ISSN 0095-4527, Cytology and Genetics, 2025, Vol. 59, No. 2, pp. 168–178. © Allerton Press, Inc., 2025.
Ukrainian Text © The Author(s), 2025, published in Tsitologiya i Genetika, 2025, Vol. 59, No. 2, pp. 31–43.

Distinct Functions of the PH Domain in BCR/ABL p210 Isoform: Interaction with Cytoskeletal and Membrane Remodeling Proteins

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Received November 3, 2024; revised November 20, 2024; accepted January 9, 2025

Abstract—The BCR/ABL fusion protein, generated by the Philadelphia chromosome translocation, drives chronic myelogenous leukemia (CML) and other myeloproliferative disorders. The p210 isoform includes a pleckstrin homology (PH) domain absent in the p190 isoform, which is linked to acute lymphoblastic leukemia (ALL). This structural difference may underlie the distinct subcellular localization and signaling profiles of the two isoforms. Here, we investigate the role of the PH domain of BCR in interactions with cortactin and FBP17, proteins involved in cytoskeletal remodeling and membrane dynamics. Using GST-pulldown assays and western blotting, we demonstrated direct interactions between the PH domain of BCR and both cortactin and FBP17. Colocalization studies, supported by confocal and STED microscopy, revealed that cortactin colocalizes with the PH domain of BCR in the centrosomal and perimembrane regions of cells. Notably, the SH3 domain of cortactin was not required for this interaction, but full-length cortactin was essential, suggesting that other domains mediate binding. These findings highlight the role of the PH domain in directing BCR/ABL to the centrosome, where it interacts with cortactin to potentially influence actin dynamics and vesicular trafficking. This centrosomal localization may spatially restrict the constitutive tyrosine kinase activity of ABL, contributing to the less aggressive phenotype of p210-associated CML compared to p190-driven ALL. Understanding the role of the PH domain as a key structural difference between p210 and p190 is critical for elucidating the molecular basis of BCR/ABL-mediated leukemogenesis. Future studies will explore the phosphorylation of cortactin and FBP17 by ABL kinase and the domains responsible for these interactions.

Keywords: BCR/ABL, cortactin, FBP17, PH domain, CML, GST-pulldown, protein-protein interactions

DOI: 10.3103/S0095452725020045

INTRODUCTION

The BCR/ABL fusion protein is a hallmark of several myeloproliferative disorders, arising from the Philadelphia chromosome translocation t(9;22) (Hecht et al., 1985). This fusion creates oncoproteins with distinct isoforms, primarily p190, p210, and p230, which differ based on the breakpoint in the BCR gene. The p190 isoform is most commonly associated with B-cell acute lymphoblastic leukemia (B-ALL), reflecting its potent oncogenic activity in lymphoid progenitors. The p210 isoform is the most prevalent in chronic myelogenous leukemia (CML), driving the disease through aberrant tyrosine kinase activity and deregulated signaling pathways that affect hematopoietic stem cell proliferation and survival. The p230 isoform, characterized by a longer BCR fragment, is linked to a rarer, more indolent form of chronic neutrophilic leukemia (CNL), with comparatively reduced kinase activity. These isoforms not only dictate disease phenotype but also influence response to targeted therapies, making their study critical for understanding disease mechanisms and improving treatment strategies (Emilia et al., 1997). The p190 and p210 isoforms of the BCR/ABL fusion protein differ significantly in their structural composition due to variations in the breakpoint within the BCR gene. The p190 isoform results from a breakpoint in the minor cluster region (m-BCR) of the BCR gene. It retains the coiled-coil domain of BCR, which is essential for the dimerization and constitutive activation of ABL kinase. However, it lacks the central domains of BCR, such as the DBL-homology (DH) domain, the pleckstrin homology (PH) domain, and the Rho guanine nucleotide exchange factor (RhoGEF) domain, which are present in the p210 isoform. The p210 isoform results from a breakpoint in the major cluster region (M-BCR) of the BCR gene (Ben-Neriah et al., 1986). In addition to the coiled-coil domain, p210 includes the DH, PH, and RhoGEF domains. These additional domains are implicated in cytoskeletal organization and signaling pathways, which contribute to the broader oncogenic and cellular transforma-

tion potential of p210 compared to p190. These structural differences have functional implications. The additional domains in p210 enhance its interactions with various cellular pathways, leading to distinct signaling outputs compared to p190 (Quintás-Cardama and Cortes, 2009). Consequently, p210 is primarily associated with chronic myelogenous leukemia (CML), while p190 is more prevalent in acute lymphoblastic leukemia (ALL), reflecting differences in disease phenotype and progression (Emilia et al., 1997; Telegeev et al., 2004).

