

SHORT COMMUNICATION

CUB-domain-containing protein 1 overexpression in solid cancers promotes cancer cell growth by activating Src family kinases

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The transmembrane glycoprotein, CUB (complement C1r/C1s, Uegf, Bmp1) domain-containing protein 1 (CDCP1) is overexpressed in several cancer types and is a predictor of poor prognosis for patients on standard of care therapies. Phosphorylation of CDCP1 tyrosine sites is induced upon loss of cell adhesion and is thought to be linked to metastatic potential of tumor cells. Using a tyrosine-phosphoproteomics screening approach, we characterized the phosphorylation state of CDCP1 across a panel of breast cancer cell lines. We focused on two phospho-tyrosine pTyr peptides of CDCP1, containing Tyr707 and Tyr806, which were identified in all six lines, with the human epidermal growth factor 2-positive HCC1954 cells showing a particularly high phosphorylation level. Pharmacological modulation of tyrosine phosphorylation indicated that, the Src family kinases (SFKs) were found to phosphorylate CDCP1 at Tyr707 and Tyr806 and play a critical role in CDCP1 activity. We demonstrated that CDCP1 overexpression in HEK293 cells increases global phosphotyrosine content, promotes anchorage-independent cell growth and activates several SFK members. Conversely, CDCP1 downregulation in multiple solid cancer cell lines decreased both cell growth and SFK activation. Analysis of primary human tumor samples demonstrated a correlation between CDCP1 expression, SFK and protein kinase C (PKC) activity. Taken together, our results suggest that CDCP1 overexpression could be an interesting therapeutic target in multiple solid cancers and a good biomarker to stratify patients who could benefit from an anti-SFK-targeted therapy. Our data also show that multiple tyrosine phosphorylation sites of CDCP1 are important for the functional regulation of SFKs in several tumor types.

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INTRODUCTION

Overexpression of CUB (complement C1r/C1s, Uegf, Bmp1) domain-containing protein 1 (CDCP1) is associated with cancer progression and poor prognosis for patients with various solid cancer types including lung,¹ breast,² kidney,³ colon,² prostate⁴ and pancreatic⁵ carcinomas. It is widely established that CDCP1 promotes cell invasion and metastasis phenotypes *in vitro* and analysis of primary tumor samples support the observation that high CDCP1 expression promotes cell proliferation as measured by Ki67 antigen levels.⁶

CDCP1 is a type I transmembrane glycoprotein with a large extracellular domain containing three CUB domains. The intracellular domain of CDCP1 contains five tyrosine phosphorylation sites (Tyr734, Tyr743, Tyr762, Tyr707 and Tyr806) and tyrosine 734 of CDCP1 has been reported as the major phosphorylation site for Src family kinases (SFKs)^{7,8} including Src, Fyn, Yes and Lyn. Structural analysis of CDCP1 has demonstrated that Tyr734 and Tyr762 phosphorylations by SFKs are required for the recruitment of PKC δ at phospho-Tyr762 CDCP1⁷ and promotes activation of AKT. Apart from this, the downstream pathway associated with CDCP1 remains unclear.

Many studies have shown that increased expression and activation of SFKs contribute to tumor proliferation in various cancers⁹ and correlate with poor prognosis for the patients. Several transmembrane proteins can provide docking sites to bind

and activate SFKs such as lymphocyte-specific protein tyrosine kinase (Lck), which interacts with CD4 and CD8¹⁰ in immune cells or Fyn and Yes which bind nephrin in podocytes of kidney glomeruli.¹¹ CDCP1 overexpression has been reported to activate SFKs in the context of metastatic melanoma¹² and constitutive activation of SFK has been shown to be due to loss of expression of negative regulators such as C-terminal src kinase (CSK)-binding protein^{13,14} or Src-like-adaptor protein.^{15,16}

In this paper, we report Tyr707 and Tyr806 as two novel tyrosine phosphorylation sites on CDCP1 for SFKs and identify phospho-signaling events downstream of CDCP1 using tyrosine phosphoproteomic analysis. Our data support the model that CDCP1 overexpression activates SFKs in cancer leading to phosphorylation of several SFK substrates involved in cellular proliferation. Analysis of breast and lung tumor samples from patients, show a consistent correlation between CDCP1 expression and SFK activity confirming our *in vitro* observations that CDCP1 signaling is pathophysiologically relevant in humans to drive tumor growth and survival.

