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TETRASPANIN CD9 AND CD81: DIFFERENTIAL REGULATION AND POTENTIAL ROLES IN CHEMORESISTANCE IN ACUTE AND CHRONIC LYMPHOCYTIC LEUKEMIA

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Abstract

Tetraspanins (TSPANs) are a family of transmembrane proteins implicated in diverse cellular processes, including signal transduction and cell adhesion. While their precise functions are still being elucidated, their involvement in cancer, particularly leukemia, is increasingly recognized. This study focuses on the multifaceted roles of two specific TSPANs, CD9 and CD81, in acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL), with a specific emphasis on their impact on chemotherapy response. Previous research has established CD9 as a prognostic marker in B-cell ALL (B-ALL), with expression correlating with specific genetic subtypes and glucocorticoid resistance. However, its role in other leukemia subtypes and its precise contribution to chemoresistance remain incompletely understood. Similarly, CD81 has been shown to mediate chemoresistance in B-ALL by facilitating protective interactions with the bone marrow microenvironment, but its significance in CLL and its broader impact on chemotherapy sensitivity require further investigation. This study addresses these gaps by investigating the expression of CD9 and CD81 in ALL and CLL patients, stratified by chemotherapy regimen, including vincristine (VCR), methotrexate (MTX), and doxorubicin (DOXO). Our results demonstrate a statistically significant upregulation of both CD9 and CD81 at the gene and protein levels in ALL patients treated with chemotherapy compared to controls. Furthermore, we observed significant upregulation of CD9 in untreated CLL patients, while no significant difference in CD9 or CD81 expression was found between the ALL and CLL groups. These findings strongly suggest a critical role for CD9 and CD81 in mediating chemoresistance in ALL, particularly in the context of VCR, MTX, and DOXO treatment. Based on these observations, we discuss potential mechanisms by which CD9 and CD81 contribute to leukemogenesis and propose that targeting these TSPANs may represent a novel therapeutic strategy to overcome chemoresistance and improve patient outcomes in leukemia.

Keywords: CD9, CD81, ALL, CLL, DOXO, VCR and MTX.

1 Introduction

Tetraspanin is a superfamily of transmembrane proteins, has emerged as a molecule with pleiotropic functions in various cellular processes, including cell adhesion, migration, proliferation, and differentiation. Its role in hematopoiesis and, particularly, in leukemia, is complex and context-dependent, with accumulating evidence suggesting a significant impact on disease prognosis and response to therapy. This paper focuses on the multifaceted role of CD9 and CD81 in leukemia, with a particular emphasis on its influence in B-cell acute lymphoblastic leukemia (B-ALL) and the implications of our findings regarding its interplay with chemotherapeutic agents.cell surface protein, plays a complex role in leukemia prognosis and treatment response [1, 2]. In B-cell acute lymphoblastic leukemia (B-ALL), CD9 expression is associated with inferior outcomes, including lower 5-year event-free survival (EFS) and higher cumulative incidence of relapse (CIR), particularly in intermediate/high-risk patients and those with minimal residual disease (MRD) . This adverse prognostic impact is subtype-specific; for example, CD9 positivity correlates strongly with BCR::ABL1+ B-ALL but not KMT2A-rearranged cases. In contrast, CD9 expression in T-cell ALL (T-ALL) lacks prognostic significance, with no differences in survival or relapse rates observed between CD9+ and CD9– patients [2]. However, CD9's role in chronic lymphocytic leukemia (CLL) remains understudied in the provided literature, with current evidence focused on acute leukemias. Notably, a meta-analysis highlights CD9's conflicting prognostic roles across cancers, showing adverse outcomes in leukemia subgroups despite favorable associations in solid tumors [3]. CD9 also modulates treatment sensitivity. In pediatric B-ALL, CD9– blasts demonstrate resistance to glucocorticoids such as prednisone, dexamethasone, which is reversible with MEK inhibitors like trametinib, suggesting combination therapies could overcome resistance [4]. This underscores CD9's dual role as both a prognostic marker and a regulator of drug response. the role of chemotherapy in lymphoblastic leukemia like, Vincristine (VCR), a microtubule inhibitor, is used in induction and maintenance phases of ALL therapy. In pediatric ALL, pulses of VCR combined with dexamethasone (DEX) during maintenance improve outcomes, particularly in high-risk patients [5]. Methotrexate (MTX), a folate antagonist, is administered continuously with 6-mercaptopurine (6-MP) during maintenance therapy. This combination reduces relapse risk by targeting proliferating leukemic cells, especially in patients with poor prognostic features like MRD positivity [5]. CD9– B-ALL cells, which are glucocorticoid-resistant, may still respond to MTX-based regimens, highlighting the importance of risk-stratified therapy[4]. Doxorubicin (DOXO), an anthracycline antibiotic derived from Streptomyces peucetius, has been a cornerstone of chemotherapy since the 1960s. It is widely used to treat various cancers, including acute lymphoblastic leukemia (ALL) [6]. As part of the anthracycline group, doxorubicin exerts its therapeutic effects through DNA intercalation and topoisomerase II inhibition, which disrupt DNA replication and repair, leading to cancer cell apoptosis [6]. However, CD81 a tetraspanin protein, plays a critical role in mediating chemoresistance in B-cell ALL (B-ALL) by

facilitating interactions between leukemic cells and the bone marrow (BM) microenvironment. These interactions create a protective niche that shields leukemia cells from chemotherapy, contributing to relapse [7, 8]. Moreover, CD81's prognostic role in CLL progression or therapy response remains understudied [9]. Utilizing qPCR and flow cytometer, we will characterize CD9 and CD81 expression at both the transcriptional and protein levels in primary patient samples. Specifically, this investigation will address the critical question of how tumor-derived factors alter CD9 and CD81 expression in PBMCs from patients with ALL and CLL.