The pleckstrin homology (PH) domain of the BCR protein is a multifunctional module implicated in cellular signaling and cytoskeletal regulation. While specific details about the role of the BCR PH domain are not fully elucidated, PH domains in general are known for their ability to bind phosphoinositides in the plasma membrane, facilitating membrane association and recruitment of proteins to specific subcellular compartments (Hirata et al., 1998; Lemmon, 2010). PH domains are found in a wide variety of proteins and typically function as lipid-binding modules. They often bind to phosphoinositides, such as phosphatidylinositol 3,4,5-trisphosphate (PIP3) or phosphatidylinositol 4,5-bisphosphate (PIP2), with specificity varying among proteins (Lemmon and Ferguson, 2001). This binding mediates the localization of proteins to membranes and facilitates their role in signaling pathways (Lenoir et al., 2015). For example, PH domain of Akt binds PIP3, targeting the kinase to the plasma membrane for activation by upstream kinases (Thomas et al., 2002). PH domain of GRB2-associated binding protein (GAB1) mediates membrane recruitment during growth factor signaling (Maroun et al., 1999, 2003). PH domain of dynamin aids in membrane curvature sensing during vesicle trafficking (Krueger et al., 2003; Itoh et al., 2005; Gu et al., 2010). Recent studies have demonstrated involvement of PH domain of BCR in protein-protein and protein-lipid interactions, which are crucial for the oncogenic activity of BCR/ABL (Reckel et al., 2017; Gregor et al., 2019). For instance, the PH domain binds phosphoinositides, such as PIP2 and PIP3, which aids in membrane localization and the recruitment of signaling complexes (Miroshnychenko et al., 2010). This function is consistent with the general role of PH domains in other proteins, where they mediate interactions with lipids and contribute to cellular localization and signal transduction pathways, such as those seen in Akt signaling and vesicle trafficking. Proteomic studies using leukemia cell lines (e.g., K562) have identified 23 co-precipitating proteins that potentially bind to the PH domain of BCR, highlighting its broader interaction network and functional implications in signaling cascades critical to leukemia progression (Miroshnychenko et al., 2010). Among these identified partners were cortactin, a regulator of actin cytoskeleton remodeling, and FBP17, a protein involved in membrane curvature and vesicular trafficking. These findings underscore the involvement of the BCR PH domain in pathways linked to cytoskeletal dynamics and membrane-associated processes.

Cortactin is a cytoskeletal protein primarily involved in the regulation of actin filaments (Wu and Parsons, 1993). It enhances the nucleation and branching of actin filaments by interacting with the Arp2/3 complex, a critical regulator of actin polymerization (Weed and Parsons, 2001; Krueger et al., 2003). This protein plays an essential role in various cellular processes, including cell motility, where it facilitates the formation of lamellipodia and invadopodia, structures crucial for cell migration and invasion (Cosen-Binker and Kapus, 2006; Schnoor et al., 2018). Additionally, cortactin is involved in clathrin-mediated endocytosis by stabilizing actin filaments at the endocytic vesicle, and it links cytoskeletal dynamics to intracellular signaling pathways through interactions with multiple signaling molecules (Zhu et al., 2005). Importantly, cortactin overexpression has been associated with enhanced metastatic potential in various cancers, highlighting its role in cancer progression (Buday and Downward, 2007; Weaver, 2008; Jeannot and Besson, 2020).

FBP17 (Formin-Binding Protein 17) is a BAR domain-containing protein critical for membrane curvature and the formation of endocytic vesicles. It plays a significant role in membrane remodeling by inducing tubular membrane structures through its binding to curved membranes, facilitating processes such as endocytosis and vesicle trafficking (Snider et al., 2021). FBP17 also coordinates actin filament growth with membrane remodeling by interacting with WASP (Wiskott-Aldrich Syndrome Protein) to recruit and activate the Arp2/3 complex (Li et al., 2023). Furthermore, it participates in signaling pathways that regulate cytoskeletal organization, influencing cell morphology and division (Tsujita et al., 2006). In the context of neuronal development, FBP17 has been implicated in dendritic spine formation and synaptic plasticity through its interaction with actin-regulating proteins (Taylor et al., 2019).

Together, these functions underscore the potential importance of cortactin and FBP17 interactions with the PH domain of BCR. These interactions likely contribute to pathways governing cytoskeletal dynamics, vesicle trafficking, and cellular signaling, with implications for understanding their roles in pathological contexts such as chronic myelogenous leukemia.

As previous proteomic studies used mass-spectrometry to identify potential interaction partners of PH domain (Miroshnychenko et al., 2010), it is important to confirm this data with more reliable methods, because mass-spectrometry can also detect indirect interactions and give false positive results (Yugandhar et al., 2019). The aim of the current research is to investigate whether there is a direct interaction between FBP17 and the PH domain of BCR, as

well as between cortactin and the PH domain of BCR. Additionally, we seek to determine whether the SH3 domain of cortactin, which is typically involved in protein-protein interactions, plays a crucial role in the interaction between cortactin and the PH domain of BCR. To complement these findings, we also aim to examine the intracellular localization of cortactin, full-length BCR, and the PH domain of BCR using fluorescence microscopy. This comprehensive approach will provide critical insights into the molecular mechanisms and cellular context of these interactions, enhancing our understanding of their role in the pathology of chronic myelogenous leukemia.