RESULTS AND DISCUSSION

CDCP1 Tyr707 and Tyr806 are novel phosphorylation sites for SFK in breast cancer cells

Quantitative phosphoproteomics of two triple-negative breast cancer cell lines, SUM159 and MDA-MB-231-LM2 and, four

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human epidermal growth factor 2 (HER2)-positive breast cancer cell lines BT474, AU565, HCC1954 and SKBR3, has identified Tyr707 and Tyr806 as two novel phosphorylation sites of CDCP1 (Supplementary Figure S1a). The CDCP1 intracellular domain contains five putative phosphorylated tyrosines Tyr707, Tyr734, Tyr743, Tyr762 and Tyr806. We hypothesized that CDCP1 may be regulated by epidermal growth factor receptor (EGFR) or HER2 based on recent evidence demonstrating that CDCP1 is a deltaHER2 effector.¹⁷ However, quantitative phosphoproteomics of HCC1954 cells treated with EGFR or EGFR/HER2 inhibitor did not affect Tyr707 and Tyr806 phosphorylation of CDCP1 (data not shown). Previous work has shown that SFK activity is a major contributor of the tyrosine phosphorylation signature characteristic of breast cancer cells,¹⁸ and that SFK is involved in the phosphorylation of Tyr734, Tyr743 and Tyr762 on CDCP1.^{19,20} Therefore, we assessed whether SFK could be involved in Tyr707 and Tyr806 CDCP1 phosphorylation and analyzed HCC1954 cells treated with the SFK inhibitor dasatinib. The decrease in phosphorylation of Tyr707, Tyr806 as well as Tyr734 were confirmed by immunoprecipitating CDCP1 followed by western

blotting (Supplementary Figure S1b). These data highlight Tyr707 and Tyr806 as two novel phosphorylation sites of CDCP1 that depend on SFK activity in cells.

CDCP1 overexpression activates SFK

To address the functional effects of CDCP1 in tumor cells, we transduced a wild-type CDCP1 construct into a CDCP1-null cell line HEK293. CDCP1 overexpression induced a strong increase in the global phosphotyrosine content (Figure 1a) correlating with a significant increase in anchorage-independent cell growth in soft agar (Figure 1b). To investigate the signaling events downstream of CDCP1, we performed phosphotyrosine profiling of HEK293 cells overexpressing CDCP1 (Supplementary Table S1). The table in Figure 1c shows a panel of 15 phosphopeptides increasing upon CDCP1 overexpression. As expected CDCP1 phosphopeptides are increased when overexpressing the protein. Interestingly, phosphopeptides containing Tyr416 from SFK are also increased upon CDCP1 overexpression together with some already known Src kinase substrates PKC δ (PRKCD) (Tyr311), p120 catenin (CTNND1) (Tyr96),

