Signal Transduction by the CEACAM1 Tumor Suppressor

PHOSPHORYLATION OF SERINE 503 IS REQUIRED FOR GROWTH-INHIBITORY ACTIVITY*

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CEACAM1 is a cell-cell adhesion molecule that mediates homophilic cell adhesion. In addition, CEACAM1 was also shown to suppress the growth of prostate, breast, and colon tumors. Structural and functional analyses showed that the adhesion activity of CEACAM1 is mediated by its extracellular domain while its cytoplasmic domain is necessary and sufficient for growth-inhibitory activity. The signal pathways leading to CEACAM1-mediated growth suppression are not known.

We studied the importance of phosphorylation of serine 503 in this growth-inhibitory signaling pathway. Full-length CEACAM1 was found to be phosphorylated in vivo in both tyrosine and serine residues. Mutation of tyrosine 488 to phenylalanine did not abolish the tumor-suppressive activity of CEACAM1, suggesting that phosphorylation at tyrosine 488 is not critical for CEACAM1's tumor-suppressive activity. Although expression of CEACAM1's cytoplasmic domain inhibited the growth of DU145 prostate cancer cells in vivo, mutation of serine 503 to alanine abolished the growth-inhibitory activity. In addition, the change of serine 503 to aspartic acid produced tumor-suppressive activity similar to that of the wild-type CEACAM1. These results suggested that phosphorylation at serine 503 is essential for CEACAM1's growth-inhibitory function in vivo.

CEACAM1† (previously known as C-CAM) is a cell-cell adhesion molecule of the immunoglobulin supergene family that has been shown to mediate homotypic cell adhesion (1, 2). The involvement of CEACAM1 in growth regulation was suggested by observations that CEACAM1 protein was down-regulated in several tumors (3–5), including breast, and colon tumors. Structural and functional analyses showed that the adhesion activity of CEACAM1 is mediated by its extracellular domain while its cytoplasmic domain is necessary and sufficient for growth-inhibitory activity. The signal pathways leading to CEACAM1-mediated growth suppression are not known.

We studied the importance of phosphorylation of serine 503 in this growth-inhibitory signaling pathway. Full-length CEACAM1 was found to be phosphorylated in vivo in both tyrosine and serine residues. Mutation of tyrosine 488 to phenylalanine did not abolish the tumor-suppressive activity of CEACAM1, suggesting that phosphorylation at tyrosine 488 is not critical for CEACAM1's tumor-suppressive activity. Although expression of CEACAM1's cytoplasmic domain inhibited the growth of DU145 prostate cancer cells in vivo, mutation of serine 503 to alanine abolished the growth-inhibitory activity. In addition, the change of serine 503 to aspartic acid produced tumor-suppressive activity similar to that of the wild-type CEACAM1. These results suggested that phosphorylation at serine 503 is essential for CEACAM1's growth-inhibitory function in vivo.

which indicated that CEACAM1 functions as a growth suppressor. In addition, direct injection of Ad-CEACAM1, an adenoviral vector that carries the human CEACAM1 gene, into DU145 tumors in nude mice significantly suppressed the growth of these tumors (11). These results suggested that CEACAM1 functions as a tumor suppressor in prostate tumors and that Ad-CEACAM1 is a potential therapeutic agent for prostate cancer.

Structure and function study of CEACAM1 revealed that the first extracellular immunoglobulin domain is important for its adhesion function (2). We found that neither the adhesion domain of CEACAM1 (13, 15) nor its extracellular and transmembrane domains are required for CEACAM1's tumor-suppressive activity (16). However, the cytoplasmic domain of CEACAM1 is essential and sufficient for growth suppression of prostate cancer cells (16), suggesting the involvement of signal transduction through the cytoplasmic domain.

