THE PI3K INHIBITOR LY294002 DOWNREGULATES AKT PHOSPHORYLATION AND REDUCES CELL PROLIFERATION WITHOUT DECREASING THE PHOSPHOCHOLINE LEVEL IN OVARIAN CANCER CELLS

E. Iorio[1], C. Alberti[2], P. Alberti[2], A. Ricci[1], M. Pisanu[1], P. Pinciroli[2], S. Canevari[2], F. Podo[1], and A. Tomasetti[2]
[1]Cell Biology and Neurosciences, Istituto Superiore di Sanità, Roma, RM, Italy, [2]Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

Introduction

Studies have recently been addressed in our laboratories to gather objective evidence regarding the evolution of aberrant phosphatidylcholine (PC) metabolism in the tumor progression from epithelial ovarian non tumoral (EONT) to epithelial ovarian cancer (EOC) cells. In particular, we found a 3- to 8-fold increase in the phosphocholine (PCho) level in EOC compared with EONT cells (Iorio E et al. Cancer Res 2005; 65:9369; Iorio E et al. Cancer Res 2010; 70:2126). Alterations of cell signalling through multiple components of the PI3K/AKT pathway are considered as a hallmark of cancer cells and have been implicated in EOC pathogenesis. Purpose of the present study was to investigate the contribution of PI3K/AKT pathway in the accumulation of PCho content in EOC by using the PI3K inhibitor Ly294002 on human EOC cell lines showing distinct phenotypes.

Methods

We selected three *in vitro* EOC cell lines at different steps of the EMT-like occurring during tumor progression: OAW42 (with an epithelial phenotype), IGROV1 (epithelial-like with unstable adherens junction) and SKOV3 (a more frankly mesenchymal phenotype).
For MRS experiments EOC cells were grown in complete medium or exposed to the PI3K inhibitor LY294002 for up to 24 h. MRS experiments were performed on ethanolic extracts on a Bruker Avance 400 spectrometer using a ^{1}H-X multinuclear inverse probehead.

Results

The selected EOC cell lines showed different sensitivity to increased concentration (0-40 μM) of LY294002 in a proliferation assay (Figure 1). Accordingly, 24 hr PI3K inhibition caused a significant increase in the G1-phase population in IGROV1 and OAW42 cells, with no significant effect on the cell cycle of SKOV3 cells. Indeed, PI3K inhibition was associated with strong and stable decrease in the phosphorylation of the PI3K downstream effector AKT in the LY294002-sensitive IGROV1 but not in resistant SKOV3 cells, while OAW42 cells showed intermediate P-AKT levels (Figure 2).

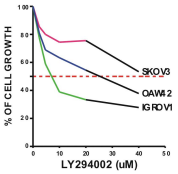

Figure 1

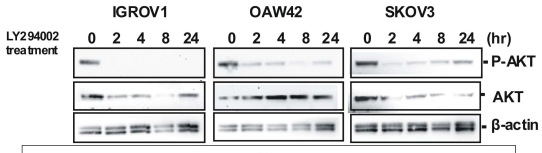

Figure 2

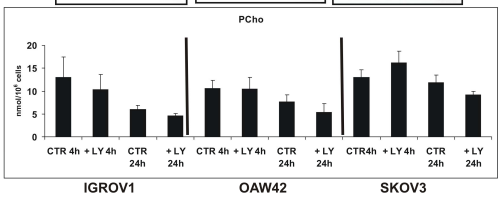

Figure 3

^{1}H MRS quantification revealed no significant changes in the levels of PCho content between untreated (CTR) cells and cells exposed to LY294002 for either 4h or 24h (Figure 3) nor between LY294002-sensitive and LY294002-resistant cells, in spite of the differences in their proliferation activity.

Conclusions

Although the PCho content is significantly increased upon transformation of ovarian cells, the level of this metabolite does not appear to represent a simple indicator of activation of the PI3K/AKT pathway, which is however confirmed to have a pivotal role in the proliferation of EOC cells. A more comprehensive characterization of the network of involved intracellular signaling pathways can probably lead to a better understanding of ovarian epithelial carcinogenesis and clearer elucidation of the molecular mechanisms responsible for MRS-detected changes of PCho levels in EOC cell lines, providing an opportunity to more effectively interfere with signal transduction targets involved in ovarian tumor cell growth, survival, and progression.