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 disease2. 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 D2O 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.

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 cells3-4.

Figure 2. PCA model of all samples exposed to either the cold methanol (left) (R2X = 0.656, Q2 = 0.328) or MilliQ freeze/thaw (right) (R2X = 0.542, Q2 = 0.248) harvesting. Red triangles represent SKOV-3 samples and tan inverted triangles originate from SKOV-3-R samples.

Figure 3. Relative changes in 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.

Conclusion

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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