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Review

Signal regulation by family conspiracy

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Abstract. The signal regulating proteins (SIRPs) are a family of ubiquitously expressed transmembrane glycoproteins composed of two subgroups: SIRP α and SIRP β, containing more than ten members. SIRP α has been shown to inhibit signalling through a variety of receptors including receptor tyrosine kinases and cytokine receptors. This function involves protein tyrosine kinases and is dependent on immunoreceptor tyrosine-based inhibition motifs which recruit key protein tyrosine phosphatases to the membrane. Negative regulation by SIRP α may also involve its ligand, CD47, in a bi-directional signalling mechanism. The SIRP β subtype has no cytoplasmic domain but instead associates with at least one other transmembrane protein (DAP-12, or KARAP). DAP-12 possesses immunoreceptor tyrosine-based activation motifs within its cytoplasmic domain that are thought to link SIRP β to activating machinery. SIRP α and SIRP β thus have complementary roles in signal regulation and may conspire to tune the response to a stimulus.

Key words. SIRP; SHPS-1; BIT; signal transduction; CD47; KARAP; DAP-12; ITIM; ITAM; inhibitory receptor, receptor tyrosine kinase; SHP-1; SHP-2; tyrosine phosphorylation; SH2 domain; tyrosine kinase; tyrosine phosphatase.

Introduction

The signal regulatory proteins (SIRPs) are a family of transmembrane glycoproteins involved in signal transduction. One subtype of the SIRP family, SIRP α, exerts a negative influence on signal transduction through receptor tyrosine kinases (RTKs) by its association with certain phosphotyrosine phosphatases and possibly tyrosine kinases [1]. This negative regulation may have a tumour-suppressive effect and is therefore of considerable medical interest. SIRPs constitute a family which is in turn a member of the inhibitory receptor superfamily, which has been primarily studied from an immunological perspective. Since SIRP family members are also expressed in many non-haematopoietic cell types, they take the concept of inhibitory receptors out of the immune system and into the realm of general signal transduction. Therefore, this review will discuss the SIRP family in a general context without special emphasis on immunology [for reviews on existing inhibitory receptor superfamily members and mechanisms, see refs 2–9].

Structural characteristics

The SIRP family currently numbers more than 15 closely related members which vary in the form of subtle amino acid differences in their extracellular domains. The gene locus is at chromosome 20p13 in humans [10, 11] and on chromosome 2 in the mouse [12]. The family can additionally be divided into two structurally distinct subtypes designated SIRP α and SIRP β which differ by the presence or absence, respec-

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tively, of an intracellular domain. To aid the reader, a summary of notable structural features of the SIRP family is supplied in figure 1.

The SIRP α subtype has an apparent molecular weight of between 85 and 90 kDa in humans and rats and 110–120 kDa in the mouse. Several groups have discovered a SIRP α-like molecule which has been designated SHPS-1, p84, BIT, MFR, MyD-1 and SIRP α 1 [1, 13–18]. For simplicity, all these molecules will be referred to here as SIRP α. SIRP αs are extensively glycosylated and have either three or one immunoglobulin (Ig)-like domains. A single transmembrane domain separates the extracellular domain from a cytoplasmic domain of approximately 100 amino acids containing four tyrosines, two of which are within a region conforming to the immunoreceptor tyrosine-based inhibitory motif (ITIM) consensus, 'I/VxYxxL'. ITIMs are present in a large group of molecules that negatively regulate cell functions. Phosphorylation of the tyrosine in an ITIM provides a binding site for SH2 domains often belonging to a tyrosine phosphatase (see below). A proline-rich region near the C terminus of SIRP α may represent a binding site for SH3 domain-containing molecules [1, 10].

The shorter subtype, SIRP β, is more than 90% identical to SIRP α in its extracellular domain excluding the signal peptide. However, SIRP β possesses a dissimilar transmembrane domain with a positively charged lysine residue and has no significant intracellular sequence.

Extracellular domain—structure and ligands

The extracellular domain of SIRP α has three Ig-like domains. The N-terminal Ig-like domain is of the Ig V type and most of the sequence variation between different α members lies in this region. The second and third domains are of the Ig C1 type and are encoded by an exon which can be removed by alternative splicing [1, 13, 16, 19, 20].