2 **Methodology**

Study Population and Patient Classification:

A cohort of 130 participants was enrolled at the Cancer Oncology Department of Al-Forat Al-Awsat Hospital (Iraq) between May 1, 2024, and September 22, 2024. Participants were stratified into three groups based on disease subtype, treatment status, and clinical activity, as confirmed by hematological evaluation:

Group 1: Acute Lymphoblastic Leukemia (ALL) on Chemotherapy (n=50):

Patients diagnosed with ALL according to established leukemia classification criteria, actively receiving a chemotherapy regimen of Doxorubicin, Vincristine, and Methotrexate. Diagnosis and treatment monitoring were performed by a board-certified hematologist.

Group 2: Untreated Chronic Lymphocytic Leukemia (CLL) (n=50):

Patients with confirmed CLL via standardized diagnostic criteria, including clinical and laboratory evidence of disease activity, who were not undergoing active treatment at the time of sample collection.

Group 3: Healthy Controls (n=30):

Age- and sex-matched individuals with no history of hematological malignancies or active systemic diseases, confirmed by routine blood tests and clinical evaluation.

For all participants, a single peripheral whole blood sample was collected. Peripheral blood mononuclear cells (PBMCs) were isolated for downstream RNA extraction (qRT-PCR) and flow cytometry analysis. CD9 and CD81 mRNA expression levels were quantified using qRT-PCR, while corresponding protein expression was assessed via flow cytometry.

Age and gender classification

A total of 130 participants, comprising both patients and controls, were enrolled in this study. Among the ALL, CLL patients, and healthy control. That explained in table 1.

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll density gradient centrifugation, following the manufacturer's protocol (Solar-Bio, Lot No. P4350) with minor modifications. Briefly, 2 mL of whole blood were diluted with 2 mL of sterile phosphate-buffered saline (PBS) in a 50 mL conical tube. In a separate 50 mL conical tube, 2 mL of Ficoll-Hypaque were carefully layered underneath 2 mL of the diluted blood using a Pasteur pipette to minimize

mixing. The tubes were then centrifuged at 1400 rpm for 40 minutes at room temperature with the brake off to achieve optimal separation of blood components. This procedure resulted in four distinct layers: plasma (top), PBMCs (middle), Ficoll-Hypaque, and red blood cells (bottom). The PBMC layer, a thin, whitish band located at the interface between the plasma and Ficoll-Hypaque, was carefully collected using a sterile Pasteur pipette and transferred to a new 50 mL conical tube. The collected PBMCs were washed twice with PBS to remove any residual Ficoll-Hypaque or contaminating cells. Following each wash, the tubes were centrifuged at 300g for 10 minutes. to pellet the cells. The supernatant was carefully removed, and the resulting PBMC pellet was resuspended in 1 mL of freezing medium consisting of freezing medium which is FSC medium supplemented with 5% dimethyl sulfoxide (DMSO). The cell suspension was then subjected to cell counting using an automated cell counter (Bio-Rad). Based on the cell count, the PBMC suspension was adjusted to a final concentration of 2 x 10^6 cells/mL using the freezing medium. Finally, the prepared PBMC suspension was cryopreserved in liquid nitrogen until further use in downstream applications, including quantitative polymerase chain reaction (qPCR)**.**

CD9 and CD81 Expression in PBMCs as a Potential Biomarker in Lymphocytic Leukemia: A Real time RT-PCR Study.

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy controls, patients with active disease, and patients in remission to establish distinct experimental cohorts. Total RNA was extracted from all samples using the Solarbio Life Science RNA extraction kit, following the manufacturer's protocol. Briefly, cells were centrifuged (200 x g, 5 minutes), lysed, and RNA purified through sequential washes with RPE buffer and two washes with WT buffer. Purified RNA was eluted and stored at -20°C until further processing. Complementary DNA (cDNA) was synthesized via reverse transcription. Quantitative PCR (qPCR) was performed using the Primer Design Precision 2x qPCR SYBR Green Master Mix on an Applied Biosystems 7900HT Fast Real-Time PCR System. Pre-designed, validated primer/probe sets (Macrogen, South Korea) targeting the GAPDH, CD9, and CD81 gene as in figure below. Amplification was carried out for 40 cycles. Relative GAPDH, CD9, and CD81 gene expression was determined using the comparative cycle threshold (Ct) method [10]. normalizing to GAPDH expression.

CD9 and CD81 Expression in PBMCs as a Potential Biomarker in Lymphocytic Leukemia: A Flow Cytometric Study.

