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CD150 AND CD180 ARE INVOLVED IN REGULATION OF TRANSCRIPTION FACTORS EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: **Sequential stages of B-cell development is stringently coordinated by transcription factors (TFs) network that include B-lineage commitment TFs (Ikaros, Runx1/Cbfb, E2A, and FOXO1), B-lineage maintenance TFs (EBF1 and PAX5) and stage specific set of TFs (IRF4, IRF8, BCL6, BLIMP1). Deregulation of TFs expression and activity is often occurs in malignant B cells.** ***The aim*** **of this study was to evaluate TFs expression in chronic lymphocytic leukemia cells taking into consideration CD150 cell surface expression. From other side we attempted to regulate TFs expression** ***via*** **CD150 and CD180 cell surface receptors.** ***Materials and Methods:*** **Studies were performed on normal peripheral blood B-cell subpopulations and chronic lymphocytic leukemia (CLL) cells isolated from peripheral blood of 67 primary untreated patients with CLL. Evaluation of TFs expression was performed on mRNA level using qRT-PCR and on protein level by western blot analysis.** ***Results:*** **Median of PAX5 and EBF1 mRNA expression was higher in cell surface CD150 positive (csCD150$^+$) compared to csCD150$^-$ CLL cases or normal CD19$^+$ and CD19$^+$CD5$^+$ B-cell subsets. Differences in mRNA expression of IRF8, IRF4 and BLIMP1 between studied groups of CLL and normal B cells were not revealed. All CLL cases were characterized by downregulated expression of PU.1 and BCL6 mRNAs in comparison to normal B cells. At the same time elevated SPIB mRNA expression level was restricted to CLL cells. Protein expression of IRF4, IRF8 and BCL6 was uniformly distributed between csCD150$^-$ and csCD150$^+$ CLL cases. PU.1 protein and CD20 that is direct PU.1 target gene positively correlated with CD150 cell surface expression on CLL cells. Ligation of CD150 and CD180 alone or in combination upregulated IRF8 and PU.1 while downregulated the IRF4 mRNA expression. Signaling** ***via*** **CD150 or CD180 alone elevated the level of BCL6 mRNA. Strong downregulation of IRF4 mRNA was observed after CD150, CD180 or CD150 and CD180 coligation on CLL cells. We found that in CLL cells CD150 is a negative regulator of SPIB while CD180 is involved in upregulation of EBF1 expression level. Moreover, CD180 ligation on CLL cells caused increase of CD150 mRNA level that is a one of the EBF1 target genes.** ***Conclusions:*** **Analysis of TFs expression profile revealed upregulated SPIB mRNA level and downregulated PU.1 in CLL cells. CD150 and CD180 receptors may modulate transcriptional program in CLL cells by regulating the TFs expression levels.**

Key Words: **transcription factors, chronic lymphocytic leukemia cells, CD150, CD180.**

Multistep B-cell development is stringently coordinated by numerous input signals from the cell surface receptors that lead to activation of unique transcriptional programs [1]. Exclusive gene expression profile is determined by interplay of specific transcriptional factors (TFs) at the every stage of B-cell development started from B-lineage diversification until terminally differentiated plasma cells [2, 3]. Cooperation of E2A, EBF1, PAX5 and LEF1 TFs moves common lymphoid progenitors to pro-B cell; tandem of IRF4 and IRF8 regulates pre-B cell stage; phenotype of transitional B cells is maintained by OBF1 and NF-κB; expression of BCL6, PAX5 and MITF is essential for germinal centre development and finally plasma cell differentiation is regulated by IRF4, BLIMP1, and XBP1 [2–4]. TFs often create mutual suppression loops that regulate alternative differentiation of B-cell subsets [2]. The block of malignant B cell maturation, as a rule, occurs at different stages of differentiation that bear phenotypic characteristic including TFs profile of corresponding normal B-cell counterparts [1]. However, TFs expression and functions are often dysregulated in B-cell derived leukaemia and lymphoma. For example, in approximately 30% of diffuse large B-cell lymphoma (DLBCL) and 15% of follicular lymphoma (FL) genetic alteration of *BCL6* gene leads to BCL6 constitutive expression and activation [4]. Overexpression of IRF4 was observed in multiple myeloma and activated B-cell like (ABC) DLBCL [5, 6]. However, it was shown that cooperation of PU.1 with IRF4 and IRF8 prevents pre-B acute lymphoblastic leukemia development [7]. Thus, IRF4 may act both as oncogene and tumor suppressor [8]. The block of terminal differentiation of malignant B cells in ABC DLBCL is linked to downregulation of BLIMP1 expression [1]. That is why finding new approaches that could modify stability, expression and activity of dysregulated TFs is a perspective direction for optimising treatment of B-cell malignancies.

