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Identification of Cadherin Tyrosine Residues That Are Phosphorylated and Mediate Shc Association

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Abstract Previously, we reported association of the adaptor protein Shc through its SH2 domain with the cytoplasmic domain of the adhesion molecule cadherin (Xu et al. [1997] J. Biol. Chem. 272:13463–13466). This association was dependent on tyrosine phosphorylation of cadherin and could be modulated by extracellular Ca^{2+} and epidermal growth factor in intact cells. There are six tyrosine residues in the cytoplasmic domain of cadherin. To define the tyrosine residue(s) that mediate Shc recognition, site-directed mutagenesis was employed to alter Tyr851 and/or Tyr883 in cadherin, which both conform to a predicted Shc SH2 domain recognition sequence. Mutation of either Tyr851 or Tyr883, but mostly the latter, decreased Src phosphorylation of cadherin and the binding of Shc to cadherin, as determined by Sepharose bead binding and gel overlay assays. Of the two tyrosine residues, Tyr883 is the major Src phosphorylation and Shc binding site. However, the double mutant (Tyr851, 883 Phe) exhibited less Shc association than the single Tyr883 Phe mutant, suggesting a role for Tyr851 also. In addition, the binding of Shc to the cadherin cytoplasmic domain was competitively inhibited by tyrosine phosphorylated peptides containing either Tyr851 or Tyr883, but not by the corresponding non-phosphorylated peptides. Mutation of Tyr851 and/or Tyr883 did not alter the capacity of the cytoplasmic domain of cadherin to bind β-catenin in vitro. However, Shc binding to cadherin did negatively influence β-catenin binding to the same molecule. J. Cell. Biochem. 75:264–271, 1999.

Key words: growth factors; signal transduction; adhesion; adaptor

Shc, an adaptor protein with no known catalytic activity, is a prominent substrate for protein tyrosine kinases and plays an important role in linking receptor tyrosine kinase signaling pathways to Ras activation [Bonfini et al., 1996]. Shc is composed of an amino-terminal phosphotyrosine binding (PTB) domain, a central collagen homology domain that contains three tyrosine phosphorylation sites and a carboxyterminal Src homology 2 (SH2) domain. Both the SH2 and PTB domains bind to phosphorylated tyrosine residues, but in structurally and mechanistically distinct ways. PTB domains recognize residues aminoterminal to a phosphotyrosine residue [van der Geer and Pawson, 1995], while SH2 domains bind to a phosphotyrosine residue based on the context of its carboxyterminal residues [Songyang et al., 1993]. While either of these phosphotyrosine recognition motifs can mediate SHC association with activated growth factor receptor tyrosine kinase, direct comparisons and mutagenesis data suggest that in many cases it is primarily the PTB domain that mediates Shc association with activated receptors [Prigent et al.,1995; Dikic et al., 1995; He et al., 1995; Craparo et al., 1995; Fournier et al., 1996; Pratt et al., 1996; Isakoff et al., 1996; Yajnik et al., 1996; Sakaguchi et al., 1998]. However, both the PTB and SH2 domains of Shc have been demonstrated to be essential for mitogenic signal transduction through the EGF receptor [Gotoh et al., 1995; Sasaoka et al., 1996; Ricketts et al., 1996; O'Bryan et al., 1998; Sakaguchi et al., 1998]. Hence, the two domains are non-redundant even within the context of one mitogenic pathway.

The possibility that Shc mediates activation of signaling pathways in addition to Ras has been suggested [Bonfini et al., 1996, Gotoh et al., 1997] and molecules that regulate cell adhe-

Abbreviations used: EGF, epidermal growth factor; SH, Src homology; PTB, phosphotyrosine binding.

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sion have been implicated as Shc interacting proteins. Evidence has been presented for Shc interaction with integrin receptors [Mainiero et al., 1995], though this interaction seems indirect [Wary et al., 1996]. Previously, we discovered through a two-hybrid screen in yeast that the cytoplasmic domain of cadherin interacted with the SH2 domain of Shc [Xu et al., 1997]. This interaction was also detected in mammalian cells and required tyrosine phosphorylation of cadherin, as evidenced by potentiation of the association in cells treated with the phosphotyrosine phosphatase inhibitor pervanadate. In vitro studies demonstrated that the interaction was direct and required Src-mediated phosphorylation of the cadherin cytoplasmic domain.

