

The effect of aminoxyacetate on metabolism of breast cancer cells

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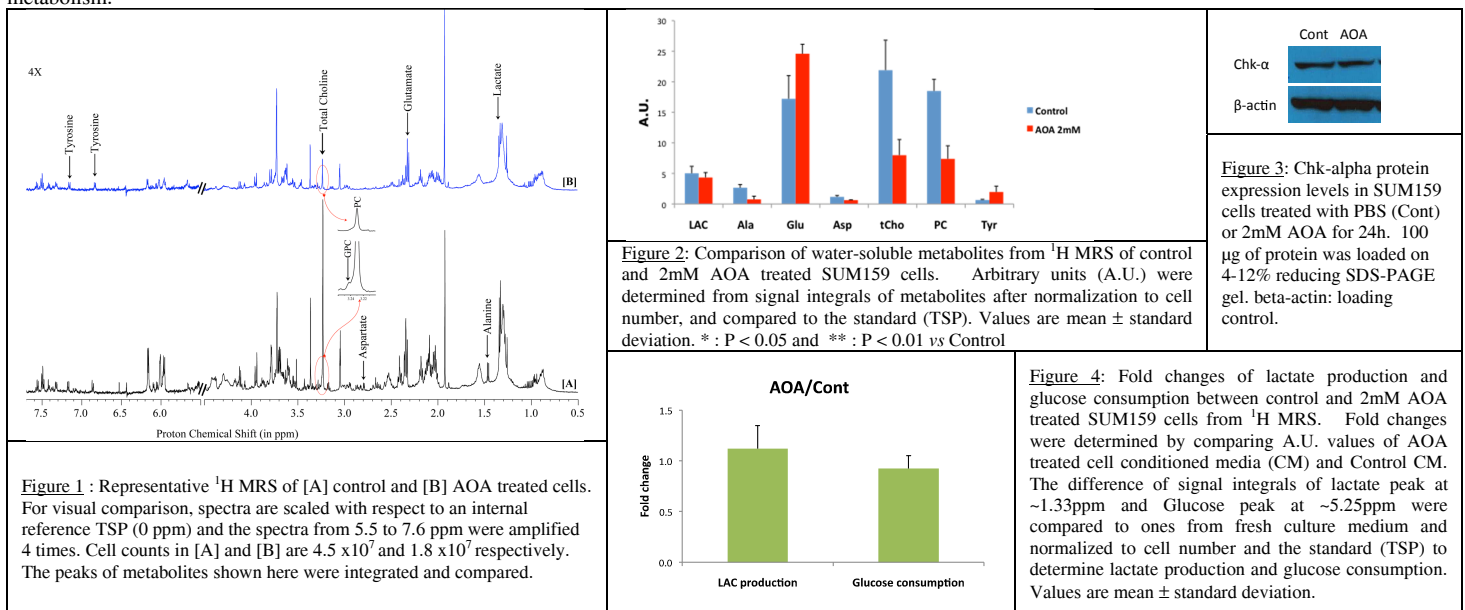
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Introduction: Triple negative breast cancer, defined by the lack of estrogen, progesterone and HER2 receptors, currently lacks effective targeted therapies. Unlike normal cells, cancer cells are exquisitely dependent on alternative pathways to generate energy and the building blocks for growth and cell division. This provides us with the opportunity to target those pathways to specifically “starve” cancer cells. Besides depending on glucose as a source for energy production, cancer cells are also addicted to the amino acid, glutamine. This alternative molecule is likely used by cancer cells to promote the efficient use of an abundant, easily available resource for energy and macromolecular synthesis. In this way, *metabolic reprogramming* aberrantly signals cell to proliferate and provides molecular building blocks for cellular replication [1]. Aminoxyacetate (AOA) is a small molecule inhibitor of the glutaminolytic pathway, which inhibits the entry of glutamine and aspartic acid into the TCA cycle. In this study we evaluated the effect of AOA on several intracellular metabolite levels including phosphocholine (PC) by ¹H magnetic resonance spectroscopy (MRS). Choline kinase (Chk) is the initial enzyme that catalyzes the transfer of a phosphate group from ATP to choline with magnesium as cofactor to PC in Phosphatidylcholine (PtdCho) biosynthesis. High levels of Chk-alpha and PC are closely related to malignant transformation, invasion, and metastasis [2-4]. PC is a precursor for PtdCho, the major phospholipid in eukaryotic membranes that plays a critical role in membrane structure and cell signaling.

Methods. Cell culture and treatment: SUM159 cells were grown in DMEM/F-12 (1:1) medium with 5% FBS, 5 µg/ml insulin, and 0.5 µg/ml hydrocortisone. Cells were treated with PBS (vehicle control) or 2mM AOA (Sigma) in culture media for 24 h. **NMR study:** Cells were collected and water-soluble as well as lipid extracts were obtained from vehicle control, and 2mM AOA treated cells using the dual-phase extraction method [5]. Fully relaxed ¹H MRS of the water-soluble phase was performed on a Bruker Avance 500 spectrometer. Signal integrals of lactate (LAC), alanine (Ala), glutamate (Glu), aspartate (Asp), total choline (tCho: PC + glycerophosphocholine (GPC) + free choline), and tyrosine (Tyr) in cell extracts, as shown in Figure 1, were determined and normalized to cell number, and compared to the standard (TSP). To determine the lactate production and glucose consumption in SUM159 cells, conditioned media (CM) were collected before and after 24h treatment, lyophilized and dissolved in D₂O. ¹H MRS was performed. **Immunoblot analysis:** 100µg of protein from cell lysates at 24 h post-treatment was resolved on 4-12% acrylamide gel using a polyclonal Chk-alpha antibody (custom made) and a monoclonal beta-actin antibody (Sigma).

Results and Discussion: ¹H MRS results from cell extracts showed significant reductions of Ala, Asp, tCho, and PC and a significant increase of Glu after the treatment with 2mM AOA for 24h (Figure 2). Although not statistically significant, an almost 3 fold increase of Tyr was also observed. Asp and Glu are involved in the reversible reaction of transaminases between TCA cycle metabolites, oxaloacetate and ketoglutarate respectively. Tyr is related to fumarate synthesis and tyrosine transaminase that produces p-hydroxyphenylpyruvate is inhibited by AOA. Ala is produced by alanine transaminase from pyruvate. These transamination reactions are inhibited by AOA. AOA treatment did not change Lac in cell extracts. PC and tCho were reduced significantly after AOA treatment (Figure 2). Chk-alpha protein levels did not change after 24h treatment with AOA (Figure 3). ¹H MRS of CM showed Lac production and glucose consumption did not change significantly with AOA treatment (Figure 4). Our results here showed that levels of metabolites that are related to the TCA cycle and phosphatidylcholine biosynthesis were affected by treatment with 2mM AOA in SUM159 cells. Chk-alpha protein that catalyzes choline to form PC was not affected by AOA, so that the decreased levels of PC and tCho are not due to the inhibition of Chk-alpha protein synthesis by AOA. Further investigation is necessary to understand the mechanism of AOA-induced changes in metabolism.



References and Acknowledgements: [1] DeBerardinis *et al*, *Cell Metab*, 7, 11 (2008); [2] Aboagye E *et al*, *Cancer Res*, 59, 80 (1999); [3] Ackerstaff E *et al*, *Cancer Res*, 61, 3599 (2001); [4] Ramirez de M *et al*, *Oncogene*, 21,4317 (2002); [5] Glunde K *et al*, *Cancer Res*, 65, (2005). This work was supported by the Susan Komen Foundation and NIH P50CA103175.