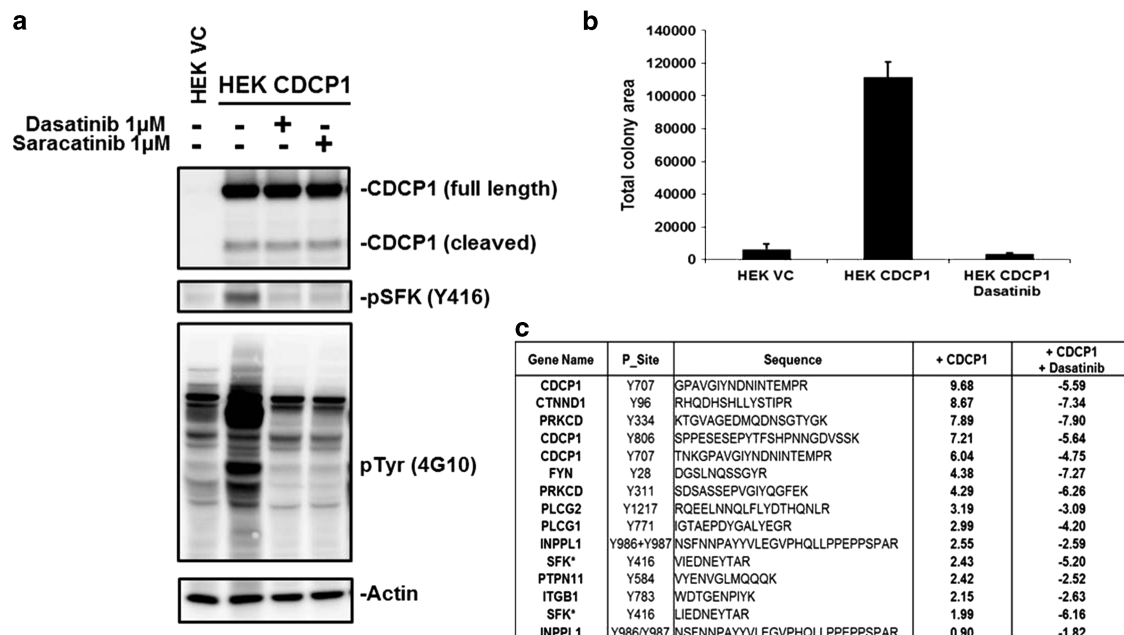


Figure 1. CDCP1 overexpression promotes anchorage-independent cell growth by activating SFK in HEK293 cells. (a) HEK293 cells were infected with CDCP1 wild-type (WT) cDNA purchased from GenScript and cloned into the retroviral vector pQCXIN. After infection, CDCP1-WT-expressing cells were isolated by G418 (400 μ g/ml) selection. HEK293-CDCP1 treated with dasatinib (1 μ M) or saracatinib (1 μ M) for 2 h. The cell lysates were collected for western blot analysis with anti-CDCP1 (Cell Signaling), global anti-phosphotyrosine (4G10, Millipore), anti-pSFK Tyr416 (Biosource) and the loading control anti-actin (Cell Signaling) antibodies. (b) Anchorage-independent cell growth in soft agar conditions at day 15; 0.8% agar medium was layered on the bottom of a 24-well plate and 2000 cells per well were seeded on the top of this layer in 0.4% agar medium. After 15–16 days colonies are stained with Hoechst 33342 viability dye (1 μ g/ml) for 12–14 h. Pictures are taken at low magnification (2 \times) using UV filter on a fluorescent microscope and quantification was done using Image-Pro software. Statistical analysis of total colony area of HEK293 cells infected with indicated viruses and treated with SFK inhibitor dasatinib 1 μ M. Data are means \pm standard deviation (s.d.) ($n > 3$). (c) Quantitative phosphoproteomics identifies a list of 15 selected phosphopeptides increasing upon CDCP1 overexpression and decreasing with dasatinib treatment (1 μ M). HEK293-VC, HEK293-CDCP1 and HEK293-CDCP1 treated for 2 h with dasatinib were harvested and lysed, and proteins were digested overnight with trypsin. Phosphotyrosine peptides were enriched from peptide mixtures using immunoprecipitation with anti-phosphotyrosine antibodies followed by TiO₂ enrichment. Purified phosphopeptides were then analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS), and LTQ-Orbitrap Elite data acquisition was done using a 'top collision induced dissociation (CID) 15 method'. Full scans were performed in the Orbitrap at 120 000 resolution with target values of 1E6 ions and 500 ms injection time, while MS/MS scans were done in the ion trap with 1E4 ions and 200 ms. Database searches were performed with Mascot Server using UniProt database (version 3.87). Mass tolerances were set at 10 p.p.m. for the full-MS scans and at 0.8 Da for MSMS. Label-free quantification was performed on duplicate LC-MS runs for each sample using Progenesis LC-MS (nonlinear dynamics software). The peptide intensities were normalized across all LS/MS runs by Progenesis software and normalized peptide intensities were summed for each unique phosphorylated peptide with mascot score exceeding 20. These intensities were then used to calculate the log₂ fold-change ratios of each unique phosphopeptide of HEK293-CDCP1 versus HEK293-VC and HEK293-CDCP1 treated with dasatinib versus HEK293-CDCP1. In case of ambiguous phosphorylation site assignments, spectra were manually interpreted for confirmation localization of the phosphorylation site using Scaffold (Proteome software). Gene name, pY site, peptide sequence, phosphopeptide intensity corresponding to Log₂ fold-change ratios are shown.

phospholipase C-gamma-1 (PLCG1) (Tyr771), Shp2 (PTPN11) (Tyr584), inositol polyphosphate phosphatase-like 1 (INPPL1) also known as SH2-domain containing phosphatidylinositol 3,4,5-triphosphate 5-phosphatase 2 (SHIP2) (Tyr986), or integrin beta-1 (ITGB1) (Tyr783).^{18,21} PKC δ Tyr311 phosphorylation was previously shown to be regulated in a CDCP1-/SFK-dependent manner.²⁰

The increase in SFK Tyr416 phosphorylation was confirmed in CDCP1-overexpressed HEK293 cells by western blot analysis (Figure 1a and Supplementary Figure S2a). Treatments with Src kinase inhibitors like dasatinib and saracatinib prevented an increase in the global phosphotyrosine content induced by CDCP1 expression (Figure 1a) and impaired the ability of HEK293-CDCP1 to develop colonies in soft agar assays (Figure 1b). CDCP1-induced SFK pathway activation was confirmed biochemically for six known Src substrates shown in the top hit list (Supplementary Figure S2a). Src, Fyn, Yes and Lyn showed an increased kinase activity with a more pronounced effect on both Lyn and Yes (Supplementary Figure S2b). To investigate the kinase responsible for CDCP1 phosphorylation, we performed an immunoprecipitation of CDCP1 in HCC1954 and HEK293 overexpressing CDCP1

followed by mass spectrometry analysis. We hypothesized that one or more members of the SFK family could directly interact and phosphorylate CDCP1. In both cell lines, we identified SRC, YES and LYN among the CDCP1-associated protein list suggesting that CDCP1 can interact with and activate all SFK members (Supplementary Table S2). Taken together, our results demonstrated a novel role of CDCP1 overexpression in the promotion of cell growth by interacting and activating SFK in HEK293 cells.