Cadherins are cell surface glycoproteins which mediate homophilic cell-cell adhesion in a calcium-dependent manner [Takeichi, 1991]. The cytoplasmic domains of classic cadherins are highly conserved and linked to actin filaments through the catenin family of proteins. Crosstalk between this adhesion molecule and the EGF receptor tyrosine kinase is suggested by several facts. β-catenin is an EGF receptor tyrosine phosphorylation substrate which mediates interaction of this receptor with the cadherin-catenin complex [Hoschvetzky et al., 1994]. As mentioned above, Shc, an EGF receptor tyrosine kinase substrate, interacts with both the EGF receptor and cadherin [Xu et al., 1997]. The extent of cadherin tyrosine phosphorylation in vivo is uncertain; however, tyrosine phosphorylation of this adhesion molecule has been reported in v-Src transformed cells [Hamaguchi et al., 1993; Behrens et al., 1993; Brady-Kalnay et al., 1998], in cells treated with the phosphotyrosine phosphatase inhibitor pervanadate [Brady-Kalnay et al., 1995; Xu et al., 1997; Soler et al., 1998], and in endothelial cells exposed to vascular endothelial growth factor [Esser et al., 1998]. Complicating the phosphotyrosine status of cadherin may be the association of a phosphotyrosine phosphatase with cadherin or the cadherin-catenin complex [Brady-Kalnay et al., 1995, 1998; Balsamo et al., 1996, Kypta et al., 1996, Balsamo et al., 1998]. Biological data showing that EGF modulates cadherin interaction with the actin cytoskeleton [Hazan and Norton, 1998] and that pervanadate decreases cadherin-dependent cell:cell adhesion [Ozawa and Kemler, 1998] have been presented.

To identify the Shc SH2 domain binding site(s) in the cytoplasmic domain of cadherin, site-directed mutagenesis and peptide competition experiments have been employed to identify the cadherin tyrosine residues responsible for Shc binding. The results show that Tyr883 of Shc is the major Src phosphorylation site and the major Shc binding site, while Tyr851 also contributes to both Src phosphorylation and Shc binding.

MATERIALS AND METHODS

Materials

Glutathione-S-transferase (GST) and pan-cadherin antibodies were purchased from Santa Cruz (Santa Cruz, CA), while phosphotyrosine and β-catenin antibodies were purchased from Transduction Laboratories. The purification of recombinant Shc from baculovirus-infected insect cells and the preparation of Shc antiserum have been described previously [Xu et al., 1997]. Antibody bound to nitrocellulose was detected with horseradish peroxidase coupled to Protein A or anti-mouse IgG and enhanced chemiluminescence. Cadherin peptides containing tyrosine/phosphotyrosine residues 851 or 883 were synthesized by Quality Controlled Biochemicals, Inc., with the following sequences: 851 peptide APPYDSLLFD; 851P peptide APP(p)YDSLLFD; 883 peptide DQDYDYLND; and 883P peptide DQD(p)YDYLND; and a control tyrosine phosphorylated peptide: DND-(p)YIIPLPDPK, derived from the sequence surrounding Tyr 1021 of the platelet-derived growth factor β receptor.

Plasmid Construction

A Bam HI/Not I DNA fragment, encoding the cytoplasmic domain of mouse N-cadherin encoding residues 792 to 906 was isolated in yeast two-hybrid screen [Xu et al., 1997] and subsequently cloned into the Bam HI/Not I site of pGEX-5X-1 (Pharmacia) as the GST cadherin C-terminal fusion protein (pGEX-5X-1 C-Cad).

Site-Directed Mutagenesis

Mutagenesis was carried out in pGEX-5X-1 C-Cad vector according to instruction manual using ExSite PCR-based site-directed mutagenesis kit (Stratagene). The sequences of PCR mutagenesis oligos are as follow: Y851F sense: 5′TTG ACT CCC TCT TAG TCT TTG ACT AC 3′; Y851F anti-sense: 5′ACG GTG GCG CCG

TGG GGT CGT TGT C 3′; Y883F sense: 5′TTG ACT ACC TGA ATG ACT GGG GAC CC 3′; Y883F anti-sense: 5′AGT CCT GGT CCC CAC CGC TAC TGG A 3′. The resulting mutations were confirmed by direct DNA sequencing. The Y851F, Y883F and the double mutant Y851FY883F mutations were introduced into both the pGEX-5X-1 C-Cad and pRK5 Myc C-Cad vectors.

In Vitro Src Kinase Assay

Wild-type or mutant GST-cadherin coupled to glutathione Sepharose 4B beads (Pharmacia) were incubated for 45 min with purified c-Src (Upstate Biotechnology Inc.) at room temperature in Src kinase buffer (20 mM Tris, pH 7.6, 40 mM MgCl2, 2 mM MnCl2 and 0.5 mM EGTA) in the presence of 10 mM ATP and 1 mM sodium orthovanadate. Following this incubation, the Sepharose beads were washed three times with phosphate buffered saline containing 1% Triton X-100 and 1 mM Na_3VO_4 (PBSTV buffer).

