Lymphocyte Antigen Receptor Signal Integration and Regulation by the SHC Adaptor

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The Shc adaptor protein transduces signals from transmembrane receptors to the Ras pathway of cell activation by providing binding sites for the recruitment to the submembrane compartment of the Grb2/Sos G-nucleotide exchange complex. The need for Shc in this process is however unclear since Grb2 can be recruited directly to phosphotyrosine containing membrane receptors through its src-homology-2 domain. Evidence from studies in lymphocytes indicates that Shc is multifunctional and is involved in the integration of independent signals to the Ras pathway. Furthermore, Shc may be a key control point at which signaling can be modulated both by interfering signals and by feedback mechanisms. Here we review recent literature to support these functions for Shc.

Key words: Antigen receptor / Lymphocytes / Signal transduction / Tyrosine kinase.

Introduction

In eukaryotic cells, Ras proteins act as molecular switches which, when activated by extracellular signals, trigger proliferation and differentiation along predetermined pathways. Activation of Ras proteins is accomplished by recruitment to the membrane compartment of Ras-specific G-nucleotide exchange factors of which the best studied is Sos. Recruitment of Sos is mediated by Grb2, a small adaptor protein which through a src homology-2 domain (SH2) binds to phosphotyrosine containing membrane receptors through its src-homology-2 domain. Evidence from studies in lymphocytes indicates that Shc is multifunctional and is involved in the integration of independent signals to the Ras pathway. Furthermore, Shc may be a key control point at which signaling can be modulated both by interfering signals and by feedback mechanisms. Here we review recent literature to support these functions for Shc.

Key words: Antigen receptor / Lymphocytes / Signal transduction / Tyrosine kinase.

Shc Is a Modular Adaptor

Shc is ubiquitously expressed in mammalian cells as two isoforms, of 52 and 46 kDa respectively, deriving from different usage of two alternative in-frame translation initiation codons (Pelicci et al., 1992). As shown in Figure 1, both isoforms share two domains which bind phosphotyrosine motifs, an amino-terminal phosphotyrosine binding (PTB) domain and a carboxy-terminal SH2 domain, separated by a region rich in proline and glycine residues, the collagen homology (CH1) domain (reviewed in Bonfini et al., 1996). In some tissues, with the conspicuous exception of hematopoietic cells (Pelicci et al., 1992), an additional isoform of 66 kDa can be detected, which is encoded by a distinct transcript generated through alternative splicing of the 5' exons. The resulting protein is identical to p52Shc but contains an additional amino-terminal region rich in proline/glycine residues, named accordingly CH2 domain (Migliaccio et al., 1997). Two sites of tyrosine phosphorylation have been mapped in the CH1 domain at tyrosines 239/240 and tyrosine 317 respectively (Egan et al., 1993; Gotoh et al., 1996; van der Geer et al., 1996). When phosphorylated, both tyrosine sets acquire the capacity to bind the SH2 domain of Grb2 and hence to recruit Sos. The central CH1 domain is emerging as an interesting candidate for phosphotyrosine independent protein-protein interactions. The high content in proline residues of the CH1 domain suggests it may interact with proteins containing SH3 domains. Indeed Shc has been shown to interact both in vivo and in vitro with the SH3 domain of PLCγ and Ras-GAP and in vitro with the SH3 domains of Src-like kinases (Bonfini et al., 1996). Hence Shc differs from the simple adaptor Grb2 in that it has at least three different domains involved in protein-protein interactions.

Shc in Antigen Receptor Signaling

Ras activation is a key step in lymphocyte antigen receptor signaling (reviewed in Izquierdo Pastor et al., 1995). Although the T cell antigen receptor (TCR) and B cell antigen receptor (BCR) are devoid of intrinsic tyrosine kinase activity, they are functionally coupled to cytoplasmic PTKs.
Antigen receptors are composed of two distinct modules, which separately subserve the functions of ligand binding and signal initiation. All the chains of the signaling module are characterized by the presence of specific motifs, the immunoreceptor tyrosine-based activation motifs (ITAMs). The tyrosine phosphorylated ITAM on another TCR/CD3/\(\zeta\) chain (Ravichandran et al., 1993). The binding affinity of Shc to tyrosine phosphorylated ITAM is however low, at least compared to ZAP-70 and, furthermore, Shc preferentially binds to singly rather than doubly phosphorylated ITAM on the Ig-\(\alpha\) and Ig-\(\beta\) chains of the activated BCR (D’Ambrosio et al., 1996). In B cells, Shc has been shown to interact with the ITAMs of the Ig-\(\alpha\) and Ig-\(\beta\) chains of the BCR through its PTB domain, or to a tyrosine phosphorylated ITAM within the same complex, thereby providing a means of receptor aggregation and signal amplification (Figure 2B).

