SHORT COMMUNICATION

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FNBP1 IN CHRONIC MYELOID LEUKEMIA: SPATIAL ASSOCIATION WITH BCR-ABL AND POTENTIAL IMPLICATIONS FOR TARGETED THERAPY

Background. Chronic myeloid leukemia (CML) develops as a result of the appearance of the oncoprotein BCR-ABL, which, due to its tyrosine kinase activity, leads to abnormal cellular signal transduction and blast transformation. FNBP1 is a protein involved in cytoskeletal remodeling, endocytosis, phagocytosis, and cell migration, but its functional role in the development of CML is unclear. **Aim.** To investigate the spatial relationship between FNBP1 and the BCR-ABL oncoprotein in CML cells and to assess the potential involvement of FNBP1 in BCR-ABL-related signaling networks. **Materials and Methods.** The subcellular localization of FNBP1 and BCR-ABL was studied using immunofluorescence staining followed by confocal microscopy in K562 cells. The obtained images were processed and analyzed using Fiji software. The bioinformatic analysis of the FNBP1 expression in different cancer types was performed using the GEPIA platform. **Results.** The bioinformatic analysis revealed a heterogeneous regulation of the FNBP1 expression in various malignancies, with the largest increase observed in leukemia. A cytoplasmic punctate distribution of FNBP1 was shown in K562 cells. Partial colocalization between FNBP1 and BCR-ABL was found predominantly in the peripheral cytoplasmic regions. **Conclusions.** The observed common spatial distribution of FNBP1 and BCR-ABL enhances the understanding of this protein complex's formation, suggesting a potential role for FNBP1 in CML development.

Keywords: chronic myeloid leukemia, BCR-ABL, FNBP1, tyrosine kinase, BCR-ABL/FNBP1 colocalization.

Chronic myeloid leukemia (CML) is a clonal hematopoietic malignancy characterized by the presence of the Philadelphia (Ph) chromosome, which arises from the reciprocal chromosomal translocation t(9;22)(q34;q11) [1, 2]. This genetic rearrangement results in the formation of the *BCR-ABL* fusion gene, encoding a constitutively active tyrosine kinase, which serves as the principal molecular driver of leukemogenesis. The BCR-ABL oncoprotein aberrantly phosphorylates numerous downstream substrates, leading to the dysregulation of the key signaling pathways controlling cell proliferation, survival, and differentiation, thereby promoting the malignant transformation of hematopoietic stem cells [3—6]. The treatment of CML is primarily based on tyrosine kinase inhibitors (TKIs), including imatinib

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and its derivatives. However, the long-term therapeutic efficacy of imatinib is frequently limited due to the development of drug resistance, which commonly arises from mutations within the BCR-ABL kinase domain [4, 7—9]. These limitations highlight the need to identify the additional molecular targets involved in CML pathogenesis and to develop novel therapeutic strategies [1, 4, 10].

Formin-binding protein 1 (FNBP1), also known as formin-binding protein 17 (FBP17), belongs to the F-BAR/EFC protein family, which is widely expressed in eukaryotic cells [11, 12]. FNBP1 is involved in multiple cellular processes, including cytoskeletal remodeling, endocytosis, phagocytosis, and cell migration, the dysregulation of which may contribute to malignant transformation [13—17]. In our previous studies, FNBP1 has been identified as a novel interacting partner of BCR-ABL [18, 19]. However, despite the well-established role of FNBP1 in membrane dynamics and cytoskeletal organization, its potential involvement in BCR-ABL-associated signaling networks remains largely unexplored.

In this study, we have analyzed the spatial relationship between FNBP1 and BCR-ABL proteins in CML cells and compared these patterns with the distribution of FNBP1 and BCR in an alternative cellular model. Investigating the spatial arrangement of proteins involved in BCR-ABL signaling provides insight into the molecular mechanisms driving CML and may facilitate identification of the novel regulatory factors with potential therapeutic relevance.

Materials and Methods

Cell culture. K562 and J774 cells were obtained from the Bank of Cell Lines from Human and Animal Tissues of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine. K562 cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich), penicillin (50 U/mL), and streptomycin (100 μg/mL). J774 cells were cultured in DMEM (Thermo Fisher Scientific) supplemented with 10% FBS (Sigma-Aldrich), penicillin (50 U/mL), and streptomycin (100 μg/mL). Cell cultures were maintained in a humidified incubator at 37 °C with 5% CO_2. The cells were subjected to immunofluorescence analysis upon reaching 80% confluency.