Sepharose Binding Assay

GST-cadherin fusion proteins were expressed using the pGEX-51–1 vector (Pharmacia) and purified from *E. coli* according to the manufacturer's instructions. The fusion proteins were coupled to glutathione Sepharose 4B beads and tyrosine phosphorylated using purified c-Src kinase as described above. After washing with PBSTV buffer, the beads were incubated with recombinant Shc in PBSTV buffer or A-431 cell lysate in TGH buffer (1% Triton X-100, 10% glycerol, 100 mM NaCl, 50 mM Hepes, pH 7.2) supplemented with 10 ng/ml leupeptin, 10 ng/ml aprotinin, 544 μM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, and 1 mM Na_3VO_4 (TGHIV buffer). After incubation and washing with PBSTV or TGHIV buffer, bound proteins were analyzed by SDS polyacrylamide gel electrophoresis (SDS PAGE) and Western blotting with Shc antibody.

Gel Overlay Assay

Tyrosine phosphorylated GST-cadherin fusion proteins were subjected to SDS PAGE and transferred to nitrocellulose filter. The filter-bound protein was denatured in 6 M guanidinium HCl and renatured by serial dilution of guanidinium HCl from 3 M to a final concentration of 0.185 M. The filter was then incubated with recombinant Shc in the presence or absence of cadherin peptides. After washing five times with TBST buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% Triton X-100), bound protein was detected by Western blot with anti-Shc.

RESULTS AND DISCUSSION

Peptide Competition Assay

In a previous study [Xu et al., 1997], we demonstrated that the binding of Shc to the cytoplasmic domain of cadherin is dependent on the tyrosine phosphorylation of cadherin in vivo and in vitro. Of the six tyrosine residues in the cadherin cytoplasmic domain, the residues carboxyterminal to Tyr851 and Tyr883 form consensus binding sites for the SH2 domain of Shc as deduced from peptide library studies [Songyang et al., 1994]. To determine whether these tyrosine residues do mediate Shc binding, peptides containing sequences flanking Tyr851 and Tyr883 were synthesized to contain either tyrosine or phosphotyrosine. The peptides were used in competition assay to measure Shc binding to the cytoplasmic domain of tyrosine phosphorylated cadherin. In these experiments, purified Src was employed to phosphorylate the cytoplasmic domain of cadherin. While it is not clear which tyrosine kinase(s) actually phosphorylates cadherin in vivo, cadherin is tyrosine phosphorylated in v-*src* transformed cells [Brady-Kalnay et al., 1998; Hamaguchi et al., 1993; Behrens et al., 1993]. Also, the presence of Src family tyrosine kinases in cadherin-containing adherins junctions has been reported [Tsukita et al., 1991].

The results of Sepharose bead binding assay and a gel overlay assay are shown in Figure 1. The Sepharose binding assay (Fig. 1A) shows that the presence of the 851 or 883 non-phosphotyrosine peptides have no effect on the binding of Shc to the immobilized tyrosine phosphorylated GST cadherin fusion protein. However, the presence of the phosphotyrosine-containing 851P or 883P peptides decreased the binding of Shc to cadherin approximately four-fold. When equimolar amounts of the 851P and 883P peptides were combined and assayed, Shc association was decreased more than with either peptide alone. This decrease was less than additive, however. The observed inhibition by the 851P and 883P peptides was not due to the presence of just a phosphotyrosine residue, as an irrelevant control phosphotyrosine peptide (control

pY), representing the sequence around Tyr1021 in the PDGFβ receptor, did not decrease the binding of Shc to cadherin. In the absence of Src phosphorylation of the GST-cadherin molecule, no Shc binding was detectable, as expected based on previous data [Xu et al. 1997].

The result of the gel overlay assay, as shown in Panel B, is consistent with that of Sepharose binding assay, i.e. only phosphotyrosine-containing 851P and 883P peptides competed for the binding of recombinant Shc to the tyrosine phosphorylated GST-cadherin fusion protein. In this experiment here was a four-fold decrease in Shc binding to GST-cadherin when either the 851P or 883P peptide was present.