In T cells, Shc is phosphorylated following TCR triggering and binds the TCR \(\zeta\) chain (Ravichandran et al., 1993). The relevance of this interaction to the formation of Shc/Grb2/Sos complexes has however remained controversial. In vitro binding studies have shown that Shc association with the \(\zeta\) chain involves the Shc SH2 domain and a tyrosine phosphorylated \(\zeta\) chain ITAM (Ravichandran et al., 1993; Osman et al., 1995). The binding affinity of Shc to tyrosine phosphorylated \(\zeta\) is however low, at least compared to ZAP-70 and, furthermore, Shc preferentially binds to singly rather than doubly phosphorylated ITAM (Osman et al., 1995), a situation which is not likely to occur in correctly activated cells, where all three \(\zeta\) chain ITAMs have been shown to be fully phosphorylated (Neumeister et al., 1998). Furthermore, the stable interaction between phospho-\(\zeta\) and ZAP-70 mediated by a dual interaction of the tandem SH2 domains of ZAP-70 with the two phosphotyrosine residues within the \(\zeta\) chain ITAM (Hatada et al., 1995) suggests a potential steric hindrance to the access of Shc.

Shc has however been shown to associate with ZAP-70 in response to TCR engagement (Milia et al., 1996). This interaction involves the Shc PTB domain and tyrosine 474 on ZAP-70 and is essential for correct TCR signaling since over-expression of the isolated Shc PTB domain or a Phe\(^{474}\) mutant of ZAP-70 inhibits TCR activation of the NF-AT transcription factor (Pacini et al., 1998). Conversely, only ZAP-70-bound Shc appears to interact with phospho-\(\zeta\) (Milia et al., 1996), suggesting that Shc might use its dual phosphotyrosine binding capacity to promote the formation of a trimeric phospho-\(\zeta\)/ZAP-70/Shc complex which would result in an enhanced stability of the interaction of Shc with the activated TCR (Figure 2A). The SH2 domain of Shc could bind either to a tyrosine phosphorylated ITAM within the same \(\zeta\) chain as the ZAP-70 molecule to which it is bound through its PTB domain, or to a tyrosine phosphorylated ITAM on another TCR/CD3/\(\zeta\) complex, thereby providing a means of receptor aggregation and signal amplification (Figure 2B).

In B cells, Shc has been shown to interact with the ITAMs of the Ig-\(\alpha\) and Ig-\(\beta\) chains of the BCR through its SH2 domain (D’Ambrosio et al., 1996) and to associate with Syk (Nagai et al., 1995), although it is not yet known if this is mediated by the Shc PTB domain. On BCR activation, Shc is phosphorylated on tyrosine residues in a Lyn and Syk dependent manner and binds Grb2/Sos complexes (Saxton et al., 1994; Lankester et al., 1994; Smit et al., 1994). Interestingly, Shc also appears to associate with nonphosphorylated ITAM on the Ig-\(\alpha\) chain of the resting BCR (D’Ambrosio et al., 1996), suggesting a mechanism for efficient recruitment of Shc to tyrosine phosphorylated ITAM following stimulation.

The capacity of Grb2 to directly interact with tyrosine phosphorylated ITAMs (Osman et al., 1995) raises the question of the actual requirement for Shc in antigen receptor signaling. One answer lies in the potential of Shc to induce higher levels of Ras activation through its twin Grb2 binding sites. In mouse B cells, both tyrosine 239 and tyrosine 313 (317 in human Shc) are phosphorylated in re-
response to BCR aggregation, and as such acquire the capacity to efficiently bind Grb2 (Harper and DeFranco, 1997). Phosphorylation of both sites is required for efficient formation of Shc/Grb2/Sos complexes (Harper and DeFranco, 1997), suggesting a cooperativity of the two Grb2 binding sites in promoting translocation of Sos to the membrane. According to the model proposed by the authors, this would be achieved by two Grb2 molecules simultaneously binding to a single Sos molecule. In support of this model, Grb2, artificially targeted to the membrane, by providing it with a membrane localization sequence, did not result in Ras activation, but membrane localization of Sos did (Holsinger et al., 1995). The failure of membrane targeted Grb2 to activate Ras could be accounted for by an insufficient clustering of the membrane targeted molecule, resulting in suboptimal Sos recruitment. Furthermore, in T-cells, Shc phosphorylation has been reported to result in enhanced formation of Grb2/Sos complexes (Ravichandran et al., 1995), suggesting a role for Shc in regulating the association between Grb2 and Sos, and implicating Shc in controlling the extent of Ras activation. Hence Shc may contribute to signal amplification in lymphocytes.

