Activity-based proteomic profiling reveals reduction of lipid hydrolase activity levels in non-small cell lung cancer tumors

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Lipids are crucial for maintenance of membrane homeostasis and many other functions of healthy cells and sufficient supply of lipids is especially important for cells that are rapidly proliferating. Cancer cells frequently show increased uptake of lipids as well as increased rate of lipogenesis, making lipid metabolism a promising therapeutic target in cancer. More recently, accumulation of neutral lipids like triacylglycerol and cholesterol esters in lipid droplets has evolved as a hallmark of aggressive cancers. In addition to increased uptake and biosynthesis of lipids, decrease in intracellular lipid hydrolysis contributes to lipid droplet accumulation. However, the role of lipid hydrolases in the context of cancer is less widely explored. By employing state-of-the-art mass spectrometry techniques, we aimed to explore lipid hydrolysis in non-small cell lung cancer (NSCLC) on the proteome, lipidome and serine hydrolase activity level. Tumors and adjacent (healthy) tissue of NSCLC patients were collected and subjected to in-depth proteomics, lipidomics and activity-based proteomic profiling (ABPP). Tissues were collected immediately after tumor resection and flash frozen, or in the case of ABPP immediately incubated with a small serine hydrolase-specific probe that is recognized by active serine hydrolases. Labeling active serine hydrolases with the probe allowed us to enrich active enzymes belonging to this class of enzymes (including lipases) and subsequently quantify the abundance of active enzymes. By ABPP we identified a number of serine hydrolases with higher activities (fold change >1.5) in healthy tissue, including monoclonal lipase (MGL), neuropathy target esterase (PNPLA6), epoxide hydrolase (EPHX1), neutral cholesterol ester hydrolase (NCEH1) and liver carboxylesterase 1 (CES1). Proteomics analysis revealed that several of those lipid hydrolases as well as a number of fatty acid binding proteins are also higher in abundance in the healthy tissue. Furthermore, a number of proposed PPARalpha targets are downregulated in tumor tissue, and lipidomics analysis on the same tissues exposed significant triacylglyceride accumulation as well as higher levels of ceramides and lysosphatidylcholines in tumors. Collectively, these data highlight the implication of lipid hydrolysis in NSCLC, and suggest that lung cancer cells shut down lipid catabolism pathways contributing to the observed changes in the lipidome.

Proteomic identification of novel kinase pathways in synaptic plasticity

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Long term potentiation (LTP) is a fundamental property of neurons that refers to the increase instrength and efficacy of synaptic connections in response to neuronal activity. LTP is critically involved not only in learning and memory but also during the activity-dependent developmental phases of neural circuit formation and refinement. While the role of calcium/calmodulin-dependent protein kinase II (CaMKII) in induction of LTP is well understood, mechanisms that are important for activity dependent neuronal development are not well studied. Using an unbiased proteomic approach, we identify novel kinase signaling pathways in a chemical-LTP paradigm in rat hippocampal neurons. Chemical-LTP was induced on day 18 in vitro rat hippocampal neuronal cultures using Tetra Ethyl Ammonium (TEA). Protein lysates from neuronal cultures before induction of LTP (pre) and at two distinct times 5min (early) and 20min (late) post LTP induction were obtained. To map temporally which proteins were phosphorylated during our LTP paradigm, we used phosphopeptide enrichment and mass spectrometry to identify phosphoproteins that significantly changed at distinct time points. We identified 9,468 phosphosites from 3,082 phosphoproteins. Strikingly, a larger proportion of the significantly different phosphoprotein fraction post LTP (p<0.05) displayed an overall increase in phosphorylation. GO analysis of phosphoproteins associated with LTP indicates a significant enrichment for proteins involved largely in the regulation of synaptic functions like endosomal transport and recycling, axonogenesis, and endomembrane system organization. Out of the differentially phosphorylated kinases during LTP, novel kinases to be identified through our study include Brsk1, Brsk2, Bmpr2, Taok1, Dclk1, Aak1, Brsk2 and Abl2. Two phosphorylation sites within kinases that involve kinase activity include T464 at Brsk2 and S9 at TAOK1. Further we found that the consensus motifs derived from the phosphosites of the differentially phosphorylated proteins exhibit a proline directed serine phosphorylation pattern. In summary, we performed an unbiased analysis of the phosphoproteome derived from rat hippocampal cultures pre and post chem-LTP induction. For future studies we would like to conduct functional characterization of the Brsk2 and TAOK1 and its mutants to further identify its role in neurodevelopmental disorders.