New method for detection of phosphoproteins reveals regulator of melanoma invasion

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Scientists have developed a new approach for surveying phosphorylation, a process that is regulated by critical cell signaling pathways and regulates several key cellular signaling events. The research, published by Cell Press in the April 10th issue of the journal *Molecular Cell*, describes the regulation of a previously uncharacterized protein and demonstrates that it plays an important role in cancer cell invasion.

Many cancers, including melanoma, are associated with mutations in the gene encoding the protein kinase B-Raf. Kinases are proteins that regulate the function of other proteins by attaching a phosphate group to them. B-RAF mutations often lead to dysregulation of protein phosphorylation by the mitogen-activated protein (MAP) kinase signaling pathway. Identification and characterization of MAP kinase target proteins is critical for understanding the mechanisms involved in cancer progression.

"In contrast to targets regulated at the level of gene expression, little is known about how proteins are modified in response to oncogenic B-Raf signaling in melanoma cells. In particular, identifying cellular targets for phosphorylation is needed to gain a more comprehensive understanding of the responses to MAP kinase pathway dysregulation in melanoma," explains senior study author Dr. Natalie G. Ahn from the Department of Chemistry and Biochemistry at the University of Colorado and the Howard Hughes Medical Institute.

Current strategies to identify phosphorylated proteins require

purification techniques to enrich phosphorylated from non-phosphorylated proteins and metabolic labeling procedures to quantify changes in phosphorylation. Unfortunately, these methods are not readily applied to all sample types. Dr. Ahn and colleagues developed a method for analyzing phosphorylated proteins in human cell extracts that does not depend on enrichment and can be performed quantitatively in a label-free manner.

Using their method, the researchers identified ninety phosphorylation events that were regulated by oncogenic B-Raf. The phosphorylated proteins included many known signaling molecules. However, one of the targets, MINERVA/FAM129B, belonged to a protein family with unknown function. Further investigation established a role for MAP kinase-dependent phosphorylation of MINERVA/FAM129B in cancer cell invasion within a three dimensional extracellular matrix environment.

"Our results revealed successful selection and sequencing of phosphopeptides in proteolytic digests without affinity enrichment, as well as label-free quantitation of regulated protein phosphorylation events," concludes Dr. Ahn. "Further, we demonstrated pathway-dependent phosphorylation of FAM129B and discovered its importance in controlling melanoma cell invasion."

Source: Cell Press (news : web)