Identification of the degradation determinants of insulin receptor substrate 1 for signaling cullin-RING E3 ubiquitin ligase 7-mediated ubiquitination.

Abstract:
Negative feedback regulation of insulin signaling involves ubiquitin-dependent degradation of insulin receptor substrate 1 (IRS1). Cullin-RING E3 ubiquitin ligase 7 (CRL7) mediates the ubiquitination of IRS1 in hyperphosphorylated form. Multisite IRS1 phosphorylation triggers interactions with CRL7 for ubiquitin modification. Insulin signaling is self-restrained when its downstream effector kinases are hyperactivated to trigger the negative feedback inhibition. Hyperactivation of mechanistic target of rapamycin complex 1 (mTORC1) and its effector kinase S6 kinase 1 (S6K1) is known to trigger multisite seryl phosphorylation of insulin receptor substrate 1 (IRS1), leading to its ubiquitination and degradation. This negative feedback inhibition functions to restrain PI3K activity and plays critical roles in the pathogenesis of cancer and type II diabetes. Recent work has implicated a role for cullin-RING E3 ubiquitin ligase 7 (CRL7) in targeting IRS1 for mTORC1/S6K1-dependent degradation. In the present study we have employed both cell-based degradation and reconstituted ubiquitination approaches to define molecular features associated with IRS1 critical for CRL7-mediated ubiquitination and degradation. We have mapped IRS1 degradation signal sequence to its N-terminal 574 amino acid residues, of which the integrity of Ser-307/Ser-312 and Ser-527, each
constituting a S6K1 phosphorylation consensus site, was indispensible for supporting CRL7-forced degradation. In vitro, S6K1 was able to support the ubiquitination of bacterially expressed IRS1 N-terminal fragment by CRL7 but at low levels. In contrast, CRL7 supported efficient ubiquitination of IRS1 N-terminal fragment in hyperphosphorylated form, which was isolated from infected insect cells, suggesting requirement of additional phosphorylation by kinases yet to be identified. Finally, removal of IRS1 amino acids 1-260 led to substantial reduction of ubiquitination efficiency, suggesting a role for this region in mediating productive interactions with CRL7. The requirement of multisite phosphorylation and the N terminus of IRS1 for its turnover may ensure that complete IRS1 degradation occurs only when mTORC1 and S6K1 reach exceedingly high levels.

Zeitschriftentitel / Abkürzung: J Biol Chem

Jahr: 2012
Band: 287
Heft / Issue: 48
Seiten: 40758-66
Sprache: eng
Print-ISSN: 0021-9258
TUM Einrichtung: r Pharmakologie und Toxikologie

Occurences: Einrichtungen > Fakultäten > Fakultät für Medizin > Kliniken und Institute > Institut für Pharmakologie und Toxikologie > 2012

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