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Minireview

Molecular mechanisms of insulin receptor substrate protein-mediated modulation of insulin signalling

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Abstract The insulin receptor substrate (IRS) proteins act as important mediators of insulin action. Their regulation serves to augment the specificity of the insulin signalling cascade. They can be regulated – both positively and negatively – at the level of phosphorylation, and signalling through these proteins can be further modulated through the actions of SOCS (suppressor of cytokine signalling) proteins. Understanding the mechanisms of IRS regulation will provide further insight into the pathophysiology of insulin resistance and type 2 diabetes.
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Key words: Insulin; Signalling; IRS; SOCS; Phosphorylation

1. Introduction

Classical receptor tyrosine kinase (RTK) signalling is initiated by ligand-induced receptor dimerisation and autophosphorylation. Phosphorylation of tyrosine residues within a specific consensus sequence of the receptors was early recognised to provide docking sites for Src homology 2 (SH2) domain-containing signalling proteins such as PI3K (phosphatidylinositol-3 kinase), PLCγ, RasGAP, Grb2, and others. This mode of signal transmission is utilised by several receptors, including EGFR, PDGFR, FGFR, the Met receptor and Trk. In addition, signalling from the above receptors is in part mediated by recruitment of docking proteins, including FRS2α and β, Gab1 and 2, and LAT. The signalling output is thus determined by the recruitment of signalling proteins to both the receptor and the various docking proteins [1]. However, not all receptors conform to this mechanism. Although the insulin and insulin-like growth factor receptors (IR, IGFR) belong to the RTK superfamily, signal transmission by these receptors mainly occurs via the insulin receptor substrate (IRS) adapter proteins (IRS-1 to -4). These adapter proteins bind to the transphosphorylated activated receptors at tyrosine docking sites, are themselves phosphorylated, and in turn recruit SH2 domain-containing signalling molecules to form the productive signalling complex [2].

The involvement of the IRS proteins in insulin and IGF receptor signalling provides possibly tighter regulation of the final response. Thus, while regulation of the signal initiated by the EGF and PDGF-receptors is mainly modulated by the opposite action of the respective agonist versus phosphotyrosine phosphatases (terminating the phosphotyrosine–SH2 domain interaction), internalisation/recycling of the receptor and in part downregulation of the receptor's tyrosine kinase activity by serine phosphorylation [3], supplementary mechanisms have been reported to act upon the control of IRS molecules. Most notably, serine/threonine (S/T) phosphorylation mediated by several protein kinases has been shown to modulate – both positively and negatively – signal transmission via IRS, while interaction with SOCS (suppressor of cytokine signalling) proteins provides a further mechanism of attenuating IRS signalling. Understanding how the action of IRS molecules is coordinated during insulin signalling is of particular interest as deregulation of these molecular events has been demonstrated to be associated with (and even causative of) insulin resistance and type 2 diabetes. We review here the recent progress in the understanding of the molecular mechanisms modulating the action of IRS proteins.

2. Modulation of IRS protein action via serine/threonine phosphorylation

2.1. Negative modulation

Initial observations indicated that treatment of isolated soleus muscle and 3T3 adipocytes with okadaic acid (a general inhibitor of serine/threonine phosphatases) reduced insulin-stimulated, IRS-1-associated PI3K activity and glucose transport while not affecting the receptor's activity [4]. Moreover, co-expression in IR-expressing CHO cells of the phospholipid- and calcium-activated protein kinase C interfered with insulin signalling by inhibiting tyrosine phosphorylation of IRS-1 and its subsequent binding to PI3K [5]. These data provided the first indication that increased S/T phosphorylation of IRS proteins could affect insulin action. Subsequently, the observation that tumour necrosis factor α (TNFα) and chronic hyperinsulinemia – two inducers of insulin resistance – trigger increased S/T phosphorylation of IRS-1 and IRS-2 provided a clue to a molecular basis of insulin resistance.