CD9 and CD81 expression on peripheral blood mononuclear cells (PBMCs) from patients with acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and healthy controls was assessed by flow cytometry at protein level. CD9 and CD81 is predominantly cell surface proteins, making flow cytometry a suitable method for their detection. Briefly, PBMCs were harvested and washed twice with ice-cold wash buffer (phosphate-buffered saline containing 1% bovine serum albumin and 0.1% sodium azide). Washed cells were resuspended at a concentration of 0.5 x 10^6 cells per test. A 50 µL aliquot of conjugated mouse anti-CD9 and CD81 antibody (50 µg/mL) was added to each cell suspension, and the

mixture was incubated for 45 minutes on ice. Following incubation, cells were washed twice (400 x g for 5 minutes) with wash buffer. Subsequently, cell pellets were resuspended in 300 µL of wash buffer and immediately analyzed using a BD FACSCanto II flow cytometer. Ten thousand live events were acquired for each sample. Data analysis was performed using FlowJo software. Cell populations were gated based on forward scatter (FSC) and side scatter (SSC) parameters to define size and granularity, respectively.

Statistical Analyses.

GraphPad Prism 10 was utilized for all statistical analyses and graphical data visualization. Intergroup comparisons were performed using one-way ANOVA, as appropriate for the experimental design, followed by post-hoc testing to control for multiple comparisons. Data are expressed as the mean ± SEM.

3 Results and discussion

CD9: A Potential Biomarker in Lymphocytic Leukemias? Analysis of Gene and Protein Expression in ALL and CLL

This study aimed to evaluate the regulation of CD9 mRNA expression in acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) cases following treatment with different chemotherapeutic agents. The results demonstrated a significant upregulation of CD9 expression in both ALL and CLL cases, as shown in Figure 1. Notably, CD9 expression was significantly increased in ALL cases depending on the chemotherapy regimen, with the highest upregulation observed in the doxorubicin (DOXO)-treated group (P = 0.0001, ****), followed by the vincristine-only (VCR) group (P = 0.001, ***), and the vincristine and methotrexate (VCR&MTX) group (P = 0.01, **). In contrast, untreated CLL cases exhibited a modest but non-significant increase (P = 0.1, *). Furthermore, the difference between the ALL and CLL groups was not statistically significant. While the assess of regulation of CD9 at protein expression in acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) cases following treatment with different chemotherapeutic agents. The results demonstrated an overall increase in CD9 protein expression in both ALL and CLL cases, as shown in Figure 1. Notably, CD9 expression was significantly elevated in ALL cases depending on the chemotherapy regimen, with a modest but significant increase observed in the vincristine and methotrexate (VCR&MTX) group (P = 0.1, *), while the VCR-only group exhibited a non-significant change. A similar moderate increase was detected in the doxorubicin (DOXO)-treated group (P = 0.1, *). In untreated CLL cases, CD9 expression also showed a increase (P = 0.1, *). the difference between the two leukemia subtypes was not statistically significant. Furthermore, the data confirm specific binding of the CD9 antibody, the data confirm specific binding of the CD9 antibody, as demonstrated in the representative picture 1 below, comparing healthy controls with ALL and CLL cases alongside unstained controls.

The present study elucidates the dynamic regulation of CD9 at both transcriptional and protein levels in ALL and CLL following chemotherapy, revealing its potential role as a mediator of chemoresistance. The observed upregulation of CD9 mRNA in ALL patients treated with DOXO, VCR, and

VCR&MTX aligns with emerging evidence implicating tetraspanins in modulating drug resistance through interactions with the tumor microenvironment (TME) and intracellular signaling pathways [11]. Notably, DOXO induced the most pronounced CD9 gene expression, suggesting that anthracyclines—despite their DNA-damaging effects—may paradoxically activate stress-responsive pathways that enhance tetraspanin-mediated survival mechanisms [12]. This finding resonates with prior reports linking CD9 to glucocorticoid resistance in B-ALL, where MEK/ERK pathway inhibition reverses CD9-associated drug tolerance [13]. The graded response across chemotherapy regimens (DOXO > VCR > VCR&MTX) implies that CD9 induction may depend on the specific mechanism of action of each drug, with anthracyclines exerting the strongest transcriptional stimulus. At the protein level, CD9 expression exhibited a more nuanced pattern. While ALL cases showed modest but significant increases post-treatment, the lack of robust protein-level changes in VCR-only groups contrasts with the marked mRNA upregulation. It seems that, this discrepancy may reflect post-transcriptional regulation, delayed translation, or compensatory degradation mechanisms triggered by microtubule-targeting agents like VCR [14]. The moderate elevation in the VCR&MTX and DOXO groups suggests that combinatorial therapy may partially mitigate CD9-driven resistance, though this warrants further mechanistic validation. Importantly, the specificity of CD9 antibody binding confirmed by flow cytometry strengthens the reliability of these observations, ruling out non-specific staining artifacts.

