Two-dimensional Liquid Chromatography Coupled to Mass Spectrometry for Mapping LKB1 Dependent Signaling Networks in Non-small Cell Lung Cancer

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Liver kinase B1 (LKB1) is a tumor suppressor gene encodes for a serine-threonine protein kinase (STK11/LKB1), and is a key regulator in cell proliferation, polarity, apoptosis, chromatin regulation, and a checkpoint for metabolic stress.[1] Somatic mutations of LKB1 lead to loss of its expression in cells[2] and is found in certain types of cancer including 30-40% non-small cell lung cancer (NSCLC), a leading cause of cancer mortality. The metabolic signaling of LKB1 mainly transduces through phosphorylation of the protein AMP-activated protein kinase (AMPK).[1] This generates a cascade of phosphorylation events in AMPK-pathway members that signals for activation or deactivation of cellular events. Understanding the molecular biology of this complex signaling will facilitate the knowledge of LKB1 as a tumor suppressor. Further, the identification of the changes of phosphorylation events associated with these proteins will reveal potential biomarkers and drug targets for NSCLC. This study is conducted as a multiomics study. Two-dimensional liquid chromatography coupled to mass spectrometry (2D LC-MS/MS) is used as the proteomics platform in protein identification. It is the goal of the study to identify signature phosphorylation events by label-free phosphoproteomics. The extent of phosphorylation, and its location on a protein will be used to distinguish differences, and to establish the pattern of these signaling events in LKB1 related carcinogenesis.

To study LKB1 signaling events we have used two LKB1-deficient cell lines, HCC15 and A549. These cell lines were transduced with a retrovirus carrying wild-type LKB1 (LKB1-WT). Control cells were prepared by transducing the cell lines with a retrovirus containing vector alone (Vector) and a retrovirus containing a kinase dead version of LKB1 (LKB1-KD)..Cell lysates were prepared by sonication, and the supernatant containing soluble proteins collected by

centrifugation. The proteins in each sample were subjected to trypsin digestion in a mild detergent-containing buffer at pH 8 and the phosphopeptides collected by enriching digested, desalted samples, using TiO_2 packed columns. The enriched samples were analyzed by 2D LC-MS/MS. The samples were fractionated online (reverse-phase/reverse-phase) at pH 10 into fifteen fractions by gradually elevating the percent organic level of the gradient in a C18 column. The separated peptides were nano-electrosprayed into the mass spectrometer, fragmented by collision-induced dissociation in an LTQ orbitarp elite (Thermo scientific) and identified with Thermo Proteome Discoverer software using SEQUEST search algorithm. Carbamidomethylation, oxidation, deamidation, phosphorylation at serine, threonine and tyrosine were used as modifications. The extent of each phosphorylation event was statistically determined by analyzing normalized spectral counts of each peptide/protein.

In this phosphoproteomics analysis of LKB1, we discovered 3430 protein groups, 10457 peptides and 8002 different phosphopeptides in all samples. These peptides contain mono-, di-, and tri- phosphorylation sites. More than 8000 phosphorylation sites are phosphor-serine, and over 6000 are phosphor-threonine. Tyrosine has the lowest frequency of phosphorylation with approximately 150 phosphorylation sites per cell line. We detect a single phosphorylation site in LKB1 at serine-31, which has been detected in different tumor types including NSCLC tumors by other research groups.[3] Furthermore, AMPK protein and its direct targets show significant differences in phosphorylated residues. AMPKα has different phosphor-serine residues in Vector, LKB1-KD and LKB1-WT samples. AMPKβ gives identical phosphor-serine residues in Vector and LKB1-KD compared to LKB1-WT indicating the changes in signaling pathway induced by the kinase activity of LKB1. In addition, some phosphorylation patterns are the same for LKB1-WT and LKB1-KD when compared to Vector control, implying functions of LKB1 that do not require kinase activity. Direct targets of AMPK pathway such as 6-phosphofructo-2-kinase show significant differences in phosphorylated residues in LKB1-WT confirming the involvement of AMPK-pathway with active LKB1. The most significant changes in phosphorylation are in proteins that are active in cell cycle control, chromatin regulation, and remodeling of epithelial junctions. More importantly, these phosphoproteins confirms the activation of AMPK pathway-related and non-related proteins associated with LKB1 expression revealing some novel links in phosphoprotein network in NSCLC.

References

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