Tyrosine Phosphorylation of Caldesmon Is Required for Binding to the Shc-Grb2 Complex*

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S3-v-erbB is a retroviral oncogene that encodes a ligand-independent, transforming mutant of the epidermal growth factor receptor. This oncogene has been shown to be sarcomagenic in vivo and to transform fibroblasts in vitro. Our previous studies (McManus, M. J., Lingle, W. L., Salisbury, J. L., and Maihle, N. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11351–11356) showed that expression of S3-v-erbB in primary fibroblasts results in the tyrosine phosphorylation of caldesmon (CaD), an actin- and calmodulin-binding protein. This phosphorylation is transformation-associated, and the phosphorylated form of CaD is associated with a signaling complex consisting of Shc, Grb2, and Sos in transformed fibroblasts. To identify the tyrosine phosphorylation site(s) in the CaD molecule and to further elucidate the functional role of CaD tyrosine phosphorylation in S3-v-ErbB oncogenic signaling, we have generated a series of mutant CaDs in which one or more tyrosine residues have been replaced with phenylalanine. Using a CaD null cell line, DF1 cells (an immortalized chicken embryo fibroblast cell line), and transient transfection assays, we demonstrated that Tyr-27 and Tyr-393 are the major sites of tyrosine phosphorylation on CaD. Interestingly, Tyr-27 is located within the myosin binding domain of CaD, and Tyr-393 is adjacent to one of the major actin binding and actomyosin ATPase inhibitory domains. Our studies also show that the tyrosine phosphorylation of CaD enhances its binding to the Shc-Grb2 complex. Specifically, replacement of Tyr-27, but not of Tyr-165 or Tyr-393, significantly reduces the ability of CaD to interact with the Shc-Grb2 complex. Together, these studies demonstrate that the major sites of tyrosine phosphorylation on CaD are located in the myosin and actin binding domains of CaD and that Tyr-27 is the major tyrosine phosphorylation site through which CaD interacts with the Shc-Grb2 complex.

In response to ligand stimulation, the epidermal growth factor receptor (EGFR) undergoes a series of changes, including homo- and/or heterodimerization, activation of the kinase domain, and autophosphorylation of tyrosine residues in its carboxyl-terminal domain (reviewed in Refs. 1–5). The activated EGFR in turn phosphorylates a variety of intracellular substrates, and the phosphotyrosine residues on the receptor itself function as docking sites for Src homology 2 (SH2) and phosphotyrosine binding domain-containing proteins such as Shc and Grb2 (6, 7). Shc, once phosphorylated on tyrosine, binds to both the activated EGFR and to the SH2 domain of Grb2 (6, 7). Subsequently, Grb2 interacts with the nucleotide exchange factor Sos through its SH3 domain, thereby recruiting the Shc-Grb2-Sos-signaling complex to the plasma membrane, leading to activation of the Ras/Raf signal transduction pathway (reviewed in Ref. 8; reviewed in Ref. 9). This EGFR-induced signaling pathway is strictly mitogenic; mere overexpression of the EGFR generally does not result in transformation (10–12).