MATERIALS AND METHODS

Vectors and plasmids. Coding sequence of cortactin was amplified by PCR using oligonucleotides CTTN-F (5'-tatagaattcAGATGTGGAAAGCTTCAGCAG) and CTTN-R (5'-tataggatccAAAGAAGGCCT-GATCTGTAGTG) and pOTB7-CTTN (kind gift of prof. A. Dubrovska) as a template. PCR conditions were (Denaturation cycle: 15 s 93°C; Annealing cycle: 30 s 60°C; Elongation cycle: 90 s 72°C; 30 cycles total and 2 min of final extension at 72°C). Amplified PCR fragment was purified from agarose gel on homemade silica spin columns packed with 8 layers of GF/C glass fiber filters (Whatman) using guanidine thiocyanate as binding agent according to standard protocol (Dowhan 2008). Purified DNA fragment was inserted into pBluescriptSKII+ vector by blunt end ligation into EcoRV site using T4 DNA ligase according to manufacturer's recommendations (Briefly, 5 units of T4 DNA ligase in manufacturer supplied ligase reaction buffer and total reaction volume of 20 μL for 16 h at +20°C with 3 : 1 insert to vector molar ratio and 200 ng total concentration of DNA in ligation reaction). Ligation reaction was transformed into *E. coli* TOP10 chemically competent cells using heat shock method and plated on agar plates with selective antibiotics followed by incubation at +37°C for 18 h (Froger and Hall, 2007). Individual colonies were used for plasmid isolation and identification of clones containing correct insert. Obtained construct pBLSK-CTTN was used as a template for further subcloning. Specifically, CTTN coding sequence was subcloned by BamHI and NotI sites into pGEX4T2 vector. FBP17-pmCherryC1 was a gift from Christien Merrifield (Addgene plasmid # 27688; http://n2t.net/addgene:27688; RRID: Addgene 27688) (Taylor et al., 2011). Coding sequence of FBP17 was cut from mCherry-FBP17 using BglII and EcoRI restriction endonuclease sites and subcloned to pGEX4T-1 vector using BanHI and EcoRI sites. Ligation reaction conditions were identical to those described previously in the text for cortactin PCR fragment and BluescriptSKII+ vector. Expression construct pGEX with GST tagged SH3 domain of cortactin was a kind gift of Serhii Kropyvko (IMBG, Kyiv, Ukraine). Expression construct pET28c-PH with polyhistidine tagged PH domain of BCR was created in previous work (Miroshnychenko et al., 2010).

Plasmid isolation. Midiprep plasmid isolation was done by alkaline lysis (Birnboim and Doly, 1979). Miniprep plasmid isolation was done by non-ionic detergent plasmid DNA isolation method (Lezin et al., 2011). Purity and quantity of isolated plasmid DNA was assessed by agarose gel electrophoresis and spectrophotometric measurements on Nanodrop 2000 UV-vis spectrophotometer (Thermo Fisher).

Reagents and materials. Pfu DNA polymerase, T4 DNA ligase, Restriction endonucleases EcoRV, EcoRI, BamHI, BglII, NotI (Thermo Fisher). Anti-polyhistidine antibodies (Sigma Aldrich). Glutathione Sepharose 4B (Pharmacia), His-select Nickel affinity gel (Sigma Aldrich), imidazole (Sigma Aldrich), Triton X-100, NaCl, Tryptone, Yeast extract, Sucrose, EDTA, acrylamide, bisacrylamide, TEMED, beta-mercaptoethanol, sodium dodecyl sulphate (SDS), luminol, coumaric acid, hydrogen peroxide, potassium acetate, NaOH, isopropanol, ethanol, Na_2HPO_4, KH_2PO_4, NH4Cl, ammonium persulphate, KCl, PMSF, Lyzozyme, G-coumassie 250, bromphenol blue, Tris Base (CHEMLABORREAKTIV, Ukraine).

Endonuclease digestion of DNA. For each restriction endonuclease digest reaction 5 units of restriction endonuclease, 1 μg of plasmid DNA, total reaction volume of 20 μL were used. Buffer was selected for optimal activity of each endonuclease according to manufacturer's recommendations (Thermo Fisher). Typical reaction conditions are 37°C for 1 h followed by thermal inactivation at 80°C for 20 min.

Protein expression and purification. Plasmids with expression constructs were transformed in BL21 Rosetta chemically competent *E. coli* cells by heat shock (Froger and Hall 2007) and plated on agar plates with 100 μg/mL ampicillin and 25 μg/mL chloramphenicol as selective antibiotics. Agar plates were incubated at +37°C for 18 h. Individual colonies were picked and inoculated into 5 mL ZYM-505 growth medium (Studier 2005) with 100 μg/mL ampicillin and 25 μg/mL chloramphenicol as selective antibiotics and incubated in shaking platform at 200 rpm at +37°C for 18 h. Following day, 0.2 mL of started culture was inoculated in 100 mL of ZYM-5052 autoinduction medium (Studier 2005) and incubated on orbital shaking platform at 200 rpm at +37°C until OD600 reached 0.4–0.5. After that temperature was reduced to ambient values (typically 20–22°C) and further incubation was performed for another 18–24 h. Small amount (~200 μL) of culture was taken before and after induction and boiled for 5 min in 4× loading buffer to compare protein expression on polyacrylamide gel electrophoresis (PAGE). Bacterial cells were harvested by centrifugation at 2000 G for 20 min. Cell pellet was vortexed and resuspended in 10 mL of

