Detection by 1H MRS of altered phosphatidylcholine metabolism in human ovarian cancer cells

E. Iorio[1], A. Ricci[1], M. Pisanu[1], R. Canese[1], M. Di Vito[1], C. Rozera[1], M. Spada[1], F. Belardelli[1], D. Mezzanzanica[2], S. Canevari[2], and F. Podo[1]

[1]Istituto Superiore di Sanità, Roma, Italy, [2]Istituto Nazionale Tumori, Milano, Italy

INTRODUCTION

Ovarian cancer is portrayed as an insidious disease or silent killer that caused no symptoms and generally can not be recognized clinically until the disease formed widespread metastases within the abdominal cavity. MRS offers powerful approaches to detect metabolic alterations associated with malignant phenotypes of tumor cells and investigate the biochemical pathways responsible for the mechanisms underlying spectral variations. More recent studies in our laboratory have attempted to gather objective evidence regarding the "evolution" of aberrant choline (Cho) phospholipid metabolism in ovary carcinoma (EOC) with respect to epithelial ovarian non-tumoral (EONT) cells (1-3). Our project is therefore aimed at a) evaluating the potential of monitoring by MRS abnormal Cho-phospholipid metabolism in ovary cancer; b) elucidating the biochemical mechanisms responsible for altered phosphatydylcholine (PC) metabolism; c) investigatingthe effect of c-erbB-2/neu overexpression in a ovarian cancer line (SKOV3.ip) both in vitro and in xenografts implanted in immunodeficient mice.

METHODS

Cells: EOC cells lines established from human ascitic or primary tumors; epithelial ovarian non-tumoral cells obtained by transfection of normal ovary surface epithelium with 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 (Bruker AVANCE). ^{1}H NMR assays have been developed in our laboratory to quantify the activity rates of PC-cycle enzymes in cell lysates, by simultaneously measuring precursor and product concentrations in cell-free preparations exposed to the appropriate substrates and cofactors (1-3) Assays on phospholipase-mediated PC hydrolysis were carried out by measuring Cho formation in cell lysates ($CaCl_2$ 10mM, pH 7.2) upon addition of monomeric 1,2-dihexanoyl-sn-glycero-3-phosphocholine (C6PC). The formation of Cho under these experimental conditions was mainly due to phospholipase D (pld) , with a minor contribution by GPC-phosphodiesterase (GPC-pd (Ca)), the final step of the deacylation pathway mediated by phospholipases A and lyso-phospholipase. The activity of pld was, therefore, expressed as a range of minimum and maximum values. The PC phospholipase C (plc) activity was measured by adding alkaline phosphatase to cell lysates in the presence of C6PC. Moreover, the GPC-pd (GPC-pd (Mg)) activity was assayed in the presence of $MgCl_2$ 10mM, pH 7.2 (optimal conditions for this enzyme).

In vivo MRI-guided ^{1}H-MRS analyses were conducted at 4.7 T on a Varian-Inova horizontal bore system. Statistical analyses were performed by ANOVA.

RESULTS

The accumulation of phosphocholine (PCho) and total choline-containing metabolites (tCho) in ovary cancer cells was associated with activation of enzymes involved in both biosynthetic and catabolic pathways (Tab.1).

The choline kinase activity (Chok) increased 12- to 19-fold (P = 0.02) in all EOC cells with respect to hTERT. Regarding PC hydrolysis we observed: a) a strong activation of plc in all investigated ovary carcimoma cell lines (P = 0.02); b) a 2- to 3-fold increase of phospholipase D (pld) in some (IGROV1 and OVCAR3) tumor cells (P < 0.001), but not in CABAI; c) an up to 4-fold increase in GPC-pd(Mg) (P = 0.02).

We established an in vivo passaged SKOV3.ip cell line (a more cancerous variant of SKOV3) from the ascites in injected SCID mice, following the protocol of Yu et al (4). We found that the SKOV3.ip cells contained about 2-fold higher PCho content vs. the parental cell line. The tCho signal detected in SKOV3.ip xenografts represented the highest resonace in vivo ^{1}H MR spectra (Fig. 1).

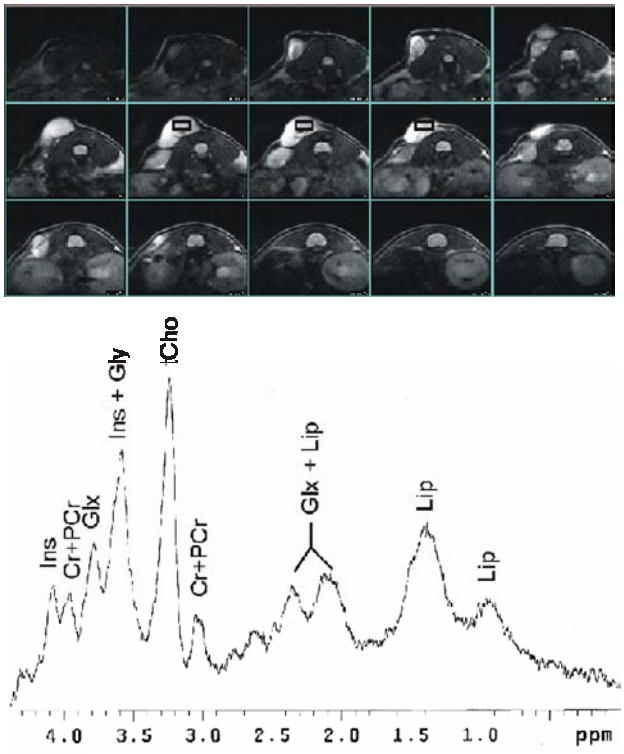

Figure 1. ^{1}H MR images and spectra obtained from tumour xenografts about 30 days after s.c. injection of 5 x 10^6 SKOV3.ip cells. Transverse images were obtained with a gradient echo technique. The ^{1}H MR spectra was collected from the voxel shown as a black rectangular in the images by using a PRESS sequence (TR/TE=1500/23 ms, VOI = 6 µl, 1024 averages).

CONCLUSIONS

PCho and tCho levels increase in human ovary carcinoma cells vs. non-tumoral immortalized ovary surface epithelium cells. Activation of both biosynthetic and catabolic enzymes occur in the PC-cycle during ovary cancer progression. This body of evidence provides grounds for possible further developments of non-invasive *in vivo* clinical methods (MRS and choline-based PET) for diagnosis and treatment follow-up as well as for the design of new therapeutic strategies in ovary cancer

REFERENCES

1) Iorio E. et al. Cancer Res 2005 20: 9369-9376.

2) Iorio E et al. ESMRMB 2006 Warsaw, e-poster 504.

3) Podo F et al. ISMRM Workshop: MR of Cancer, Pocono, 13-16 October 2006.

4) Yu D etal. Cancer Res 1993:891-898.

Table1. Fold-increase of PCho contents (nmoles/10^6 cells) and of mean activity rates of enzymes (nmoles/10^6 cells.h) in EOC vs EONT.

Cells	PCho	ChoK	plc	pld	GPC-pd
hTERT	2.24 ± 0.26	0.64 ± 0.14	0.45 ± 0.29	5.6-6.8 ± 1.2	3.8 ± 0.44
SKOV3	3.6 x	11.6 x	n.d.	n.d.	n.d.
CABA1	6.6 x	16.0 x	11.1 x	0.5-0.9 x	1.6 x
IGROV1	6.1 x	12.3 x	16.8 x	1.9- 2.5 x	3.2 x
OVCAR3	5.5 x	18.7 x	10.3 x	3.2 -4.0 x	4.6 x