Tyr707, Tyr734 and Tyr806 play an important role in CDCP1 oncogenic properties

Given that CDCP1 promotes cell growth by activating Src kinases, we investigated the functional role of Tyr707, Tyr734 and Tyr806 of CDCP1 overexpression in cell viability. The CDCP1 Y707F, Y734F, Y806F and YF3 mutants were transduced into HEK293 cells and a decrease in Tyr734 and Tyr806 phosphorylation was observed (Figure 2a). Interestingly, the mutation at Tyr734 also had an effect on the level of Tyr707 phosphorylation. However, Tyr806 phosphorylation was not modulated by the mutation at Tyr734

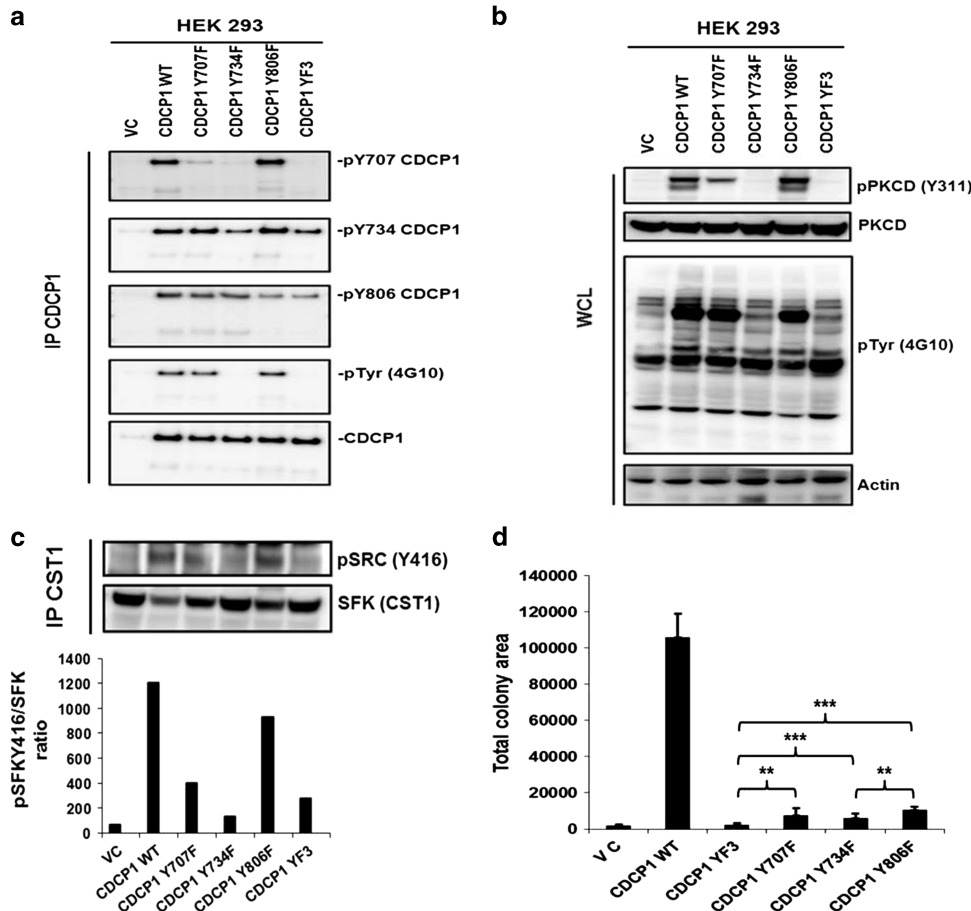


Figure 2. Tyr707, Tyr734 and Tyr806 are involved in the promotion of CDCP1-dependent cell growth. (a) HEK293 cells were infected with CDCP1 wild-type (WT) and mutated cDNA (Y707F, Y734F, Y806F and YF3) purchased from GenScript and cloned into the retroviral vector pQCXIN. After infection, CDCP1 WT and mutants expressing cells were isolated by G418 (400 μ g/ml) selection. Cell lysates from HEK293 cells infected with indicated viruses were collected for an overnight immunoprecipitation of CDCP1 followed by western blot analysis with anti-CDCP1, anti-phospho-Tyr707, -Tyr734, -Tyr806 CDCP1-specific antibodies (Cell Signaling) and global anti-phosphotyrosine antibody (4G10, Millipore). (b) Cell lysates from HEK293 cells infected with indicated viruses were collected for western blot analysis with anti-pPKC δ Tyr311, anti-PKC δ -specific antibodies (Cell Signaling), global anti-phosphotyrosine antibody (4G10, Millipore) and the loading control anti-actin (Cell Signaling) antibody. (c) Upper panel: cell lysates from HEK293 cells infected with indicated viruses were collected for an overnight immunoprecipitation of SFK with the CST1 antibody (gift from Dr Serge Roche, CRBM, University of Montpellier, France) followed by western blot analysis with same anti-total SFK and anti-pSFK Tyr416 (Biosource). Lower panel: bar charts representing ratios of Tyr416 phosphorylation level of SFK over total SFK level after quantification using the ImageJ software. (d) Anchorage-independent cell growth in soft agar conditions at day 15 as described previously in Figure 1b. Statistical analysis of total colony area of HEK293 cells infected with viruses expressing indicated CDCP1 constructs. Data are means \pm s.d. ($n > 3$, ** $P < 0.01$ and *** $P < 0.001$; Student's *t*-test).