Immunofluorescence analysis. K562 cells were fixed with 4% paraformaldehyde in PBS containing 0.2% Triton X-100 for 20 min to preserve cellular architecture and permeabilize membranes. Non-specific binding sites were blocked with 2% BSA in PBS for 1 h at room temperature. Cells were subsequently incubated with primary anti-FBP17 (Santa Cruz Biotechnology, USA) and anti-Bcr-Abl (Thermo Fisher Scientific, USA) antibodies for 1 h at room temperature. After washing, samples were incubated with the secondary antibodies conjugated to DyLight 550 and DyLight 488 (Thermo Fisher Scientific, USA) for 1 h in the dark to prevent photobleaching. Nuclear DNA was counterstained with DAPI for 2 min. To preserve fluorescence and stabilize the specimens for imaging, the slides were mounted using CitiFluor™ AF1 mounting medium (Science Services, Germany).

Confocal microscopy. Fluorescent imaging was performed using a confocal laser scanning system from Leica Microsystems (Germany) with a 90× oil immersion objective (NA 1.3). The excitation and emission settings were individually optimized with the Leica LAS X software to minimize the cross-excitation and fluorescence signal bleed-through.

Bioinformatic analysis. The analysis of FBP17 expression across different cancer types was performed using the GEPIA platform, which integrates transcriptomic data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases — http://gepia.cancer-pku.cn/detail.php?gene=FNBP1.

Image analysis. The quantitative analysis of colocalization was performed using the Fiji software and the JaCOP plugin [20]. Automated Costes thresholding was applied for the Pearson correlation coefficient [21]. For Manders M1 and M2, a coefficient threshold was selected manually only to include pixels relevant to the cell region of interest and exclude the background [22].

Results and Discussion

The analysis of FNBP1 expression across different cancer types, performed using the GEPIA platform, revealed marked variability in FNBP1 expression among the tumors compared to their corresponding normal tissues, suggesting a potential association with oncogenesis or tumor progression (Fig. 1, *a*). The elevated FNBP1 expression was observed in several malignancies, including diffuse large B-cell