Unlike the wild-type EGFR receptor, mutant receptor proteins encoded by the retroviral oncogene v-erbB exhibit ligand-independent tyrosine kinase activity, and most of these viral oncoproteins cause transformation in a tissue-specific manner (reviewed in Refs. 13 and 14). For instance, E1-v-ErbB, which lacks the extracellular ligand binding domain, only elicits transformation in erythroblasts (reviewed in Ref. 14). S3-v-ErbB, which has deletions in both the extracellular domain and the carboxyl-terminal region, is sarcomagenic but not leukemogenic (reviewed in Ref. 14). Tissue-specific transformation by either E1-v-ErbB or S3-v-ErbB is correlated with tyrosine phosphorylation events that are distinct from those induced by the normal interactions that occur between wild-type EGFR and its ligands (15). These observations indicate that the oncogenic signal transduction pathways induced by E1-v-ErbB and S3-v-ErbB may be different from the mitogenic signaling pathways stimulated by ligand binding to the wild-type receptor. This idea has been reinforced by the recent finding that caldesmon (CaD), a regulatory protein that modulates actin-myosin interactions and the reorganization and stability of actin stress fibers, becomes phosphorylated on tyrosine in S3-v-ErbB-transformed cells but not in E1-v-ErbB-expressing cells or in cells stimulated with ligand (16). Although both E1-v-ErbB and S3-v-ErbB expression induces the formation of a Shc-Grb2-Sos-signaling complex, the tyrosine-phosphorylated form of CaD only associates with the Shc-Grb2-Sos complex in S3-v-ErbB-transformed cells (16). This distinct pattern of tyrosine phosphorylation events in S3-v-ErbB-transformed fibroblasts may be associated with the molecular basis for transformation by this receptor. However, it remains to be determined whether the tyrosine phosphorylation of CaD is required for its interaction with the Shc-Grb2-signaling complex and what functional role CaD tyrosine phosphorylation may play in S3-v-ErbB-induced oncogenic signaling.
CaD is a ubiquitous actin- and calmodulin-binding protein (reviewed in Refs. 17 and 18). Two isoforms, h-CaD and l-CaD, have been identified, which result from alternative splicing of mRNA transcripts arising from a single gene (19, 20). h-CaD is present only in smooth muscle cells (19, 20). In contrast, l-CaD is widely distributed in a variety of nonmuscle cells and is one of the major components of actin stress fibers (19, 20). Although it lacks the central helical region that is present in h-CaD, l-CaD shares all of the other structural and functional features of h-CaD, including a rod-like molecular shape, heat stability, ability to bind actin, myosin, tropomyosin, and calmodulin, and inhibition of actomyosin ATPase activity (Refs. 19 and 20; reviewed in Refs. 17 and 18). There is increasing evidence that CaD plays a regulatory role in microfilament reorganization during cell division as well as in actin-based cell motility in interphase cells (Refs. 21–23; reviewed in Refs. 18 and 24). In mitotic cells, the binding of CaD to actin stress fibers is inhibited by the serine phosphorylation of CaD via the cell cycle-dependent kinase p34^cdc2, resulting in stress fiber disassembly (25, 26). In interphase cells, overexpression of a CaD carboxy-terminal fragment, which contains the actin, calmodulin, and tropomyosin binding domains of CaD, increases the number of tropomyosin-associated actin filaments and also causes an increase in cytochalasin B resistance due to the increased stability of microfilaments (21). In addition, in recent studies by Lamb et al. (27), inhibition of CaD via microinjection of a CaD-specific antibody can lead to disruption of actin stress fibers.

In this study, we have concentrated on structurally mapping the sites of CaD tyrosine phosphorylation in transformed fibroblasts and on identifying the functionally relevant site(s) of tyrosine phosphorylation in the CaD molecule. Using a series of CaDs bearing point mutations, a CaD null cell line (DF1), and transient transfection assays, we confirm previous results demonstrating that CaD is tyrosine-phosphorylated only in transformed cells, and we identify tyrosine 27 and tyrosine 393 as the major sites of CaD tyrosine phosphorylation. In further experiments, our studies demonstrate that the association of CaD with the S3-v-ErbB-induced Shc-Grb2 signaling complex is dependent on the tyrosine phosphorylation of CaD.

MATERIALS AND METHODS

Reagents and Antibodies—Anti-human CaD monoclonal antibody and anti-chicken gizzard CaD monoclonal antibody were from Transduction Laboratories (San Diego, CA) and Sigma, respectively. Both anti-human Shc and anti-phosphotyrosine monoclonal antibody 4G10 were obtained from Transduction Laboratories (San Diego, CA) and Sigma, respectively. Both anti-chicken gizzard CaD monoclonal antibody were from Transduction Laboratories (San Diego, CA) and Sigma, respectively. Both anti-chicken gizzard CaD monoclonal antibody were from Transduction Laboratories (San Diego, CA) and Sigma, respectively.

Plasmid Construction and Mutagenesis—A recombinant pBluescript CaD plasmid containing the entire coding sequence of chicken gizzard nonmuscle CaD (from Joseph Bryan, Baylor College of Medicine) was used as the template for our site-directed mutagenesis reactions. The oligonucleotides used to create the tyrosine-to-phenylalanine mutations were as follows: 5′-GCAAGAGAAGTCCCTCAGGAAGATGTA-3′ for changing Tyr-27 to Phe; 5′-ACAGTTACGAAATCTGCACCAA-3′ for changing Tyr-165 to Phe; 5′-AGTACAGTGA-3′ for changing Tyr-393 to Phe. The underlined sequences indicate the mutated regions. The mutant forms of CaD, in which one or more tyrosine residues were replaced with phenylalanine, were generated using a polymerase chain reaction-based site-directed mutagenesis system (Stratagene, La Jolla, CA), according to the manufacturer's instructions.

