Evaluation of harvesting protocols for metabolic profiling of epithelial ovarian cancer cells*

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Aim

- Develop a reliable protocol for NMR-based profiling of polar metabolites in tumor cells evaluated based on reproducibility and classification power.
- Apply the protocol to the parental ovarian cancer SKOV-3 cell line and a multiresistant sub-line (SKOV-3-R)[1] to examine resistance-specific metabolic changes.

Background

Epithelial ovarian carcinoma (EOC) is a major cause of cancer death; although treatment may initially be successful, many patients relapse into treatment-resistant disease[2]. Metabolic profiling provides novel tools for analyzing cells and is of great future importance for understanding the connections between metabolic alterations and responses to chemotherapy. Although the use of *in vitro* cell models is emerging in the field of metabolomics, no current "gold standard" exists for the entire workflow. As the purpose of metabolomics investigations is to analyze snapshots of on-going biological processes, this requires optimized protocols for cell harvesting to ensure reproducibility and biologically meaningful data.

Methods

SKOV-3 cells and the resistant sub-line, SKOV-3-R were cultured in cell culture plates and harvested using either a cold methanol or a MilliQ water freeze/thaw protocol (Figure 1). For each cell line, six biological replicates were harvested in parallel. Metabolite extraction was performed through liquid-liquid extraction and the polar phase was evaporated and reconstituted in buffered D_2O prior to NMR analysis on a 600 MHz instrument. Spectra were binned, normalized to total NMR signal, Pareto scaled and analyzed with SIMCA-P+ by supervised and unsupervised methods.

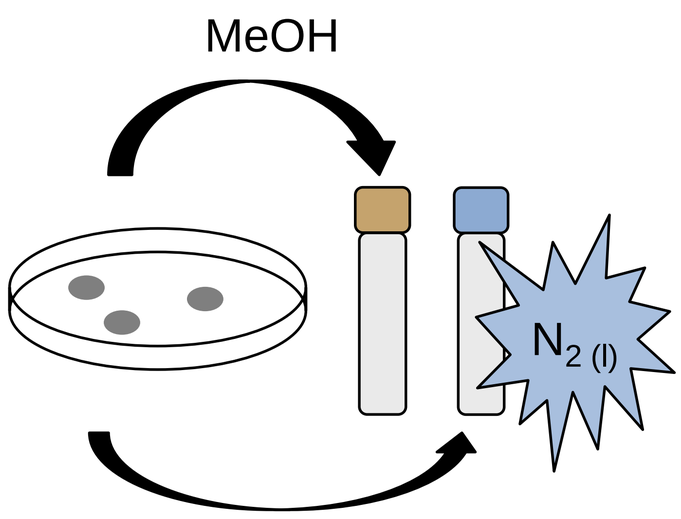

Figure 1. For both harvesting protocols, growth medium was removed, cells were washed and detached using a cell scraper. When MilliQ water was used, the detached cells were collected in cold MilliQ water and snap-frozen in liquid N_2 followed by thawing. The freeze-thaw cycle was then repeated once with subsequent sonication on ice.

1. Wintzell M, Lofstedt L, Johansson J, Pedersen AB, Fuxe J, Shoshan M: Repeated cisplatin treatment can lead to a multiresistant tumor cell population with stem cell features and sensitivity to 3-bromopyruvate. *Cancer Biol Ther* 13(14), 1454-1462 (2012).
2. Landen CN, Jr., Birrer MJ, Sood AK: Early events in the pathogenesis of epithelial ovarian cancer. *J Clin Oncol* 26(6), 995-1005 (2008).
3. Alison MR, Lin WR, Lim SM, Nicholson LJ: Cancer stem cells: in the line of fire. *Cancer Treat Rev* 38(6), 589-598 (2012).
4. Bapat SA, Mali AM, Koppikar CB, Kurrey NK: Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. *Cancer Res* 65(8), 3025-3029 (2005).

Results

- Experimental variability was lower for the harvesting protocol with MilliQ water and freeze/thaw cycles as compared with cold methanol harvesting.
- From a statistical standpoint, both protocols yield highly similar profile differences between the two cell lines, as well as similar profiles within each cell line (Figure 2).
- Compared with the parental cell line, the chemoresistant cell line showed a significantly different profile as shown in Figure 3. This profile is compatible with, and extends, what is known about the metabolism of chemoresistant, *i.e.*, more progressed and possibly tumor-initiating cells[3-4].