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Abbreviations used: CLL – chronic lymphocytic leukemia; DLBCL – diffuse large B-cell lymphoma; FL – follicular lymphoma; HL – Hodgkin's lymphoma; PBMCs – peripheral blood mononuclear cells; TFs – transcription factors.

The present study is focused on evaluation of expression levels of key B-cell regulatory TFs in chronic lymphocytic leukemia (CLL) cells compared to their normal counterparts. Previously, we showed that cell surface receptors CD150 and CD180 are involved in activation of pro-survival Akt and MAPK signaling pathways in CLL cells. However, stimulation of both receptors on CLL cells led to strong inhibition of Akt-mTOR, p38MAPK and JNK pathways [9]. Since, many of TFs are downstream targets of Akt and MAPK pathways we also tested a hypothesis whether it is possible to regulate TFs expression *via* CD150 and CD180 cell surface receptors in CLL cells.

MATERIALS AND METHODS

Isolation of normal B-cell subsets and malignant CLL cells. Population of $CD19^+$ B cells and $CD19^+CD5^+$ B-cell subpopulation were enriched from T-cell depleted peripheral blood mononuclear cells (PBMCs) of healthy individuals by magnetic separation using Dynabeads coated with sheep anti-mouse IgG (Dynal Biotech ASA, Norway) according to manufacturer's protocols. Mouse anti-CD5 (10.2) and anti-CD19 (HD37) mAbs were kindly provided by Prof. Edward A. Clark (University of Washington, Seattle, WA, USA). Peripheral blood of four healthy individuals was obtained from the Blood Transfusion Centre (Kyiv, Ukraine). PBMCs of previously untreated 67 CLL patients were obtained from the Department of Oncohematology of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine (IEPOR NASU), Kyiv, Ukraine. All patients were thoroughly informed about the study that was approved by the Committee on Bioethics at IEPOR.

Flow cytometry. PBMCs of all CLL patients were immunophenotyped on the subject of CD150 and CD180 expression as well as common B cell markers (CD19, CD20, CD22, CD37, CD38, CD40, CD48, CD95) and CLL marker (CD5, CD23, CD43). Detailed description of immunophenotyping procedure and immunophenotyping results were published earlier [9]. PBMCs from CLL patients were used for *in vitro* stimulation assay only when the level of $CD19^+$ cells was more than 95% and these cells coexpressed CD150 and CD180 receptors.

In vitro cell stimulation. To initiate signaling *via* CD150 and CD180 receptors $10 \cdot 10^6$ of $CD150^+CD180^+$ PBMCs from CLL patients were incubated with anti-CD150 (IPO3, IEPOR NASU) or/and anti-CD180 (G28-8, kindly provided by Prof. Edward Clark, University of Washington, Seattle, WA, USA) mAbs both at final concentration 10 µg/ml in RPMI-1640 medium supplemented with 10% FCS. In experiments with CD150 mRNA upregulation $CD150^-CD180^+$ PBMCs from CLL patients were used. PBMCs cultivated in medium alone were used as a negative control. Stimulation was stopped after 4 h of incubation with ice cold PBS + 0.01% NaN_3.

Western blot analysis. Cell lysis, gel electrophoresis, western blot analysis were performed as described early [10]. The list of followed antibodies was used for western blot analysis: goat anti-IRF4, anti-IRF8, anti-Actin, anti-BLIMP1, rabbit anti-BCL6 (all from Santa Cruz Biotechnology, USA), rabbit anti-PU.1 (Cell Signaling Technology, Beverly, MA, USA). Secondary goat anti-rabbit and donkey anti-goat HRP-conjugated antibodies were from Santa Cruz Biotechnology (USA). Clarity Western ECL substrate (Immuno-Star HRP, BioRad, USA) and medical X-ray film (AGFA, Belgium) were used for visualization.