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Abbreviations: RTK, receptor tyrosine kinase; SH2, Src homology 2; IRS, insulin receptor substrate; IR, insulin receptor; IGF-1R, insulin-like growth factor-1 receptor; PI3K, phosphatidylinositol-3 kinase; AMPK, AMP-activated protein kinase; PKB, protein kinase B; SOCS, suppressor of cytokine signalling; TNF, tumour necrosis factor

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The uncoupling of IRS proteins from productive insulin signalling has been shown to depend on various mechanisms, most likely acting in a concerted manner. It has been shown that: (i) elevated S/T phosphorylation of IRS-1 and -2 inhibits their binding to the juxtamembrane region of the activated IR, impairing their ability to undergo tyrosine phosphorylation [6]; (ii) specific phosphorylation of serine and threonine residues adjacent to YxxM SH2 domain-binding motifs decreases the binding affinity of the cognate molecule to the phosphotyrosine [7]; and (iii) S/T phosphorylation commits both IRS-1 and -2 to proteasomal degradation [8].

The search for the kinase(s) responsible for mediating IRS protein S/T phosphorylation has been the focus of several laboratories. Based on the fact that high IRS S/T phosphorylation leads to an insulin-resistant state, inhibition of such kinase(s) would provide a possible means to reduce insulin resistance. However, as detailed in Table 1, there are several kinases that have been described to be potentially capable of IRS-1 phosphorylation. Some of these actions are yet to be confirmed in vivo, for example direct phosphorylation of IRS-1 by PI3K in vivo has not been demonstrated. Given the number of IRS-1-directed kinases, it is likely that each kinase can partially contribute to the overall S/T phosphorylation status of IRS-1, with the relative importance of each kinase determined by its expression level in a given tissue, the cell type, as well as its degree of activation (dependent on the extracellular insulin-resistance-inducing stimuli, such as hyperinsulinemia, TNFα or other cytokines, glycated molecules, fatty acids, etc.).

2.2. Positive modulation

While a broad consensus has emerged over the past 10 years regarding the negative regulatory function of S/T phosphorylation of IRS proteins, more recent data indicate that a certain phosphoserine and phosphothreonine content on particular residues is necessary for a functional IRS protein. In fact, in quiescent cells, basally phosphorylated IRS-1 and -2 display a certain degree of S/T phosphorylation and are readily tyrosine-phosphorylated by IR or IGF-1R upon stimulation with the respective agonist. However, in an in vitro system, fully dephosphorylated recombinant IRS-1 and IRS-2 undergo defective IR-mediated tyrosine phosphorylation, indicating that a basal S/T phosphorylation level is necessary and promotes subsequent tyrosine phosphorylation by the receptor [9]. In keeping with this observation are two recent reports demonstrating that AMP-activated protein kinase (AMPK) (by phosphorylating S789) and protein kinase B (PKB) (by phosphorylating serines 265, 302, 325 and 358) exert a positive regulatory role by increasing the IRS-1-associated PI3K activity and tyrosine phosphorylation after insulin stimulation, respectively [6,10]. In Fig. 1, we summarise the mode of activation and positive versus negative modulatory action of the various IRS kinases described to date.