The signal pathway leading to tumor suppression by CEACAM1 is largely unknown. The cytoplasmic domain of CEACAM1 is relatively short (71 amino acids) and does not contain strong homology to the kinase or phosphatase domains, suggesting that the cytoplasmic domain may not possess kinase or phosphatase activity typical of growth factor receptors. However, several tyrosine kinases, including Lyn (17), Hck (17), and Src (18), were reported to associate with the human homologue of CEACAM1, CD66a. Similarly, Najjar et al. (19) reported that rat CEACAM1 is a substrate of the insulin receptor kinase. Binding of SH2-containing phosphatase (SHIP-1) to mouse CEACAM1 in a tyrosine phosphorylation-dependent fashion was also reported (20). The binding of tyrosine kinases and phosphatases to CEACAM1 may be caused by the presence of a partial immunoreceptor tyrosine-based activation and inhibition motif in the CEACAM1 cytoplasmic domain (20). These observations suggested that tyrosine phosphorylation of the CEACAM1 cytoplasmic domain may be critical for CEACAM1's function.

In addition to tyrosine phosphorylation, CEACAM1 was shown to be phosphorylated at serine by an in vivo labeling study (21). Whether phosphorylation at serine is required for CEACAM1's growth-suppressive activity is a critical issue that has not been addressed. Here we present results obtained with use of a site-specific phosphorylation-negative CEACAM1 mutant and a putative phosphorylation-equivalent mutant to test the biological significance of serine 503 phosphorylation in CEACAM1-mediated tumor-suppressive activity.

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors and Generation of Recombinant Adenovirus—Generation of adenovirus containing the full-length wild-type CEACAM1 cDNA in the sense (Ad-CEACAM1) and antisense (Ad-AS) orientations has been described previously (22). The Ad-CA
Y488F virus, in which Tyr-488 was mutated to Phe, was generated as follows. The CAM1-Y488F fragment with flanking HindIII-NotI sites was generated by polymerase chain reaction (PCR) with oligonucleotides 5′-TGCAGAACCAGTTGGAGACTGCTGTCGTCGTC-3′ and 5′-GGGATCCGAGCTGATGAGCTGATTATAG-3′. The HindIII and NotI sites are underlined and the residues corresponding to Phe488 are in bold. The fragments were ligated into the EcoRI and HindIII sites of pSR-CAM3 as template. The 1.6-kb product was subcloned into pCRII to yield pCRII-Adeno-Y488F. The DNA fragment coding for CAM1-Y488F was isolated from pCRII-Adeno-Y488F by digestion with HindIII and NotI, and the fragment was inserted into the adenoviral shuttle vector pXCMV at the HindIII-NotI site to generate pXCMV-CAM1-Y488F. The CAM1-Y488F virus, with six amino acids in its cytoplasmic domain, was generated in the same manner as for Ad-CAM1-Y488F, except for the use of template pSR-CAM3. The Ad-CAM1-S503A virus, which contains a Ser-503 to Ala mutation, was generated in the same manner as for Ad-CAM1-Y488F, except that oligo 200, which contains nucleotides 63 to 40 (25) and a HindIII site, and oligo 201, which contains nucleotides 1504–1580 with a TCA to GCA mutation at the serine 503 and a NotI restriction site, were used as the PCR primers, and a full-length CEACAM1 cDNA was used as the template.