Quantitative RT-PCR. A detailed description of total RNA isolation, cDNA synthesis and real-time PCR were reported elsewhere [10]. The following forward (For) and reverse (Rev) primers for real-time PCR were used: IRF4: For 5′-CCACTACCTCCTTTCCTATC-3′, Rev 5′-CCGTTCCTTTTCAGAGTCCT-3′; IRF8: For 5′-CCAACAGATCACCGTCTAA-3′, Rev 5′-AAGTG-CAAAGTAAGGCATC-3′; PU.1: For 5′-CTTCCAGTTCTC-GTCCAA-3′, Rev 5′-GAGCTTCTTCTTCACCTTC-3′; SPIB: For 5′-GCATACCCCACGGAGAACT-3′; Rev 5′-GGCTGTCCAACGGTAAGTCT-3′, BCL6: For 5′-CTC-CGTGCCCATGTGCTTA-3′; Rev 5′-GAGTCTGAAGGT-GCCGGAAA-3′, EBF1: For 5′-GTACCATGCTGGTCTG-GAGTG-3′; Rev 5′-GTGTGACTTCCACAACACCAGG-3′, PAX5: For 5′-GTCCCAGCTTCCAGTCACAG-3′; Rev 5′-CGGAGACTCCTGAATACCTTCG-3′. As internal control gene for normalization was chosen TATA-box binding protein (TBP): For 5′-CCACTCACAGACTCT-CACAAC-3′; Rev 5′-CTGCGGTACAATCCCAGAACT-3′. The PCR cycling conditions were the following: 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 40 s at 60 °C. The threshold cycle (Ct) values were determined for the internal control (TBP) and the tested genes at the same threshold level in the exponential phase of the PCR amplification curves. The Ct method was used to calculate test genes expression level normalized to the endogenous control (in case of TFs expression in CLL cells and normal B cells subsets). Experiments with receptors ligation were additionally normalized to untreated control cells. Results were presented in relative units or fold change. Dissociation curve analysis was performed after every run to check the specificity of the reactions. For all types of cell samples 3–5 reactions (each in triplicates) were run for each genes with internal control and standard error of the mean (SEM) was calculated.

Statistical analysis. Obtained data were analysed by unpaired Mann — Whitney U test using Prism software Version 4.0. Statistical significance between groups was assessed as $p \leqslant 0.05$. Pearson's coefficient was used for determination of correlation between variables. Box plots showed the results of TFs mRNA expression level where whiskers means maximum and minimum values, the line within the rectangle shows the median, and the top and bottom of the rectangle represent the third and first quartile, respectively.