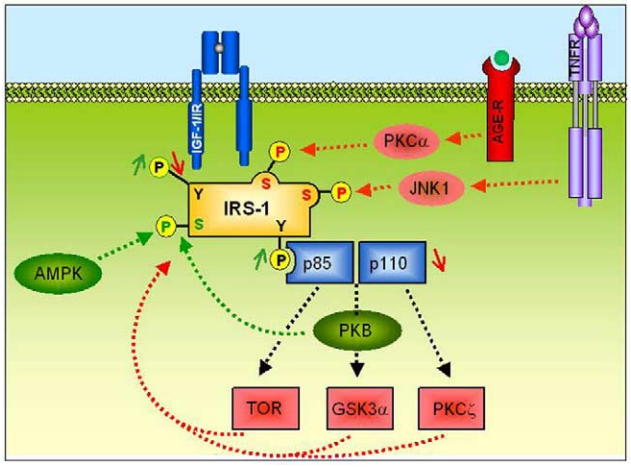

Fig. 1. Modulation by S/T phosphorylation of the signalling potential of IRS-1. Tyrosine-phosphorylated IRS-1 recruits heterodimeric p85/p110 PI3K (and other signalling proteins such as Grb2, PLCγ, SHP2, rasGAP, not shown here). IRS-1 tyrosine phosphorylation and association with PI3K is positively regulated by AMPK and PKB-mediated S/T phosphorylation (green arrows), negative regulation is mediated by PKCα and ζ, JNK1, TOR and GSK-3 (red arrows).

3. Animal models

Most of the research related to the identification of IRS-1 S/T kinases has been performed in cell culture systems which are more amenable to biochemical investigation than in vivo models. The ensuing challenge is to define the precise role that

Table 1
Summary of the protein S/T kinases demonstrated to function as IRS-1 kinases

Kinase	Mode of activation	Phosphorylated residues	References
Inhibitory S/T phosphorylation			
PKCα	PMA, human glycated albumin	S612, Rn[a]	[26][b]
MAPK	n.d.[c]	n.d.	[27]
GSK-3	Insulin	n.d.	[28]
PKCζ	Insulin	n.d.	[29]
JNK	TNFα	S307, Mm	[11]
TOR	TNFα	S636, S639, Rn	[30]
PI3K	Insulin	n.d.	[31]
Rho kinase α	Hypertension	n.d.	[32]
IKK	TNFα	S312, Hs	[33]
Casein kinase II	Insulin	S99, T502, mM	[34]
Activatory S/T phosphorylation			
AMPK	AICAR	S789, Rn	[10]
PKB	Insulin	S265, 302, 325, 358, Mm	[6]

The mode of activation and the specific residues which are phosphorylated are indicated.
[a]The numbering of S/T residues refers to the rat (Rn), mouse (Mm) or human (Hs) sequence.
[b]Miele, C., Riboulet, A. et al., submitted.
[c]Not determined.

each of these kinases plays in the pathophysiology of insulin resistance and diabetes. In this respect, the biochemical observation of inhibition of insulin action by JNK-mediated IRS-1 phosphorylation on S307 [11] has obtained physiological confirmation from a JNK1-deficient mouse model in which the absence of JNK1 (i) leads to a decreased IRS-1 S307 phosphorylation, (ii) protects the genetically predisposed *ob/ob* mice from developing obesity, and (iii) ameliorates all the metabolic parameters associated with insulin resistance [12].

Similarly, mice lacking Akt-2/PKB2 display insulin resistance and a diabetes mellitus-like syndrome [13]; however, this phenotype is likely generated by the absence of PKB downstream signalling, and the contribution of the lack of positive regulation of PKB still remains to be investigated. With the recent availability of antibodies directed against specific phosphorylated residues of IRS-1, rapid progress is likely to occur towards our understanding of how each kinase contributes to the modulation of IRS-1 action in animal models.

4. Modulation of IRS protein action via interaction with SOCS proteins

The SOCS family of proteins has been shown to play a regulatory role in insulin signalling mediated, at least in part, by effects (direct and indirect) on IRS proteins. The SOCS family of proteins boast similar structural characteristics, including a unique NH2-terminal domain of variable length, a central SH2 domain and a COOH-terminal 'SOCS box', with this structural resemblance reflecting functional similarities. Initial reports of the role of the SOCS proteins showed them acting as negative regulators of cytokine action via inhibition of JAK/STAT signalling. Subsequent work has shown that, as one would expect from efficient signalling modulators, they are capable of modulating signalling events at different stages of the signal transduction process. Three mechanisms have thus far been identified: (i) SOCS proteins bind and inactivate Janus kinases (JAKs), (ii) they can impede access of other crucial signal transduction mediators such as STATs and SHP2 to receptor binding sites, (iii) more recent evidence suggests that the 'SOCS box' acts as an adapter to facilitate the ubiquitination of signalling proteins and their subsequent targeting to the proteasome.