The putative N-glycosylation motif (NXS/T) is present 15 times in mouse [16] and 5 times in human SIRP α. Different levels of glycosylation are one likely reason for the difference in size seen in SDS-PAGE between mature full-length proteins of different species. The pattern of glycosylation is probably necessary for molecular recognition (see below).

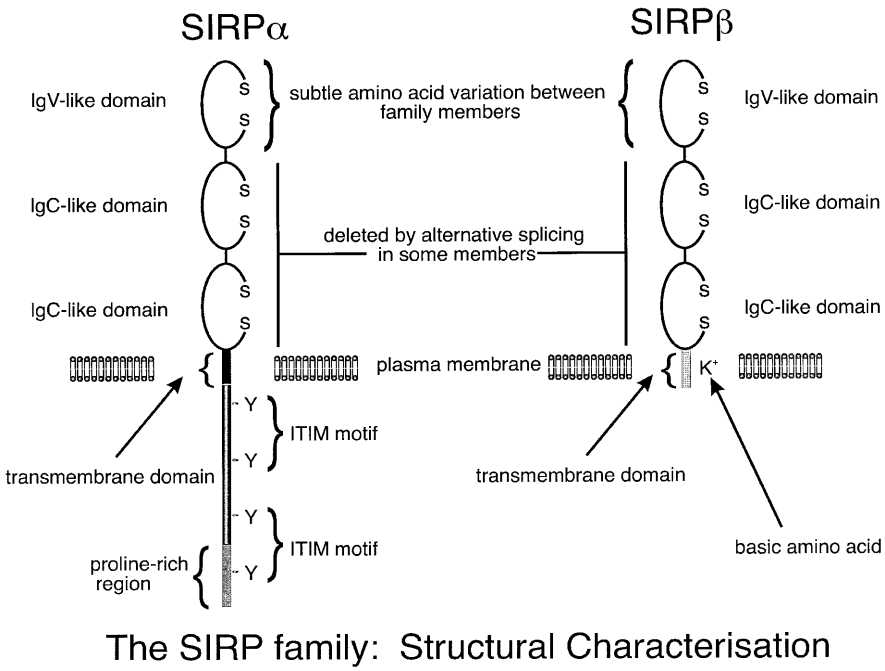

Figure 1. The SIRP family consists of two subtypes that differ by the presence or absence of an intracellular domain. The SIRP α subtype possesses a long cytoplamic domain with a proline-rich region near the C terminus and two ITIM motifs that can bind SH2 domain-containing phospatases. SIRP β has no cytoplamsic domain but has a charged lysine residue within its transmembrane domain that is relevant to its association with DAP-12 (see text). Both subtypes are similar in their extracellular domains which contain three Ig-like domains. The indicated Ig C-like domains are sometimes removed by alternative splicing. Individual amino acid differences are evident in the single Ig V-like domain when different SIRP family members are compared.

CD47, or 'integrin-associated protein', was recently identified as a ligand for SIRP α [21, 22]. CD47 is a transmembrane glycoprotein present in a variety of cells of haematopoietic lineage and other tissues, including the brain. CD47 is co-expressed with SIRP α in retinal cells [23]. The pre-mRNA is alternatively spliced, leading to protein isoforms with intracellular deletions of different sizes [24–27]. CD47 has been implicated in cell adhesion and transepithelial cell migration. It is also associated with the $\alpha_v\beta_3$ integrin (vitronectin receptor) [28], with proteins linking IAP with the cytoskeleton (PLICs) and intracellularly with thrombospondins [24].

How CD47 binds to the extracellular domain of SIRP α is not yet known. Recent evidence with cell adhesion assays suggests, however, that the N-terminal Ig V-like domain of SIRP α is both necessary and sufficient for the interaction with CD47 [29]. There may nevertheless be additional ligands, possibly reflecting the variation seen among SIRP α molecules. CD47 as a single ligand does not explain the sequence variation concentrated in the Ig V-like domain of SIRP α. One strong hypothesis assumes that the variation among SIRPs has functional significance, i.e. to define an individual affinity for a single receptor or group of receptors. A similar hypothesis has already been speculated in the case of the KIR family and different species of MHC class I ligands [30]. SIRP α resides constitutively in a complex with the CSF-1 receptor in macrophages [31]. There are numerous examples of other ITIM-containing proteins clustering around receptors. A 'one SIRP for each receptor' hypothesis may be valid and would imply more SIRP αs are to be found.