CEACAM1 mutants containing only the cytoplasmic domain (CAM1-cyto), cytoplasmic domain with a serine 503 to alanine mutation (CAM1-cyto-S503A), or cytoplasmic domain with a serine to aspartic acid mutation (CAM1-cyto-S503D), were constructed as follows. A translational in frame deletion (ATG) was introduced at the N terminus of the cytoplasmic domain by PCR. An oligonucleotide (oligo 55, AAGCTTAGGATGAGCAAGACATTGGCGGGCGG) that contained the HindIII restriction site, a sequence encoding methionine and glycine, and nucleotides 1345–1365 of CEACAM1 (25), was synthesized and used as the 5′-primer. For the CAM1-cyto, the 3′-oligonucleotide primer (oligo 54) contained a sequence complementary to nucleotides 1540–1560 of CEACAM1 and a NotI restriction site. For the CAM1-cyto-S503A mutant, the 3′-oligonucleotide primer (oligo 201) contained nucleotides 1504–1560 with a TCA to GCA mutation at the serine 503 and a NotI restriction site. Using oligo 55 as the 5′-primer and corresponding 3′-primer and full-length CEACAM1 cDNA as the template for PCR, 256-bp (CAM1-cyto, CAM1-cyto-S503A, and CAM1-cyto-S503D) products were obtained. The PCR products were subcloned into a pCRII plasmid, and the nucleotide sequence of the double-stranded DNA was determined to confirm that no nucleotide substitution had occurred. The fragments were digested with HindIII and NotI and inserted into the adenoviral shuttle vector pXCMV at HindIII-NotI sites to generate pXCMV-CAM1-cyto, pXCMV-CAM1-cyto-S503A, and pXCMV-CAM1-cyto-S503D. Recombinant adenoviruses containing cDNAs coding for mutant CEACAM1 sequences were generated in 293 embryonic kidney cells by cotransfection of XCMV1 and XCMV2 (26) with the respective plasmid. The materials bound to protein G-Sepharose were eluted by boiling the protein G-Sepharose in SDSPAGE sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis (28). After electrophoresis, proteins were transferred to nitrocellulose membranes, exposed to rabbit anti-cytoplasmic domain antibody anti-C2 (27) and secondary antibody, and detected by an enhanced chemiluminescence assay.

**Western Immunoblot Analysis of CEACAM1 Cytoplasmic Domain Mutant Proteins Expressed in DU145 Cells**—Aliquots of the cell lysate were boiled in SDS sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis (28). After electrophoresis, proteins were transferred to nitrocellulose membranes, exposed to rabbit anti-cytoplasmic domain antibody anti-C2 (27) and secondary antibody, and detected by an enhanced chemiluminescence assay.

**Measurement of in Vivo Tumor Growth from Recombinant Adenovirus-infected DU145 Cells**—DU145 cells were infected with recombinant adenovirus at an m.o.i. of 10 for 48 h. The cells were harvested by trypsin treatment and resuspended in MEM. Cells (2 × 106 cells in a tumor volume of 100 μl) were injected subcutaneously into the flanks of nu/nu mice. The sizes of tumors that developed from these DU145 cells were determined weekly with calipers to measure length, width, and height of the tumor nodules. Tumor sizes were calculated according to the formula of Rockwell et al. (29).

**Statistical Analysis**—The non-linear regression approach (30) with random effects was used to analyze the longitudinal growth data of tumors in different treatment groups. The tumor growth rates were modeled according to the exponential curve with the formula: tumor size (mm3) = C × e(k×t) + Δ, where C is a constant, k is tumor growth rate, and Δ is measurement error. In this analysis, tumor growth rates are functions of treatment effects and mouse variability, which arises from biological or genetic factors. This mixed-effects model accounts for both within-subject and between-subject variations.

**RESULTS**

**Phosphorylation of CEACAM1**—DU145 cells, derived from human prostatic carcinoma metastasized to brain (31), were previously shown to be deficient in CEACAM1 expression, and re-expression of CEACAM1 by adenoviral-mediated gene transfer was able to inhibit their tumorigenicity, as tested in vivo in a nude-mouse xenograft model (16). To examine whether CEACAM1 was phosphorylated when expressed in DU145 cells, the cells were infected with control adenovirus (Luc) or Ad-CEACAM1. Following metabolic labeling with [32P]orthophosphoric acid, the full-length wild-type CEACAM1 was immunoprecipitated with polyclonal antibody against CEACAM (27). That CEACAM1 could be identified as a phosphoprotein suggested that CEACAM1 was phosphorylated in DU145 cells (Fig. 1A). In addition, no phosphorylation was detected on the CEACAM1 mutant with deletion of the cytoplasmic domain, i.e. CAM1-G454, suggesting that the phosphorylation sites were located within the last 65 amino acids of the cytoplasmic domain (Fig. 1A).

