Epithelial Ovarian Cancer: searching for new modulators of drug resistance

Introduction
Epithelial Ovarian Cancers (EOC) represent the most lethal gynaecological malignancies in the Western world. Most patients (75%) have advanced disease when diagnosed due to the lack of reliable screening tests and specific diagnostic markers. Although many patients respond initially to standard therapy based on platinum drugs, the majority of women will develop drug-resistant recurrences and die in 5 years. Therefore, the identification of novel genes involved in chemo-resistance is mandatory for the design of new therapeutic strategies.

Aim of the work
Alterations of different cellular pathways, such as DNA repair, apoptosis and p53, have been identified as involved in chemoresistance mechanisms. Performing a high-throughput shRNA-based screening, we evaluated cell survival arising from the combination of gene silencing of 680 genes related to these three pathways and platinum drug treatment in different EOC cell lines. Bioinformatics analysis and validation screening unveiled 8 genes which could represent new modulators of platinum drug resistance in EOC. Our aim is to analyze the role of one of the identified genes, SGK2 (serum and glucocorticoid inducible kinase 2), on EOC cell survival and sensitivity to platinum drugs.

Conclusions and applications
SGK2 functions are quite unknown. Some evidences have linked SGK2 to lung cancer and myeloid malignancies, no information about SGK2 and ovarian cancer exists. We have found that SGK2 inhibition (by silencing or pharmacological inhibition) sensitizes EOC cells to platinum treatment, whereas SGK2 overexpression confers higher resistance in vitro and in vivo growth rate and an increased resistance to platinum drugs in vitro, confirming the role of SGK2 in platinum sensitivity and tumor cell growth. The combination of SGK2 silencing/inhibition and platinum drugs may represent a promising strategy to improve the management of EOC patients.

Future perspectives
► Elucidate if the mechanism linking SGK2 to observed synthetic lethality could be connected to autophagy, an interesting pathway for therapeutic intervention.
► Evaluate the effect of GSK + CBDCA treatment in ovarian tumour growth (xenografts) in nude mice.

Figure 1: SGK2 is a member of a family of serine/threonine kinases. The SGK family consists of three distinct but highly homologous genes: SGK1, SGK2 and SGK3. Schematic representation of SGK2 and its isoforms. SGK kinase family is involved in the regulation of cellular metabolism in response to stress stimuli and it is induced by factors such as glucocorticoids and signals activating PI3K (phosphatidylinositol 3-kinase). All SGK isoforms have at least two key regulatory sites, a threonine in the activation loop of the catalytic domain and a serine in the C-terminal hydrophobic domain, both of which require phosphorylation for complete activity.

Figure 2: SGK2 has a role in platinum sensitivity in EOC cells. (A) SGK2 silencing increased sensitivity of MDAB cells to CBDCA treatment. SGK2 PknA unh. did not change the expression of the other two isoforms. (B) OVCA2 cells became more resistant to platinum treatment when exogenous SGK2 was overexpressed.

Figure 3. SGK2-overexpressing OVCA2 cells present an increased in vitro and in vivo growth rate. (A) The growth curve underneath a higher growth rate of SGK2-overexpressing OVCA2 cells respect the control. (B) Preliminary experiment with nude mice inoculated with 2x10^6 cells/tumor. Tumour growth was monitored for a month, measuring tumor volume twice/week. At the end, tumors were removed. (C) When treated with GSK and GSK+CBDCA, SGK2-expressing cells display cytoplasmic vesicles, that seem to be linked to autophagy. (D) Indeed, p62 and LC3, two important autophagy markers, increased with GSK and GSK+CBDCA treatments. This p62 modulation, in particular, was abolished and the ratio LC3II/LC3I was inverted in the control when SGK2 was overexpressed, while p62 increase became more marked (and LC3III followed the same trend of p62) with SGK2 silencing.

Figure 4. The SGK1 (SGK1 kinase inhibitor, GSK650694A, is able to make SGK2-expressing cells (MDA4, TOV21G, TOV112D, GSK3, OVCA4) more sensitive to platinum treatment. The same response is not present in cell lines with no SGK2 expression (CaOVA, COV318, OVA90H, KURAMOCHI) (Data not shown). GSK650694A allowed (also at lower concentrations) to decrease concentration of platinum drug, maintaining the increase of cell sensitivity with combined treatment.

Figure 5. (A) When treated with GSK and GSK+CBDCA, SGK2-expressing cells display cytoplasmic vesicles, that seem to be linked to autophagy. (B) Indeed, p62 and LC3, two important autophagy markers, increased with GSK and GSK+CBDCA treatments. This p62 modulation, in particular, was abolished and the ratio LC3II/LC3I was inverted in the control when SGK2 was overexpressed, while p62 increase became more marked (and LC3III followed the same trend of p62) with SGK2 silencing.

Figure 6. SGK2 interacts with p62. SGK2 (in green) and p62 (in red) co-localized in particular around cytoplasmic vesicles when cells were treated with GSK and GSK+CBDCA, as noticed by Immunofluorescence analysis, and they co-immunoprecipitated (Data not shown).