Choline Phospholipid Characterization of Hox B7 Overexpressing MCF-7 Cells

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Introduction: Hox genes are transcription factors responsible for tissue remodeling. HOX genes play an important role in the proliferation and differentiation of cells. Besides their function in embryogenesis inappropriate Hox gene expression has been found in many neoplasias including renal, lung, breast, prostate, ovarian, colorectal, colon, and neuroblastoma (1). Several studies have shown that Hox genes are involved in a multistep neoplastic process by regulating cell cycle, angiogenesis and apoptosis (2,3). Expression and function of Hox genes in tumors appears to be tissue specific as no specific expression pattern of the Hox gene across tumors has been found. HoxB7, one of the members of the Hox genes, when overexpressed in multiple subpopulations of hematopoietic progenitors, modulated the proliferative/differentiative processes inducing a prolonged proliferation of these blast cells and granulo-monocytic oriented cells (3). HoxB7 has been shown to be constitutively expressed in both melanoma primary lesions and cell lines (4). Recent studies of homeobox genes in breast cancer cells and primary tumors indicate that they may play a contributory or causal role in tumorigenesis by regulating cell cycle, apoptosis, angiogenesis, and/or metastasis (4). We have, over the past decade, characterized changes in choline phospholipids metabolites with specific molecular alterations (5, 6,7) to build a framework for understanding the role of choline phospholipid metabolism in tumor progression and metastasis. Here we have investigated the effect of HoxB7 overexpression on the choline phospholipids metabolites of the MCF-7 human breast cancer cell line. An understanding of the metabolic effects of Hox B7 gene function would provide an important complement to existing data.

Materials and Methods: Hox B7 overexpressing MCF-7 cells and MCF-7 cells transfected with empty vector were a kind gift from Dr Sara Sukumar, Sydney Kimmel Cancer Center, Johns Hopkins. Real time PCR was carried out to confirm the overexpression of HoxB7 gene in the MCF7 cells. Typically RNA was isolated from the cells. Hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene was used for normalizing for input RNA amount. For high resolution proton NMR spectroscopy studies cells were grown in culture medium to 60–70% confluency before extraction. Cells were extracted with methanol, chloroform, and water (1:1:1, v/v/v). To determine concentrations, peak integration from various metabolites were compared to that of the internal standard TMSP and corrected for cell numbers and cell size. Data were expressed as mean ± SD. Mann Whitney U test was carried out to compare the metabolite levels. $P < 0.05$ was considered statistically significant. Five separate extracts each from HoxB7 overexpressing and empty vector transfected MCF-7 cells were analyzed.

Results and Discussion: Real time PCR showed a significantly higher mRNA expression of HoxB7 between control empty vector and Hox B7 overexpressing MCF7 cells. 1H MRS of cell extracts demonstrated the presence of three water-soluble, choline-containing [-$N(CH_3)_3$] metabolites, i.e., choline, phosphocholine (PCho), and glycerophosphocholine (GPC). These metabolites resonate at ~3.2 ppm downfield of the internal standard and chemical shift reference TMSP. There were no significant differences in these metabolites between Hox B7 MCF7 overexpressing cells and empty vector MCF 7. However, the phosphocholine/glycerophosphocholine (PC/GPC) ratio was significantly higher in Hox B7 overexpressing cells compared to control empty vector MCF 7 cells ($p < 0.02$) (Figure 1 b). Representative spectra from the choline region in control empty vector and HoxB7 overexpressing MCF 7 cells are shown in Figure 1a. A PC/GPC switch has been shown to occur with oncogenic transformation in human mammary epithelial cells (8). The PC/GPC switch in HoxB7 MCF 7 cells observed here is consistent with the role of HoxB7 in increasing the malignancy of these cells.

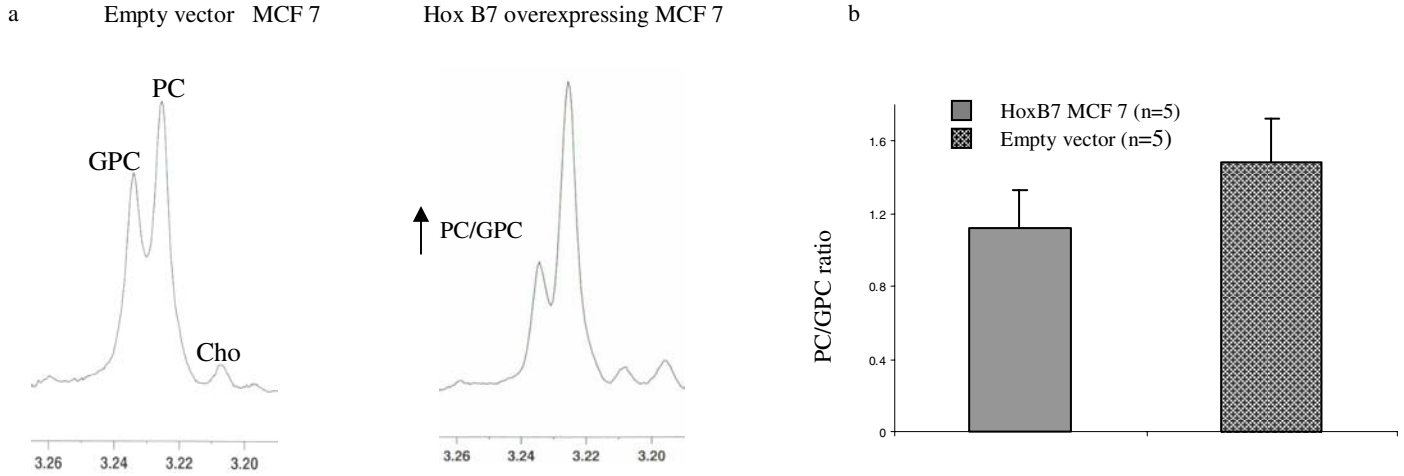

Figure 1: PC/GPC switch in HoxB7 over expressing MCF 7 Breast carcinoma cells

References

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