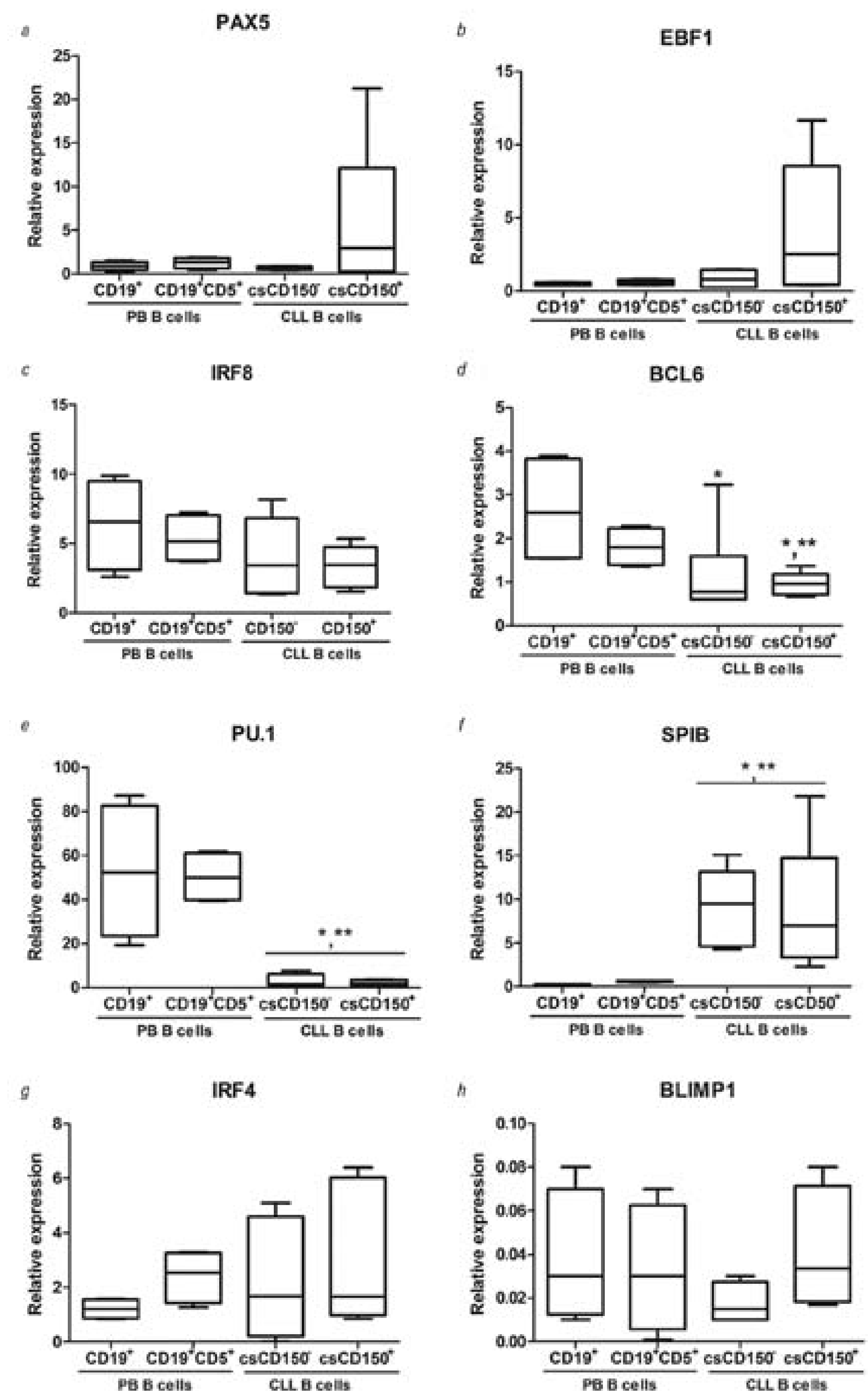

Fig. 1. TFs mRNA expression levels in CLL cells (qRT-PCR). The $csCD150^-$ CLL cells (n = 6) and $csCD150^+$ CLL cells (n = 6) were compared to $CD19^+$ and $CD19^+CD5^+$ PB B cells (4 donors). $^*p \leqslant 0.05$ compared to $CD19^+$ normal B cells. $^{**}p \leqslant 0.05$ compared to $CD19^+CD5^+$ normal B cells

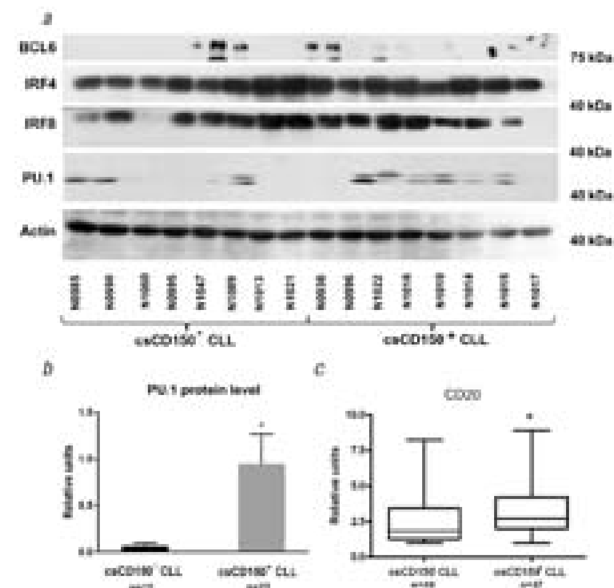

Fig. 2. TFs protein expression in CLL cells: *a* — protein expression of BCL6, IRF4, IRF8, and PU.1 in csCD150⁻ and csCD150⁺ CLL cases. Results of western blot analysis; *b* — densitometry analysis of PU.1 expression level in csCD150⁻ and csCD150⁺ CLL cases. Results were normalized to actin expression and presented in relative units; *c* — cell surface CD20 expression level in csCD150⁻ and csCD150⁺ CLL cases. Flow cytometry analyses. Results are presented as GeoMean MFI ratio of antigen to isotype control. $^*p = 0.05$ compared to csCD150⁻ CLL cases

RESULTS AND DISSCUTION

CLL cells is clonal malignant B cells that according to gene expression signature is closely related to CD5⁺ peripheral blood B-cell subpopulation [11]. That is why peripheral blood CD19⁺ B cells and CD19⁺CD5⁺ B cells obtained from healthy individuals were used in our study for comprehensive analysis of TFs profile expression between CLL cells and normal B cell counterparts. The present study is focused on the TFs that maintain B-cell identity (EBF1 and PAX5) and regulate differentiation from naïve B cell stage toward plasma cells (IRF4, IRF8, PU.1, SPIB, BCL6, and BLIMP1). CLL cases were grouped according to the cell surface CD150 expression. We tested the hypothesis that cell surface CD150 positive (csCD150⁺) and CD150 negative (csCD150⁻) CLL cells may stop their maturation at the different stages of B-cell development and therefore could have diverse TFs expression profiles.