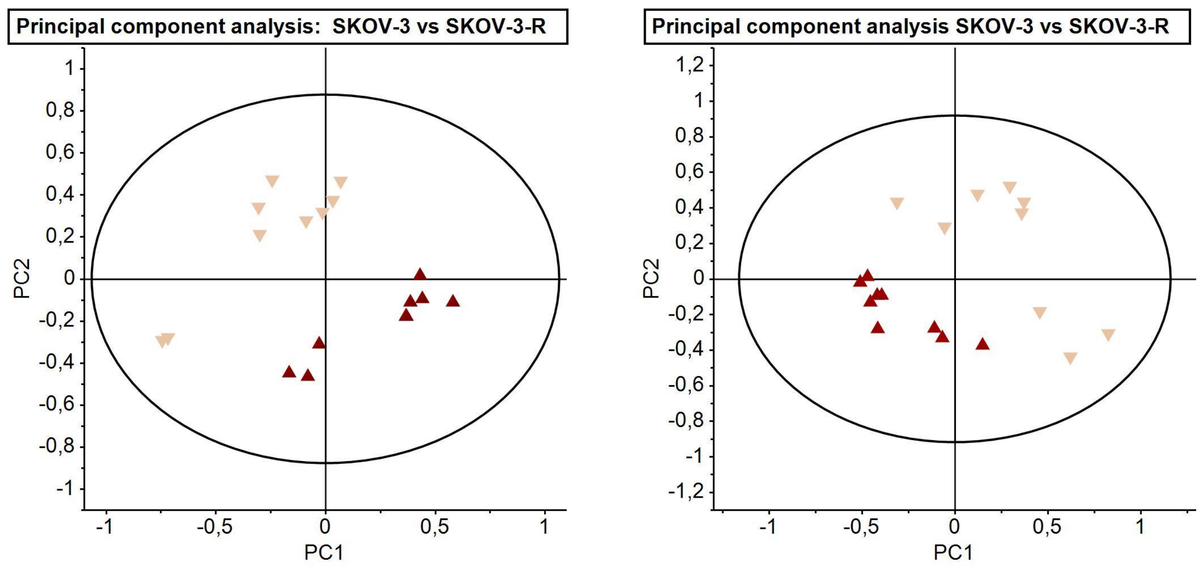

Figure 2. *PCA model of all samples exposed to either the cold methanol (left) (R_2X = 0.656, Q2 = 0.328) or MilliQ freeze/thaw (right) (R_2X = 0.542, Q2 = 0.248) harvesting. Red triangles represents SKOV-3 samples and tan inverted triangles originate from SKOV-3-R samples.*

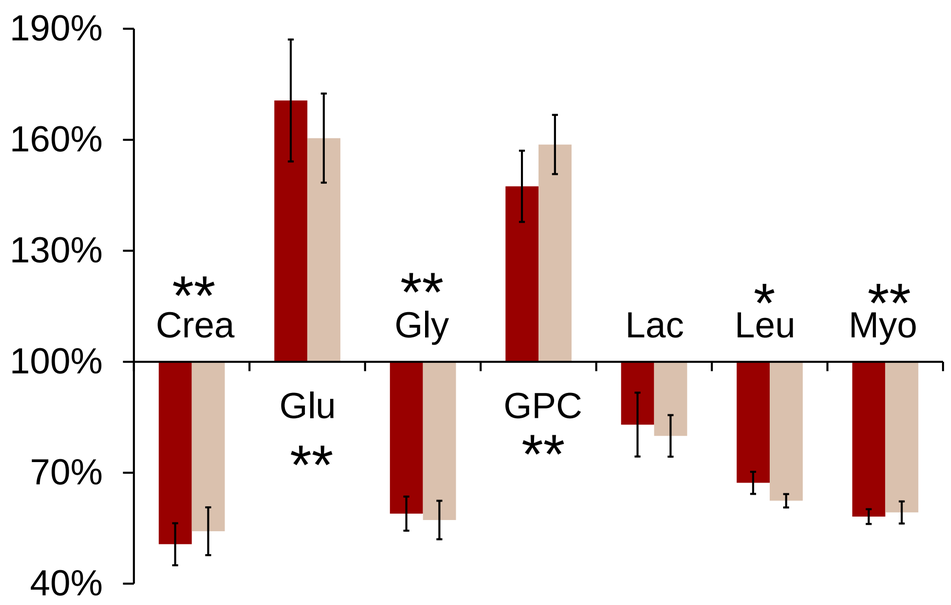

Figure 3. *Relative changes in averaged normalized bin integrals for some metabolites in SKOV-3-R cells compared with SKOV-3 (defined as 100%). Red bars represent the MilliQ water freeze-thaw protocol while methanol harvesting are displayed in tan. Altered metabolites include creatine (Crea), glutamate (Glu), glycine (Gly), glycerophosphocholine (GPC), lactate (Lac), leucine (Leu) and myo-inositol (Myo). * p-value <0.01, ** p-value <0.001.*

Conclusions

The MilliQ water freeze/thaw protocol is preferred over methanol harvesting due to higher reproducibility and increased sample preparation options. The resulting metabolic profiles summarize metabolic alterations in chemoresistant cells consistent with a progressed and aggressive phenotype.

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