

Phosphatidylcholine-specific phospholipase C contributes to the increase of phosphocholine in ovarian cancer cells

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the leading cause of death in women with gynaecological malignancies. The existing gaps in the knowledge of the molecular mechanisms responsible for EOC progression lead to the current limitations of therapy regimens, mainly restricted to the use of cytotoxic drugs and often associated with side effects and onset of drug resistance. Gene expression and protein activity have consequences on metabolic profiles, whose detection by sufficiently powerful non-invasive molecular imaging approaches may lead to the identification of novel functional biomarkers of in vivo tumor progression and therapy response. Our group recently focused attention on alterations of the total choline containing metabolites (tCho) spectral profile in human EOC cells [1,2], an aspect which received only scarce attention in the past. Quantification of phosphatidylcholine (PC) metabolites in cell extracts showed higher levels of phosphocholine (PCho, 3- to 8 x, $P < 0.0001$) and tCho (2.0 to 4.4 x, $P < 0.0001$) in EOC as compared to non tumoral (normal or immortalized) cells (EONT). Purpose of this work was to investigate the enzyme activities responsible for aberrant choline metabolism in EOC cells, in order to possibly identify novel therapy targets in ovary cancer.

METHODS

Cells: human EOC cell lines established from ascitic fluid or from primary tumors; epithelial ovarian non-tumoral cells (EONT) 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). High resolution MRS analyses were performed on cell extracts at 16.4 or 9.4 T.

RESULTS

The increase in PCho content in cancer cells was associated with activation of enzymes involved in both biosynthetic and catabolic pathways (Figure 1). In particular, we observed a strong increase (up to 20-fold) in the activity of choline kinase (chok) with respect to non tumoral hTERT cells. The two catabolic pathways mediated by phospholipase D (pld) and glycerophosphocholine-phosphodiesterase (GPC-pd) contributing to the direct formation of free choline increased at least 2-4 times in some (but not in all) EOC as compared to EONT cells. This study showed for the first time a striking increase (up to 17-fold) in the activity of PC-specific phospholipase C (plc) in EOC cells. The order of increase in PC-plc activity was comparable to that measured for chok. Exposure of OVCAR3 cells to tricyclodecan-9-yl-potassium xanthate (D609, 24h) led to a decrease in PC-plc activity, associated with a drop in PCho content ($P = 0.028$) and reduced ($P = 0.001$) cell proliferation (Figure 2), in the absence of apoptosis.

CONCLUSIONS

Activation of both biosynthetic and catabolic enzymes occur in the PC-cycle during ovary cancer progression. Evidence of abnormal PC metabolism has implications in cancer biology and may provide an avenue to the development of non-invasive *in vivo* clinical methods (MRS and choline-based PET) for diagnosis and treatment follow-up. PC-plc may represent a novel target for the design of new therapeutic strategies in ovary cancer.

REFERENCES

- 1) Iorio E. et al. *Cancer Res* 2005; 20: 9369-9376
- 2) Podo F et al. *Current Med Imaging Rev* 2007; 3:123-137

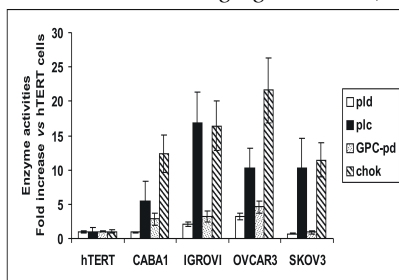


Figure 1

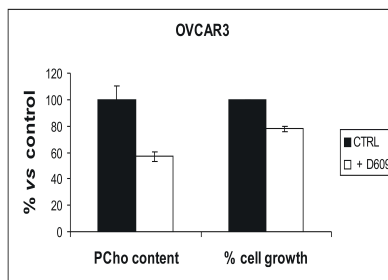


Figure 2

Figure 1 Basal enzyme activities of the biosynthetic and catabolic pathways in the PC cycle in human serous ovarian carcinoma cell lines (CABA1, IGROVI, OVCAR3, SKOV3) as compared to non tumoral hTERT cells.

Figure 2 .Relative quantification of PCho content and percent of cell growth in OVCAR3, exposed to D609 (24h) as compared to untreated cells.