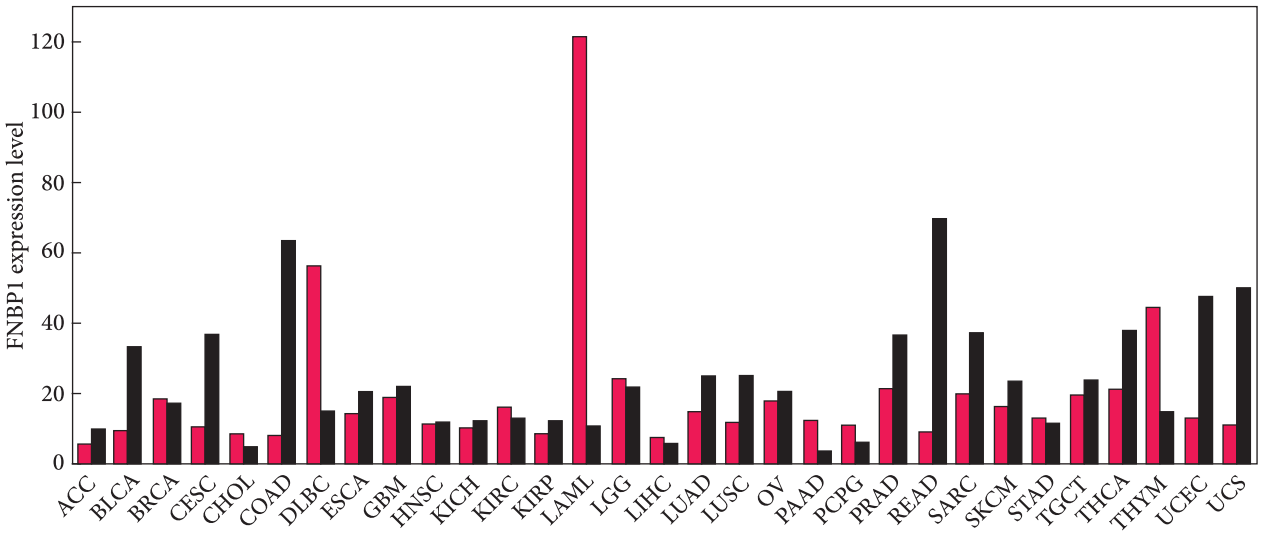

Fig. 1. The role of FNBP1 in cancer. FNBP1 expression in normal samples (gray box) and tumor samples (red box) from patients with cancer compiled with GEPIA data from the Cancer Genome Atlas and the Genotype Tissue Expression databases. ACC — adrenocortical carcinoma, BLCA — bladder urothelial carcinoma, BRCA — breast invasive carcinoma, CESC — cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL — cholangiocarcinoma, COAD — colon adenocarcinoma, DLBC — diffuse large B-cell lymphoma, ESCA — esophageal carcinoma, GBM — glioblastoma multiforme, HNSC — head and neck squamous cell carcinoma, KICH — kidney chromophobe carcinoma, KIRC — renal clear cell carcinoma, KIRP — renal papillary cell carcinoma, LAML — acute myeloid leukemia, LGG — low grade glioma, LIHC — hepatocellular carcinoma, LUAD — lung adenocarcinoma, LUSC — lung squamous cell carcinoma, OV — ovarian serous cystadenocarcinoma, PAAD — pancreatic adenocarcinoma, PCPG — pheochromocytoma and paraganglioma, PRAD — prostate adenocarcinoma, READ — rectal adenocarcinoma, SARC — sarcoma, SKCM — cutaneous melanoma, STAD — stomach adenocarcinoma, TGCT — testicular germ cell tumors, THCA — thyroid carcinoma, THYM — thymoma, UCEC — uterine corpus endometrial carcinoma, UCS — uterine carcinosarcoma

lymphoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, and thymoma. The most pronounced increase in FBP17 expression was observed in acute myeloid leukemia, where its level was tenfold higher than in normal hematopoietic cells, representing the highest differential expression among all analyzed malignancies. This hyperexpression may indicate a leukemia-specific regulatory role of FNBP1 in cytoskeletal remodeling or intracellular signaling pathways. Notably, in many other cancer types, the FBP17 expression was reduced compared to normal tissues, including bladder urothelial carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, colon adenocarcinoma, lung adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, rectal adenocarcinoma, sarcoma, cutaneous melanoma, thyroid carcinoma, uterine corpus endometrial carcinoma, and uterine carcinosarcoma. In other malignancies, the differences in the FNBP1 expression between tumor and normal tissues were relatively modest.

Overall, this analysis demonstrates heterogeneous regulation of the FNBP1 expression across different malignancies, indicating a context-dependent role of this protein in tumor biology. These findings highlight the importance of further investigating FNBP1-mediated signaling networks, particularly in hematological malignancies, including CML.

Our previous studies identified FNBP1 as one of the interacting partners of the BCR-ABL oncoprotein [18, 19]; however, the mechanism underlying the formation of this protein complex remained unclear. To further study the molecular mechanisms of the interaction between these target proteins, we investigated their subcellular localization in K562 cells using an immunofluorescence analysis followed by confocal microscopy (Fig. 2, *a*). FNBP1 exhibited a cytoplasmic distribution characterized by discrete punctate structures. The merged images revealed the regions of the partial overlap between the fluorescence signals of FNBP1 and BCR-ABL. These colocalization sites were predominantly detected in peripheral cytoplasmic regions, as was demonstrated by a multichannel pixel intensity plot profile in a selected region of interest (Fig. 2, *b*), suggesting the site of FNBP1/BCR-ABL complex formation and indicating the potential involvement of these proteins in the shared signaling networks and intracellular com-