To further examine the amino acids that were phosphorylated, phosphorylated CEACAM1 protein was immunoblotted with anti-phosphotyrosine antibody. As shown in Fig. 1B, phosphorylated CEACAM1 protein was reactive with anti-phosphotyrosine antibody, suggesting that CEACAM1 is at least phosphorylated on tyrosine residues. To test whether CEACAM1 is phosphorylated on residues other than tyrosines, immunoprecipitation of CEACAM1 was carried out in a lysis buffer without the tyrosine phosphatase inhibitor vanadate. As shown in Fig. 1A, CEACAM1 was labeled with 32P, but at a lower level when compared with the CEACAM1 prepared in the presence of vanadate. In addition, this phosphorylated CEACAM1 was not reactive with antibody specific to anti-phosphotyrosine (Fig. 2).
overnight in phosphate-free MEM containing 0.5 mCi of \([32P]\)phosphoric acid. Immunoprecipitation of CEACAM1 protein (C-CEACAM1) was performed as described under "Experimental Procedures." The immunoprecipitates were analyzed on SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. A, PhosphorImager analysis of phosphorylated proteins; B, Western immunoblot analysis of proteins with anti-phosphotyrosine antibody (anti-pY) in 1 to 1000 dilution. Arrow indicates the location of CEACAM1 proteins in SDS-polyacrylamide gel.

Fig. 1. Phosphorylation of CEACAM1 in DU145 cells. DU145 cells were infected with control virus (Ad-Luc), Ad-CEACAM1, or Ad-CAM1-G454 virus, respectively, for 24 h. The cells were then incubated overnight in phosphate-free MEM containing 0.5 mCi of \([32P]\)phosphoric acid. The cells were then incubated overnight in phosphate-free MEM containing 0.5 mCi of \([32P]\)phosphoric acid. The cells were washed, lysed, and immunoprecipitated with Ab669 in the absence (A and B) or presence (C and D) of vanadate as described under "Experimental Procedures." A and C, PhosphorImager analysis of phosphorylated proteins; B and D, Western immunoblot analysis of proteins with anti-phosphotyrosine antibody (anti-pY).

S503A, we estimate that about 85% of total Ser/Thr phosphorylation in CEACAM1 occurred on Ser-503. Western blot analysis using Ab669 showed that similar amounts of CEACAM1 proteins were expressed among different mutants (data not shown).

There are two tyrosine residues in the cytoplasmic domain of CEACAM1, i.e. Tyr-488 and Tyr-513 (25). Mutation of Tyr-488 to Phe resulted in a significant decrease in phosphotyrosine, suggesting that Tyr-488 is a major tyrosine phosphorylation site in CEACAM1 (Fig. 2D). However, Tyr-513 is also phosphorylated in vivo, albeit to a lesser extent, because mutation of Tyr-488 to Phe did not completely abolish tyrosine phosphorylation (Fig. 2D). By quantifying the intensity of CAM1-Y488F with that of wild type CEACAM1 using a densitometer, we estimated that about 80% of total tyrosine phosphorylation occurred on Tyr-488.

The relative extent of total Ser/Thr phosphorylation versus total Tyr phosphorylation on CEACAM1 can be estimated from the radioactivities on the wild-type CEACAM1 proteins immunoprecipitated in the absence and presence of vanadate (Fig. 2, A and C). From this comparison, we find that Ser/Thr phosphorylation accounts for about 40% and Tyr phosphorylation about 60% of total phosphorylation on CEACAM1.

Effect of Tyrosine 488 Mutation on CEACAM1's Tumor-suppressive Activity—To investigate the effect of tyrosine phosphorylation on CEACAM1-mediated tumor suppression, Ad-CAM1-Y488F was used to infect DU145 cells. Expression of the full-length wild type CEACAM1 in DU145 cells, which were previously shown to be deficient in CEACAM1 protein expression (11), effectively inhibited tumor growth in vivo when tested in a mouse xenograft model (Fig. 3). Similarly, expression of CEACAM1 with a Tyr-488 to Phe mutation (CAM1-Y488F) in DU145 cells, though not as effective as that of wild-type CEACAM1, showed significant growth-inhibitory activity in two separate experiments (Fig. 3, A and B). Statistical analysis showed that the growth rates of Ad-CAM1-Y488F-

1B). This observation suggested that CEACAM1 is phosphorylated in serine/threonine residues in addition to tyrosine residues.