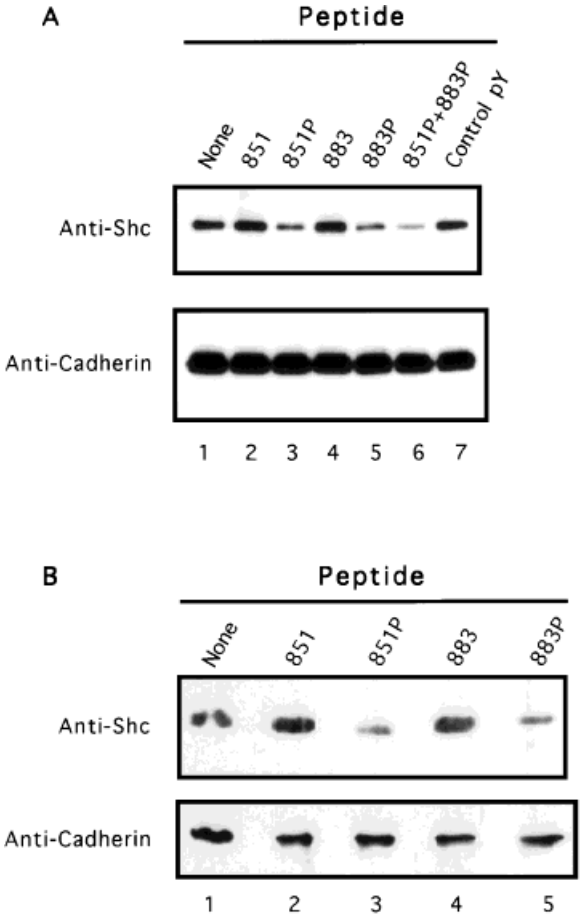

Fig. 1. Capacity of phosphotyrosine peptides to compete with Shc for cadherin binding. **A:** Peptide competition in a Sepharose binding assay. GST or GST-cadherin proteins were immobilized on Sepharose beads and incubated with purified Src. After washing, the beads were incubated for 2 h with recombinant Shc (2 μg/ml) in the presence or absence of the indicated peptide (100 μg/ml). After incubation, the Sepharose beads were washed and bound proteins were subjected to SDS-PAGE and Western blotting using either anti-Shc or anti-GST. **B:** Peptide competition in a gel overlay experiment. After incubation with Src, tyrosine phosphorylated GST-cadherin fusion proteins were subjected to SDS-PAGE and transferred to a nitrocellulose filter. Bound proteins were denatured with 6M guanidinium hydrochloride and renatured by serial dilution. The filter was then incubated with recombinant Shc (1.6 μg/ml) in the absence or presence of the indicated peptides (100 μg/ml). After incubation for 16 h, the filter was washed with TBST buffer and bound proteins were detected by a Western blotting with either anti-Shc or anti-cadherin.

Site-Directed Mutagenesis Analysis

To confirm the importance of Tyr851 and Tyr883 in this interaction, site-directed mutagenesis of these residues was employed to produce single or double Tyr → Phe mutant cadherins. The Wild-type and mutant cadherin cytoplasmic domains were then expressed as GST fusion proteins and subjected to a Src kinase assay. The results, shown in Figure 2 (top panel), indicate that Tyr883 is the major tyrosine phosphorylation site for Src as the Y883F single mutation dramatically decreased the capacity of cadherin to function as a substrate for Src. The data also suggest that Tyr851 is a minor tyrosine phosphorylation site for Src. The Y851F mutant showed a slight decrease in tyrosine phosphorylation, but when the double mutant (Y851, 883F) was assayed, there was a

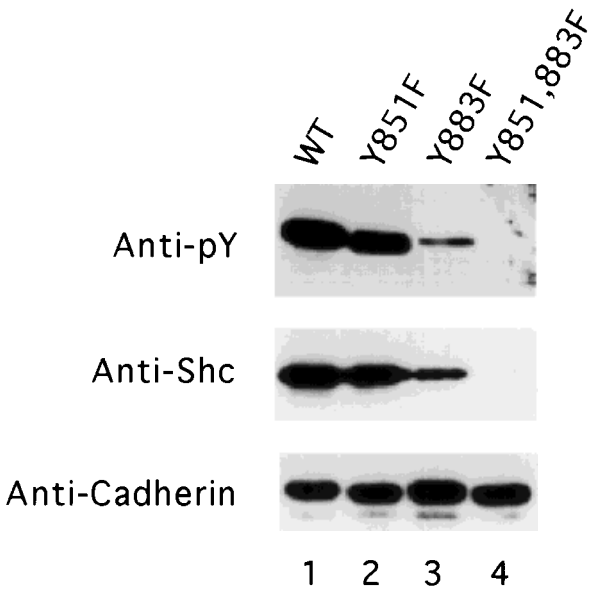

Fig. 2. Influence of mutagenesis of Tyr851 and Tyr883 on tyrosine phosphorylation of cadherin and Shc association. GST-cadherin fusion proteins (Wild-type or mutants) were immobilized on Sepharose beads and incubated with Src kinase. The beads were then washed and incubated for 30 min with recombinant Shc protein (2.4 μg/ml). After washing, bound proteins were subjected to SDS-PAGE and western blotting using anti-Shc, anti-PY or anti-cadherin as indicated.

further decrease in tyrosine phosphorylation compared with the Y883F mutant.

