Genomic expression and biochemical characterization of enzymes contributing to the phosphocholine MRS signal in ovary cancer

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Introduction

Elucidation of the mechanisms responsible for the aberrant phosphatidylcholine (PC) metabolism in epithelial ovarian cancer (EOC) cells may allow identification of novel biomarkers of tumor progression and design of new targeted therapies. Our previous studies showed that the pool of choline-metabolites (tCho) increased from about 2.0 mM in normal ovary surface epithelial (OSE) cells and non-tumoral immortalized cell variants (collectively indicated as EONT cells) to 4.0-7.0 mM in epithelial ovarian cancer cells (EOC) with parallel 3- to 8-fold increases in phosphocholine (PCho), the most abundant tCho component (Iorio et al Cancer Res, 2005, 20: 9369).

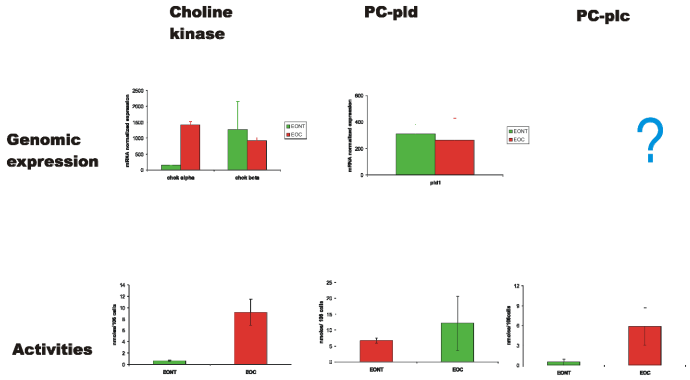
The purpose of this study was to investigate activities and genomic expression of enzymes responsible for PCho accumulation in EOC cells.

Methods

Human EOC cell lines (OVCAR3, IGROV1, SKOV3, CABA1) were established from ascitic fluid or from primary tumors; EONT cells were either isolated from normal ovary surface epithelium (OSE), or immortalized by OSE transfection with SV-40 large T-antigen (IOSE) or by SV-40 large T-antigen *plus* cDNA encoding for human telomerase (hTERT). MRS analyses were performed on cell extracts at 16.4 or 9.4 T. Microarray-based gene expression was evaluated by Gene Set Enrichment Analysis on EOC and EONT data sets, focusing attention on genes involved in choline metabolism.

Results

The observed increases in intracellular tCho and PCho contents in ovarian cancer cells may result from activation of both different biosynthetic and/or catabolic PC-cycle pathways in combination with enhanced choline transport. Genomic analysis showed that: a) choline kinase (chok) alpha was upregulated (about 10 x), while chokβ was practically unaltered in cancer cells; b) of the two cytidylyltransferase (ct) isoforms, ct1α expression decreased by 30% and that of ct1β increased by 38%; c) cholinephosphotranferase (CPT1) increased by 40%. The genomic changes of chok were confirmed by western blot experiments with higher level of expression of chok alpha in EOC. A strong increase (up to 20-fold) in the activity of choline kinase (chok) was measured in EOC with respect to hTERT cells. In the phospholipase-mediated pathways the overall expression of pld family genes decreased by about 20%. In catabolic pathways, a striking increase (up to 17-fold) was only found in the activity of PC-specific phospholipase C (plc), while the activities of phospholipase D (pld) and glycerophosphocholine-phosphodiesterase (GPC-pd) increased at least 2-4 times in some (but not all) EOC cells. Furthermore, regarding PC-plc, for which no genomic information is as yet available, MRS analyses confirmed its role in modulating the PCho profile in cells exposed to PC-plc inhibitor. In fact, exposure of OVCAR3 cells to tricyclodecan-9-yl-potassium xanthate (D609, 24h) led to a decrease (90%) in PC-plc activity, associated with a drop (50%) in PCho content (P = 0.028) and reduced (P = 0.001) cell proliferation, in the absence of apoptosis.



Conclusions

The results show that major contributions to PCho accumulation in EOC cells likely derive from both upregulation/activation of chokα and increase in PC-plc activity. The emerging knowledge of the genetic and molecular regulation of PC-cycle enzymes that are altered in EOC cells may open novel possibilities for novel targeted anticancer therapies.

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