mRNA expression level of B-cell identity markers PAX5 and EBF1 was similar in studied normal B cell subsets and csCD150⁻ CLL cases (Fig. 1, *a, b*). However, median of PAX5/EBF1 expression was higher in csCD150⁺ than in csCD150⁻ CLL cases. CLL cells showed the similar mRNA expression levels of IRF8 and BCL6 regardless of csCD150 expression (Fig. 1, *c, d*). BCL6 mRNA expression was higher in normal B cells that in CLL cells ($p \leqslant 0.03$) (Fig. 1, *d*). All tested CLL cases were characterised by lower level of PU.1 mRNA compared to normal B-cell subpopulations ($p \leqslant 0.02$) (Fig. 1, *e*). On the other hand, the level of SPIB mRNA was significantly higher in csCD150⁻ as well as csCD150⁺ CLL cells than in normal B cell subsets (Fig. 1, *f*). Expression level of IRF4 was similar in CLL and normal B cells (Fig. 1, *g*). mRNA expression level of BLIMP1 — master regulator of plasma cell development, was low in all tested normal B cell subsets and CLL cells (Fig. 1, *h*). According to obtained results CLL cells has similar profile in mRNA expression of IRF4, IRF8, EBF1, and PAX5 TFs to normal peripheral blood B-cell subpopulations. Unique feature of malignant CLL cells is upregulated mRNA expression of SPIB and downregulated PU.1 and BCL6 TFs.

To clarify whether csCD150⁻ and csCD150⁺ CLL cells have any differences in TFs expression on protein level we used western blot analysis. High expression levels of IRF4 and IRF8 proteins were detected in all CLL cases regardless of csCD150 expression (Fig. 2, *a*). All CLL cases were negative for BLIMP1 protein expression (data not shown). BCL6 protein was revealed only in 31% of CLL cases despite the presence of BCL6 mRNA in all studied CLL cases. Moreover, BCL6 expression did not correlate with cell surface CD150 expression. At the same time, PU.1 protein was predominantly expressed in csCD150⁺ CLL cases regardless of the uniform expression of PU.1 mRNA in CLL (see Fig. 2, *a*, Fig. 1, *e*). Furthermore, PU.1 protein level positively correlated with cell surface CD150 expression ($r = 0.4$, $p < 0.05$). The level of PU.1 protein was 14 times higher in csCD150⁺ than in csCD150⁻ CLL cases ($p = 0.002$) (Fig. 2, *b*). It was shown that CD20 receptor is one of the PU.1 target genes [12]. We checked whether any correlations exist between cell surface CD150 and CD20 expression in CLL cases. Indeed we found positive correlation between CD150 and CD20 cell surface expression in CLL cases ($r = 0.45$, $p \leqslant 0.05$). Median of CD20 cell surface expression was higher in csCD150⁺ CLL cases (Fig. 2, *c*) ($p = 0.01$). Thus, upregulated levels of PU.1 protein expression and its target CD20 were correlated with cell surface CD150 expression in CLL cells.

Numerous data suggest that CD150 is involved in activation of Akt and MAPK signaling in normal and malignant B cells [9, 13–15]. In case of CLL, CD150 could realize its signaling properties alone or together with CD180 receptor [9]. Since many of downstream targets of Akt and MAPK pathways are TFs, we test the hypothesis whether CD150 and/or CD180 are involved in regulation of TFs expression in CLL cells. To check this we performed *in vitro* ligation of CD150 and CD180 alone or simultaneously on CLL cells with followed evaluation of TFs mRNA levels. These receptors were not involved in B-lineage maintenance TF PAX5 expression level regulation (Fig. 3, *a*). Only CD180 ligation on CLL cells leaded to upregulation of EBF1 mRNA level and CD150 receptor did not abrogate this effect of EBF1 (Fig. 3, *b*). Expression level of IRF8 mRNA was significantly upregulated in CLL cells after ligation of any if these receptors alone or in combination (Fig. 3, *c*). CD150 or CD180 alone, but not CD150 and CD180 ligation upregulated BCL6 expression in CLL cells (Fig. 3, *d*). In opposite, crosslinking of CD150 or CD180 alone and simultaneous ligation of these receptors on CLL cells resulted in strong downregulation of IRF4 mRNA (Fig. 3, *g*). Coligation of CD150 and CD180 did not have any additive effect on IRF4 mRNA downregulation. PU.1 and SPIB as the partners could act in cooperation with IRF4 and IRF8 TFs [16]. Stimulation of CLL cells *via* CD150 or CD180 receptors alone or their combination resulted in increased PU.1 mRNA level in CLL cells with additive effect of CD150 and CD180 coligation (Fig. 3, *e*). At the same time, CD150 ligation led to downregulation of SPIB mRNA level in malignant B cells, while CD180 and CD150 + CD180 ligation did not have any impact on regulation of SPIB expression level (Fig. 3, *f*). Taken together, CD150 and CD180 receptors are involved in regulation of TFs mRNA expression levels in CLL cells. CD150 and CD180 receptors alone or their combination had unidirectional effect on downregulation of IRF4, up-

