S636, S666 AND S736 PHOSPHORYLATIONS OF INSULIN RECEPTOR SUBSTRATE 1 (IRS1) DECREASE THE VIABILITY OF HELA CELLS VIA INHIBITING TYROSINE PHOSPHORYLATION OF IRS1

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Insulin receptor substrat 1 (IRS1) protein is the main adaptor molecule of the Insulin (IR) and Insulin like Growth Factor Receptor (IGFR) signaling. Upon insulin binding, insulin receptor is autophophorylated and IRS1 binds to IR. IR-bound IRS1 is phosphorylated by IR. Tyrosine phosphorylation of IRS1 triggers the insulin cascade and it activates the main downstream targets, AKT and ERK proteins. IRS1 signaling is regulated by post-translational modifications. Although, tyrosine phosphorylation of IRS1 positively regulates the signaling pathways, Serine/Threonine phosphorylation is generally inhibits. Increased expression/activation of IRS1 is associated with cell proliferation and survival. Therefore, understanding the effects of posttranslational modifications of IRS1 in cancer is important. In this study we aimed to determine the effects of S/T phosphorylations on viabilityof HeLa cells. In this respect we converted Ser616, S636, S666 and S736 residues in human IRS1 to Alanine and Glutamic Acid with side directed mutagenesis by using pcDNA3.1flag-tagged-human-IRS1 expression vector as a template. All mutants were confirmed by DNA sequencing. We transfected HeLa cells with mutant IRS1 vectors. After serum starvation, HeLa cells treated with insulin for 30 minutes and we determined the phosphorylation levels of IRS1 and ERK1/2 in all groups. Viability levels of all groups were determined by MTT analysis. MTT analysis showed that ectopic expressions of S636A, S666A and S736A mutants increased the cell viability howeverthe viability levels of glutamic acid mutants of these groupsdecreased. We observed thattyrosine phosphorylation of IRS1 and activation of ERK1/2 significantly increased in HeLa cells that expressed Alanine mutants of IRS1 compare to WT-IRS1 without insulin treatment. After insulin treatment, IRS1 tyrosine phosphorylation seven and three fold increased in S666A, S636A and S736A respectively compare to WT-IRS1 expressed cells. Tyrosine phosphorylation levels of S636E, S666E and S736E expressed cells decreased compare to Alanine mutants. Although we did not observe significant changes in ERK phosphorylation in S736A compare to WT-IRS1, phosphorylation of ERK decreased in S636A and S636 and S666A after insulin treatment. Interestingly, S616A did not show any significantchanges in activations of IRS1 and ERK1/2 compare to WT-IRS1 but tyrosine phosphorylation of IRS1 and ERK activation significantly increased in S616E expressed HeLa cells compare to WT-IRS1 without insulin treatment. Our results showed that posttranslational modifications of IRS1 has a pivotal role in cell viability. S/T phosphorylations inhibits the cell viability depends on the phosphorylation motif sites through triggering the different signaling pathways.

Keywords - Insulin signaling, IRS1, HeLa, IGFR