^{1}H NMR of colorectal cancer tumor progression organoid extracts

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Introduction

Organoids are 3D cellular structures that potentially can bridge the gap between cancer genetics and time consuming patient studies to enable personalised therapy. Tumor organoids can possibly be used to discover biomarkers of malignant transformation and therapy response. Here we studied aqueous extracts of colorectal Tumor Progression Organoids (TPOs, i.e. a model system for the development of cancer) by ^{1}H NMR and show how the metabolism changes upon malignant transformation from healthy colon tissue 'Wild Type' (WT, i.e. TPO0) to TPO4. Eventually, this could lead to suitable biomarkers for early stage monitoring of therapy response in patients by in vivo MRS.

Methods

CRC TPOs (TPO4:APC/KRAS/p53/SMAD4; TPO3: APC/KRAS/p53; TPO2: APC/KRAS; TPO1: APC; TPO0: Wild Type (WT)) were grown in matrigel on expansion medium (37 °C, 5% CO_2, air) until an average size range of 40 < r < 70 μm. Harvesting was done on ice, by suspending the organoids from 2 x 2 growing wells (2 wells per NMR sample, all experiments in duplo) using ice cold normal saline, centrifuging at 1200 rpm and resuspending several times to remove all medium and matrigel. Metabolites of organoids were extracted using the Folch method and sonication. The extracts were dried using a speedvac at 40 °C and stored at -80 °C until NMR measurement. Prior to ^{1}H NMR measurement, samples were dissolved in 50 mM phosphate buffer with 10% D_2O and 0.004 mM trimethylsilylpropanoic acid TSP as a chemical shift reference. A 1D gradient pulsed NOESY sequence with interscan delay of 5 s, using spectral width of 12 ppm and acquisition time of 4s was used to measure the samples on a Bruker 600 MHz Avance III NMR system at 298K. Spectra were processed and metabolites quantified after signal deconvolution using Chenomx software.

Results & Discussion

In figure 1 results are shown for several metabolites that are involved in phospholipid metabolism, glycolysis and glutaminolysis as a function of TPO mutation grade. Levels of total choline (tCHo), phosphocholine (PC), phosphoethanolamine (PE), ethanolamine (Eth), glutamate and glutamine are significantly higher in the TPOs than in WT organoids. Counterintuitively, the amount of lactate decreases in these organoids going from WT to TPO4, which is probably caused by accumulation of lactate in the lumen of WT and TPO1, while the TPOs with more mutations are more solid and have smaller lumen.

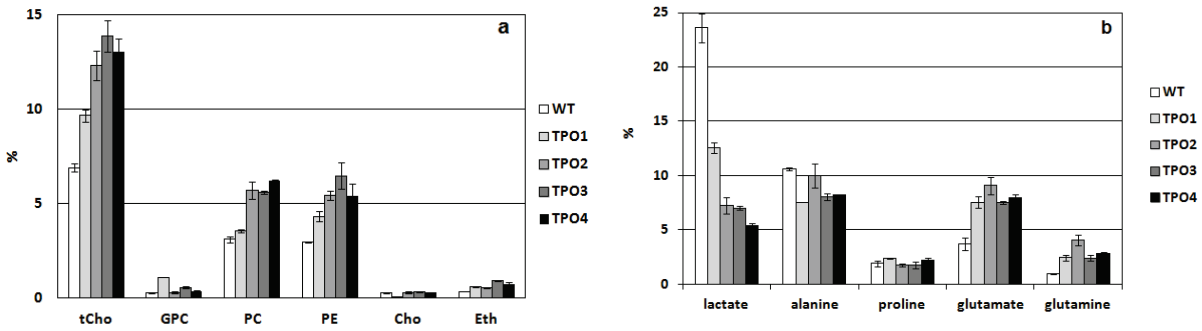

Figure 1. Relative concentration of metabolites expressed as % of total metabolite concentration for:
a. Metabolites involved in phospholipid metabolism; **b.** Metabolites related to glycolysis and glutaminolysis.

Conclusion

Our ^{1}H NMR study of CRC TPOs clearly indicates several biomarkers for malignant transformation in colorectal organoids. These biomarkers have also been observed previously in various cancer types within cell lines, animals and humans, confirming the potential usage of tumor organoids for cancer diagnostics. Next we will study TPOs grown on differentiation medium and the response of patient-derived tumor organoids to chemotherapy by ^{1}H NMR.