There is a growing body of evidence supporting the important role of the SOCS proteins in insulin signalling. In addition to being induced by insulin treatment in a number of tissues and cell lines, SOCS-1, -2, -3 and -6 have been shown to interact with the IR and IGFR in vitro, in vivo and in the yeast-2-hybrid system [14–18]. Expression of SOCS-1, -3 and -6 has been shown to have effects on insulin-induced IRS-1–p85 association and on activation of PKB, mitogen-activated protein (MAP) kinase and STAT5 [15,18,19]. How the SOCS proteins achieve these effects is under debate. One of the suggested mechanisms is through inhibition of IR activity, although this observation remains somewhat controversial [18]. The effect of ectopic SOCS on the level of tyrosine phosphorylation of the IRS molecules, via the three canonical mechanisms of inhibition is more clear, with extant demonstrations of the inhibition of JAK activity [20], inhibition via competition with receptor binding sites [15] and induction of proteosomal degradation (see Fig. 2) [21].

While SOCS-1 has been shown to directly bind with high affinity, and thus potently inhibit, JAK, the affinity of SOCS-3 for JAK is relatively low and inhibition occurs only after binding JAK-proximal sites on the receptor. In the case of insulin signalling, Kawazoe and colleagues have demonstrated that overexpression of both SOCS-1 and -3 inhibits the insulin-induced activation of JAKs [20]. However, the physiological importance of JAK inhibition in this setting is placed in question by the likely overall small contribution of JAK-mediated IRS phosphorylation, which is believed to be primarily mediated by the IR itself.

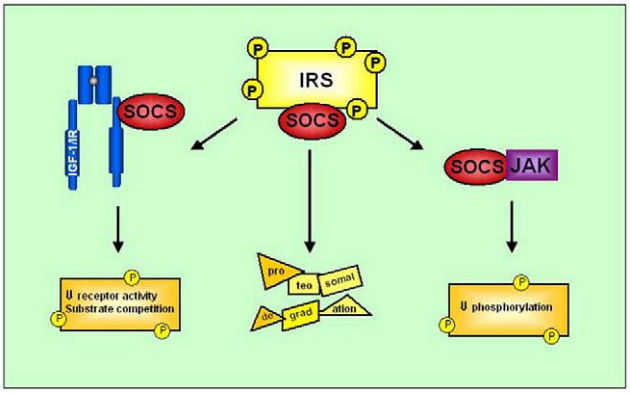

Fig. 2. Modulation by SOCS of the signalling potential of IRS. SOCS has been shown to modulate IRS signalling potential via at least its three canonical mechanisms of inhibition, as shown. In the context of signalling via the IR/IGF1R, SOCS introduces competition for receptor binding sites, inhibits JAK activity and induces proteosomal degradation of IRS.

Of probably more physiological significance is the demonstration that SOCS proteins introduce competition for crucial receptor binding sites. In addition to directly binding and inhibiting the catalytic activity of JAKs, SOCS-3 has been shown to bind to the Y960 residue of the IR, a common docking site for other substrates of the IR, including IRS, SHC and STAT5 [15]. Thus, association between SOCS-3 and IR may inhibit the association between IRS and IR, hence reducing the phosphorylation of IRS.

There is evidence that the SOCS proteins may also mediate effects on insulin signalling distal to the IR itself. SOCS proteins have been shown to play a role in proteosomal targeting of associated proteins by complexing with Elongins B and C (for review, see [22]). It has recently been demonstrated that binding of IRS-1 and -2 by SOCS-1 and -3 (but not SOCS-2) promotes their subsequent ubiquitination and degradation, an effect mediated by the SOCS box [21].