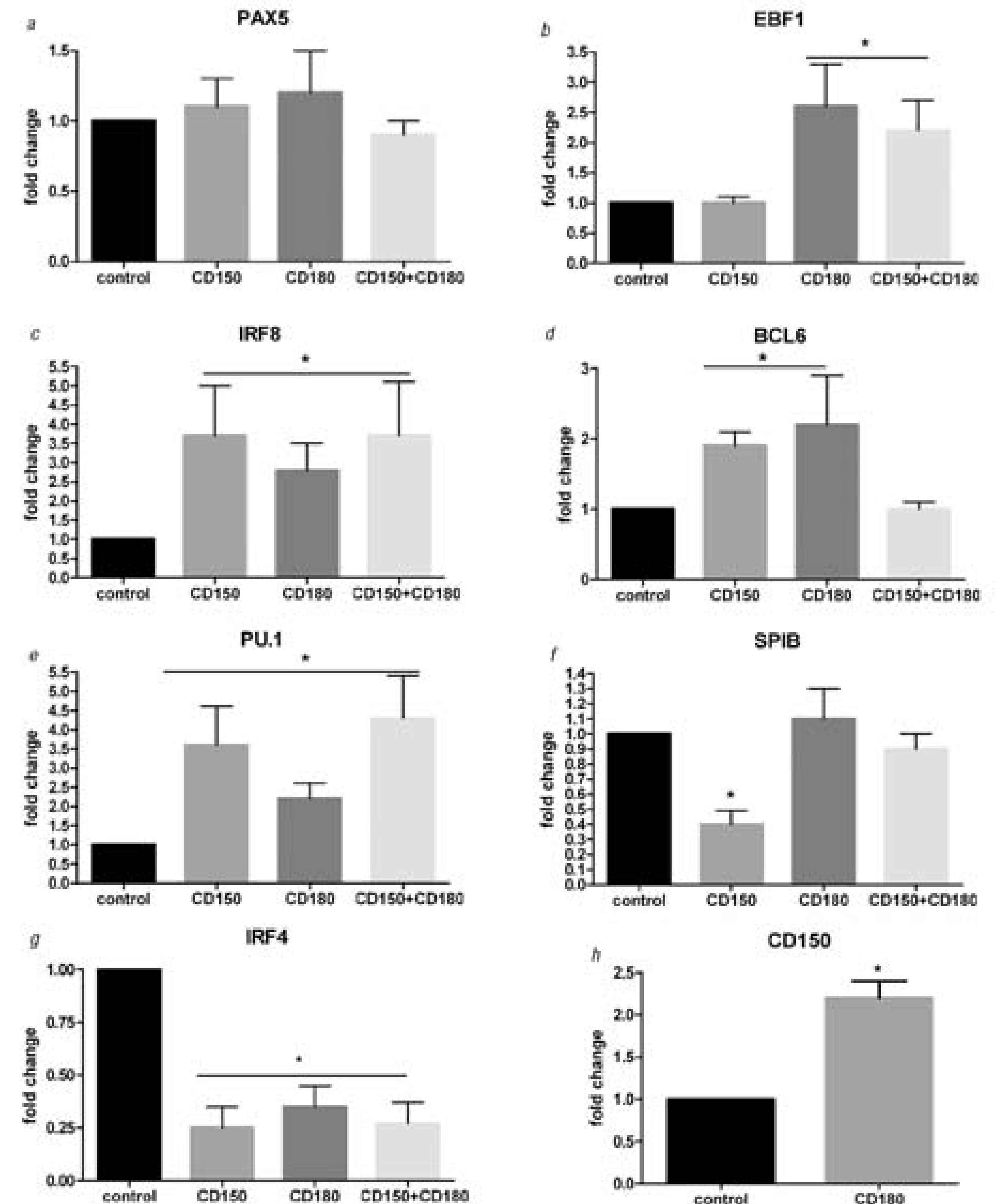

Fig. 3. CD150 and CD180 mediated regulation of PAX5 (*a*), EBF1 (*b*), IRF8 (*c*), BCL6 (*d*), PU.1 (*e*), SPIB (*f*), and IRF4 (*g*) mRNA expression. Results are presented as a mean of mRNA expression in three independent CD150⁺CD180⁺ CLL cases. (*h*) CD150 mRNA expression level in csCD150⁻ CLL cells after CD180 ligation in three independent CLL cases. $^*p \leqslant 0.05$ compared to control (CLL cells cultivated in medium alone)

regulation of IRF8 and PU.1 mRNA in CLL cells. Also, signaling *via* CD150 as well as CD180 increase the level of BCL6 mRNA expression. In addition, CD150 receptor is negative regulator of SPIB mRNA expression while CD180 is a positive regulator of EBF1 mRNA in CLL cells.

CLL is a disease of $CD5^+CD19^+CD23^+$ B cells, which are accumulated in peripheral blood, bone marrow and second lymphoid organs, resulting from disbalance between cell proliferation and apoptotic death [17]. Cellular origin of CLL cells is still controversial. Existence of two CLL subtypes with presence or absence of somatic hypermutations in a variable region of heavy chain immunoglobulin genes (*IGHV*) indicates that malignant B cells in near half of CLL cases derived from germinal center (GC)-experienced B cells, while other half of CLL cases stoped their differentiation at the stage of naïve B cells [11]. Based on the latest data $CD5^+CD27^-$ peripheral blood B cells are the most adequate normal B cell analogue for CLL cells with unmutated *IGHV*. Rare subpopulation of $CD5^+CD27^+$ memory B cells could give rise for CLL cells that carry mutated *IGHV* [11]. It was revealed earlier that expression levels of lymphoid enhancer-binding factor 1 (LEF1), receptor tyrosine kinase-like orphan receptor 1 (ROR1), fibromodulin (FMOD), T-cell leukemia/lymphoma 1 (TCL1), Ataxin (ATXN1), early B-cell factor 1 (EBF1) and p27 are significantly different in CLL cells compared to normal B-cell analogues [18]. In our study, in addition to evaluation of B-cell stage specific TFs mRNA expression, we revealed deregulation in expression of E26-transformation specific (ETS) TFs PU.1 and SPIB in CLL cells. We found upregulated mRNA expression level of SPIB and downregulated PU.1 