Fig. 2  Three Possible Models of Shc Interactions during T-cell Antigen Receptor Activation. (A) Shc interacts through its PTB domain with ZAP-70 and through its SH2 domain with the CD3-ζ chain, thus stabilizing the trimolecular interaction. (B) Shc interacts with ZAP-70 in one TCR/CD3 complex and with the ζ-chain of another thus contributing to TCR clustering and receptor capping. (C) Shc interacts with ZAP-70 of the TCR/CD3 complex and through its SH2 domain with p56Lck associated with the CD4 coreceptor thus favouring TCR-CD4 interaction with antigen presented on MHC class II. The models are not necessarily mutually exclusive.
Integration of Antigen Receptor and Coreceptor Signals

Although the antigen receptor provides the primary signal for lymphocyte activation, this signal is not sufficient. Antigen presentation involves a cognate interaction between the antigen coreceptors CD4/CD8 on T cells, and MHCII/MHC1 on the antigen presenting cell. Coreceptor coengagement is a key requirement for the generation of a productive signal. Furthermore, dissection of the CD4 signal transduction pathway has revealed a striking similarity in the signaling events triggered by TCR and CD4 (Baldari et al., 1995a; Milia et al., 1997, and references therein), supporting the concept that signal coordination and integration are essential features of the T cell response to antigen. Owing to their modular structure, adaptors appear as ideal candidates for this function.

Shc has been shown to be phosphorylated on tyrosine residues and to recruit Grb2/Sos complexes in response to CD4 engagement (Baldari et al., 1995b). Furthermore, co-clustering of CD3 and CD4 induces enhanced Shc phosphorylation (Ravichandran et al., 1993), suggesting that Shc might represent a point of convergence of the signals triggered by TCR and CD4. The CD4 associated PTK Lck appears responsible for Shc recruitment and, potentially, for its phosphorylation (Baldari et al., 1995b). Shc is required for CD4 signal transduction since overexpression of the Shc SH2 domain exerted a transdominant negative effect on activation of a reporter construct under the control of the NF-AT transcription factor. The effect was paralleled by a reduction in CD4 dependent Shc phosphorylation. Interestingly, while only the p52 isoform is phosphorylated in response to TCR engagement, an epitope specific phosphorylation of either the p52 or the p46 isoform, or both, was observed in response to CD4 engagement (Baldari et al., 1995a; Milia et al., 1997). The capacity of Shc to use its SH2 domain to bind CD4/Lck and its PTB domain to bind ZAP-70 suggests a mechanism where a single Shc molecule could bind simultaneously phospho-ζ-bound ZAP-70 and CD4-bound Lck, thus contributing to TCR/CD4 clustering, a feature which implies both a stabilization of the surface interactions and physical proximity of the TCR and CD4 signaling components (Figure 2C).

A synergistic phosphorylation of Shc has also been shown to occur in response to coligation of the TCR/CD3 complex with the costimulatory receptor CD2, which is also functionally coupled to a Src family PTK (Umehara et al., 1996).

Similarly in B cells, co-ligation of the BCR and the costimulatory receptor, CD19, has been shown to lower the threshold for the induction of a mitogenic signal by the BCR (Carter and Fearon, 1992). A role for Shc has been proposed in the integration of the signals triggered by the BCR and CD19. Coengagement of the BCR and CD19 has indeed been shown to enhance both the tyrosine phosphorylation of Shc and the formation of Shc/Grb2/Sos complexes, compared with ligation of the BCR alone (Lanekester et al., 1994). Although the mechanism of Shc recruitment to CD19 has not been investigated, the physical and functional coupling of CD19 to Src family PTKs suggests that the latter might subserve this function. Thus Shc collects and integrates signals generated by antigen receptors and costimulatory receptors.

Shc in Negative Signaling

B cell activation and proliferation are negatively regulated by the Fc receptor for IgG, FcγRII. Coligation of BCR and FcγRII has been proposed to occur in vivo when soluble IgG interacts with BCR-bound antigen, resulting in suppression of antibody production (Goroff and Finkelman, 1988). Negative signaling involves activation and membrane recruitment of the tyrosine phosphatase SHP-1 through an interaction involving the SHP-1 SH2 domain and a phosphotyrosine residue located in an immunoreceptor tyrosine-based inhibition motif (ITIM) within the intracellular domain of FcγRII (D’Ambrosio et al., 1995). The tyrosine phosphatase activity of SHP-1 is believed to downregulate signaling mediated by phosphotyrosine-based interactions. A puzzling feature of these data is that Shc phosphorylation occurs to the same extent in conditions of positive or negative signaling (Triandapani et al., 1997a). Accordingly, Shc phosphorylated under positive or negative signaling conditions is equally competent to bind Grb2 in vitro. Inhibition, however, correlated with a reduced formation of Shc/Grb2/Sos complexes in vivo (Triandapani et al., 1997a).