cold PBS containing 1 mM PMSF as protease inhibitor and 100 μg/mL lysozyme. Lysate was incubated on ice for 30 min followed by ultrasonic disruption (typically 10 s pulse followed by 10 s rest period repeated for 6 times). Lysed cells were centrifuged at 16000 G for 20 min at +4°C. Supernatant was collected into a new tube. Portion of a sample was collected and boiled in a 4× loading buffer for further analysis of proteins contained in soluble fraction of lysate. The subsequent affinity purification is different for his-tagged and GST tagged proteins. Polyhistidine tagged PH domain was purified on a column packed with His-select Nickel affinity gel and equilibrated with PBS. Briefly, 200 μL of nickel affinity resin was packed into 8 mL gravity flow column and washed several times with PBS. Cleared lysate containing recombinant PH domain of BCR was applied to the column and let to flow through by gravity. Next, 10 column volumes of wash buffer (PBS containing 10 mM imidazole) were used to wash column from unspecific binding of *E. coli* endogenous proteins. Following that, bound PH domain was eluted by 3 column volumes of elution buffer (PBS containing 300 mM of imidazole) and collected in a new tube. Eluate was desalted against PBS buffer using PD-10 desalting column according to manufacturer's instructions. Concentration and purity of eluted and desalted protein was determined by linearized Bradford assay (Ernst and Zor, 2010) and PAGE. Cleared lysate containing GST tagged recombinant proteins were mixed with 200 μL of PBS equilibrated glutathione Sepharose 4B and incubated 4 h at +4°C on rotary laboratory shaker (Biosan). Slurry was pelleted by centrifugation at 500 G for 1 min at +4°C. Supernatant was discarded and slurry was resuspended in 1 mL of PBS, centrifuged for 1 min at 500 G at +4°C, supernatant was discarded to wash off remaining unbound proteins. Wash procedure was repeated 3 times. The remaining slurry contained GST-bound proteins that are ready for GST pulldown experiment.

GST pulldown. Equal amounts of purified PH domain (5 μg) were applied to glutathione Sepharose slurry containing protein of interest and GST protein only (negative control and incubated overnight at +4°C on rotary laboratory shaker (Biosan). Following incubation slurry was pelleted by centrifugation at 500 G for 1 min at +4°C, portion of supernatant was saved for further analysis and the rest was discarded. Slurry was washed 3 times with PBS as was described previously in protein expression and purification section. GST bound proteins were eluted from glutathione Sepharose by incubating for 3 hours with GST elution buffer (PBS containing 10 mM reduced glutathione) at +4°C on rotary laboratory shaker (Biosan). Slurry was pelleted by centrifugation at 13000 G for 10 min at +4°C. Supernatant was collected in a new tube for further analysis and experiments. Protein concentration in eluted samples was determined by linearized Bradford assay (Ernst and Zor, 2010).

Protein transfer and western-blot analysis. Polyacrylamide gel electrophoresis (PAGE) was performed in BioRad protean Tetra Cell vertical gel electrophoresis system in 10% resolving gel according to standard protocol (Litovchick, 2018). Equal amounts of eluted proteins (10 μg) after GST-pulldown experiments in PAGE loading buffer were applied to each well of a gel along with 5 μL of prestained wide range molecular weight marker (Cleaver Scientific, CSL-BBL). Electrophoresis was performed at 120 V until dye migration front run out from the gel into buffer tank. Polyacrylamide gel was sandwiched with 0.22 μm nitrocellulose membrane and protein transfer was performed in BioRad wet transfer chamber for 2 h at 200 mA current. Effectiveness of transfer was assessed by transfer of prestained molecular weight marker (Cleaver Scientific, CSL-BBL) on a membrane. Membrane was incubated in 10 mL of 5% non-fat dry milk in PBS for 1 h at ambient temperature at agitation platform for blocking of nitrocellulose membrane. After blocking, 10 mL of 1 : 400 dilution of anti-polyhistidine antibodies (Sigma Aldrich, MAB3844) in 5% non-fat dry milk in PBST was added on top of membrane and incubated overnight at +4°C on agitating platform. Following incubation with primary antibodies, membrane was incubated with PBST buffer for 10 min at room temperature following discarding of PBST buffer to wash membrane from unspecific binding. Washing procedure was repeated 3 times. 10 mL of HRP-conjugated goat anti-mouse secondary antibodies (Abclonal) diluted 1 : 5000 in PBST buffer was added to the membrane and incubated 1 h at ambient temperature on agitating platform. Membrane was washed 3 times from secondary antibodies with PBST buffer as was described earlier. Membrane was incubated for 1 min with 1 mL of homemade enhanced chemiluminescence (ECL) buffer containing 100 mM Tris pH 8.8, 2.5 mM luminol, 0.4 mM coumaric acid, and 0.02% H_2O_2 and chemiluminescence signal was detected using BioRad Chemidoc gel documentation system with exposure times optimized for best signal to noise ratio.

Cell culturing and fluorescence microscopy. HEK-293T cells were transfected with pmCitrine-C1-PH plasmid with polyethyleneimine (PEI) and stained with mouse anti-cortactin (Santa-Cruz Biotechnology, sc-55579) and rabbit anti-CLTA antibodies (Abclonal, A3793). K562 cells were stained with mouse anti-cortactin (Santa-Cruz Biotechnology, sc-55579) and rabbit anti-BCR antibodies (Abclonal, AA0068). For fluorescence detection anti-mouse Star580 (Abberior Instruments) and anti-rabbit StarRED (Abberior Instruments) secondary antibodies were used. Leica SP8 STED-3D microscope with 100× oil lens and 1.4 numerical aperture was used to obtain images of microslides in different fluorescence channels. Citrine-PH fluorescent protein was excited with 515 nm laser, Star580 and StarRED fluorophores were excited with 564 and 633 nm laser wavelengths,

respectively. For stimulated emission depletion 594 nm STED laser was used to deplete Citrine and 775 nm STED laser was used to deplete StarRED and Star580 fluorophores. Protocols for cell culturing conditions, transfection, cell fixation, immunofluorescence and mounting have been described previously (Gurianov et al., 2021a). Image processing and deconvolution was done in DeconvolutionLab Fiji plugin as described previously (Gurianov et al., 2021b).