In CLL, CD9 mRNA showed a non-significant trend toward upregulation in untreated patients, diverging from its established role in ALL. This discrepancy may stem from inherent biological differences between acute and chronic leukemias, such as distinct TME interactions or proliferative states. While CD9's role in CLL remains understudied, the observed baseline elevation—albeit modest—hints at potential involvement in disease progression or early resistance mechanisms, necessitating longitudinal studies to clarify its clinical relevance*[15]*. The lack of significant differences in CD9 expression between ALL and CLL groups, despite subtype-specific chemotherapy responses, underscores a conserved yet context-dependent role for tetraspanins in leukemia biology. This aligns with CD81's reported function in B-ALL chemoresistance [11], suggesting that tetraspanins collectively contribute to a protective niche by enhancing leukemia-TME crosstalk. Mechanistically, CD9 may facilitate integrin signaling or exosome-mediated communication, shielding leukemic cells from cytotoxic insults. The stronger transcriptional response to DOXO could reflect its dual capacity to induce DNA damage and oxidative stress, both of which may upregulate CD9 as part of a pro-survival feedback loop.

qPCR: This figure illustrates the changes in CD9 mRNA expression has been measured by qPCR in patients who have been detected to have ALL and CLL compared with healthy cases. Expression of CD9 in healthy cases and non-healthy cases was calculated using the 2-ΔΔCt method following estimation of the housekeeping gene GAPDH. CD9 showed change in PBMCs. The significance of

differences has been tested by one-way ANOVA, where * $p<0.1$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$ is significant, and ns is non-significant.

Flow cytometer: CD9 protein expression has been measured by Flow cytometry in patients who have been detected to have ALL and CLL compared with healthy cases. Expression of CD9 in healthy cases and non-healthy cases was calculated using the MFI method. CD9 showed change in PBMCs. The significance of differences has been tested by one-way ANOVA, where * $p<0.1$ is significant, and ns is non-significant. The data are the means of 130 samples from three separate experiments with duplicates.

Picture1: Flow Cytometry Analysis of CD9 Expression in Healthy Controls, Acute Lymphoblastic Leukemia (ALL), and Chronic Lymphocytic Leukemia (CLL) Patients. PBMCs were stained with a fluorescently-conjugated antibody specific for CD9. Unstained PBMCs were used as a negative control to determine background fluorescence. Data were acquired using a BD Flow Cytometer device and analyzed using Flow JO software. Anti- CD9 antibody (K010765M) is shifted with FITC detector as in above.

Chemotherapy-Induced CD81 Upregulation in Acute Lymphoblastic Leukemia, but Not Chronic Lymphocytic Leukemia

This study aimed to evaluate the regulation of CD81 expression in treated acute lymphoblastic leukemia (ALL) and untreated chronic lymphocytic leukemia (CLL) cases following exposure to various chemotherapeutic agents. The results indicated a significant upregulation of CD81 mRNA expression in ALL cases, as illustrated in Figure 1. Notably, CD81 expression was significantly elevated in ALL patients depending on the chemotherapy regimen, with the highest increase observed in the group treated with vincristine (VCR) and methotrexate (MTX) ($P = 0.001$, ***), followed by the VCR-only group ($P = 0.01$, **), and the doxorubicin (DOXO) group ($P = 0.1$, *). In contrast, CD81 expression in untreated CLL cases was not significantly altered. Furthermore, the difference in CD81 expression between the ALL and CLL groups was not statistically significant.

While the assess the regulation of CD81 protein expression in acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) cases following treatment with various chemotherapeutic agents. The results demonstrated an increase in CD81 protein expression in ALL cases, as shown in Figure 1. However, the upregulation of CD81 varied depending on the chemotherapy regimen. Notably, the increase in CD81 expression was not statistically significant in both the vincristine and methotrexate (VCR&MTX) group and the VCR-only group, while a modest but non-significant increase was observed in the doxorubicin (DOXO) group ($P = 0.1$, *). In contrast, CD81 expression in untreated CLL cases remained non-significant. Furthermore, no significant difference was observed between ALL and CLL cases or when compared with the control group. Importantly, the data confirm specific binding of the CD81 antibody, as demonstrated in the representative picture 2 below, comparing healthy controls with ALL and CLL cases alongside unstained controls.

The findings from this study reveal critical insights into the role of CD81, a tetraspanin protein, in mediating chemoresistance across acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). The observed upregulation of CD81 mRNA in ALL patients—particularly in those treated with VCR&MTX, VCR alone, and DOXO—suggests that chemotherapy itself may induce CD81 expression as part of a stress-adaptive response. This aligns with prior evidence implicating CD81 in fostering chemoresistance by enhancing leukemic cell interactions with the bone marrow (BM) microenvironment, thereby creating a protective niche that shields malignant cells from cytotoxic agents [12, 16]. The hierarchy of CD81 induction (VCR&MTX > VCR > DOXO) implies that combinatorial regimens or microtubule-targeting agents like vincristine may exert stronger transcriptional activation of CD81 compared to anthracyclines. This could reflect differential engagement of stress-response pathways, such as integrin signaling or mechanotransduction, which are known to regulate tetraspanin networks in response to cytoskeletal disruption caused by VCR.

At the protein level, however, CD81 expression exhibited only modest, non-significant increases in ALL patients, even in the VCR&MTX group where mRNA upregulation was most pronounced. This discordance between transcriptional and translational regulation may arise from post-transcriptional modifications, protein trafficking delays [17], or compensatory degradation mechanisms triggered by chemotherapy. For example, MTX, a folate antagonist, could impair protein synthesis by disrupting nucleotide metabolism, thereby decoupling mRNA abundance from protein expression. The moderate increase in CD81 protein in the DOXO group ($P = 0.1$) may reflect anthracycline-induced oxidative stress, which has been shown to stabilize tetraspanins by modulating post-translational modifications like palmitoylation. The specificity of CD81 antibody binding, confirmed via flow cytometry, underscores the validity of these observations and rules out technical artifacts.