Stimulation of SIRP α tyrosine phosphorylation and association with SH2 domain-containing phosphatases

A variety of different stimuli result in tyrosine phosphorylation of SIRP α. These include epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin, neurotrophins, lysophosphatidic acid (LPA), adhesion to fibronectin, growth hormone (GH), colony-stimulating factor and serum [1, 13, 31–39]. SIRP α tyrosine phosphorylation is also induced by light in the suprachiasmatic nucleus of the brain and may therefore be involved in modulating the circadian rhythm [40]. Despite this apparent promiscuity, leukaemia inhibitory factor and interferon gamma are unable to induce SIRP α tyrosine phosphorylation in fibroblasts [37].

Tyrosine phosphorylation of SIRP α following insulin stimulation of RAT1-IR cells occurs mainly on the last two tyrosines (Y449 and Y473) [41]. Mutating either of these tyrosines abolishes most of the tyrosine phosphorylation of SIRP α and its association with SHP-2. Which tyrosines become phosphorylated may vary with the nature of the stimulation. Tyrosine phosphorylation of SIRP α leads to the recruitment of either of the two SH2 domain-containing tyrosine phosphatases SHP-1 and SHP-2 [1, 31, 42]. The association of SIRP α with the phosphatase is mediated by the SH2 domains of the phosphatase and the phosphorylated tyrosine motifs of SIRP α. Binding stimulates the catalytic activity of the phosphatase. SIRP α molecules are also substrates of these phosphatases [1, 31, 33, 35]. Y449 has specific affinity for the N-terminal SH2 domain of SHP-2, while Y473 prefers the C-terminal SH2 domain [41]. In vitro analysis using phosphopeptides concluded that two molecules of SHP-2 can bind in tandem to one molecule of tyrosine-phosphorylated SIRP α and that the ITIM motifs (tyrosines one and three) are necessary and sufficient for induction of SHP-2 catalytic activity [15]. Structural data following the crystallisation of SHP-2 is supportive of this model revealing that the N-terminal SH2 domain regulates activity following binding to a phosphotyrosine moiety, while the C-terminal domain plays an accessory role [43]. Nevertheless, while the phosphatase is activated by certain tyrosines, Takeda et al. [41] reported that all four phosphotyrosine motifs act in vitro as substrates for activated SHP-2.

Despite their similar structures, SHP-1 and SHP-2 appear to possess different biological functions, SHP-1 with an inhibitory, and SHP-2, a seemingly opposite role [reviewed in refs 44, 45]. One function of tyrosine-phosphorylated SIRP α in haematopoietic cells may therefore be to direct SHP-1 to a receptor, e.g. the CSF-1R [31], where it dephosphorylates target members of the complex.An explanation for SHP-2 association is less clear. Recruitment to SIRP α from the cytosol to the membrane following stimulation [34, 41] may sequester SHP-2 away from areas where it has a positive effect. SHP-2 could activate proteins by dephosphorylation of e.g. src family kinases [46]. The SH3 domain of c-src associates with SHP-2. SHP-2 dephosphorylates Y527 in vitro [47]. Overexpression of SHP-2 causes an increase in c-src activity but this is independent of SHP-2 catalytic activity, and dephosphorylation of Y527 is not observed [48]. Activation of SHP-2 through binding to SIRP α was not utilised in these experiments. Finally, other proteins associate with SIRP α in a manner dependent on SIRP α tyrosine phosphorylation (see below). SIRP α may rely on these proteins for negative signalling. Dephosphorylation of SIRP α by SHP-2 would dismantle such a negative signalling complex.

