

Effects of downmodulation of Choline Kinase on MRS choline profile and transcriptome in ovarian cancer cells

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Introduction

Epithelial Ovarian Cancer (EOC) remains the leading cause of death in women with gynecologic malignancies, due to late diagnosis and early relapse associated with development of chemoresistance. Detection of the abnormal phosphatidylcholine (PC) metabolism in EOC by analysis of magnetic resonance spectroscopy (MRS) profile, showed a significant increase in phosphocholine (PCho) content in EOC cells compared with non tumoral counterparts (Iorio E et al, Cancer Res 2005; 65:9369), associated with an altered activity profile of some PC-cycle enzymes, including 12-to 25-fold activation of choline kinase (ChoK), responsible for PCho production, following choline phosphorylation in the PC biosynthetic Kennedy pathway (Iorio E et al, Cancer Res 2010; 70:2126). The alpha-isoform of ChoK (Chok-alpha) has an essential role in growth control and signal transduction and has been implicated in carcinogenesis. Aims of the present study are to evaluate the biological relevance of ChoK expression and activity in EOC and to define the possible role of MRS profiles in providing non invasive biomarkers to monitor the effectiveness of agents selectively targeted against ChoK-alpha activity.

Methods

To investigate the role of ChoK-alpha in EOC cell growth and tumor progression, we specifically silenced CHKA gene expression by transient RNA interference in two EOC cell lines (SKOV3 and OVCAR3). We then evaluated the main biological effects related to cell cycle regulation, cell proliferation, alterations of global gene expression and changes of the MRS-detected total choline profile. MRS experiments were performed on cell ethanolic extracts using a Bruker Avance 400 spectrometer equipped with a ¹H-X multinuclear inverse probehead.

Results and Discussion

Inhibition of ChoK-alpha mRNA expression was associated with a significant reduction of overall ChoK protein expression and an about 70% drop in PCho content. We observed a 20% inhibition of cell growth associated with a consistent increase in cells blocked in the G1-phase of cell cycle. Comparative evaluation of the global transcriptome, showed 440 genes differentially expressed (FDR<0.25, P<0.05) in CHKA-silenced compared with controls cells, equally distributed among induced and repressed genes. Interestingly, among the most relevant co-repressed genes we found CyclinA1, related to regulation of cell cycle progression and cytokines genes (IL6 and IL8) related to inflammation and EOC aggressiveness, whose functional role is currently under further investigation. Our observations, confirming a main role for ChoKα in deregulated choline metabolism in EOC tumors, warrant further investigations on the upstream and downstream signaling and metabolic alterations associated with ChoK activation and suggest this enzyme molecule as a promising target for alternative therapeutic approaches.