or Tyr707. These results indicate that Tyr707 requires Tyr734 phosphorylation to be phosphorylated by Src kinases. It appears that CDCP1 tyrosine phosphorylation occurs in an ordered manner, where Tyr734 is a key precursor phosphorylation event allowing Tyr707 and Tyr806 to be subsequently phosphorylated. We then evaluated the role of these three CDCP1 tyrosine phosphosites by assessing the downstream signaling of pTyr416 SFK and pTyr311 PKC δ , which were decreased in HEK293 Y734F and in HEK293 YF3 compared to HEK293-CDCP1 wild-type cells (Figures 2b and c). In contrast, CDCP1 Y806F did not show any effect on CDCP1 downstream signaling. It appears that HEK Y707F partially decreased pTyr416 SFK, pTyr311 PKC δ and the global phosphotyrosine content (Figures 2b and c). These results indicate that Tyr734 is the first step in a cascade of phosphorylation events leading to the activation of PKC δ and SFK. Surprisingly, despite the important role of Tyr734 in CDCP1 downstream signaling, mutation of one of these three tyrosine sites is sufficient to fully abrogate anchorage-independent cell growth in soft agar conditions induced by CDCP1 in HEK293 cells (Figure 2d). If Tyr734 appears to be the most important CDCP1 phosphosite to drive SFK

activity and its downstream signaling, Tyr707 and Tyr806 play a critical role in promoting CDCP1 growth-enhancing properties. Thus, intensity of the phosphopeptide-containing Tyr734 and Tyr707 may serve as a biomarker in breast and lung cancer patient samples to predict the functional activity of CDCP1.

CDCP1 overexpression promotes solid cancer cell growth by amplifying SFK oncogenic signaling

Many studies have shown that CDCP1 regulates cell adhesion and promotes metastasis development in cancer such as melanomas or breast carcinomas.^{12,19} Engineered CDCP1 overexpression has been validated in several studies to be a driver of oncogenic growth but its endogenous role in cancer was not clearly understood. We wanted to address the contribution of CDCP1 overexpression in the context of multiple solid cancers. The HER2⁺ breast cancer cell line HCC1954 and two non-small cell lung cancer cells SKMES-1 and NCI-H2122 were selected for analysis because of their high phosphorylation levels of CDCP1 (Figure 3a). CDCP1 was inactivated in those cells by stably expressing