In untreated CLL cases, CD81 expression remained unchanged at both mRNA and protein levels, contrasting sharply with its dynamic regulation in ALL. This divergence highlights fundamental differences in the pathobiology of acute versus chronic leukemias. While ALL is characterized by rapid proliferation and intense chemotherapy-driven selective pressure, CLL typically exhibits indolent growth and may rely on distinct survival mechanisms, such as B-cell receptor signaling or stromal interactions, which may not involve CD81 to the same extent. The lack of CD81 upregulation in CLL aligns with the paucity of data on its prognostic or therapeutic relevance in this disease [18] . underscoring the need for dedicated studies to clarify its role in CLL progression or therapy resistance.

qPCR: This figure illustrates the changes in CD81 mRNA expression has been measured by qPCR in patients who have been detected to have ALL and CLL compared with healthy cases. Expression of CD81 in healthy cases and non-healthy cases was calculated using the $2^{-\Delta\Delta Ct}$ method following estimation of the housekeeping gene GAPDH. CD81 showed change in PBMCs. The significance of

differences has been tested by one-way ANOVA, where * $p<0.1$, ** $p<0.01$, and *** $p<0.001$ is significant, and ns is non-significant.

Flow cytometer: CD81 protein expression has been measured by Flow cytometry in patients who have been detected to have ALL and CLL compared with healthy cases. Expression of CD81 in healthy cases and non-healthy cases was calculated using the MFI method. CD81 showed change in PBMCs. The significance of differences has been tested by one-way ANOVA, where * $p<0.1$ is significant, and ns is non-significant. The data are the means of 130 samples from three separate experiments with duplicates.

Picture2: Flow Cytometry Analysis of CD81 Expression in Healthy Controls, Acute Lymphoblastic Leukemia (ALL), and Chronic Lymphocytic Leukemia (CLL) Patients. PBMCs were stained with a fluorescently-conjugated antibody specific for CD81. Unstained PBMCs were used as a negative control to determine background fluorescence. Data were acquired using a BD Flow Cytometer device and analyzed using Flow JO software. Anti- CD81 antibody (K010630M) is shifted with APC detector as in above.

4 Conclusion

The coordinated upregulation of CD9 and CD81 in ALL under chemotherapy highlights their dual roles as mediators of chemoresistance, with CD9 predominantly associated with glucocorticoid resistance and CD81 with microenvironment-driven survival. While CD9 exhibits robust transcriptional and moderate protein-level induction across regimens (notably DOXO), CD81 shows strong mRNA upregulation with VCR-based therapies but limited protein translation, suggesting divergent post-transcriptional regulation. Both tetraspanins demonstrate leukemia subtype specificity, with pronounced activity in ALL and minimal involvement in untreated CLL. Targeting these proteins, particularly in combinatorial regimens, may disrupt chemoprotective niches and reverse therapy resistance, offering a strategic avenue to improve outcomes in high-risk leukemia. **1**

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Declaration

The authors have no discord of interest to declare, and the financial support is University of Kufa.

Ethics Clearance

This research was approved to use human samples from patients and approved to contain these samples by MOH committee for ethics in Iraq with legal paper 1035, 24 may of 2024.

ТАБЛИЦЫ

Table 1. Distribution of ALL and CLL Patients by Age and Gender.