The reduced interaction of Shc with Grb2 occurring in vivo following coligation of BCR and FcγRII could be accounted for by recruitment into the signaling complex of an SH2 domain containing protein competing with Grb2 for binding to phosphorylated Shc. Indeed such a role has been attributed to the inositol phosphatase SHIP.

Under conditions of negative signaling, SHIP associates with the phosphorylated FcγRII ITIM through its SH2 domain, is phosphorylated and associates with Shc (Ono et al., 1996; Triandapani et al., 1997a, b). This could have the dual effect of competitively inhibiting Grb2/Sos interaction with Shc - and hence reducing Ras activity – and recruiting the phosphatase into the BCR signaling complex.

T cell activation is negatively regulated by CTLA-4. T cells from CTLA-4 deficient mice exhibit both constitutive phosphorylation of Shc and TCR ζ chain and a constitutive interaction of these molecules with each other and with Grb2 (Marengère et al., 1996). Also MAP kinase, which is a well established effector of the Ras pathway, was found to be constitutively active in CTLA-4 deficient T cells (Marengère et al., 1996). The tyrosine phosphatase SYP was proposed to negatively regulate Shc in normal T cells expressing CTLA-4, as it was associated with CTLA-4 and showed phosphatase activity directed towards Shc (Marengère et al., 1996).
Shc in Alternative Signaling Pathways

Although in lymphocytes Shc has been investigated mainly as a potential regulator of Ras, some indication of other functions have begun to emerge in other systems. Shc has been shown to interact with F-actin in nerve cells upon stimulation with NGF (Thomas et al., 1995). This growth factor-induced association of Shc with the cytoskeleton suggests a role for Shc in membrane ruffling and actin fiber reorganization. Shc appears to be also involved in endocytosis, as it is recruited to endocytic structures such as coated pits and endosomes in response to growth factor receptor stimulation (Lotti et al., 1996). This potential role is supported by the finding of an interaction of Shc, mediated by the CH1 domain, with α- and β-adaptin components of plasma membrane adaptor proteins that are believed to be involved in receptor endocytosis (Obyayashi et al., 1996). These possibilities are highly attractive, as both membrane ruffling and endocytosis imply the activation of small GTP binding proteins. Thus Shc might regulate these processes by recruiting alternative guanine nucleotide exchange factors, either through Grb2 or through other SH3/SH2/SH3 adaptors, such as Crk or Nck.

Recent data suggest a role for Shc in cell survival. Shc has been shown to interact with cadherin, a transmembrane protein involved in the Ca^{2+} dependent regulation of cell adhesion. Furthermore, Shc is functionally coupled to β1 integrins, which mediate cell adhesion to the extracellular matrix, possibly through caveolin (Wary et al., 1996). Only a subset of integrins is coupled to Shc, which is necessary and sufficient for integrin dependent activation of the MAP kinase pathway. Interestingly, adhesion mediated by integrins which are not coupled to Shc results in cell cycle arrest and apoptosis (Wary et al., 1996), suggesting a role for Shc in cell cycle progression and survival. A Ras independent survival pathway involving Shc has been identified in hematopoietic cells. In this study expression of a dominant negative SH2-Shc mutant was found to induce apoptosis in IL-3 dependent cells. The anti-apoptotic effect of Shc was dependent on tyrosines 239/240 and is supported by the finding of an interaction of Shc, mediated by integrins which are not coupled to Shc results in cell cycle arrest and apoptosis (Wary et al., 1996), suggesting a role for Shc in cell cycle progression and survival. A Ras independent survival pathway involving Shc has been identified in hematopoietic cells. In this study expression of a dominant negative SH2-Shc mutant was found to induce apoptosis in IL-3 dependent cells. The anti-apoptotic effect of Shc was dependent on tyrosines 239/240 and involved c-myc gene expression (Gotoh et al., 1996).

Conclusions

Shc is clearly capable of interacting with a number of different proteins during lymphocyte activation and hence integrate both positive and negative parallel signaling pathways. Each Shc molecule can, however, only interact with a subset of its possible partners at any one time. This raises the question of the timing of each interaction. Does Shc interact sequentially with different proteins during the process of signal propagation? Alternatively, given the interactions between some of its partners, Shc may play a structural role in the assembly of a multimeric signal integration and amplification complex and could contribute to receptor capping. In this light, recruitment of phosphatases involved in signal extinction could have the dual role of terminating specific pathways by competing for limiting binding sites on positive effectors and disrupting the transducing complex by dephosphorylation of critical tyrosine residues.

Acknowledgements

The experimental work from our laboratories cited in this review was generously supported by the Italian Association for Cancer research (AIRC). The support of Telethon and the MURST (quota 60%) is also gratefully acknowledged.

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