Role of choline kinase and phosphatidylcholine phospholipase C in aberrant choline metabolism in human epithelial ovarian cancer

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Introduction

Detection and characterization by magnetic resonance spectroscopy (MRS) of aberrant phosphatidylcholine (PC) metabolism in tumors fostered in the last decade novel areas of investigation in cancer cell biology and allowed identification of new indicators of in vivo tumor progression by application of choline-based MRS and PET diagnostic approaches. Recent characterization in our laboratories of abnormal PC metabolism in ovarian cancer by MRS showed a significant increase in phosphocholine (PCho) content in epithelial ovarian cancer (EOC) cells compared with non tumoral counterparts (Iorio et al, Cancer Res 2005; Iorio et al, submitted 2009). Elucidation of the molecular mechanisms underlying alterations of PC metabolism may contribute to the identification of new prognostic factors and novel therapeutic targets to improve EOC treatment and follow-up.

Purposes of this study were a) to investigate the molecular mechanisms underlying the aberrant choline metabolism in ovary cancer; b) to evaluate the role of choline kinase (ChoK) and PC-specific phospholipase C (PC-plc) in surgical samples isolated from patients, as a basis for the possible design of new therapies.

Methods

Human EOC cell lines (OVCAR3, IGROVI, CABAI, SKOV3) 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 *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. Western blot experiments were performed by using polyclonal rabbit anti-human Chok antibody [Glunde et al, Cancer Res 2005] and polyclonal rabbit anti-*Bacillus cereus* antibody (Spadaro et al, Cancer Res 2008).

Results and Conclusions

Choline kinase (ChoK) was over-expressed at protein level (about 3x) and activated (9-25 x) in EOC cells. Moreover, the mRNA level of ChoKα isoform was constitutively over-expressed in cancer cells, in the presence of unaltered levels of the others enzymes of the Kennedy pathway. PC-specific phospholipase C (PC-plc) protein was also over-expressed (about 3x) and activated (up to 17-fold) in EOC cells. These results indicate that other factors, likely depending upon oncogene-driven signaling alterations, may influence ChoK and PC-plc activity. Chok down-modulation by RNA silencing reduced PCho level by 70% in OVCAR3 and SKOV3 cells. Since pharmacological inhibition of PC-plc was associated with PCho reduction of about 38% , we could conclude that both ChoK and PC-plc are the major enzymes for PCho accumulation in these cancer cells. Increased ChoKα mRNA, as well as ChoK and PC-plc protein expression, were also detected in surgical specimens isolated from EOC patients.
In conclusion, the peak of PCho provides an endogenous reporter on the activity of these enzymes, and can be used to detect and monitor in ovarian cancer the effects of the downregulation or inhibition of these enzymes as novel possible therapeutic strategies.

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