RESULTS AND DISCUSSION

Western blot analysis of GST-pulldown samples demonstrated that full-length recombinant FBP17 protein directly interacts with PH domain of BCR (Fig. 1). This finding aligns with our previous work, which showed that FBP17 colocalizes with BCR near the peripheral regions of cells during phagocytosis in the murine macrophage cell line J774 (Antonenko et al., 2023). These results suggest that the BCR portion of the BCR/ABL fusion protein plays an active role in membrane dynamics. Another important participant of membrane and cytoskeleton remodeling is cortactin. Here we show that it colocalizes with BCR in centrosomal and perimembrane region of K562 cells (Fig. 2), and with PH domain of BCR and clathrin in HEK293T cells (Fig. 3).

Such intracellular distribution of cortactin and BCR indicates that BCR is involved in vesicular transport and membrane remodeling. Colocalization was confirmed using STED microscopy, with proteins appearing closer than the effective resolution of the technique (~40 nm), which suggests a potential direct interaction. To validate this hypothesis, we performed a GST-pulldown assay followed by western blotting, which confirmed a direct interaction between the purified recombinant PH domain of BCR and full-length cortactin (Fig. 4a). Protein-protein interactions are often mediated by Src homology (SH) domains. Cortactin contains an SH3 domain, prompting us to investigate whether this domain mediates its interaction with the PH domain of BCR. Interestingly, no interaction was detected between the recombinant SH3 domain of cortactin and the PH domain of BCR in our GST-pulldown and western blot experiments (Fig. 4b). This result suggests that other domains of cortactin, such as the actin-binding or NTA domains, might mediate this interaction. Alternatively, the specific conformation of full-length cortactin may be critical for its interaction with the PH domain, a structural context that cannot be replicated using the isolated SH3 domain. The confirmed interactions between the PH domain of BCR and both cortactin and FBP17 highlight the important role of BCR in membrane and cytoskeletal remodeling processes, particularly during clathrin-mediated endocytosis and early endosome formation. Since both cortactin and FBP17 are involved in these processes, their interactions with the PH domain could explain the distinct subcellular localization of the p210 isoform of BCR/ABL (Wetzler et al., 1993). Unlike the p190 isoform, which is more diffusely distributed in the cytoplasm, the p210 isoform appears more restricted to membrane-associated regions (Wetzler et al., 1993; Telegeev et al., 2004). We propose that these interactions help anchor BCR/ABL p210 to specific cellular compartments, potentially limiting the constitutive tyrosine kinase activity of the ABL portion of the fusion protein to these local domains. This restricted localization may underlie the less aggressive phenotype of the myeloproliferative disorder associated with p210 compared to the p190 isoform, which lacks the PH domain and exhibits a broader intracellular distribution. Furthermore, phosphorylation of cortactin and FBP17 by the ABL kinase could disrupt their functions, perturbing cytoskeletal remodeling and vesicular trafficking, and contribute to oncogenic transformation of hematopoietic cells (Wang et al., 2008). Future studies should experimentally determine whether cortactin and FBP17 are phosphorylated by ABL kinase, as this has only been predicted through bioinformatics in our prior work (Gurianov et al., 2016). Additionally, deletion mutants of cortactin and FBP17, lacking key domains, could help pin-

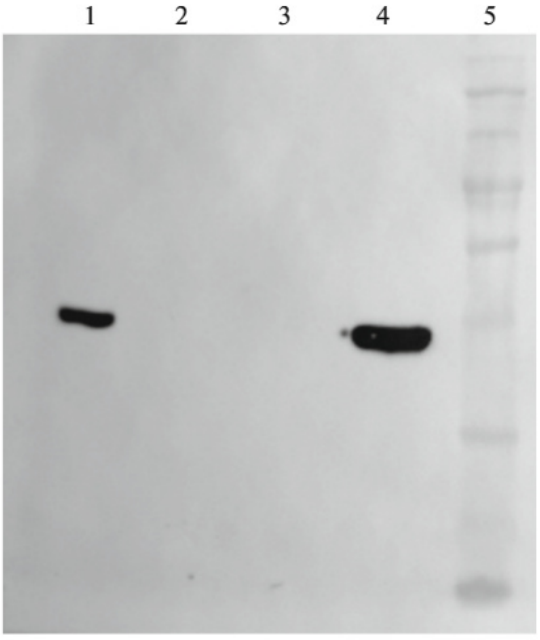

Fig. 1. Western blot analysis of GST pulldown of recombinant GST-tagged FBP17 with his-tagged PH domain of BCR. 1—Elution fraction of his-tagged PH domain of BCR; 2—total lysate of E. coli BL21 Rosetta expressing recombinant GST-FBP17; 3—fraction eluted with GST protein that was bound to glutathione Sepharose (negative control); 4—fraction eluted with GST-FBP17 that was bound to glutathione Sepharose; 5—molecular weight marker. Detection of band on lane 4 and its absence on lane 3 indicates direct interaction between FBP17 and PH domain of BCR.

