Choline phospholipid metabolism in human ovarian tumor progression: a MRS study

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the gynecologic neoplasia with the highest death rate, partly because of limited understanding of its patho-physiology mechanisms. Early diagnosis of EOC is critically important in disease prognosis and patient survival. Non-invasive detection of response to treatment and relapse are crucial to improve patient management. Detection by MRS of abnormal levels of choline (Cho) phospholipid metabolites has recently allowed identification of novel fingerprints of tumor progression in breast, prostate and colon carcinomas and in brain tumors (1, 2). Our project is therefore aimed at evaluating the potential of monitoring by MRS abnormal Cho-phospholipid metabolism in ovary cancer.

METHODS

Cells: EOC cells lines established from human ascitic or primary tumors or EOC cells isolated from patient ascitic exudate; 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 (Bruker AVANCE). In vivo MRI guided ^{1}H-MRS analyses were conducted at 4.7 T on a Varian-Inova horizontal bore system. Confocal laser scanning microscopy (CLSM) analyses were performed on a Leica TCS SP2 AOBS system.

RESULTS

Quantification of PC metabolites in cell extracts showed higher levels of phosphocholine (PCho, 3- to 8 x, $P< 0.0001$) and of total choline-metabolites (Cho, 2.0 to 4.4 x, $P< 0.0001$) in EOC than in EONT cells. The increase in PCho contents in cancer cells was associated with activation of enzymes involved in both biosynthetic and catabolic pathways (12-to 24 x increase in basal choline kinase and an average 3.5-fold increase in choline production by PC degradation in cell lysates).

CLSM analyses showed massive externalization of PC-specific phospholipase C (PC-plc) on the plasma membrane surface of ovary cancer cells (Fig. 1), in agreement with previous studies by our group on other cancer cells types and on mitogen-stimulated fibroblasts (3).

Preliminary in vivo MRI-guided MRS on subcutaneous (s.c.) ovarian tumour models in SCID mice allowed non-invasive detection of a number of metabolites, among which Cho gave a particularly intense signal (Fig. 2). These models will allow further investigations on the significance of Cho signals as possible pharmaco-dynamic end-points.

CONCLUSIONS

PCho and Cho levels increase in the malignant progression of human ovary epithelium cells. Activation of both biosynthetic and catabolic enzymes occur in the PC-cycle during ovary cancer progression (4). The PC-specific plc (whose sub-cellular localization and activity had been previously shown by our group to be sensitive to mitogenic cell stimulation and to oncogenic transformation) was massively externalized on the surface of plasma membrane of EOC cells. 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.

REFERENCES

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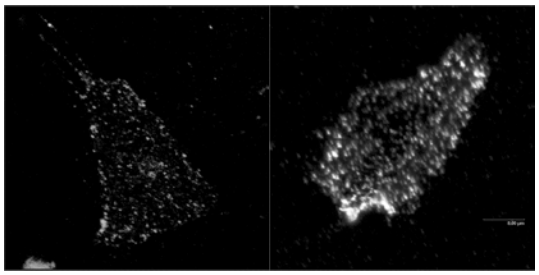

Fig.1 Comparison of PC-plc externalization on plasma membrane of EOC (from patient ascite, *right*) and OSE cells.

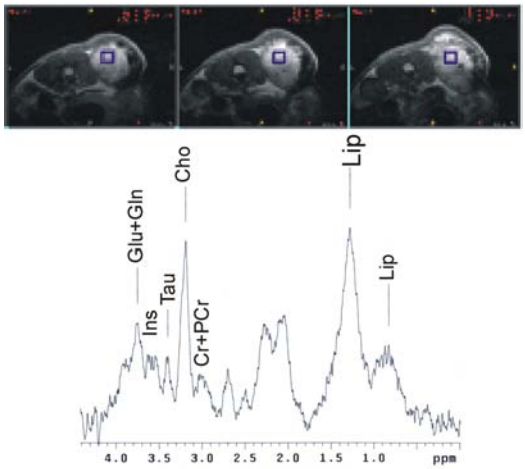

Fig. 2 Localized PRESS spectrum from selected VOI (9.2 µl) in s.c. EOC in SCID mouse.