The observation that CEACAM1 was phosphorylated in DU145 cells in vivo suggested that phosphorylation modification of CEACAM1 may be involved in the growth-suppressive function mediated by CEACAM1. A previous study by Sippel et al. (21) showed that CEACAM1 was phosphorylated at Tyr-488 and Ser-503 residues when expressed in COS cells. Thus, the phosphorylation of CEACAM1 observed in DU145 cells most likely occurred at the Tyr-488 and Ser-503 residues. To test this possibility, we have generated recombinant adenoviruses containing the full-length CEACAM1 with either a Tyr-488 to Phe mutation (Ad-CAM1-Y488F) or a Ser-503 to Ala mutation (Ad-CAM1-S503A) and used them to infect DU145 cells. Following metabolic labeling with \([32P]\)orthophosphoric acid, the wild-type and mutant CEACAM1 proteins were immunoprecipitated with anti-CEACAM1 antibody Ab669 in the absence or presence of orthovanadate, an inhibitor of phosphotyrosine phosphatase. As shown in Fig. 2B, no tyrosine phosphorylation was detected in the wild-type or mutant CEACAM1 when immunoprecipitation was performed in the absence of vanadate. On the other hand, tyrosine phosphorylation of CEACAM1 could be detected when immunoprecipitation was performed in the presence of vanadate (Fig. 2D). Thus, the phosphorylation of CEACAM1 in the absence of vanadate is on Ser/Thr rather than on Tyr. In the absence of vanadate, a significant decrease in CEACAM1 phosphorylation was observed with the CAM1-S503A mutant, whereas the CAM1-Y488F mutant exhibited only slightly reduced phosphorylation as compared with that of the wild-type CEACAM1 (Fig. 2A). This observation suggests that Ser-503 is a major phosphorylation site. By quantifying the radioactivities in the wild-type CEACAM1 and CAM1-$ \text{CAM1-G454}$ virus, respectively, for 24 h. The cells were then incubated overnight in phosphate-free MEM containing 0.5 mCi of $[32P]$phosphoric acid. Immunoprecipitation of CEACAM1 protein (C-CEACAM1) was performed as described under "Experimental Procedures." The immunoprecipitates were analyzed on SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. A, PhosphorImager analysis of phosphorylated proteins; B, Western immunoblot analysis of proteins with anti-phosphotyrosine antibody (anti-pY) in 1 to 1000 dilution. Arrow indicates the location of CEACAM1 proteins in SDS-polyacrylamide gel.

Fig. 2. Phosphorylation of CEACAM1 mutants. DU145 cells were infected with recombinant adenoviruses as indicated. After 24 h of incubation, the media were removed and the cells were then incubated overnight in phosphate-free MEM containing 0.5 mCi of $[32P]$phosphoric acid. The cells were washed, lysed, and immunoprecipitated with Ab669 in the absence (A and B) or presence (C and D) of vanadate as described under "Experimental Procedures." A and C, PhosphorImager analysis of phosphorylated proteins; B and D, Western immunoblot analysis of proteins with anti-phosphotyrosine antibody (anti-pY).
treated tumors differed significantly from no virus- or control virus-treated tumors (Table I, A and B). This observation suggests that phosphorylation at Tyr-488 is not critical for CEACAM1’s growth-suppressive activity, although it may have a role in regulating its efficiency.