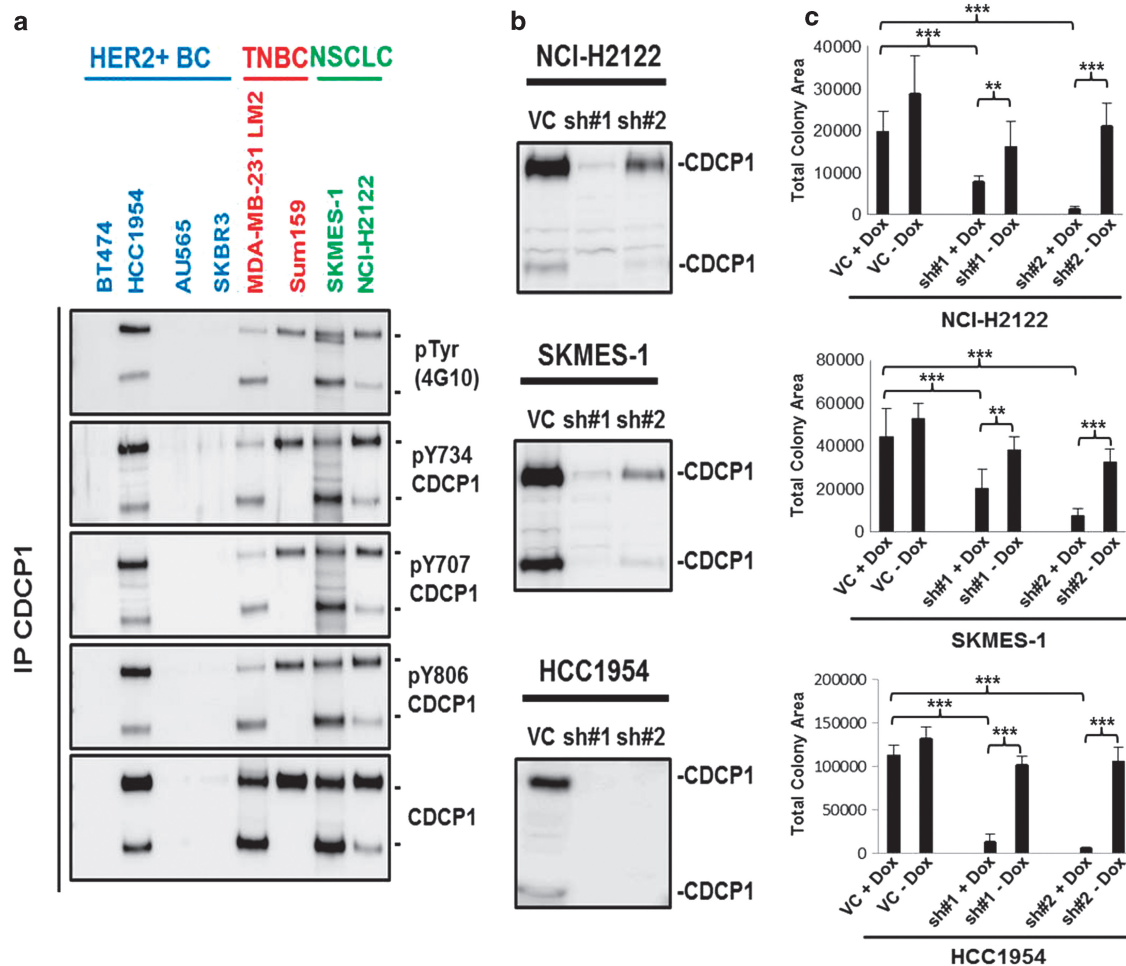


Figure 3. CDCP1 downregulation abrogates breast and lung cancer cell growth properties in a SFK-dependent manner. **(a)** Cell lysates from SUM159, MDA-MB231-LM2, BT474, HCC1954, AU565, SKBR3, SKMES-1 and NCI-H2122 cells were collected for an overnight immunoprecipitation of CDCP1 followed by western blot analysis with anti-CDCP1, anti-phospho-Tyr707, -Tyr734, -Tyr806 CDCP1-specific antibodies (Cell Signaling) and global anti-phosphotyrosine antibody (4G10, Millipore). **(b)** NCI-H2122, SKMES-1 and HCC1954 cells were infected with two shRNAs targeting CDCP1, CDCP1 sh#1 (CCTGTTACATCGTCATTCTA) and CDCP1 sh#2 (GAATAGTCTTTACTTTAGCT). shRNA were cloned into the doxycyclin (Dox)-inducible lentiviral vector pLKO-TetON-U6. After infection, shRNA-expressing cells were isolated by puromycin (1–3 μ g/ml) selection. Cell lysates from NCI-H2122, SKMES-1 and HCC1954 cells infected with indicated viruses were collected for western blot analysis with anti-CDCP1 antibody (Cell Signaling) to assess knockdown efficiency. **(c)** Anchorage-independent cell growth in soft agar conditions at day 16 as previously described in Figure 1b; 2000 SKMES-1 and NCI-H2122 and 4000 HCC1954 cells per well were seeded in those experiments. Statistical analysis of total colony area of NCI-H2122, SKMES-1 and HCC1954 cells infected with viruses expressing indicated doxycycline-inducible CDCP1 shRNA (ON-Dox or OFF-Dox). Data are means \pm s.d. ($n > 3$, ** $P < 0.01$ and *** $P < 0.001$; Student's t -test).

