Phospholipid metabolism, but not energy metabolism is affected by expression of the multidrug resistance transporter ABCB5 in G3361 melanoma stem cells

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Target audience

Purpose
Cancer stem cells (CSCs), also known as tumor-initiating cells, have been identified in several human malignancies, including melanoma. We recently found that the ATP-binding cassette (ABC) transporter ABCB5, a cell-surface marker for malignant melanoma initiating cells (MMICs) 1, is functionally required for melanoma growth. 2 CSC typically reside in hypoxic niches of tumors. 3 Hyoxia-inducible factors (HIFs) regulate cellular biochemistry, notably energy and membrane phospholipid (PL) metabolism. 4 This prompted us to investigate whether these and other metabolic pathways are directly altered by ABCB5 expression, i.e. if the functional role of ABCB5 may include ‘priming’ specific metabolic processes essential to melanoma cell survival and proliferation.

Methods
In this pilot study, we employed metabolomic high-resolution NMR spectroscopy of melanoma cell extracts to quantify a broad range of metabolites as a function of ABCB5 expression. Two groups of G3361 melanoma cells with or without ABCB5 expression (n=4 each) were analyzed for differences in metabolite profiles, with an emphasis on energy and PL metabolism. The ABCB5-negative variant had been generated by stable ABCB5 knockdown of wild-type G3361 cells. For each analysis, about 3x10^6 cells were cultured to near confluency in flasks containing RPMI 1640 medium (Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum (Gibco Life Technologies, Carlsbad, USA) under standard conditions (37 °C, 5% CO2 atmosphere). Cells were quickly rinsed with ice-cold saline and frozen by pouring liquid nitrogen into each flask. Upon nitrogen evaporation, 4 mL ice-cold methanol was added, which froze rapidly to the flask bottom. Cells were scratched into the methanol as it began to thaw. The melted mixture was transferred to a glass tube and 4 mL chloroform was added, followed by 4 mL water. Phase separation, NMR sample preparation, and high-resolution 1H and 31P NMR spectroscopy at 9.4 T (Avance 400 and TopSpin software, Bruker, Rheinstetten, Germany) closely followed a previously published protocol. 6 Total protein content was determined by the bicinchoninic acid (BCA) assay. Absolute metabolite concentrations were expressed as nmole/mg protein, and relative concentrations as % of total phosphate (totP) or total proton (totH, the water resonance being excluded). Significance of differences between the two G3361 melanoma groups was determined by using the non-parametric Mann-Whitney test (Prism software, GraphPad, La Jolla, USA).

Results
A broad range of phosphorylated metabolites were detected by 31P NMR spectroscopy, and were quantitated by referencing to methylene diphosphonate (MDP) contained in an insert in the NMR sample tube (Figure 1). Relative metabolite concentrations resulted in somewhat lower standard deviations than absolute concentrations because the latter are influenced by errors in total protein determination. However, both methods consistently yielded major increases in phosphodiester (PDE) levels for ABCB5+ vs. ABCB5− G3361 melanoma cells. The PDEs, glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE), were twice as concentrated in ABCB5+ as in ABCB5− cells (Table 1, Figure 2; means ± s.e.). These differences were clearly significant (p < 0.05) for relative concentrations, and very close to significance (p = 0.057) for absolute concentrations. Glycerophosphoglycerol (GPG), a PDE generally occurring at much lower quantities 7, was quantifiable for the ABCB5+ group, but was below detection threshold for all ABCB5− samples. Phosphocholine (PC), high-energy (NTP, PCr and UDP-hexoses) and low-energy (P) metabolite levels were very similar between ABCB5+ and ABCB5− cells (p > 0.2). Evaluation of further metabolites based on 1H NMR spectra of our cell extracts is currently underway, as is the analysis of PLs based on 31P NMR spectra of the organic phase of these extracts.

Discussion
Our pilot study shows that under normoxic conditions, the PDE levels of G3361 melanoma cells are significantly increased by expression of the MMIC marker ABCB5. PDEs are formed by PL degradation through phospholipases involved in membrane PL turnover. In agreement with a mechanism described elsewhere 8, enhanced PDE levels may indicate that PL homeostasis is ‘primed’ by ABCB5 to facilitate membrane PL synthesis under conditions where CSCs thrive, e.g. under hypoxia. Indeed, hypoxia has been shown to increase the expression of a PL metabolism enzyme that is linked to cell survival and proliferation (choline kinase). 3

Conclusion
Expression of the MMIC marker ABCB5 modifies PL metabolism of G3361 melanoma cells in a characteristic manner under normoxic conditions. Future studies will show whether this finding is positively linked to facilitated PC formation, membrane PL synthesis and cell proliferation under hypoxic conditions, for these and other cancer cells. If our hypothesis is confirmed, PL homeostasis should be considered as an additional putative target for overcoming CSC resistance to treatment.

References