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Investigating differences in lung cancer cells with induced and de-induced cisplatin resistance by ^{1}H HR-MAS NMR metabolomics

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INTRODUCTION

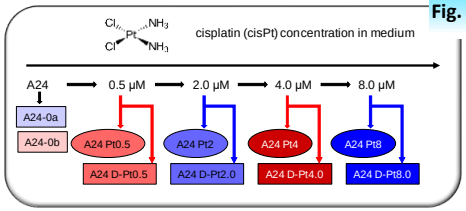
Cisplatin (cisPt)-resistance poses a major clinical problem in the treatment of non-small cell lung cancer (NSCLC).[1, 2] However, the mechanisms accounting for metabolic adaptations in cisPt-resistant cells are not well understood. In cultured NSCLC cells with induced cisPt resistance a long-term resistance is retained after de-induction.[3] High resolution magic angle spinning (HR-MAS) NMR spectroscopy allows to metabolically characterize biological samples like cells or tissue.[4-6]

METHODS

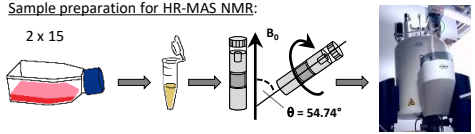
cisPt-resistant cell lines

- Generated from cisPt-sensitive WT-lung adenocarcinoma cells (A24)[3]
- Exposure to stepwise increasing concentrations of cisPt in the culture medium ranging from 0.5µM to 8µM (Fig.1)
- For de-induction cells branched off and grown in the absence of cisPt

Fig. 1



Sample preparation for HR-MAS NMR:



A24 cells/sublines cultured (RPMI 1640) 2 batches (a,b), 2x15 samples

5x10^6 cells Lysed, heated 70°C (20 min)

^{1}H HR-MAS NMR 500 MHz, 3kHz MAS, T=276K 1D-PROJECT[7], TE=400 ms, ns=512 water-presaturation

Data analysis:

- Spectra subdivided into 309 individually sized buckets
- Probabalistic quotient normalization; mean centering, pareto scaling
- Principal Component Ananlysis (PCA) and orthogonal Partial least squares analysis (o-PLS) unsing PLS-Toolbox (Eigenvector)

AIMS

- Systematically investigate metabolic alterations in cultured NSCLC-cells with incremented induced and de-induced cisPt-resistance
- Apply HR-MAS NMR-based metabolomics to study the metabolome using cisPt-sensitive NSCLC-cells (A240286S, A24) as controls
- Identify A24 cell metabolites and potential markers of cisPt-resistance
- Address the question if the maintenance of cisPt-resistance in de-induced NSCLC cells is also reflected in the metabolic profile

RESULTS

Fig. 2

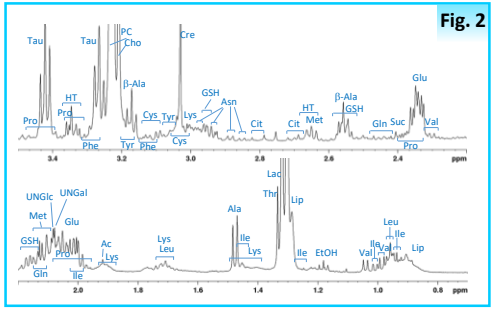


Fig. 2: 1D PROJECT spectrum (excerpt for 0.5-3.5 ppm spectral region)
More than 50 metabolites were assigned including:
Amino acids, peptides, organic acids, amines, choline containing compounds, nucleobases, nucleosides, nucleotides, phosphate sugars and lipids

Fig. 3A: Unsupervised PCA on all samples (30 x 309) demonstrates:

- Close clustering of replicates
- Scores along PC-2 correlate with increasing cisPt resistance
- De-induced samples are close to their induced counterparts
- Clear separation of the two batches along PC-1 (possibly due to passage and media differences)

Fig. 3C: PLS model prediction performs very well

- For all samples, the predicted resistance is close to the measured one

RESULTS

Fig. 3 A - D

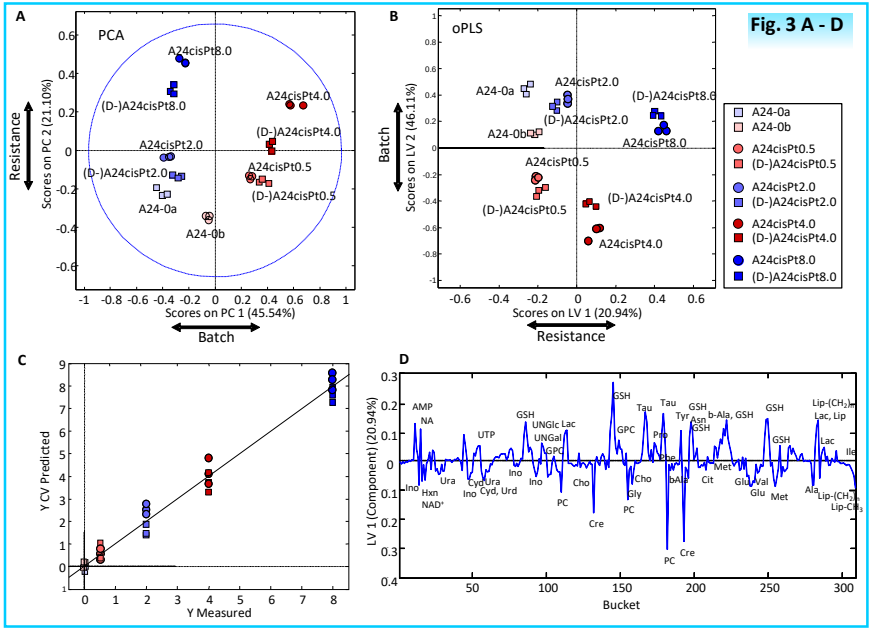


Fig. 3B: oPLS on all samples (30 x 309) demonstrates:

- Close clustering of replicates
- Scores along LV-1 correlate with increasing cisPt resistance
- De-induced samples are metabolically similar to their induced counterparts
- Batches separate along LV-2

Fig. 3D: Loading plot for oPLS LV-1

- Glutathione (GSH) and taurine (Tau) increased in cisPt-resistant cells
- Creatine (Cr) and Phosphocholine (PC) appear reduced

CONCLUSIONS

- cisPt resistance is reflected in metabolic alterations
- GSH and Tau may serve as biomarkers with elevated levels in cisPt resistant cells
- GSH and Tau may function as reactive oxygen species scavenger and for cellular defense (anitapoptotic)[8,9]
- Developed cisPt-resistance is an adaptation that is maintained even after cisPt removal and indicates a metabolic long-term memory of the cells

References: ***1****: Fennell, D.A. et al. Cancer Treat. Rev. 2016, 44, 42–50.* ***2****: Chen, S.H. et al. Int. J. Mol. Sci. 2019, 20, 4136.* ***3****: Ruprecht, N. et al. Pharmaceuticals 2020, 13, 109.* ***4****: Lindon, J.C. et al. Prog Nucl Mag Res Sp. 2009; 55: 79–100.* ***5****: Vermathen, M. et al. PLoS ONE 2015, 10(5): e0128478.* ***6****: Primasovà, H. et al. Metabolites 2019, 9, 146.* ***7****: Aguilar J.A. et al. Chem. Commun. 2012; 48:811-813.* ***8****: Brozovic A. et al. Critical Reviews in Toxicology, 2010; 40(4): 347–359.* ***9****: Sørensen B.H. et al. Am J Physiol Cell Physiol 2014; 307: C1071–C1080.*

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