The structure of all mutant CaDs was confirmed by DNA sequencing. The wild-type CaD cDNA and each of the mutant CaD constructs were introduced into the BomIII and ApoI sites of the pcDNA3 expression vector (Invitrogen, Carlsbad, CA).

Cells and Viruses—Primary cultures of chicken embryo fibroblasts (CEFs) were prepared and cultured as described (15, 16). DF1, an immortalized cell line, was derived from CEFs (28, 29) and maintained in Dulbecco's modified Eagle's medium containing 4.5 g of glucose/liter and supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (50 μg/ml), and 0.1% amphotericin B (Fungizone). A cDNA encoding S3-v-ErbB was inserted into the recombinant avian leukosis (RCAN) vector (30), and the avian recombinant retrovirus was produced by transfecting the recombinant RCAN vector into CEF cells that were derived from blast transformation is closely correlated with the tyrosine phosphorylation of CaD, a known regulator of actin stress fiber stability (16). To identify the major tyrosine phosphorylation site(s) in the CaD molecule, we have taken advantage of the avian fibroblast cell line, DF1, a stable, nontransformed cell line that exhibits anchorage-dependent growth and normal

RESULTS

We demonstrated previously that S3-v-ErbB-induced fibroblast transformation is closely correlated with the tyrosine phosphorylation of CaD, a known regulator of actin stress fiber stability (16). To identify the major tyrosine phosphorylation site(s) in the CaD molecule, we have taken advantage of the avian fibroblast cell line, DF1, a stable, nontransformed cell line that exhibits anchorage-dependent growth and normal...
fibroblastic morphology (28, 29). DF1 cells, although derived from CEFs, have a much longer life span in culture than CEFs (28, 29) and contain no detectable CaD (see Fig. 1B). After infecting DF1 cells with the recombinant helper-independent retroviral vector RCAN containing S3-v-erbB cDNA, S3-v-ErbB was expressed at high levels, similar to the levels seen in S3-v-erbB-infected CEFs (Fig. 1A). We then tested whether transient transfection could be used to overexpress CaD in S3-v-erbB-infected DF1 cells. As shown in Fig. 1B, transient transfection of CaD in DF1 cells resulted in an exceptionally high level of CaD expression (5–10-fold greater than the level detected in CEF cells, data not shown). Because of the lack of endogenous CaD in DF1, we chose DF1 cells for our analysis of the tyrosine phosphorylation sites in the CaD molecule.

There are three potential tyrosine phosphoacceptor sites in avian f-CaD. As illustrated in Fig. 2, we constructed a series of CaD mutants in which one or more of the three tyrosine residues were replaced with phenylalanine. These mutant forms of CaD included three with single replacements (CaDY27F, CaDY165F, and CaDY393F), three with double replacements (CaDY27/165F, CaDY165/393F, and CaDY27/393F), and one in which all three tyrosines were replaced (CaDY27/165/393F). The amino-terminal tyrosine of CaD (Tyr-27) is located just within the major myosin-binding site (33), and the carboxyterminal tyrosine (Tyr-393) is located close to one of the actin binding domains (34–39). Both Tyr-27 and Tyr-165 lie within a YQXXN sequence context, similar to the consensus YQNX sequence recognized by the SH2 domain of Grb2 (40). In contrast, Tyr-393 lies within a sequence that does not conform to any known phosphotyrosine binding motif.

As illustrated in Fig. 3A, tyrosine phosphorylation of CaD was detectable only in DF1S3 cells transfected with the recombinant CaD vector (Fig. 3A, DF1S3+CaD). In contrast, the tyrosine-phosphorylated form of CaD was not detectable in any of the control samples (Fig. 3A, DF1, DF1S3, or DF1+CaD). Immunoblot analysis of the same samples with anti-CaD (Fig. 3, panel B) showed that equivalent amounts of CaD were present in the immunoprecipitates from these transfectants (Fig. 3B). These data strongly suggest that in DF1 cells, CaD tyrosine phosphorylation is dependent on S3-v-ErbB expression.