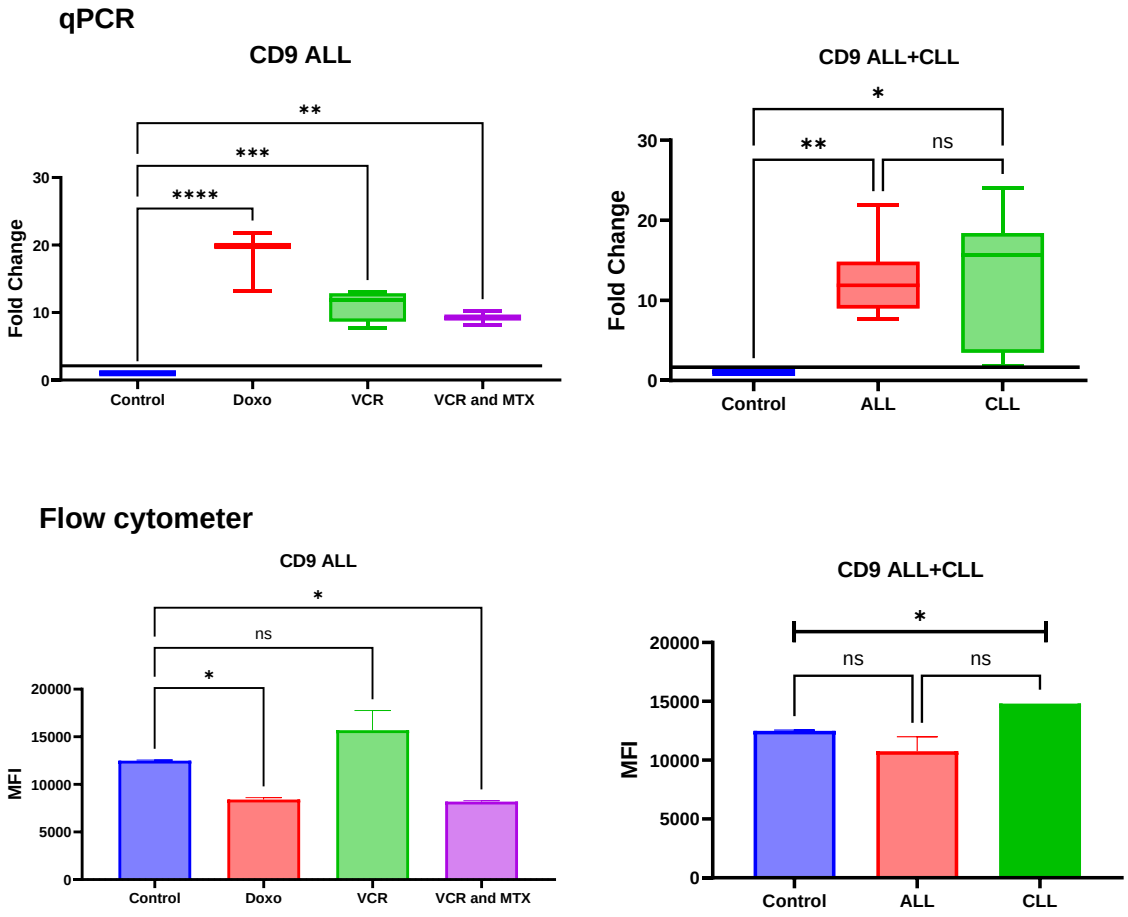
Age	Patients No ALL	Patients (%) ALL	Patient No CLL	Patients CLL (%)
≤ 15	46	92%	0	0%
>15	4	8%	100	100%
Gender	Patients No ALL	Patients ALL (%)	Patients No CLL	Patients CLL (%)
Female	26	52%	16	(34%)
Male	24	48%	34	(66%)

Table 2. List of Human Primers and PCR Product Sizes.

Name	Human primers	PCR product
CD9 F	GCAGCCTTGCTAGACCATTC	169
CD9 R	CCGATCAGAATATAGACTCCTCC	169
CD81 F	CTTCCACGAGACGCTTGACT	125
CD81 R	GGTGGCAGTCCTTGAAGAGG	125
GAPDH F	GTCGGAGTCAACGGATTTGG	165
GAPDH R	GACGGTGCCATGGAATTTGC	165

РИСУНКИ

Figure 1. The changes in the CD9 at protein and gene levels in response to ALL and CLL at different types of chemotherapies.



qPCR: This figure illustrates the changes in CD9 mRNA expression has been measured by qPCR in patients who have been detected to have ALL and CLL compared with healthy cases. Expression of CD9 in healthy cases and non-healthy cases was calculated using the 2-ΔΔCt method following estimation of the housekeeping gene GAPDH. CD9 showed change in PBMCs. The significance of differences has been tested by one-way ANOVA, where * $p<0.1$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$ is significant, and ns is non-significant.

Flow cytometer: CD9 protein expression has been measured by Flow cytometry in patients who have been detected to have ALL and CLL compared with healthy cases. Expression of CD9 in healthy cases and non-healthy cases was calculated using the MFI method. CD9 showed change in PBMCs. The significance of differences has been tested by one-way ANOVA, where * $p<0.1$ is significant, and ns is non-significant. The data are the means of 130 samples from three separate experiments with duplicates.

Figure 2. Flow Cytometry Analysis of CD9 Expression in Healthy Controls, Acute Lymphoblastic Leukemia (ALL), and Chronic Lymphocytic Leukemia (CLL) Patients. PBMCs were stained with a fluorescently-conjugated antibody specific for CD9. Unstained PBMCs were used as a negative control to determine background fluorescence. Data were acquired using a BD Flow Cytometer device and analyzed using Flow JO software. Anti- CD9 antibody (K010765M) is shifted with FITC detector as in above.