Since the capacity of Shc to interact with cadherin is dependent on the tyrosine phosphorylation of cadherin [Xu et al. 1997], cadherin mutants were assayed for Shc association using GST-cadherin immobilized on Sepharose beads (Fig. 2, middle panel). The binding of Shc to cadherin in this assay correlates well with the tyrosine phosphorylation of different cadherin mutants. Compared to Shc association with wild-type GST-cadherin, mutagenesis of Tyr883 significantly decreased Shc association, while mutagenesis of Tyr851 alone had a minor influence on Shc association. However, Tyr851 does seem to contribute to Shc binding, as the Y851, 883F double mutant bound Shc to a lesser extent than the Y883F mutant. The lower panel in Figure 2 verifies that there was a similar amount of GST-cadherin in each sample. Since neither Tyr851 nor Tyr 883 is preceded by residues that constitute a consensus site for PTB domains, it seems likely that the observed Shc association with tyrosine phosphorylated cadherin in this system is due to the SH2 domain of Shc and not the PTB domain.

Binding of Shc and β-Catenin to Cadherin

It is well known that the cytoplasmic domain of cadherin is linked to actin filaments through β-catenin and other proteins and that β-catenin is the major regulatory component of cadherin-mediated cell-cell adhesion. The Tyr to Phe mutations at residues 851 and 883 that decrease the capacity of cadherin to act as a Src substrate and to bind Shc are close to the β-catenin binding site on cadherin located at residues 832–862 in E-cadherin [Stapport and Kemler, 1994]. Therefore, the influence of these mutations on the binding of β-catenin to cadherin was tested (Fig. 3). Cell lysates containing β-catenin were tested for their capacity to associate with immobilized GST-cadherins. After incubation with lysate and washing, the GST-cadherins were probed with anti-β-catenin. The results show that Wild-type, Y883F, and Y851,883F GST-cadherins associate with β-catenin to a similar extent. In this assay, when the GST-cadherin was not prephosphorylated, β-catenin association with cadherin was not affected. These data indicate that neither Tyr851 nor Tyr883 is essential for β-catenin binding to cadherin.

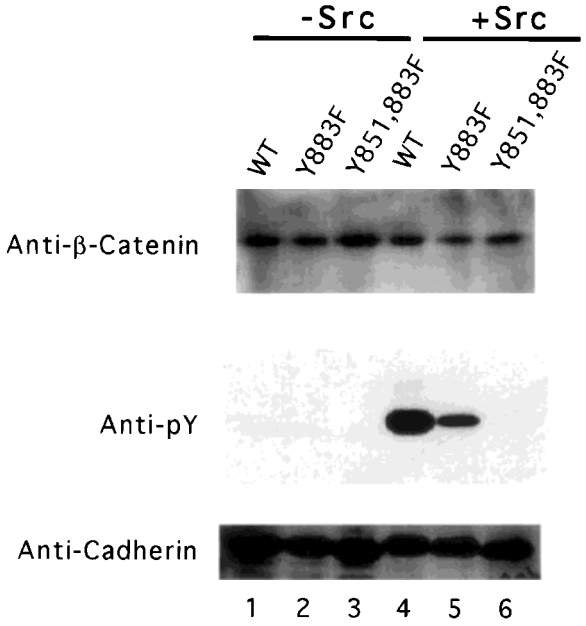

Fig. 3. The binding of β-catenin to the cytoplasmic domain of Wild-type and mutant cadherin. GST-cadherin cytoplasmic domain fusion proteins (Wild-type or tyrosine mutants, as indicated) were immobilized on Sepharose beads and incubated with or without purified Src kinase. After washing, the beads were incubated for 2 h with 1 mg A-431 cell lysate, as a source of β-catenin. The beads were then washed and bound proteins were subjected to SDS-PAGE and Western blotting using anti-β-catenin, anti-PY or anti-cadherin as indicated.

The data in Figure 3 do not allow a conclusion as to whether Shc binding to cadherin may influence β-catenin binding to the same molecule. Therefore, a competition experiment was performed to determine whether the pre-binding of Shc to cadherin interferes with the binding of β-catenin. Tyrosine phosphorylated GST-cadherin fusion protein was incubated with an A-431 cell lysate, as a source of β-catenin, in the absence or presence of increasing amounts of recombinant Shc. Thereafter the amount of β-catenin associated with cadherin was assayed. As the data in Figure 4 demonstrate, with increased concentrations of recombinant Shc there was an increased amount of Shc bound to cadherin and a decreased amount of β-catenin associated with cadherin. These results suggest that the Shc and β-catenin binding sites on cadherin may overlap, such that Shc binding to cadherin either provides a steric hindrance to β-catenin binding or alters the conformation of cadherin such that β-catenin is not recognized. While an interaction between Shc and β-catenin (not involving cadherin) has not been reported, such an interaction might

