Ph.D.Thesis of Salahaddin University-Erbil Academic Staff Studied Abroad

Title of thesis: ***Role of AKT/PKB and 14-3-3 in the Regulation of B Cell Receptor Signaling and Signalosome Assembly***

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Summary(Abstract):

AKT/PKB is an oncogenic serine/threonine kinase regulated via the PI3K pathways. 14-3-3s represent a large group of adaptor proteins that are known to interact with a plethora of signaling proteins and regulate diverse signal transduction pathways. The B-cell antigen receptor (BCR) activation and signalosome assembly are dynamic processes controlled by protein phosphorylation. The signaling events share their functions in controlling cell proliferation, differentiation, and/or apoptosis.

In Paper I, the first characterized protein is 14-3-3ζ, which was found to be a new regulator of BTK. Two 14-3-3ζ binding-sites were found to be phosphorylated by AKT/PKB and mapped to phospho-serine pSer51 in the PH domain and to phospho-threonine pThr495 in the kinase-domain. The PI3K inhibitor LY294002 abolished S51/T495 phosphorylation and disrupted the interaction. Moreover, inhibitors targeting 14-3-3 (BV02) and BTK (Ibrutinib) compromised interaction between the two proteins. Nuclear translocation of BTK was promoted following down regulation of 14-3-3ζ. Furthermore, the loss-of-function mutant S51A/T495A displayed reduced tyrosine-phosphorylation and inability to bind to 14-3-3ζ. Conversely, the gain-of-function mutant S51D/T495D exhibited intense phosphorylation, enhancing interaction of BTK with 14-3-3 ζ. Phosphorylation of this mutant was associated with ubiquitination and degradation of the protein, presumably, contributing to the termination of the B-cell receptor signaling.

In Paper II, we identified a new BTK-partner, ankyrin repeat domain 54 protein (ANKRD54) that binds to the BTK SH3 domain. Our results suggest that ANKRD54 specifically mediates nuclear export of both BTK and another TEC family kinase member, TXK/RLK. The interaction site was mapped to the Cterminus of the BTK SH3 domain, since a synthetic peptide covering this region, ARDKNGQEGYIPSNYVTEAEDS, was sufficient for mediating this interaction. ANKRD54 is the first protein reported to specifically influence nucleo-cytoplasmic shuttling of BTK. ANKRD54 probably belongs to a novel group of proteins carrying out this activity in a Crm1-dependent manner.

In Paper III, using proteomics, we identified 446 proteins, containing 186 novel AKT-associated-motif (RXRXXS/T) phosphorylation events. B-cell receptor induction leads to up regulation of 85 proteins and down regulation of 277 proteins. Proteins related to ribosome biogenesis, DNA binding, transcription and translation regulation were mainly up regulated. Conversely, down regulated proteins were mainly involved in RNA binding, mRNA splicing and mRNP export. Immunoblotting of two proteins RBM25 and MEF-2D were positively validated in the mass spectrometry data. Consistent with these findings, the AKT-inhibitor (MK-2206) remarkably reduced phosphorylation of the target proteins on the RXRXXpS/T motif, while the mTORC2-inhibitor (PP242) totally blocked this phosphorylation.

In Paper IV, we found that AKT/PKB induces BLNK and SYK phosphorylation, which promotes the 14-3-3 binding in a Ser/Thr phosphorylation-dependent manner. Using an in vitro phosphorylation screening assay, we identified BLNK and SYK as excellent substrates of AKT. Moreover, the AKT/PKB inhibitor MK2206 reduced phosphorylation of BLNK and SYK. Additionally, 14-3-3 regulates the stable interaction between SYK and BLNK and sustains phosphorylation of SYK and BLNK. Furthermore, 14-3-3 compromises binding of SYK to Importin 7 thereby abrogating shuttling of the protein to the nucleus. Alanine substitutions of T256, S295 or S297 sites resulted in abrogation of SYK binding to Importin 7. Interestingly, BLNK phosphorylation at Y84 appears to correlate with the degree of tyrosine phosphorylation of SYK at position(s) Y525/526.

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