Ser-660 Phosphorylation of Protein Kinase C beta II (PKCβII) by mammalian target of rapamycin complex 2 (mTORC2) regulates High Glucose (HG)-induced Mesangial Cell hypertrophy

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Protein kinase C beta II (PKCβII) has been implicated in Diabetic Nephropathy (DN). Mesangial cell (MC) hypertrophy is a pathologic feature of DN. PKCβII undergoes phosphorylation at the hydrophobic motif site Ser-660 for its activity. We have shown that mTOR Complex 1 (C1) regulates MC hypertrophy. How activation of PKCβII by Ser-660 phosphorylation fits into mTOR signaling to control MC hypertrophy is not known. HG significantly increased phosphorylation of PKCβII at Ser-660 in a PI 3 kinase-dependent manner. siRNAs against PKCβII, dominant negative PKCβII and nonphosphorylatable mutant of PKCβII, PKCβIIS660A, blocked mTORC1 activity due to lack of PRAS40 phosphorylation, resulting in significant inhibition of HG-induced MC protein synthesis and hypertrophy. Also, PKCβIIS660A attenuated phosphorylation of Akt at Ser-473, a putative mTOR complex 2 (C2) site. Specific inhibition of mTORC2 by shRNAs against rictor or Sin1, two exclusive and required components for its activity, suppressed HG-induced phosphorylation of PKCβII Ser-660 and Akt Ser-473, resulting in attenuation of mTORC1 activity leading to inhibition of MC hypertrophy. Also, PKCβIIS660A reversed phosphorylation of Akt Ser-473 by shRictor- or shSin1-mediated inhibition of HG-induced Akt Ser-473 phosphorylation. Furthermore, CA PKCβII reversed the shRictor- or shSin1-mediated inhibition of HG-stimulated Akt Ser-473 phosphorylation and MC hypertrophy. Finally, we show increased phosphorylation of PKCβII Ser660, PRAS40 and Akt Ser-473 in association with activation of mTORC1 in renal cortices of OVE26 mice with type 1 diabetes. These results provide the first evidence that HG-induced activation of mTORC2 phosphorylates and activates PKCβII to increase the phosphorylation of Akt at Ser-473 to finally activate mTORC1 to induce MC hypertrophy. Thus, we uncover a specific role of mTORC2 for Akt/mTORC1 activation via PKCβII Ser-660 phosphorylation.

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