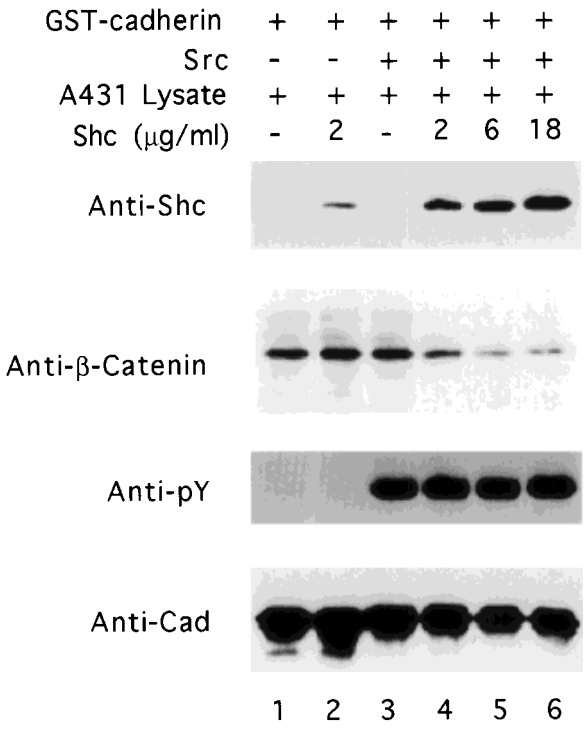

Fig. 4. The competitive binding of Shc and β-catenin to the cytoplasmic domain of cadherin. GST-cadherin cytoplasmic domain fusion protein was immobilized on Sepharose beads and incubated with or without purified Src kinase as indicated. The beads were then incubated for 2 h with A-431 cell lysate (1 mg) in the absence or presence of increasing concentrations of recombinant Shc. After incubation and washing, bound proteins were subjected to SDS-PAGE and Western blotting using anti-Shc, anti-β-catenin, anti-PY, or anti-cadherin as indicated.

account for the observed decrease in β-catenin binding to cadherin when increased recombinant Shc was added to the lysates. However, we have been unable to detect a Shc:β-catenin interaction in these lysates by co-precipitation assays (data not shown).

Analysis of phosphotyrosine peptide libraries with the SH2 domain of Shc lead to the conclusion that a consensus recognition site for this SH2 domain would contain phosphotyrosine followed at the +1 position by a hydrophobic or negatively charged residue and either Ile or Leu at the +3 position [Songyang et al., 1994]. The data predicted no selectivity would exist at the +2 position and the strongest determinant in the association would be the residue at the +3 position. The high resolution structure of the Shc SH2 domain has been determined by NMR [Zhou et al., 1995] and X-ray crystallography [Mikol et al., 1995]. In the former case the SH2 structure was determined in complex with a phosphotyrosine-containing peptide derived from the chain of the T cell receptor. This peptide sequence contained the sequence pTyr GlnGlyLeu, which conforms at the +3 position to the consensus sequence developed with peptide libraries. In the cadherin cytoplasmic domain, we have identified Tyr883 and to a lesser extent Tyr851 as recognition sites for the SH2 domain of Shc. The sequence at residue 883 is pTyrAspTyrLeu, which conforms well to the Shc SH2 consensus sequence at both the +1 and +3 positions. All cadherins have Leu at the +3 position and, with a few exceptions, all have Asp at the +1 position. In two cases. XB/U-cadherin and EP-cadherin, there is Asn at the +1 position, which is comparable to the sequence in the T cell receptor chain. Tyr851 in cadherin is followed by well conserved Asp and Leu residues at the +1 position and the +3 position, respectively, which conforms well to the predicted recognition sequence for the SH2 domain of Shc. That Tyr883 is the major Shc association site compared to Tyr 851 reflects its higher selectivity as a Src phosphorylation site. Unlike Tyr851, Tyr883 in cadherins is preceded by acidic residues at the -1 and -3 positions, which often denote tyrosine kinase recognition sites [Songyang et al., 1995].

While the biologic significance of Shc interaction with cadherin remains uncertain, use of the cadherin mutants described herein may provide a valuable approach to address this issue.

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REFERENCES

Balsamo J, Leung TC, Ernst H, Zanin MKB, Hoffman S, Lilien J. 1996. Regulated binding of a PTP1B-like phosphatase to N-cadherin: control of cadherin-mediated adhesion by dephosphorylation of β-catenin. J Cell Biol 134:801–813.