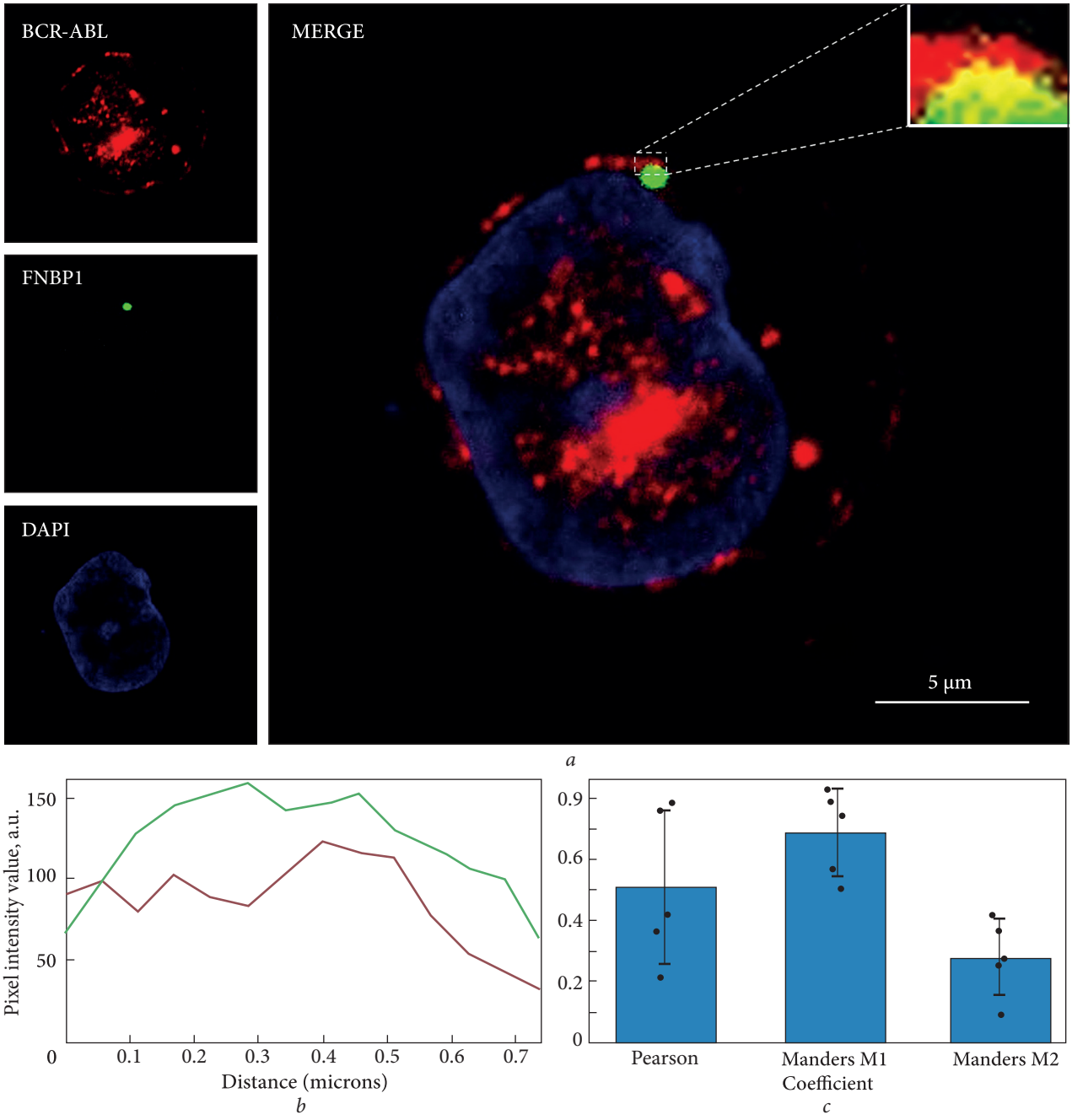

Fig. 2. Colocalization of FNBP1 with BCR-ABL in K562 cells. *a* — confocal images of cells stained for FNBP1 (green) and BCR-ABL (red). Nuclei are counterstained with DAPI (blue). The overlap of the localization signals is seen (yellow); *b* — the multichannel plot profile of the overlapping localization signals of BCR-ABL and FNBP1 inside the region of interest (Fig. 2, *a*). The Y axis indicates pixel intensity values for each channel; the X axis indicates distance in microns for the selected area (Fig 2, *a*); *c* — the quantitative analysis of BCR-ABL and FNBP1 colocalization

partments, A quantitative image analysis was performed using the Pearson correlation coefficient and the Manders overlap coefficient (Fig. 2, *c*).

Our previous studies demonstrated the colocalization of FNBP1 and BCR during phagocytosis in J774 cells [18]. A comparative analysis of these results with the data obtained for K562 cells (Table) showed that the incorporation of BCR into the BCR-ABL oncogenic protein is accompanied by a change in the pattern of its spatial association with FNBP1, which may reflect the reorganization of the intracellular signaling networks in leukemic cells. In particular, a reduced correlation was found between FNBP1 and BCR-ABL compared to BCR. There was also a marked reduction in the M1 coefficient (the overlap of BCR-ABL with FNBP1) com-