Next we sought to determine the major sites of tyrosine phosphorylation on CaD by performing the experiments described above using the mutant forms of CaD with substituted tyrosines. Fig. 3, A and C, show that the mutation of either Tyr-27 (DF1S3+CaDY27F) or Tyr-393 (DF1S3+CaDY393F) significantly decreased the level of tyrosine phosphorylation of CaD, whereas the mutation of Tyr-165 (DF1S3+CaDY165F) resulted in only a slight reduction in the tyrosine phosphorylation of CaD (Fig. 3C). As expected, the triple substitution mutant was not tyrosine-phosphorylated (Fig. 3A, DF1S3+CaDY27/165/393F). These immunoblot results suggest that the tyrosine residues Tyr-27 and Tyr-393 of CaD are the major sites of tyrosine phosphorylation in vivo.

To verify these results, DF1 cells transfected with wild-type CaD and DF1S3 cells transfected with wild-type CaD or the series of CaD double mutants in which only one CaD tyrosine residue was retained were radiolabeled with 32P, and immunoprecipitated with anti-CaD antibody, and the immunoprecipitates were resolved by SDS-PAGE. The resulting radiolabeled CaD proteins were subjected to acid hydrolysis, and the products were analyzed by two-dimensional phosphoamino acid mapping. Fig. 4A shows the phosphorylation pattern of CaD in DF1 cells expressing recombinant wild-type CaD. Two major 32P-labeled spots comigrated with the phosphoserine and phosphothreonine standards, but no radioactivity comigrated with the marker for phosphotyrosine (Fig. 4A). In contrast, in DF1S3 cells expressing recombinant wild-type CaD, three radiolabeled spots were detectable, which comigrated with the phosphoserine, phosphothreonine, and phosphotyrosine standards (Fig. 4B). This observation was consistent with our earlier finding that CaD is only tyrosine-phosphorylated in DF1 cells expressing S3-v-ErbB (as in CEFS3 (16)). In addition, both CaDY27/165F (Fig. 4C) and CaDY165/393F (Fig. 4E) were tyrosine-phosphorylated in DF1S3 cells; however, the level of tyrosine phosphorylation for both of these CaD mutants was relatively low compared with that of wild-type CaD (Fig. 4B). In contrast, the CaDY27/393F mutant was not tyrosine-phosphorylated (Fig. 4D). As predicted, no detectable tyrosine phosphorylation was present on the CaD triple mutant (Fig. 4F), even though this form was still heavily serine/threonine-phosphorylated. The relative level of tyrosine phosphorylation of wild-type CaD and each of the mutant CaDs was determined by calculating the ratio of phosphotyrosine to phosphoserine (pY/pS Ratio, Fig. 5). Substitution of either Tyr-27 or Tyr-393, but not Tyr-165, significantly reduced the Tyro/(Ser+Thr) ratio, and the Tyro/(Ser+Thr) ratio for CaDY165/393F was much lower than that observed for CaDY27/165F (Fig. 5), suggesting that the stoichiometry of Tyr-393 phosphorylation is greater than that of Tyr-27. Taken together, these results clearly demonstrate that Tyr-27 and Tyr-393, but not Tyr-165, are the major sites of tyrosine phosphorylation in the CaD molecule in vivo.

To further investigate whether tyrosine phosphorylation of CaD is required for its interaction with the Shc-associated complex as our previous results indicated (16), we immunoprecipitated Shc from transfected DF1 or DF1S3 cells and performed immunoblot analysis with anti-CaD. Surprisingly, we observed that both the wild-type recombinant CaD overexpressed in DF1 cells and the CaD triple mutant were able to interact with Shc at low but detectable levels (Fig. 6A, DF1+CaD and DF1S3+CaDY27/165/393F). However, tyrosine phosphorylation of CaD significantly enhanced its binding to this complex (Fig. 6A, DF1S3+CaD). Importantly, the substitution of Tyr-27 sharply decreased the binding of CaD to Shc in DF1S3 cells (Fig. 6A, DF1S3+CaDY27F). Finally, the mutation of either Tyr-165 or Tyr-393 showed no effect on the binding of CaD to Shc (Fig. 6A, DF1S3+CaDY165F and DF1S3+CaDY393F). Densitometric analysis indicated that the...
phosphorylation of CaD caused a 4–5-fold increase in its binding to the Shc-associated complex when compared with the triple mutant expressed in DF1S3 or wild-type CaD expressed in DF1 (data not shown). Together, these observations suggest that in the absence of S3-v-erbB expression there is a low level of CaD binding to the Shc-associated complex and that this binding is significantly enhanced by the tyrosine phosphorylation of CaD on tyrosine 27 but not on tyrosine 393.