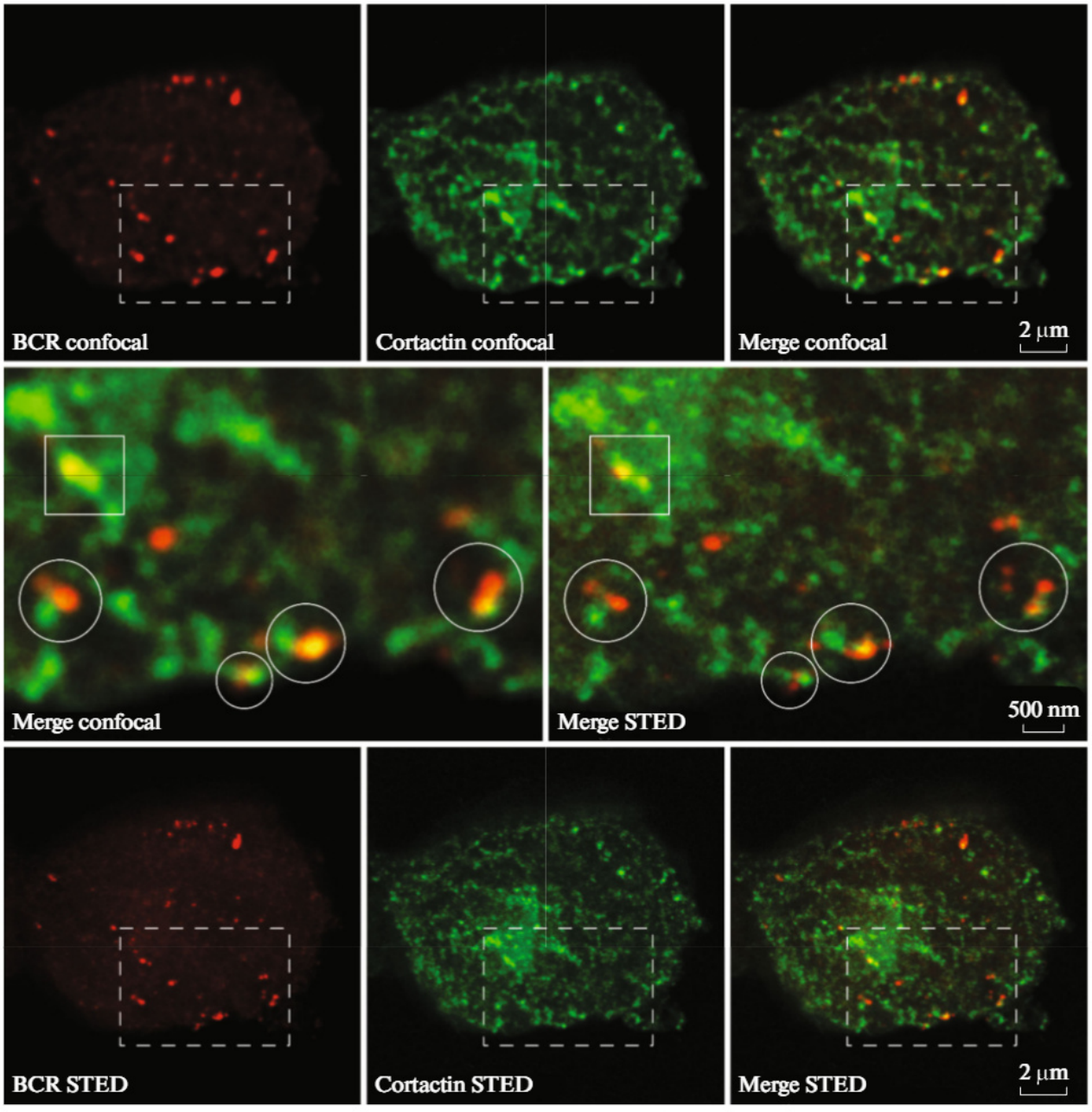

Fig. 2. Fluorescence microscopy of confocal and super-resolution STED images of fixed K562 cells stained with anti-BCR and anti-CTTN and their corresponding immunofluorescent secondary antibodies. Enlarged region shows colocalization between cortactin and BCR in presumably centrosomal region (squared region of interest) and perimembrane space near cell periphery (circled regions of interest).

point the regions responsible for their interactions with the PH domain and their roles in determining the specific subcellular localization of BCR/ABL p210. In both the current study and our previous work (Gurianov et al., 2021b) we observed that colocalization between cortactin, clathrin, and BCR predominantly occurs near the cell nucleus, likely within the centrosomal region. The ability of the PH domain alone to colocalize with these proteins in the centrosomal region strongly suggests that this domain is responsible for the targeting of BCR and the BCR/ABL fusion protein to the centrosome. We can speculate that if the BCR/ABL fusion protein is anchored to the centrosomal region by its PH domain and interacts with proteins such as cortactin, FBP17, and clathrin, it could have profound effects on cellular signaling pathways. The centrosome serves as a major signaling hub, orchestrating processes like cell division, cytoskeletal organization, and intracellular trafficking (Conduit et al., 2015). Its association with oncogenic proteins

