCLUSIONS: GDPD5 and GDPD6 down-regulation in breast cancer cells resulted in increased GPC levels, suggesting that these genes are involved in the regulation of cellular GPC level. Our study shows that GDPD5 and GDPD6 siRNA treatment did not cause apoptosis. In a recent study, GDPD6 siRNA was shown to decrease migration but did not affect proliferation [2]. In this study, we observed a significant decrease in cell proliferation of MCF7, but not MDA-MB-231 at 72 hours of GDPD6 siRNA treatment. Interestingly, GDPD5 silencing resulted in decreased cell proliferation in both cell lines at 72 hours. The effect of GDPD5 siRNA on cell proliferation was more severe in the less malignant breast cancer cell line. Cell invasion was also affected by GDPD5 and GDPD6 down-regulation. Lower cell invasion was observed in GDPD5 compared to GDPD6 siRNA treated cells. Further investigation of the molecular and metabolic effects of GDPD5 and GDPD6 silencing alone or combined are necessary to uncover their roles in choline metabolism and malignancy of breast cancer. We are currently investigating the association between the GDPDs and breast cancer recurrence and survival in patients.

REFERENCES [1] Cao M.D. et al, NMR in Biomed 2012. [2] Stewart JD, Proc Natl Acad Sci U S A. 2012. [3] Glunde et al, Cancer Res 64, 4270-6, 2004. Acknowledgements: This work was supported by NIH R01 CA134695.