Balsamo J, Arregui C, Leung TC, Lilien J. 1998. The nonreceptor protein tyrosine phosphatase PTP1B binds to the cytoplasmic domain of N-cadherin and regulates the cadherin-actin linkage. J Cell Biol 143:523–532.

Behrens J, Vakaet L, Friis R, Winterhager E, Van Roy F, Mareel MM, Birchmeier W. 1993. Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/β-catenin complex in cells transformed with a temperature-sensitive v-Src gene. J Cell Biol 120:757–766.

Bonfini L, Migliaccio E, Pelicci G, Lanfrancone L, Pelicci PG. 1996. Not all Shc's roads lead to Ras. TIBS 21:257–261.

Brady-Kalnay SM, Rimm DL, Tonks NK. 1995. Receptor protein tyrosine phosphatase PTPμ associates with cadherins and catenins in vivo. J Cell Biol 130:977–986.

Brady-Kalnay SM, Mourton T, Nixon JP, Pietz GE, Kinch M, Chen H, Brackenbury R, Rimm DL, Del Vecchio RL Tonks NK. 1998. Dynamic interaction of PTPμ with multiple cadherins in vivo. J Cell Biol 141:287–296.

Craparo A, O'Neill TJ, Gustafson TA. 1995. Non-SH2 domains within insulin receptor substrate-1 and SHC mediate their phosphotyrosine-dependent interaction with the NPEY:motif of the insulin-like growth factor I receptor. J Biol Chem 270:15639–15643.

Dikic I, Batzer AG, Blaikie P, Obermeir A, Ullrich A, Schlessinger J, Margolis B. 1995. Shc binding to nerve growth factor receptor is mediated by the phosphotyrosine interaction domain. J Biol Chem 270:15125–15129.

Esse S, Lampugnani MG, Corada M, Dejana E, Risau W. 1998. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. J Cell Sci 111:1853–1865.

Fournier E, Rosnet O, Marchetto S, Turck CW, Rottapel R, Pelicci PG, Birbaum D, Borg J-P. 1996. Interaction with the phosphotyrosine binding domain/phosphotyrosine interacting domain of SHC is required for the transforming activity of the FLT4/VEGFR3 receptor tyrosine kinase. J Biol Chem 271:12956–1263.

Gotoh N, Muroya K, Hattori S, Nakamura S, Chida K, Shibuya M. 1995. The SH2 domain of Shc suppresses EGF-induced mitogenesis in a dominant negative manner. Oncogene 11:2525–2533.

Gotoh N, Toyoda M, Shibuya M. 1997. Tyrosine phosphorylation sites at amino acids 239 and 240 of Shc are involved in epidermal growth factor-induced mitogenic signaling that is distinct from ras/mitogen-activated protein kinase activation. Molec Cell Biol 17:1824–1831.

Hamaguchi M, Matsuyoshi N, Ohnishi Y, Botoh B, Takeichi M, Nagai Y. 1993. $p60^{v\text{-}src}$ causes tyrosine phosphorylation and inactivation of the N-cadherin-catenin cell adhesion system. EMBO J 12:307–314.

Hazan RB, Norton L. 1998. The epidermal growth factor receptor modulates the interaction of E-cadherin with the actin cytoskeleton. J Biol Chem 273:9078–9084.

He W, O'Neill T, Gustafson TA. 1995. Distinct modes of interaction of SHC and insulin receptor substrate-1 with the insulin receptor NPEY region via non-SH2 domains. J Biol Chem 270:23258–23262.

Hoschuetzky H, Aberler H, Kemler R. 1994. β-catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. J Cell Biol 127:1375–1380.

Isakoff SJ, Yu YP, Su Y-C, Blaikie P, Yajnik V, Rose E, Weidner KM, Sachs M, Margolis B, Skolnik EY. 1996. Interaction between the phosphotyrosine binding domain of Shc and the insulin receptor is required for Shc phosphorylation by insulin in vivo. J Biol Chem. 271: 3959–3962.

Kypta RM, Su H, Reichardt LF. 1996. Association between a transmembrane protein tyrosine phosphatase and the cadherin-catenin complex. J Cell Biol 134:1519–1529.

Mainiero F, Pepe A, Wary KK, Spinardi L, Mohammadi M, Schlessinger J, Giancotti FG. 1995. Signal transduction by the $\alpha_6\beta_4$ integrin: distinct β_4 subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes. EMBO J 14:4470–4481.

Mikol V, Baumann G, Zurini MG M, Hommel U. 1995. Crystal structure of the SH2 domain from the adaptor protein SHC: a model for peptide binding based on X-ray and NRM data. J Mol Biol 254:86–95.

O'Bryan JP, Lambert QT, Der CJ. 1998. The src homology 2 and phosphotyrosine binding domains of the ShcC adaptor protein function as inhibitors of mitogenic signaling by the epidermal growth factor receptor. J Biol Chem 273:20431–20437.