inducible shRNA leading to 60–90% reduction of protein level upon doxycycline treatment (Figure 3b). As expected, CDCP1 knockdown in breast and lung cancer cell lines resulted in a suppression of growth on plastic (data not shown) and in colony formation in anchorage-independent growth assays (Figure 3c). Our observations suggest that CDCP1 could become a potential therapeutic target for combination therapies in lung or breast cancers. Global phosphotyrosine profiles were performed across the panel of cell lines to decipher the signaling events downstream of CDCP1 in these cancer types (Supplementary Figure S3). As expected, CDCP1 phosphopeptides decreased upon CDCP1 inactivation confirming that the knockdown was effective. The SFK downstream effectors previously identified upregulated in HEK293-CDCP1 wild-type overexpression phosphoproteomics were found among the top decreasing phosphopeptides. Although a decrease in the level of phosphorylation of these effectors is not always consistent in all the three cell lines; we clearly observe a global decrease in the SFK downstream signaling pathways upon CDCP1 knockdown. Taken together, our data suggest that overexpression of CDCP1 in solid cancer cells triggers an important cascade of tyrosine phosphorylation events leading to the activation of signaling network including SFKs, PKC, integrins, catenins and ephrins to promote cell growth and survival.

Furthermore, phosphopeptides containing Tyr416 from SFK did not show decrease in intensities in all cell lines. We suspect that additional mechanisms of SFK hyperactivation^{13,14,16} could be unique depending on the genetic background of the cells and pathways that support SFK activation in the absence of CDCP1. This was not observed in HEK293 wild-type cells where SFKs are in

an inactive state in the absence of endogenous CDCP1 expression. Loss of function of the transmembrane protein, CSK-binding protein (CBP)/phosphoprotein associated with GEMs (PAG) in colon cancer cells prevents the recruitment of the tyrosine kinase CSK, and therefore the phosphorylation of Tyr530 of SFK, which bring them back in a close inactive conformation.^{13,14} Recently, the adapter Src-like-adaptor protein has been shown to display tumor suppressor function in colorectal cancer by controlling SRC/EPHA2/AKT signaling via destabilization of the SRC substrate EPHA2.¹⁶ In contrast to these loss of function models, we propose another way SFKs can be activated in solid cancers is by CDCP1 overexpression. CDCP1 appears to play a key role in promoting SFK signaling and CDCP1 depletion inhibits SFK-mediated cell growth. The mechanism of CDCP1 protein upregulation is not entirely clear and a better understanding of its expression will be important to better understand the role of CDCP1 in cancer.

CDCP1 is specifically expressed in human cancer tissues and its expression correlates with SFK activity

Histological examination of CDCP1 in human cancer samples indicates that 30% of solid tumors express a significant high level of CDCP1, which correlates with poor prognosis for the patients on chemotherapy regimens¹⁹ similar to patients with high-SFK activity. Here, we confirm that CDCP1 is overexpressed in cancer tissues and weakly expressed in normal tissues suggesting that CDCP1 overexpression is relevant for primary tumor growth (Figure 4a). Furthermore, a high expression and/or phosphorylation of CDCP1 observed in two primary breast cancer and two lung cancer samples strongly correlated with high-Tyr416SFK and