In addition, we have performed experiments to investigate the functional role of these Tyr-to-Phe mutant CaDs in S3-v-erbB-transformed fibroblasts. Specifically, we co-infected CEFs with envelope subtype-specific RCAS/RCAN retroviral vectors (41) containing S3-v-erbB and wild-type CaD or the mutant CaD in which all the tyrosine residues were replaced with phenylalanine to see if co-expression of S3-v-ErbB with CaD or the CaD triple mutant could inhibit anchorage-independent growth in soft agar. The overexpression of either wild-type CaD or the CaD triple mutant resulted in cell death in a majority of the fibroblasts, with no evidence of expression over the endogenous levels in the small population of surviving cells. Thus, it appears that the expression of CaD is under tight regulation in CEFs and that the overexpression of CaD from exogenous sources may be toxic to cells. This conclusion is further supported by our observation that stable clones of DF1 cells that overexpress CaD could not be isolated or propagated (data not shown) and by the results of Warren et al. (21) using Chinese hamster ovary cell lines.

**DISCUSSION**

We previously showed that S3-v-ErbB expression results in the development of a Shc-associated phosphoprotein complex in transformed CEFs (16). This observation is particularly interesting, because a tyrosine-phosphorylated form of CaD, a known regulator of actin-myosin interactions, is selectively recruited to this complex only in transformed fibroblasts. Consistent with these studies, in this report we observed that CaD only becomes tyrosine-phosphorylated in DF1 cells expressing S3-v-ErbB (Fig. 2). The fact that there is no detectable endogenous CaD in DF1 cells (Fig. 1) greatly facilitated our phosphoamino acid mapping analysis and allowed us to test the in vivo structure and function of our CaD tyrosine substitution mutants (Fig. 2) without interfering effects from endogenous CaD.

Domain analysis of CaD and CaD fragments produced from limited proteolysis, chemical cleavage, and expression systems has led to the precise localization of various functional domains within the CaD molecule (33–39). For example, the myosin-binding site was first localized to the amino-terminal region of CaD (42, 43) and was recently mapped to the sequence between residues 24 and 53 (33). The crucial actin and calmodulin binding and actomyosin ATPase inhibitory motifs have been narrowed down to three segments that reside between residues 419–433, 459–466, and 487–498 in the carboxyl terminus of CaD (34–39). Using phosphotyrosine immunoblot analysis and phosphoamino acid mapping, we demonstrated here that Tyr-27 and Tyr-393 are the major sites of tyrosine phosphorylation on CaD (Figs. 3 and 4). Interestingly, Tyr-27 is located within the myosin-binding site of CaD, and Tyr-393 lies just upstream of the major actin binding and actomyosin ATPase inhibitory motifs and just downstream of the tropomyosin binding domain (Fig. 2).

It has been documented that CaD can regulate the reorganization and stability of actin stress fibers directly by binding to actin filaments (reviewed in Refs. 17 and 18) or indirectly by modulating the functions of either the actin-severing protein, gelsolin (23), or of the actin-bundling protein, fascin (44). In addition, CaD can modulate cell motility by binding to myosin through its amino-terminal region and by binding to actin through its carboxyl-terminal region. Support for this role for CaD in cell motility comes from our previous observation that CaD decreases actin filament velocity over myosin in an in vitro motility assay (33) and from the recent observation that CaD can inhibit cell contractility in vitro and can block the retrograde movement of cadherin-coated beads attached to lamellipodia (45). In addition to binding to actin and myosin, CaD also interacts with many other proteins including tropomyosin, tubulin, and calmodulin (Refs. 46–49; reviewed in Ref. 17). Moreover, CaD has been shown to be a substrate for many serine/threonine protein kinases (26, 50–53), including Ca2+-calmodulin-dependent protein kinase II (51), casein kinase II (52), protein kinase C (53), and mitogen-activated protein kinase (50). In these instances the serine/threonine phosphorylation of CaD down-regulates CaD function. For example, CaD serine phosphorylation by p34cdc2 kinase, protein kinase C, and mitogen-activated protein kinase decreases the binding affinity of CaD for actin (26, 50, 53), whereas the serine phosphorylation of CaD by either Ca2+-calmodulin-dependent protein kinase II or casein kinase II weakens its affinity for myosin (51, 52). Given that the functional interactions of CaD can be modulated by serine phosphorylation, that Tyr-27 is located within the myosin binding domain, and that Tyr-393 is adjacent to...
both the actin binding and tropomyosin binding domains of CaD, it is conceivable that the phosphorylation of these two tyrosine residues may reduce the binding affinity of CaD for myosin or actin or tropomyosin, respectively, thereby promoting the instability, disassembly, or breakdown of actin stress fibers, a known characteristic of transformed fibroblasts. Future studies will be designed to test these possibilities.