There are also two SH2 domain-containing phosphoinositol phosphatases, SHIP 1 and SHIP 2 that associate inducibly with other ITIM-containing molecules [see refs 49 and 50 for reviews]. Neither has yet been shown to be involved with SIRP α. To assist understanding of the multiplicity of interactions described in this review, all currently known binding partners and their potential

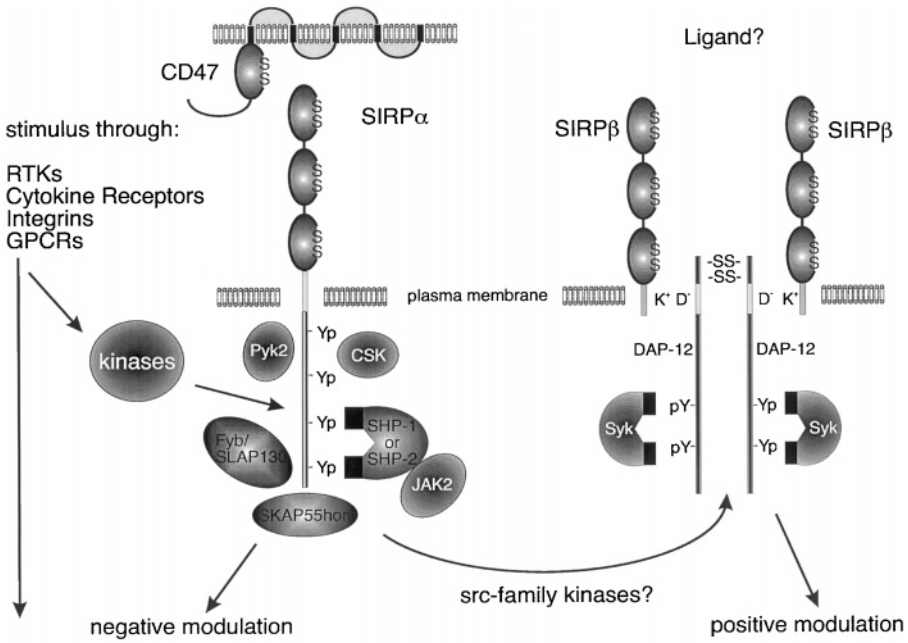

Figure 2. The SIRP family: known interactors and their putative influence upon signal transduction. Stimulation of a variety of receptors results in SIRP α tyrosine phosphorylation, thought to be catalysed by cytosolic tyrosine kinases. The consequent binding and activation of SHP-2 is necessary for an inhibitory effect upon the signalling generated by this stimulus. SIRP α associates with numerous adaptor proteins and tyrosine kinases that may participate in negative regulation of the signal itself, or the complex containing SIRP β. SIRP β associates constitutively with DAP-12 via an ionic transmembrane domain interaction, and when Syk associates with the phosphorylated ITAM motif of DAP-12, an active complex is assembled that may potentiate signalling.

involvement in signal regulation by SIRPs are depicted in figure 2.

Involvement of protein tyrosine kinases in SIRP α signalling

Endeavours have been made to identify the kinase or kinases responsible for direct tyrosine phosphorylation of SIRP α. Tyrosine phosphorylation of SIRP α occurs as early as 1–2 min after stimulation by EGF or PDGF [1]. Additionally, purified EGF receptor [34] and semi-purified insulin receptor [33] can phosphorylate SIRP α in vitro. Therefore, following EGF, PDGF or insulin stimulation of cells, the respective receptors are either directly responsible for tyrosine phosphorylation, or the kinase responsible is an intimate part of the complex.

However, SIRP α is also tyrosine phosphorylated after stimulation of receptors without tyrosine kinase activity. Stimulation of integrins by adhesion of cells to fibronectin leads to SIRP α tyrosine phosphorylation after 15 min [39, 46]. This tyrosine phosphorylation is reduced when cell lines without either c-src, fyn or FAK are used. A similar effect is seen in cell lines overexpressing CSK. CSK is a tyrosine kinase that inhibits src family kinases by phosphorylating Y527, a regulatory tyrosine present at the C terminal [for reviews, see refs 51, 52]. c-src, but not FAK, phosphorylates SIRP α in vitro [39]. Fgr, another src family kinase, has been reported to potentiate the ability of SIRP α to bind SHP-1 and downregulate phagocytosis in macrophages, but Fgr does not phosphorylate SIRP α and may therefore act as an adaptor molecule [53].