**Effect of Serine 503 to Alanine Mutation on Tumor-suppressive Activity**—We have previously shown that expression of the cytoplasmic domain of CEACAM1 is sufficient to elicit its tumor suppressive activity in vivo (16). To examine the role of serine phosphorylation on CEACAM1-mediated tumor suppression, the serine residue (S503) located in the CEACAM1 cytoplasmic domain was mutated to alanine to generate a recombinant adenovirus (Ad-CAM1-cyto-S503A) containing cDNA coding for the cytoplasmic domain with the mutation (Fig. 4A). Expression of the mutant protein was achieved by infecting DU145 prostate cancer cells with Ad-CAM1-cyto-S503A. Western blot analysis using antipeptide antibodies against the CEACAM1 cytoplasmic domain (anti-C2) (27) showed that the wild-type CEACAM1 cytoplasmic domain protein (CAM1-cyto) had a molecular mass of 7–8 kDa and was expressed with a level similar to that of full-length CEACAM1 (Fig. 4B). Mutant CAM1-cyto-S503A protein has a molecular mass similar to that of CAM1-cyto (Fig. 4C).

The effect of CAM1-cyto-S503A mutant protein on the tumorigenicity of DU145 cells in vivo was examined in a nude mouse xenograft model. DU145 cells were infected with Ad-CAM1-cyto-S503A with an m.o.i. of 10, and the cells were injected subcutaneously into nude mice 2 days after viral infection. Expression of the wild-type CEACAM1’s cytoplasmic domain inhibited the growth of DU145 prostate cancer cells in vivo, as evidenced by the reduction in tumor incidence and size (Fig. 5A). However, mutation of serine 503 to alanine abolished the growth-inhibitory activity (Fig. 5A). When the tumor growth rate was calculated by using an exponential curve, Ad-CAM1-cyto-S503A-treated tumors had a growth rate of 14.744 ± 0.491, similar to that of Ad-AS- or Ad-Luc-treated tumors that showed growth rates of 14.209 ± 0.508 and 13.229 ± 0.547, respectively. Statistical analysis showed that the growth rates of Ad-CAM1-cyto-S503A- and control virus-treated tumors did not differ significantly (Table II A).

These results suggest that phosphorylation at S503 may be critical for CEACAM1’s growth-suppressive activity.

**Effect of Serine 503 to Aspartic Acid Mutation on Tumor-suppressive Activity**—Conversion of serine to aspartic or glutamic acid has been shown to imitate serine phosphorylation-induced changes in the function of several proteins, including polymeric immunoglobulin receptor (32), myosin heavy chain (33), and bovine prolactin (34). Therefore, serine 503 in the CEACAM1’s cytoplasmic domain was changed to aspartic acid, and the corresponding recombinant adenovirus (Ad-CAM1-cyto-S503D) was generated (Fig. 4A). The effect of the

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**Table I**

| Treatment            | Growth rate | Standard error | p value |
|----------------------|-------------|----------------|---------|
| (A)                  |             |                |         |
| No virus             | 12.16       | 0.38           |         |
| Ad-AS                | 12.05       | 0.39           | 0.702a  |
| Ad-CEACAM1           | 0           | <0.001a        |         |
| Ad-CAM1-Y488F        | 6.02        | 1.16           | <0.001a |
| (B)                  |             |                |         |
| Ad-Luc               | 9.69        | 0.924          |         |
| Ad-CEACAM1           | 0           | <0.001b        |         |
| Ad-CAM1-Y488F        | −5.70c      | 12.39          | <0.0001b|

a p value for comparison to no-virus group.
b p value for comparison to Ad-Luc group.
c Negative growth rate resulted from tumors that showed initial growth and subsequently regressed.

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Fig. 3. Effect of tyrosine mutation on CEACAM1’s growth-suppressive activity. DU145 cells (no virus) or DU145 cells infected with Ad-AS (antisense, control), Ad-CEACAM1 (sense), or Ad-CAM1-Y488F (Tyr-488 mutated to Phe) were injected subcutaneously into the flanks of nu/nu mice at 2 × 10⁶ cells/site. Eighteen sites were injected for each virus-infected cells. Tumor sizes were measured at 20 and 30 days post-injection; average tumor sizes ± S.E. from each group are shown. A and B represent two independent experiments.
CAM1-cyto-S503D mutant on the tumorigenicity of DU145 cells was compared with those of CAM1-cyto and CAM1-cyto-S503A. In contrast to CAM1-cyto-S503A, expression of the CAM1-cyto-S503D mutant in DU145 cells completely suppressed DU145 tumor growth in vivo (Fig. 5B), similar to that of wild-type CAM1-cyto. In this experiment, the Ad-CAM1-cyto-S503A-treated tumors had a growth rate of 17.481 \pm 0.764, similar to that of Ad-AS-treated tumor, which was 17.561 \pm 0.782. The growth rates for Ad-CAM1-cyto- and Ad-CAM1-cyto-cyto-S503D-treated tumors were zero and significantly different from those of Ad-AS- or Ad-CAM1-cyto-S503A-treated tumors (Table II B). These results strongly suggest that phosphorylation at serine 503 is critical for CEACAM1-mediated tumor suppression.