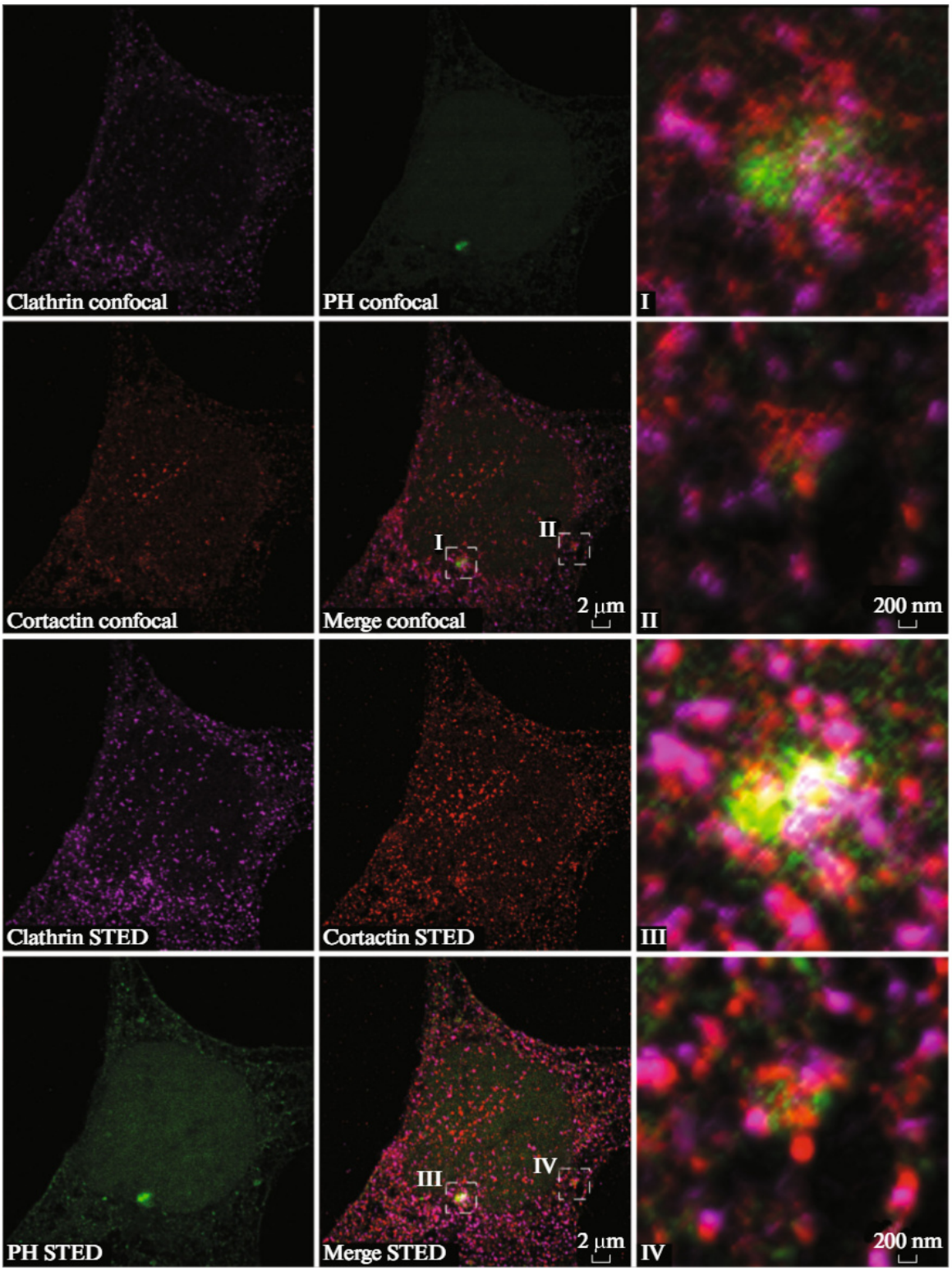

Fig. 3. Fluorescence microscopy of confocal and STED images of HEK293T cells transfected with pmCitrine-C1-PH and immunofluorescently labeled with antibodies against cortactin and clathrin A. Enlarged areas in confocal (I and II) and STED image (III and IV) indicate colocalization between PH domain of BCR, cortactin, and clatrhin in centrosomal region near cell nucleus (I and III) and on peripheral region of a cell (II and IV). This indicates that CTTN and PH domain of BCR could be involved in both clathrin-mediated endocytosis and centrosome-associated processes.