Similar effects on SIRP α tyrosine phosphorylation are seen with LPA, which stimulates cells through G protein-coupled receptors (GPCRs) [36]. It is important to note that examples of ligand-independent RTK stimulation following activation of integrins or GPCRs are both documented [54, 55]; therefore, SIRP α could be phosphorylated following RTK activation by a form of lateral signalling.

Stimulation of cells with GH leads to SIRP α tyrosine phosphorylation. The GH receptor has no intrinsic tyrosine kinase activity and signalling through it is expected to be mediated by the cytosolic tyrosine kinase JAK2. Stofega et al. [56] propose that JAK2 phosphorylates SIRP α because JAK2 can tyrosine phosphorylate SIRPs in vitro. Furthermore, JAK2 complexes with SIRP α when both JAK2 and SIRP α are overexpressed, an association which requires neither the C-terminally located proline-rich region of SIRP α nor SIRP α ty-

rosine phosphorylation. Moreover, both JAK2 and SIRP α associate directly with SHP-2 [37]. Finally, overexpression of SIRP α negatively regulates GH-induced phosphorylation of MAP kinase and STAT family members.

Two further cytosolic tyrosine kinases are relevant to SIRP α. CSK associates endogenously with SIRP α [42] in macrophages but the physiological significance is unclear. SIRP α may downregulate signalling through CSK by inhibiting src family protein tyrosine kinases.

PYK2, a cytosolic tyrosine kinase related to FAK, was recently shown to be present in an in vivo complex with SIRP α in mouse macrophages. PYK2 represented a significant proportion of the kinase activity in this complex [57]. Whether or not CSK and PYK2 are relevant to SIRP α tyrosine phosphorylation, they are likely to be participants in SIRP-related signalling.

Distribution of SIRPs among tissue and cell types

SIRP α molecules are expressed in a variety of tissue types and at different levels. Two transcripts of 4.4 and 2.4 kb have been detected by Northern hybridisation in heart, brain, placenta, lung, liver, spleen and spinal cord [20, 42]. RNAse protection analysis also reveals evidence of two transcripts [42]. These two transcripts probably reflect the two known variants generated by alternative splicing.

SIRP α protein is highly expressed in myeloid cells [17, 22, 42]. Macrophages may constitute a useful experimental system because they express a single SIRP α far more predominantly than other members [16, 31, 42, 57, 58]. High levels of SIRP α protein are also seen in neurons and specific areas of the brain [14, 19, 20, 59]. In Western blot, one study collectively detected proteins at 120, 85–95, 50 and 42 kDa in different tissues and cell lines [42]. Following enzymatic deglycosylation, only two bands, at 65 and 35 kDa, are detectable [1, 42]. Glycosylation is therefore probably critical for determining a repertoire of roles for family members.

Approaches using detection with monoclonal antibodies have been informative but occasionally misleading, suggesting that expression is highly restricted to certain tissues. The variation observed is probably due to sensitivity to a glycosylation pattern [16]. SIRP α glycosylation is therefore likely to vary between cell types and may reflect the masking or creation of potential ligand-binding sites.

Role of SIRPs in biological processes

The recently found association between the two adaptor proteins FyB/SLAP 130 and SKAP55hom [57] has led to the hypothesis that SIRP α is a scaffold protein that recruits signalling molecules to the membrane at the site of receptors. In this context, it is interesting to note that SIRP α can also associate with Grb2 in vitro [1].

NIH3T3 cells overexpressing SIRP α have a slower growth rate than mock-infected cells and are resistant to focus formation and growth following transformation by v-fms. Furthermore, MAP kinase activation is inhibited following EGF or insulin stimulation in these cells [1]. All these effects are strongly indicative of tumour suppression. On the other hand, a positive effect of SIRP α on MAP kinase activation following insulin stimulation has been reported [34].

SIRP α expression increases during macrophage fusion. Additionally, macrophage fusion is inhibited by the addition of monoclonal antibodies against SIRP α [16]. Similar experiments show that monoclonal antibodies against SIRP α inhibit the migration of neutrophils through endothelium [22]. In neuronal cells, SIRP α is documented as an adhesion molecule and implicated in neuronal outgrowth and axon guidance [14]. SIRP α may therefore be relevant to macrophage fusion, cell migration or the navigation of neurons during development, but these findings, taken together with the wide tissue expression of SIRP α suggest that its role is more fundamental, perhaps lying in specific cell recognition.