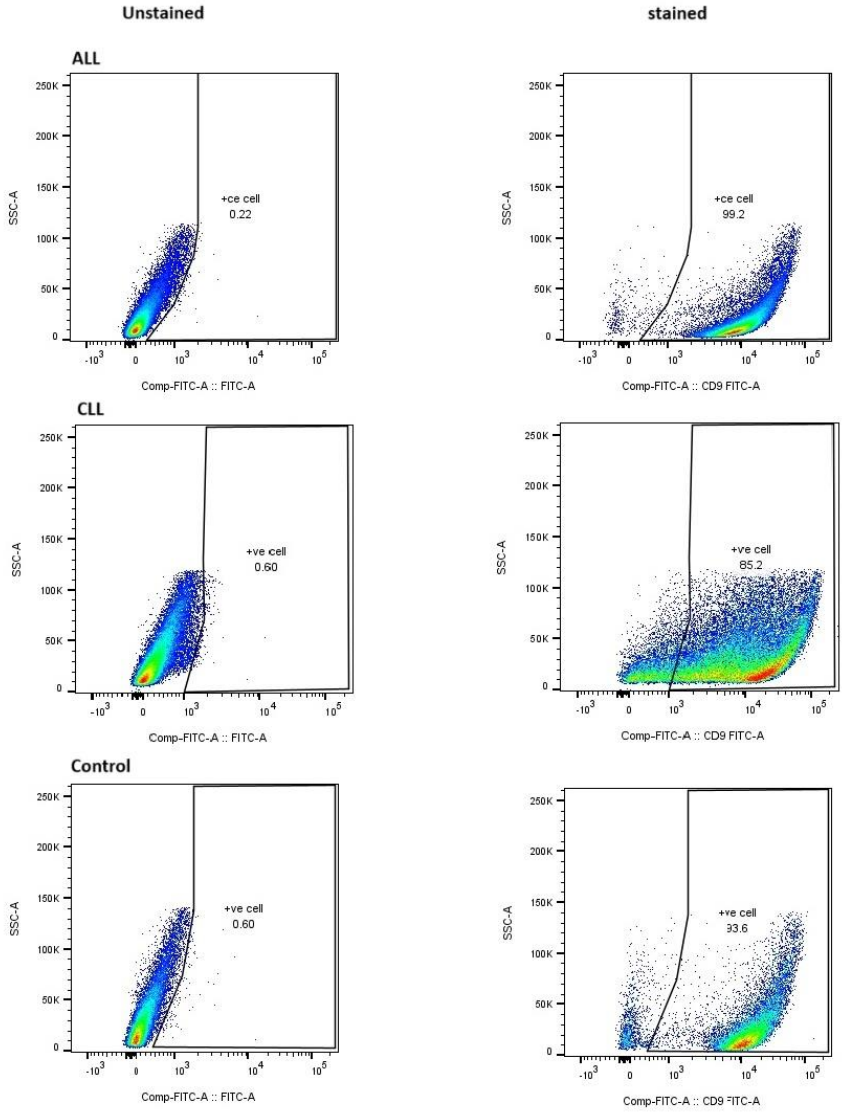
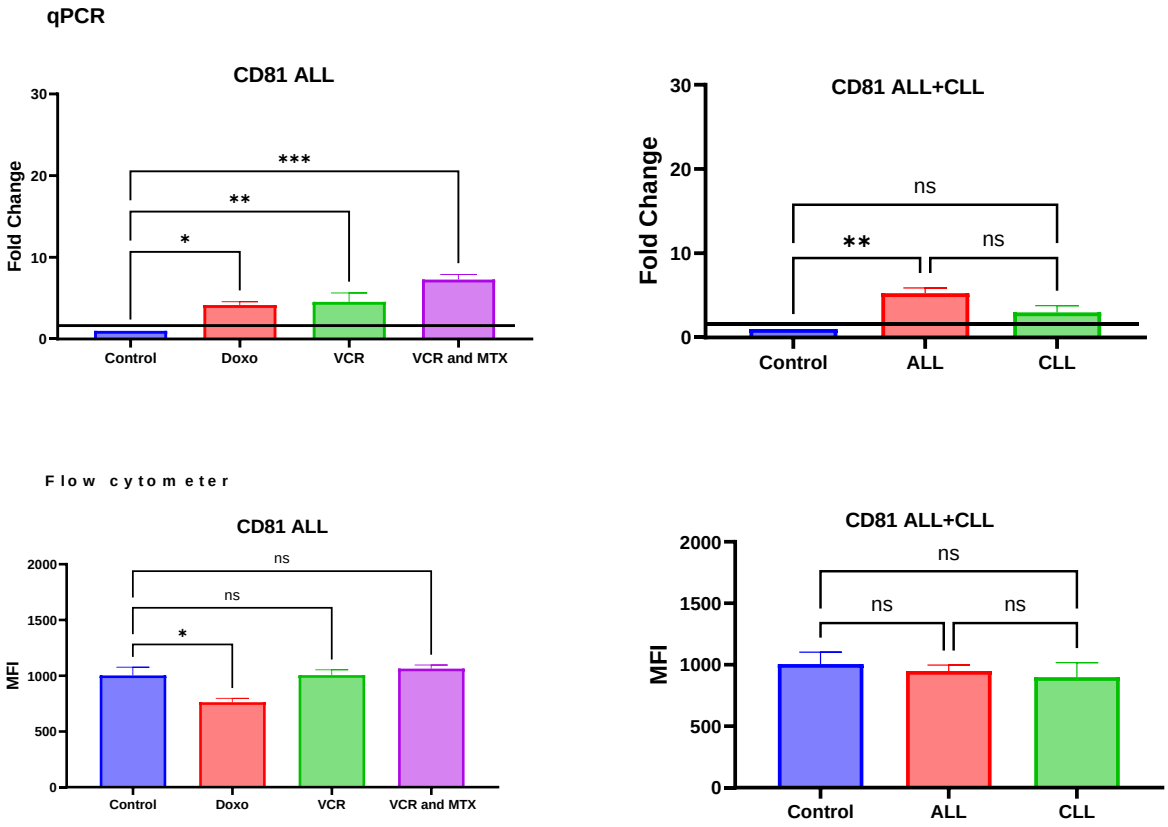


Figure 3. The changes in the CD81 at protein and gene levels in response to ALL and CLL at different types of chemotherapies.