It is, however, difficult to interpret the biological significance of many of the studies on SOCS protein action as they often involve in vitro experiments, and ectopic and supraphysiological levels of expression. However, studies in whole animals lend added credence to the story. Thus, SOCS-3 has been implicated in TNFα-induced insulin resistance, with the demonstration that SOCS-3 is overexpressed in the adipose tissue of obese mice, is induced by TNFα injection and is decreased in *ob/ob* mice lacking TNFα receptors [19]. In addition, SOCS-1 deficiency in mice causes hypersensitivity to insulin action [20], resulting in prolonged IRS-1 phosphorylation upon insulin treatment, further implicating SOCS-1 in negative regulation of the insulin signalling pathway. This group interpret SOCS action to be via the suppression of IRS-1 phosphorylation, as their experiments overexpressing SOCS-1 and -3 did not lead to reductions in overall IRS

expression levels. Conversely, enforced adenoviral-driven SOCS-1 expression in liver of mice leads to disturbances in glucose homeostasis, with dramatic reductions in hepatic IRS-1 and -2 levels, resulting in hyperglycaemia, hyperinsulinaemia and resistance to insulin injections and presumably due to proteosomal degradation of IRS [21]. Which of these mechanisms contribute to a negative regulation of the pathway in a physiological state, to what extent, and which SOCS proteins play the major role remains to be established and may well depend, at least in part, on the level of SOCS expression. It is thus possible that, at low levels of expression, SOCS-3 may inhibit signalling through IRS by competition mechanisms, while at higher levels of expression, SOCS-3 may inhibit signalling by direct antagonisation of receptor autophosphorylation, or through induction of IRS degradation.

Until very recently, the majority of the studies of SOCS proteins in insulin signalling focussed on the action of SOCS-1 and -3, and little was known about the role of SOCS-6. It has now been shown that SOCS-6 has binding affinity toward IRS-2 and IRS-4 [23] and that ectopically expressed SOCS-6 associates with the IR and inhibits signal transduction in vivo [18]. In addition, SOCS-6 has also been reported to directly inhibit IR kinase activity in vitro. However, although mice deficient in SOCS-6 have decreased in body weight, they do not exhibit defects in glucose homeostasis. Clearly, further studies are warranted in order to clarify the significance of the association of SOCS-6 with IRS proteins.

Thus, although the SOCS proteins are induced by a gamut of cytokines, and show a degree interfamilial mechanistic overlap, evidence is accumulating of the potential of specific SOCS proteins to modulate insulin signalling with exquisite control.

5. Conclusions and perspectives

Our understanding of the regulation of insulin signalling has improved in recent years with the discovery that regulatory S/T phosphorylation events, induction of SOCS expression and degradative pathways all converge on IRS molecules and modulate their activities. Moreover, a link between dysregulation of the above events and the pathophysiology of insulin resistance and diabetes has been established. However, the picture is still incomplete. Firstly, the IRS S/T residues phosphorylated by several kinases have not yet been defined (see Table 1); determination of these sites will in turn allow mutagenesis studies and eventually assign a precise function to each S/T residue, be it positive or negative. Given that S/T phosphorylation pathways can converge on the same residue (e.g. both the JNK and PI3K/PDK pathways target S307 [24]), further studies on animal models lacking relevant IRS S/T kinases should help to elucidate the relative contribution of each pathway towards the phosphorylation of a target serine or threonine residue. In addition, while we have a relatively complete picture of the S/T phosphorylation events taking place on IRS-1, much less is known on the regulation of IRS-2, which is possibly the most important adapter for insulin's metabolic action, as mice lacking IRS-2 develop diabetes [25]. Although the exact relative contribution of the mechanisms leading to IRS modulation (by phosphorylation, substrate competition and degradation) remains to be established, it is clear that the IRS proteins play a central role in the transmission of the insulin signal, and that elucidation of the means by which this signal is modulated will be of great significance for our understanding of glucose homeostasis in normal and pathophysiological states.

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