**DISCUSSION**

By demonstrating that phosphorylation at serine 503 is required for CEACAM1-mediated tumor-suppressive activity, this study raises the possibility that phosphorylation at Ser-503 is directly involved in the signal transduction pathways...
that lead to tumor suppression. The study also indicates the involvement of kinase and phosphatase activities in the regulation of CEACAM1’s growth-suppressive function.

Phosphorylation of target proteins as a mechanism of recruiting proteins into signaling complexes has become one of the paradigms for signal transduction (35, 36). Phosphorylation at a specific amino acid residue enables the phosphorylated protein to interact with proteins that contain phosphoprotein-binding domains (36). SH2 and PTB domains are known to bind to tyrosine-phosphorylated target proteins that have functional domains (36). The discovery of the mechanism by which tyrosine phosphorylation mediates tumor suppression is critical for unraveling CEACAM1’s downstream signaling pathways.

In this study we showed that phosphorylation of Tyr-488 was not critical for CEACAM1-mediated tumor suppression. This result is contrary to that reported by Izzi et al. (46). There are several possibilities that may explain this discrepancy. First, Izzi et al. (46) used mutant CEACAM1 proteins expressed in mouse colon carcinoma cell lines to study their effects in BALB/c syngeneic mice, whereas we used human prostate carcinoma cells injected in a nude-mouse xenograft model in our study. Thus, both the cancer cell lines and the mouse models are different. In addition, they transfected the CEACAM1 gene into the colon carcinoma cells by retroviral-mediated infections followed with G418 selection, whereas we used adenovirus vectors to achieve high efficiency protein expression without gene integration. Finally, it is also possible that phosphorylation of tyrosine 513 can functionally compensate for the loss of tyrosine 488. As shown in our study (Fig. 2), tyrosine 513 was phosphorylated in DU145 cells although to a much lesser extent than that of tyrosine 488. Therefore, the discrepancy between our study and that of Izzi et al. (46) may arise from differences in the extent of tyrosine 513 phosphorylation in the different cancer cell lines used.

The observation that phosphorylation of serine 503 is required for growth-inhibitory activity is important for searching the downstream mechanisms associated with CEACAM1-mediated tumor suppression. Previous efforts to identify CEACAM1-interacting proteins with the yeast two-hybrid approach using wild-type CEACAM1 cytoplasmic domain and with the protein-pull down assay using GST-CAM1-cyto fusion protein containing the wild-type CEACAM1 cytoplasmic domain fused to GST protein did not yield useful information (data not shown). In light of the present study, the failure to identify molecules that interact with the CEACAM1 cytoplasmic domain may be due to the absence of phosphorylation at the serine residue. Because the S503D mutant, which presumably mimics the phosphorylated state of CEACAM1, allows the interacting protein to bind and resume CEACAM1’s tumor-suppressive activity, the S503D mutant may be used to identify CEACAM1-interacting protein in both yeast two-hybrid and protein-pull down assays.

Taken together, the results of our study indicated that post-translational regulation at serine 503 is critical for CEACAM1’s tumor-suppressive activity. It will be interesting to identify the kinase that phosphorylates serine 503 and the adaptor protein that binds to the specific phosphorylated motif. Understanding the molecular mechanism of tumor suppression by CEACAM1 will enable us to use this molecule more effectively in the prevention and treatment of cancer.

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