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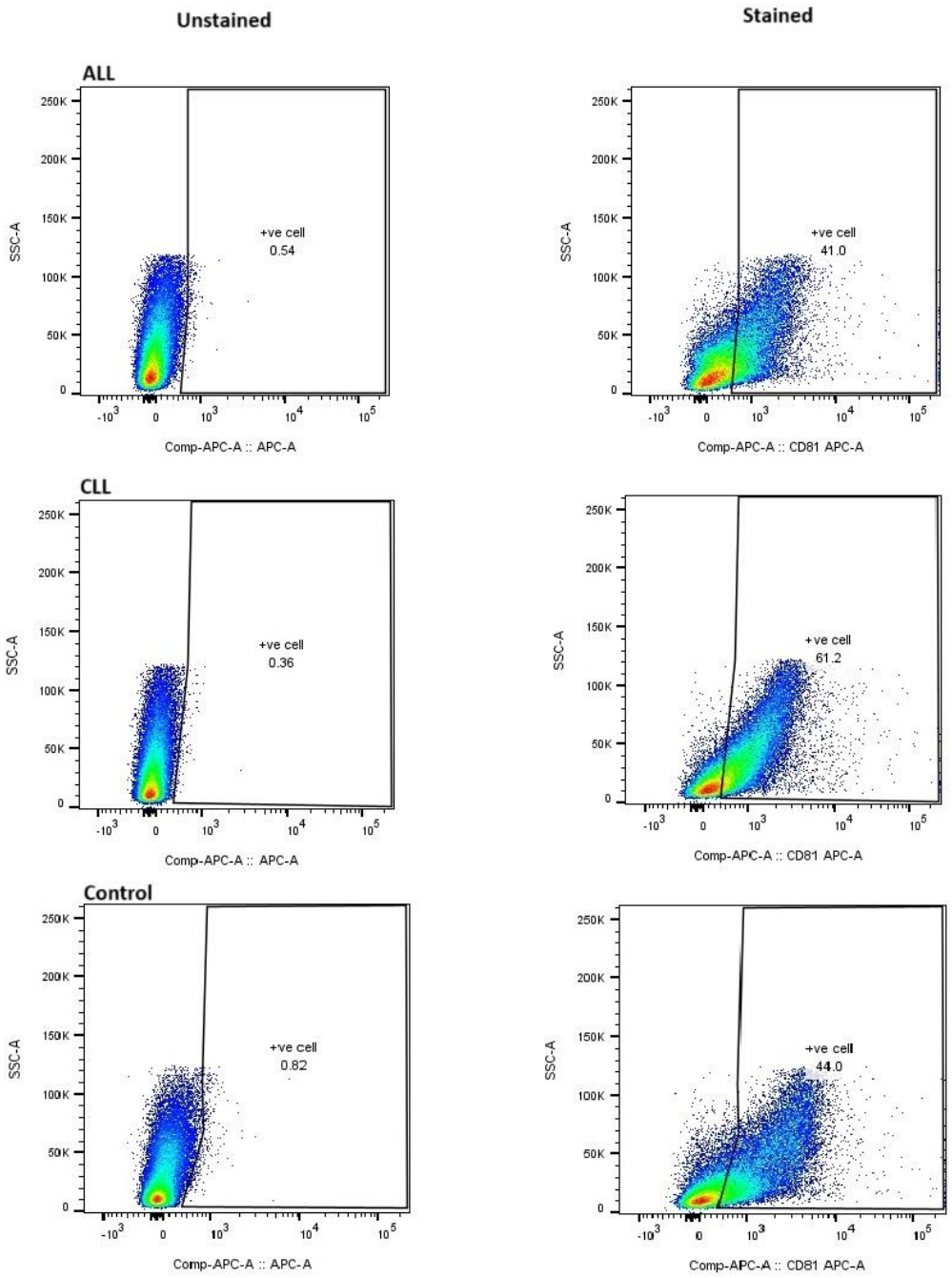
Flow cytometer: CD81 protein expression has been measured by Flow cytometry in patients who have been detected to have ALL and CLL compared with healthy cases. Expression of CD81 in healthy cases and non-healthy cases was calculated using the MFI method. CD81 showed change in PBMCs. The significance of differences has been tested by one-way ANOVA, where * $p<0.1$ is significant, and ns is non-significant. The data are the means of 130 samples from three separate experiments with duplicates.

Figure 4. Flow Cytometry Analysis of CD81 Expression in Healthy Controls, Acute Lymphoblastic Leukemia (ALL), and Chronic Lymphocytic Leukemia (CLL) Patients. PBMCs were stained with a fluorescently-conjugated antibody specific for CD81. Unstained PBMCs were used as a negative control to determine background fluorescence. Data were acquired using a BD Flow Cytometer device and analyzed using Flow JO software. Anti- CD81 antibody (K010630M) is shifted with APC detector as in above.



ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

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Блок 3. Метаданные статьи
TETRASPANIN CD9 AND CD81: DIFFERENTIAL REGULATION AND POTENTIAL ROLES IN CHEMORESISTANCE IN ACUTE AND CHRONIC LYMPHOCYTIC LEUKEMIA

Сокращенное название статьи для верхнего колонтитула:
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СПИСОК ЛИТЕРАТУРЫ

Reference Sequence Number	Authors, Title of a Publication and Source Where It Was Published, Publisher's Imprint	Full Name, Title of a Publication and Source in English	Reference's URL
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