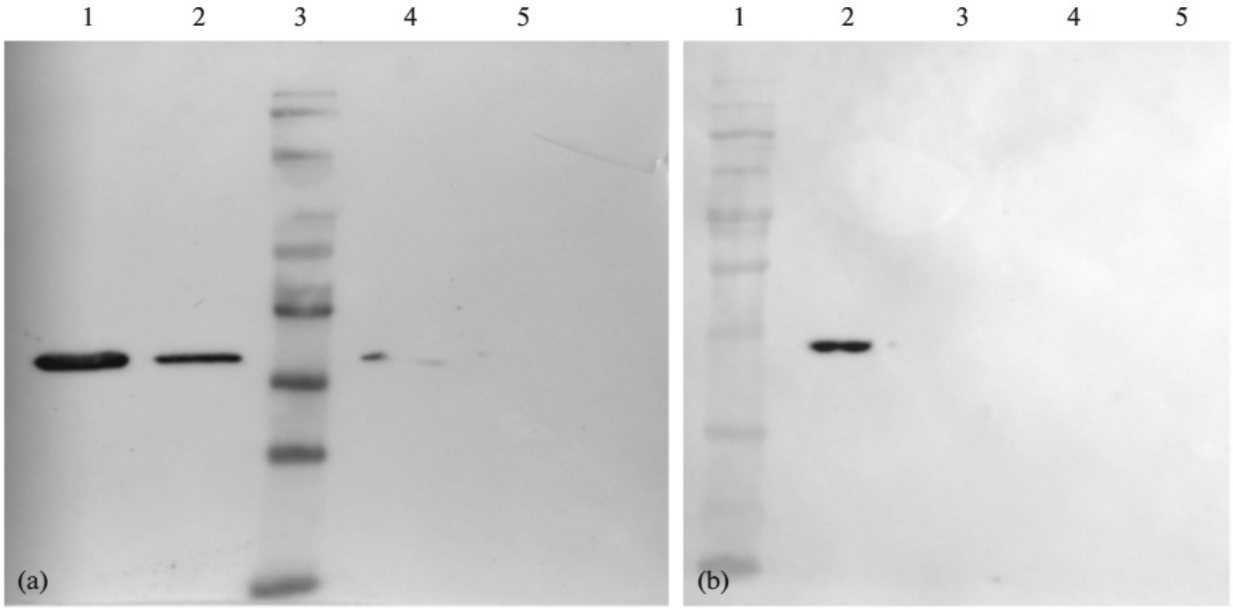

Fig. 4. Western blot analysis of GST pulldown of recombinant GST-cortactin (a) and GST-tagged SH3 domain of cortactin (b) with his-tagged PH domain of BCR. Image a: 1—Eluate of his tagged PH domain; 2—purified his-tagged PH domain that was incubated with GST-tagged proteins bound to glutathione Sepharose; 3—molecular weight marker; 4—Fraction eluted with GST-cortactin that was bound to glutathione Sepharose; 5—fraction eluted with GST protein that was bound to glutathione Sepharose (negative control). Image b: 1—molecular weight marker; 2—elution fraction of his-tagged PH domain of BCR; 3—total lysate of *E. coli* BL21 Rosetta expressing recombinant GST-tagged SH3 domain of CTTN; 4—fraction eluted with GST protein that was bound to glutathione Sepharose (negative control); 5— fraction eluted with GST-tagged SH3 domain of CTTN that was bound to glutathione Sepharose. Detection of bands corresponding to his-tagged PH domain of BCR in pulldown fraction of full-length recombinant GST-fused cortactin and absence of such detection in pull-down fraction of GST-tagged SH3 domain of cortactin demonstrates presence of direct interaction between cortactin and PH domain of BCR, but such interaction does not occur through SH3 domain of CTTN, indicating that some other part of cortactin is responsible for such interaction.

like BCR/ABL could therefore alter normal cellular functions and contribute to malignant transformation. Firstly, centrosomal localization of BCR/ABL might disrupt cell cycle regulation (Patel and Gordon 2009). The centrosome plays a pivotal role in mitotic spindle formation, and perturbations in its function often lead to genomic instability, a hallmark of cancer (Nigg, 2006). By interacting with cortactin, which is known to regulate actin cytoskeleton remodeling and microtubule dynamics, BCR/ABL could influence spindle assembly or orientation, potentially leading to abnormal cell division and aneuploidy (Wang et al., 2008). Secondly, FBP17 and clathrin interactions in the centrosomal region may enhance vesicular trafficking and membrane dynamics at this site, impacting the distribution of key signaling molecules. This could result in the spatial restriction of tyrosine kinase activity, confining the phosphorylation of substrates to the centrosomal vicinity (Lee et al., 2014). Such localized signaling might selectively activate pathways involved in centrosome-mediated cell proliferation or survival while sparing other pathways, potentially contributing to the less aggressive phenotype of CML compared to ALL. Moreover, the centrosome's role as a scaffold for various kinases suggests that BCR/ABL localization there might hijack or modify normal centrosome-associated signaling. For example, centrosomes regulate pathways like the PI3K/AKT (Zhao et al., 2014) and MAPK cascades (Pancione et al., 2021), critical for cell growth and survival. Aberrant interactions of BCR/ABL with these pathways at the centrosome could amplify oncogenic signals. Additionally, clathrin-mediated vesicle formation in this region could modulate the recycling of signaling receptors or the sequestration of inhibitory regulators, further sustaining abnormal proliferation and resistance to apoptosis. Finally, the centrosomal localization could facilitate interactions between BCR/ABL and DNA repair machinery, given the proximity of the centrosome to nuclear structures during interphase. Such interactions might inhibit proper DNA repair processes, increasing the mutation burden and contributing to leukemia progression (Giehl et al., 2005). These potential effects underscore the significance of the observed colocalization and highlight the need for further investigation into the molecular consequences of BCR/ABL anchoring at the centrosome. Future research should explore how these interactions affect downstream signaling cascades and contribute to leukemogenesis, offering potential avenues for targeted therapeutic interventions.

In conclusion, our results represent an important step toward understanding the molecular mechanisms of BCR/ABL-associated oncotransformation in hematopoietic cells. These findings provide new insights into the development of chronic myelogenous leukemia and the molecular distinctions between acute lymphoblastic leukemia and chronic myelogenous leukemia. This work could serve as a foundation for developing novel diagnostic tools and therapeutic approaches, offering potential alternatives to existing protein kinase inhibitors.

FUNDING

This work was supported by Ukrainian state funding and I-Next grant (project ID: 8230) within the Horizon 2020 program.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies involving animals or human participants performed by any of the authors.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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