Metabolomic study of AKT activation

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Introduction: AKT (protein kinase B) is a downstream effector of the phosphatidylinositol-3-kinase (PI3K) signalling pathway, which plays important roles in cell proliferation, cell growth and protecting cells from apoptosis. It is also involved in mediating the effects of insulin, such as lipogensis, glucose uptake and conversion of glucose into fatty acids and cholesterol. Constitutive activation of the PI3K/AKT pathway is found in many tumour types. The aim of this study is to examine the metabolic changes that are associated with AKT activation. A human retinoic pigment epithelial (RPE) cell line that expresses an inducible version of AKT (in which AKT can be constitutively activated by the addition of 4-hyroxy-tamoxifen) was examined.

Methods: RPE cells were treated with either 100nM 4-hyroxy-tamoxifen or vehicle (ethanol) for 48 hours. The control and AKT-activated RPE cells were harvested and extracted using a dual phase extraction method. High resolution NMR was performed on both lipid and water-soluble metabolites. The culture media from both cell types were also collected and examined using high-resolution NMR.

Results: Increased levels of many lipid (Fig. 1) and water-soluble (Fig. 2) metabolites were found in AKT-activated RPE cells when compared with controls. Increased uptake and production of several metabolites (Fig. 3) were also observed in the culture media of the AKT-activated cells when compared with controls.

Discussion: AKT activation induces increased levels of intracellular amino acids, lactate, phospholipid metabolites and adenosine-containing compounds and causes increased uptake of glucose, amino acids and free choline and production of lactate. These metabolic changes indicate that AKT activation stimulates glucose transport, glycolysis, protein synthesis and membrane turnover. AKT-activated cells also express lipogenic enzymes via activation of SREBP *[Porstmann et al, Oncogene 24: 6465-6481 (2005)]* which causes the accumulation of cellular fatty acids and membrane phospholipids. Hence, our findings are consistent with the important roles that AKT plays in cell growth and proliferation.

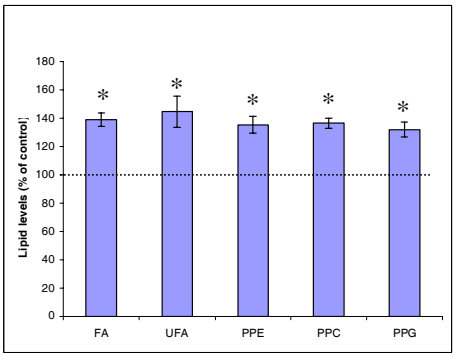

Fig. 1: Lipid metabolites (% to control) in AKT-activated RPE cells.
* significantly different from controls, $p \leq 0.05$.
FA: saturated fatty acids; UFA: unsaturated fatty acids;
PPC: phosphatidylcholine; PPE: phosphatidylethanolamine;
PPG: phosphatidylglycerol

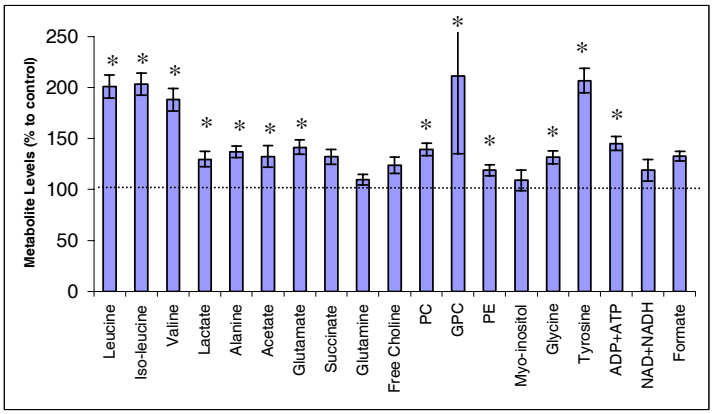

Fig. 2: Water-soluble metabolites (% to control) in AKT-activated RPE cells.
* significantly different from controls, $p \leq 0.05$.
PC: phosphocholine; GPC: glycerophosphocholine;
PE: phosphoethanolamine.

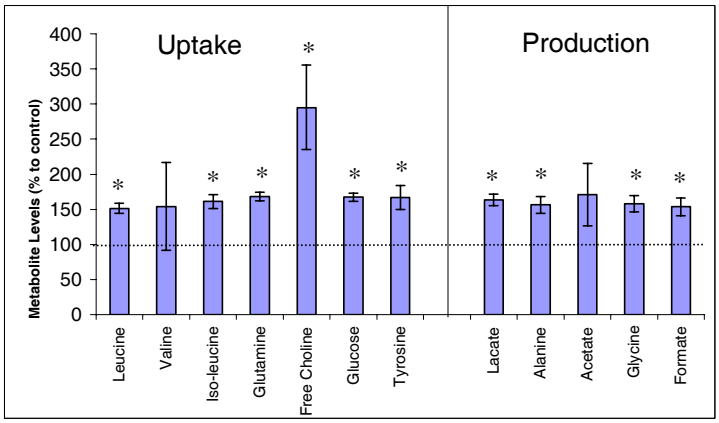

Fig. 3: Metabolites uptake and production (% to control) in AKT-activated RPE cells.
* significantly different from controls, $p \leq 0.05$.

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