in CLL cells. Abundantly expressed SPIB mRNA in CLL B cells was also showed by Stratowa et al. [19]. SPIB is expressed in normal B cells starting from pre-B cells and is upregulated in GC B cells [20]. Similarly to PU.1, it is involved in BCR mediated signaling, GC formation and inhibits plasma cell differentiation [21, 22]. However, in contrast to *PU.1* knockdown, deletion of *SPIB* gene did not lead to defects in B-cell development that fully abrogates B cell formation [23, 24]. Both PU.1 and SPIB could form a heterodimers with interferon regulatory factors — IRF4 and IRF8 [8, 16]. Nevertheless, SPIB is preferential partner for IRF4 [24]. SPIB is a subject for translocation and amplification in ABC DLBCL, where together with IRF4 it promotes malignant B cells survival *via* activation of NF-κB signaling and inhibiting of INFβ expression [6]. Highly expressed IRF4 in all examined CLL cases more likely interacts with SPIB rather than with PU.1 binding partner because expression level of PU.1 is low and heterogeneous in CLL cases despite the uniform high expression of IRF4 and SPIB (Fig. 1, *f, g*; Fig. 2, *a*). Propagation of CLL cells similarly to ABC DLBCL B cells is mostly dependent on BCR tonic signaling that is main activator of IRF4 expression [25]. So, IRF4/SPIB-mediated pro-survival program may also be realised in CLL cells. Here we showed that CD150 together with CD180 are negative regulators of IRF4 expression. However, only CD150 is involved in downregulation of SPIB (Fig. 3, *f*). So, CD150-mediated downregulation of IRF4 and SPIB expression could diminished their dimer formation and therefore inhibit transcription program orchestrated by IRF4/SPIB in CLL cells. The role of IRF4 in CLL pathogenesis is controversial. In the mouse model *IRF4* knockout in B1 subpopulation is sufficient for development of CLL [26, 27]. At the same time, several mutations in IRF4 DNA binding domain in primary CLL patients are associated with better prognosis [28]. IRF4 function in CLL development and maintaining could be different and depends on signaling background and expression level of binding partner. That is why, the role of IRF4, SPIB and PU.1 in CLL B-cell pathobiology needs further investigation.