Ozawa M, Kemler R. 1998. Altered cell adhesion activity by pervanadate due to the dissociation of α-catenin from the D-cadherinßcatenin complex. J Biol Chem 273:6166–6170.

Pratt JC, Weiss M, Sieff CA, Shoelson SE, Burakoff SJ, Ravichandran KS. 1996. Evidence for a physical association between the Shc-PTB domain and the β_c chain of the granulocyte-macrophage colony-stimulating factor receptor. J Biol Chem 271:12137–12140.

Prigent SA, Pillar TS, Ravichandran KS, Gullick CJ. 1995. Binding of Shc to the NPXY motif is mediated by its N-terminal domain. J Biol Chem 270:22097–22100.

Ricketts WA, Rose DW, Shoelson S, Olefsky JM. 1996. Functional roles of the Shc phosphotyrosine binding and src homology 2 domains in insulin and epidermal growth factor signaling. J Biol Chem 271:26165–26169.

Sakaguchi K, Okabayashi Y, Kido Y, Kimura S, Matsumura Y, Inushima K, Kasuga M. 1998. Shc phosphotyrosine-binding domain dominantly interacts with epidermal growth factor receptors and mediates ras activation in intact cells. Mol Endrocinol 12:536–543.

Sasaoka T, Ishihara H, Sawa T, Ishiki M, Morioka H, Imamura T, Usui I, Takata Y, Kobayashi M. 1996. Functional importance of amino-terminal domain of Shc for interaction with insulin and epidermal growth factor receptors in phosphorylation-independent manner. J Biol Chem 271:20082–20087.

Soler C, Rousselle P, Damour O. 1998. Cadherin mediated cell-cell adhesion is regulated by tyrosine phosphatases in human keratinocytes. Cell Adhesion Comm 5:13–25.

Songyang Z, Shoelson SE, Chaudhuri M, Gish G, Pawson T, Haser WG, Lechleider RJ, Neel BG, Birge RB, Fajardo JE, Chou MM, Hanafusa H, Schaffhausen B. Cantley LC. 1993. SH2 domains recognize specific phosphopeptide sequences. Cell 72:767–778.

Songyang Z, Shoelson SE, McGlade J, Olivier P, Pawson T, Bustello XR, Barbacid M, Sabe H, Hanafusa H, Yi T, Ren R, Baltimore D, Ratnofsky S, Feldman RA, Cantley LC. 1994. Specific motifs recognized by the SH2 domains of Csk, 3BP2, fps/fes, GRB-2, HCP, SHC, Syk and Vav. Mol Cell Biol 14:2777–2785.

Songyang Z, Carraway KL III, Eck MJ, Harrison SC, Feldman RA, Mohammadi M, Schlessinger J, Hubbard SR, Smith DP, Eng C, Lorenzo MJ, Ponder BAJ, Mayer BJ, Cantley LC. 1995. Catalytic specificity of protein-tyrosine kinases is critical for elective signalling. Nature 373:536–539.

Stappert J, Kemler R. 1994. A short core region of E-cadherin is essential for catenin binding and is highly phosphorylated. Cell Adhesion Comm 2:319–327.

Takeichi M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251:1451–1455.

Tsukita S, Oishi K, Akiyama T, Yamanashi Y, Yamamot T, Tsukita S. 1991. Specific proto-oncogenic tyrosine kinases of the *src* amily are enriched in cell-to-cell adherens junctions where the level of tyrosine phosphorylation is elevated. J Cell Biol 113:867–879.

van der Geer P, Pawson T. 1995. The PTB domain: a new protein module implicated in signal transduction. TIBS 20:277–280.

Wary KK, Mainiero F, Isakoff SJ, Marcantonio EE, Giancotti FG. 1996. The adaptor protein Shc couples a class of integrins to the control of cell cycle progression. Cell 87:733–743.

Xu Y, Guo D-F, Davidson M, Inagami T, Carpenter G. 1997. Interaction of the adaptor protein Shc and the adhesion molecule cadherin. J Biol Chem 272:13463–13466.

Yajnik V, Blaikie P, Bork P, Margolis B. 1996. Identification of residues within the SHC phosphotyrosine binding/phosphotyrosine interaction domain crucial for phosphopeptide interaction. J Biol Chem 271:1813–1816.

Zhou M-M, Meadows RP, Logan TM, Yoon HS, Wade WS, Ravichandran KS, Burakoff SJ, Fesik SW. 1995. Solution structure of the Shc SH2 domain complexed with a tyrosine-phosphorylated peptide from the T-cell receptor. Proc Natl Acad Sci USA 92:7784–7788.