Quantitative colocalization analysis of FNBP1 with BCR and BCR-ABL proteins

Protein pair analyzed	Coefficients		
	Pearson correlation coefficient	Manders overlap coefficient	
		M1: BCR/BCR-ABL overlapping with FNBP1	M2: FNBP1 overlapping with BCR/ BCR-ABL
FNBP1 and BCR in J774 cells	0.75 ± 0.05	0.70 ± 0.03	0.63 ± 0.16
FNBP1 and BCR-ABL in K562 cells	0.51 ± 0.25	0.28 ± 0.14	0.68 ± 0.12

pared to the BCR/FNBP1 pair in J774 cells, which is explained by the fact that only a relatively small fraction of the total BCR-ABL pool associates with FNBP1. It should be emphasized that the expression level of FNBP1 in K562 cells is relatively low compared to J774 cells. The reduced amount of this adaptor protein potentially limits the proportion of BCR-ABL molecules that are able to participate in co-protein complexes, which is consequently reflected in the decrease in the M1 coefficient value.

Another factor that may contribute to this difference is the distinct cellular context and functional roles of these proteins. In macrophages, FNBP1 is involved in membrane remodeling and actin cytoskeleton dynamics during phagocytosis, a process that is likely facilitated by its interaction with BCR. In K562 leukemia cells, the constitutively active BCR-ABL kinase is involved in multiple oncogenic signaling pathways and is distributed across different intracellular compartments. As a result, only a fraction of the BCR-ABL molecules can colocalize with membrane structures or endocytic domains containing FNBP1.

At the same time, the M2 coefficient (overlap of FNBP1 with BCR-ABL) remained high, indicating that a significant portion of FNBP1 is spatially associated with both BCR and BCR-ABL, which may indicate an important role of BCR in the FNBP1 function.

Taken together, these data support the existence of a partial spatial association between FNBP1 and BCR-ABL in leukemic cells while suggesting that the interaction occurs in specific cytoplasmic microdomains rather than throughout the entire BCR-ABL pool. Such a compartmentalized colocalization is consistent with the proposed role of FNBP1 as a membrane-associated adaptor protein involved in actin remodeling and vesicular trafficking, processes which may contribute to the spatial regulation of BCR-ABL signaling. Thus, the identification of BCR-ABL–associated signaling networks is essential for understanding the molecular mechanisms underlying CML pathogenesis and may provide a rationale for the development of novel therapeutic strategies targeting specific protein complexes formed by this oncoprotein.

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FNBP1 ПРИ ХРОНІЧНІЙ МІЄЛОЇДНІЙ ЛЕЙКЕМІЇ: ПРОСТОРОВА АСОЦІАЦІЯ З BCR-ABL ТА ПОТЕНЦІЙНІ МОЖЛИВОСТІ ДЛЯ ТАРГЕТНОЇ ТЕРАПІЇ

Стан питання. Хронічна мієлоїдна лейкемія (ХМЛ) розвивається в результаті появи онкопротеїну BCR-ABL, який завдяки своїй тирозинкіназній активності призводить до порушення передачі клітинних сигналів та бластної трансформації. FNBP1 — це білок, який бере участь у ремоделюванні цитоскелета, ендоцитозі, фагоцитозі та міграції клітин, але його функціональну роль у розвитку ХМЛ не з'ясовано. **Мета.** Дослідити просторовий зв›язок між FNBP1 та онкопротеїном BCR-ABL у клітинах ХМЛ та оцінити потенційну участь FNBP1 у сигнальних мережах, пов'язаних з BCR-ABL. **Матеріали та методи.** Субклітинну локалізацію FNBP1 та BCR-ABL вивчали за допомогою імунофлуоресцентного аналізу з подальшою конфокальною мікроскопією в клітинах K562. Отримані зображення обробляли та аналізували за допомогою програмного забезпечення Fiji. Біоінформатичний аналіз експресії FNBP1 у різних типах раку проводили за допомогою платформи GEPIA. **Результати.** Біоінформатичний аналіз виявив гетерогенну регуляцію експресії FNBP1 у різних злоякісних новоутвореннях, причому найбільш виражене збільшення спостерігалося при лейкемії. У клітинах K562 було показано цитоплазматичний точковий розподіл FNBP1. Часткова колокалізація між FNBP1 та BCR-ABL була виявлена переважно в периферичних цитоплазматичних ділянках. **Висновки.** Виявлений спільний просторовий розподіл FNBP1 та BCR-ABL покращує розуміння формування цього білкового комплексу та свідчить про потенційну роль FNBP1 у розвитку ХМЛ.

Ключові слова: хронічна мієлоїдна лейкемія (ХМЛ), BCR-ABL, FNBP1, тирозинкіназа, колокалізація BCR-ABL/FNBP1.