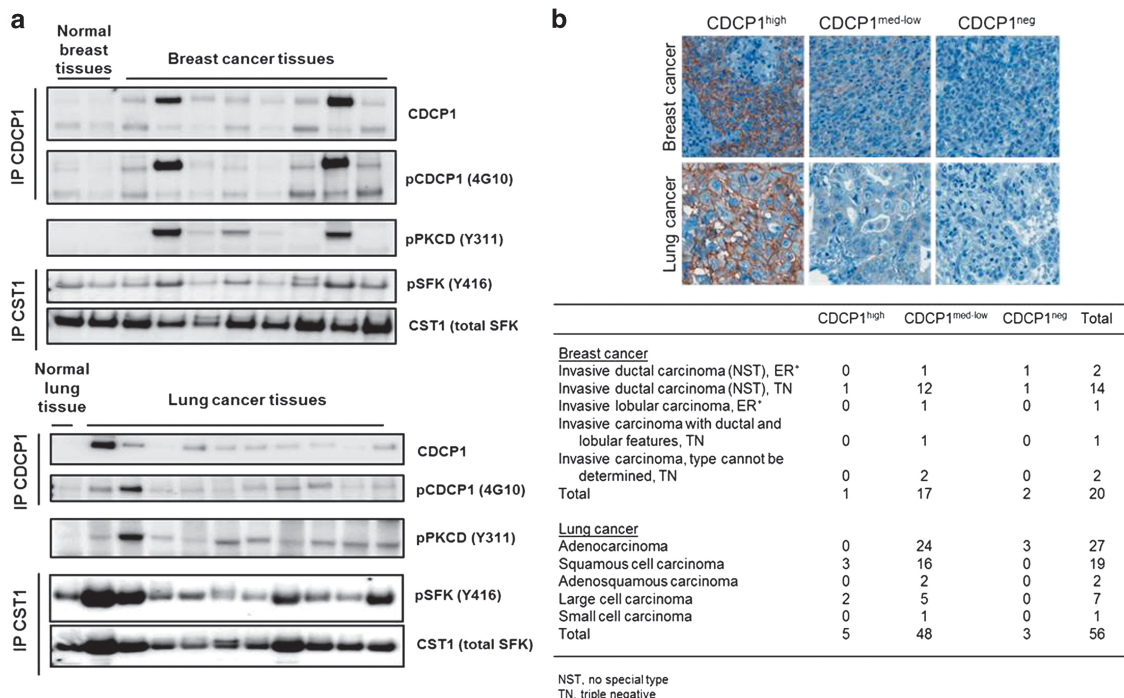


Figure 4. CDCP1 expression/phosphorylation correlates with SFK and PKC δ activity in human cancer tissues. **(a)** Total protein extracts from normal breast and breast cancer tissues, and normal lung and lung cancer tissues were collected for an overnight immunoprecipitation of CDCP1 or total SFK followed by western blot analysis with anti-CDCP1 (Cell Signaling), anti-phosphotyrosine antibody (4G10, Millipore), anti-pSFK Tyr416 (Biosource), anti-total SFK (gift from Dr Serge Roche) and anti-PKC δ Tyr311 antibodies. **(b)** CDCP1 is expressed in human breast and lung cancers. Representative images of CDCP1 immunohistochemistry (IHC) staining in breast cancers (Upper) or lung cancers (Lower), demonstrating high, medium-low or negative membranous staining. Archival formalin-fixed, paraffin-embedded human breast cancer specimens were procured from Cureline (South San Francisco, CA, USA) and Asterand (Detroit, MI, USA). Archival formalin-fixed, paraffin-embedded human lung cancer specimens were procured by Maine Institute for Human Genetics and Health and Dahl-Chase Pathology Associates (Bangor, ME, USA) and Cytomyx (Lexington, MA, USA). Immunohistochemical staining was performed on the Ventana Discovery system. The primary antibody used was CDCP1 (Cell Signaling). CDCP1-stained tumors were scored by a pathologist (MEM).

Tyr311PKC δ phosphorylation levels. The CDCP1 feed-forward loop may supersede loss of negative regulators of SFKs in breast and lung tumors and act as the main driver for SFK activity. Previous studies demonstrated that proteolytic cleavage was necessary to induce SFK binding and CDCP1 phosphorylation by active SRC.²² Our data did not confirm this conclusion as we observed a strong proportion of CDCP1 tyrosine phosphorylation in the full-length 135-kDa glycoprotein both in cancer cell lines and human tumor samples (Figures 3a and 4a). The level of full-length protein expression and phosphorylation of CDCP1 seems to be more critical than the cleavage status for its role in activating tumor promoting signaling events. Furthermore, immunohistochemical analysis revealed that CDCP1 is expressed in the majority of breast cancers (90%, 18/20) and lung cancers (94.6%, 53/56) examined (Figure 4b and Supplementary Figure S4). Confirmation of CDCP1 overexpression and activated SFK signaling in a subset of primary breast and lung tumor samples suggests that CDCP1 is an essential component of these oncogenic signaling pathways in cancer. If testing this possibility in a broader set of primary tumor samples is warranted, our data suggest that CDCP1 could become a reliable biomarker to predict SFK pathway activation in breast and lung cancers and aid in the stratification of patients who could benefit from a SFK- or CDCP1- targeted therapy.

In summary, we have characterized CDCP1 Tyr707 and Tyr806 as two novel phosphorylation sites for SFK in solid cancer cells and identified downstream phospho-signaling events using an unbiased tyrosine phosphoproteomic analysis. We demonstrated that CDCP1 overexpression activates SFK leading to an increase in the global phosphotyrosine content and the promotion of anchorage-independent growth. Examination of human tumor samples from patients revealed a correlation between CDCP1 expression/phosphorylation, SFK and PKC activities suggested that CDCP1 could potentially be used as a biomarker for breast and lung tumors that may benefit from SFK- or CDCP1- targeted therapies.

CONFLICT OF INTEREST

All authors are employees of Novartis. CL is a presidential postdoctoral fellow at the Novartis Institutes for Biomedical Research.

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