Although both Tyr-27 and Tyr-393 were found to be major sites of tyrosine phosphorylation on CaD, only the phosphorylation of Tyr-27 appeared to enhance the binding of CaD to the Shc-associated complex in DF1 cells expressing S3-v-ErbB (Fig. 6), suggesting that CaD interacts with one (or more) of these SH2 or phosphotyrosine binding domain-containing proteins via its tyrosine-phosphorylated Tyr-27 residue. Indeed, this S3-v-erB-induced signaling complex contains both Shc and Grb2 (15, 16). Shc contains a carboxy-terminal SH2 domain and an amino-terminal phosphotyrosine binding domain (6), whereas Grb2 contains an SH2 domain flanked by two SH3 domains (8). It has been well established that the Shc-Grb2-Sos complex that forms in response to stimulation by a number of different receptor tyrosine kinases is involved in the activation of the major signaling pathway associated with mitogenic signaling, i.e. the Ras/Raf/mitogen-activated protein kinase cascade (reviewed in Ref. 9). However, our recent data demon-
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state that S3-v-ErbB expression suppresses rather than activates Ras/Raf signaling (54) even though the S3-v-ErbB-induced signaling complex contains Shc, Grb2, and Sos. Why doesn’t the S3-v-ErbB-induced ShcGrb2Sos complex function as an activator of Ras/Raf signaling? It is possible that the association of this complex with CaD interrupts the normal ligand-stimulated interaction between Shc, Grb2, or Sos, which is required for the activation of Ras/Raf/MAP kinase signaling.

It is also possible that the tyrosine phosphorylation of CaD contributes to the transformed phenotype by promoting the disruption or instability of actin stress fibers in interphase fibroblasts. Future studies using Shc and Grb2 fusion constructs as well as various altered forms of CaD with truncation and site-directed point mutations may help us to determine whether the tyrosine-phosphorylated form of CaD interacts with the S3-v-ErbB-induced signaling complex by directly binding to Shc and/or Grb2. Such studies should shed new light on the mechanism by which CaD contributes to oncogenic signaling in S3-v-ErbB transformed fibroblasts.

It is conceivable that tyrosine phosphorylation of CaD may play an important role in the transformation of CEFs by S3-v-ErbB. We have put much effort into studying the effect of wild-type CaD and the CaD triple mutant, which lacks all three tyrosine residues, on the S3-v-ErbB-associated transformed phenotype. Unfortunately, we have been unable to obtain stable CEF cell lines that express either recombinant wild-type or mutant CaD. This is likely due to the toxicity of, or inhibition of, cell growth and survival by overexpressed CaD. It should be noted that the expression of recombinant full-length CaD has an inhibitory effect on the growth and/or survival of other cell types (COS and Chinese hamster ovary cells). For example, Warren et al. (21) were unable to obtain stable Chinese hamster ovary cell lines expressing full-length CaD. Because of these technical difficulties, alternative experimental strategies will need to be pursued.

In summary, we have identified Tyr-27 and Tyr-393 as the major sites of tyrosine phosphorylation on CaD. The tyrosine phosphorylation of CaD is dependent upon the expression of S3-v-ErbB. Functionally, the phosphorylation of Tyr-27, but not Tyr-393, is required for the binding of CaD to a Shc-associated complex in S3-v-ErbB-transformed fibroblasts. Recruitment of tyrosine-phosphorylated CaD to this complex may interfere with the normal signaling functions of the Shc, Grb2, and Sos complex and also may be a mechanism for disrupting actin stress fiber stability, thereby contributing to the S3-v-ErbB-induced anchorage-independent growth characteristic of transformed fibroblasts.

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