

Identification of nuclear substrates of Akt/PKB by functional proteomics: prohibitin 2 is a target of Akt phosphorylation in human promyelocytic leukemia cells

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The serine/threonine protein kinase Akt is a major signal transducer of the phosphoinositide 3-kinase (PI 3-K) pathway in all cells and tissues and plays a pivotal role in the maintenance of cellular processes including cell growth, proliferation, survival, metabolism and development of many malignancies including acute myeloid leukemia. The frequent aberrant activation of the PI 3-K/Akt pathway in human cancer has made it an attractive therapeutic target. Therefore, the study of effector proteins downstream of Akt could clarify the role of Akt in the development of myeloid leukemia. Although both localization and activity of Akt in the nuclear compartment are well documented, most Akt substrates identified so far are located in the cytoplasm, while nuclear substrates have remained elusive. In this study, we applied a proteomic approach to identify novel Akt substrates by using an antibody that recognized a consensus motif phosphorylated by Akt (K/RXK/RXXS/T) when phosphorylated on S/T (anti-phospho-Akt substrate antibody). NB4 cells were treated with ATRA, and the putative Akt substrate proteins were isolated by immunoprecipitation with the anti-phospho-Akt substrate antibody. The proteins were separated on SDS-PAGE and analyzed by ESI-Q-TOF mass spectrometry.

This analysis indicated prohibitin 2, a potential tumor suppressor protein with potent transcriptional functions in the nucleus, as a putative substrate of Akt in the nucleus of NB4 cells. The putative Akt-Prohibitin 2 interaction was validated by reverse in vivo immunoprecipitation from nuclear protein of NB4 cells. In vitro phosphorylation of endogenous prohibitin 2 by recombinant Akt further validated this result.

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