The adaptor protein APPL2 inhibits insulin-stimulated glucose uptake through both Akt and its downstream target TBC1D1 in skeletal muscle

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Introduction: APPL1 and APPL2 are the two intracellular adaptor proteins containing a PH domain, a PTB domain, and a Leucine zipper motif. Mounting evidence demonstrates that APPL1 is a positive regulator of insulin sensitivity by acting as a common relay of both adiponectin and insulin signalling pathways. However, the cellular functions of APPL2 and its relationships with APPL1 remain poorly understood. The objective of this study was to investigate the molecular relationships of APPL1 and APPL2 in insulin-mediated glucose uptake in skeletal muscle cells.

Methods: Proteins physically associated with APPL1 or APPL2 are retained by affinity purification and co-immunoprecipitation, followed by mass spectrometry–based proteomic identification. The effects of APPL1 and APPL2 in regulating insulin signaling are measured by Akt phosphorylation and in-vitro or ex-vivo glucose uptake assay.

Results: APPL1 potentiates, but the Bar domain of APPL2 inhibits insulin-stimulated phosphorylation of the protein kinase Akt and subsequent glucose uptake in both skeletal muscles isolated from the transgenic mice and cultured myotubes. Proteomic analysis demonstrates that APPL2 but not APPL1 interacts with TBC1D1, which is a key player for contraction and insulin-stimulated glucose transport in skeletal muscle. Moreover, insulin stimulates the disassociation of APPL1 and APPL2 heterodimers, but facilitates the interaction of TBC1D1 with APPL2 through Akt activation. Furthermore, insulin-evoked binding of APPL2 with TBC1D1 on Serine 229 suppresses phosphorylation of TBC1D1 on Threonine 590, leading to further suppression of glucose uptake.

Conclusion: APPL1 and APPL2 act as a pair of 'Yin-and-Yang' regulators of insulin signalling and glucose uptake in skeletal muscle. APPL2 inhibits insulin-stimulated glucose uptake through its dual effects via the Bar domain on both APPL1 and TBC1D1. These findings shed new light on our understanding of the molecular mechanisms for the metabolic actions of insulin in skeletal muscle.

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