Coligation of a chimera containing the C terminal of SIRP α with the high-affinity IgE receptor FcεRl inhibits tyrosine phosphorylation and cell activation through FcεRl. The chimera becomes tyrosine phosphorylated during coligation and binds SHP-1 and SHP-2 [60]. This may imply an ITIM-dependent mechanism relating SIRP α to other ITIM-containing receptors in haematopoietic cells. The structure of SIRP α closely resembles other inhibitory receptor superfamily members. Although the sequences are highly divergent between SIRPs and, for example, KIRs, Fcγ receptors and other subfamilies, the ITIMs maintain their integrity. Inhibitory receptor superfamily members have suppressive effects on cell activation in cells from a haematopoietic lineage, primarily natural killer cells, T cells and B cells. Where possible, these inhibitory effects were shown to be directed upon the positive signals generated by receptors containing immunoreceptor tyrosine-based activation motifs (ITAMs), for example the BCR Ig α and Ig β, CD3 ε, CD3 γ, TCR ζ, and FcεRl γ. The ability of SIRP α to have a similar effect on non-ITAM-containing receptors would appear to extend the issue.

SIRP β: the activating counterpart of SIRP α?

SIRP β, the second structural subtype of the SIRP family, has recently been assigned an activating function via its association with DAP-12 in myeloid cells, and is now one of a number of 'inhibitory activating

receptors' [61]. Members of this group of transmembrane receptors have no significant intracellular domain and rely on association with another molecule for their ability to transduce a signal [3]. Two such adaptor molecules have been identified so far and named DAP-12 [62], also known as KARAP [63–66], and DAP-10 [67] which is also called KAP-10 [68]. The antigen receptor FcεRI is also related to this pair of molecules [69]. DAP-12 exists as a homodimer and possesses one ITAM motif [6, 7, 70] per monomer. Tyrosine phosphorylation of both tyrosines within the ITAM leads to its association with Syk, a cytosolic tyrosine kinase critical for antigen receptor signalling [71]. The association between SIRP β and DAP-12 is likely to be an ionic interaction between single amino acids of opposing charge within the transmembrane domains. This type of association resembles that of other inhibitory activating receptors, notable examples being among the KIRs [3, 72].

Coligation of the SIRP β/DAP-12 complex results in MAPK activation and cellular activation in myeloid cells [61]. SIRP β would appear to have an opposite effect on cell activation to that of SIRP α and one might envisage a possible co-operative relationship between SIRP α and SIRP β in the modulation of a signal.

Final observations

Although many cDNAs encoding SIRP family members have been cloned, there is work to do to reveal the true multiplicity of the family, and the extent to which the SIRPs are specific for different receptors. The relationship between SIRP α and SIRP β must be addressed, since the effect of SIRP α on the ITAM motif-containing FcεRl signalling [60] calls for an examination of its effect on SIRP β/DAP-12. The requirement for coligation of SIRP α with FcεRl to elicit inhibitory effects indicates that ITIM- and ITAM-containing molecules co-operate to modulate signals from a position of tight intimacy. We therefore need to know which molecules known to be involved in SIRP α function, including ligands, are also relevant to SIRP β.

Future work will undoubtedly address the relationship of the SIRP family to disease. A CCA trinucleotide repeat exists in the 3′ untranslated region of the SIRP α transcript [13, 19] and such elements have been long known to affect gene regulation specifically or globally, and to be medically relevant. Furthermore, the role of SIRP α as a potential tumour suppressor should be of critical interest in cancer research.

SIRP is interesting because it highlights a fundamental concept in biology. The inhibitory receptor superfamily had been thought to be specific to the immune system. In B and T cells, antigen receptor signalling is controlled by co-receptors recruiting cytosolic complexes in response to stimulation with antigen. SIRPs show that this concept applies not only to antigen receptors, but to RTKs, cytokine receptors, GPCRs, integrins and other membrane receptor systems. Immunology, nevertheless, provides an increasingly valuable source of analogy to which we can look for inspiration.

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