TF PU.1 is essential for generation of myeloid cell lineage and B-cells development [23]. In B cells PU.1 is a positive regulator of immunoglobulin heavy and light chains, CD79, CD72, and CD20 expression [23]. Downregulated PU.1 mRNA and differential protein expression in CLL cells compared to normal B-cell analogues may be linked to decreased cell surface IgM, CD20 and CD79 expression in CLL cells. Expression of PU.1 is deregulated in numerous of B-cell malignancies, including Hodgkin's lymphomas (HL), FL, DLBCL, and multiple myeloma [29, 30]. Upregulation of PU.1 expression in HL and multiple myeloma cells lead to apoptosis of malignant B cells, moreover, higher PU.1 level is associated with better clinical outcome in FL patients, lower PU.1 level was observed in aggressive ABC DLBCL [29–32]. CD38 and ZAP-70 double negative CLL cases are characterised by higher PU.1 mRNA expression that supposed to consider PU.1 expression as surrogate prognostic marker of favourable clinical outcome [29]. All these data indicate that PU.1 is functioning as a tumor suppressor for malignant B cells. For the first time we revealed correlation between expression levels of PU.1 protein and cell surface CD150 in CLL cells. Moreover, expression level of CD20 that is a PU.1 target was also elevated in $csCD150^+$ CLL cases. Expression level and density of CD20 on CLL cells is very important, since anti-CD20 monoclonal antibodies rituximab, ofatumumab and obinutuzumab are included in first-line therapy of CLL [33]. As follows, the higher protein levels of PU.1 and CD20 in $csCD150^+$ CLL cases are additional indirect evidences for association of CD150 expression with favourable CLL outcome. Moreover, we showed that CD150 alone or together with CD180 led to strong upregulation of PU.1 mRNA expression level in CLL cells (Fig. 3, *e*). In multiple myeloma cells PU.1 directly binds to IRF4 promoter and repress it activity [30]. So, in CLL cells PU.1 upregulation after CD150 or/and CD180 ligation could be linked to decreased level of IRF4. Further exploration of CD150/CD180-PU.1-CD20 axis will reveal the perspectives of transcriptional program regulation in CLL cells *via* cell surface receptors.

Described here regulation of TFs expression *via* cell surface receptors is only one side of crosstalk between TFs and cell surface receptors. Thus, number of TFs such as EBF1, E2A, PU.1, IRF4, STAT6, and NF-κB (RELA) could bind to *SLAMF1* promoter and regulate its activity [34]. The early B-cell factor 1 (EBF1) interacts with specific binding sites both in the promoter and in the enhancer regions of the *SLAMF1* gene and is a key regulator of *SLAMF1* expression in human and mouse B cells [34, 35]. In CLL cells CD150 cell surface expression is significantly downregulated compared to normal B lymphocytes [36]. Previously it was shown that EBF1 protein expression was not detected in CLL cases both with mutated and unmutated *IGHV* [11]. In our study EBF1 mRNA level was higher in $csCD150^+$ CLL cells than in $csCD150^-$ CLL cells, but whether EBF1 mRNA is translated in these CLL cases was not addressed. Absence or low level of EBF1 expression could result in significantly decreased cell surface CD150 expression level in CLL cells compared to normal peripheral blood B cells. Our study showed that CD180 is specific positive regulator of EBF1 mRNA expression. Moreover in normal B cells CD180 is a most potent upregulator of CD150 expression [37]. That is why we tested a hypothesis whether it is possible to regulate CD150 expression *via* CD180 receptor in CLL cells. Indeed, ligation of CD180 on CLL cells led to more than two fold upregulation of CD150 mRNA expression (Fig. 3, *h*). According to results published earlier cell surface expression of CD150 and CD180 is positively correlated and these receptors coexpression is observed in 59% of CLL cases. However, 11% of CLL cases were positive only for csCD180 expression [9]. Since, cell surface CD150 expression is associated with favourable clinical outcome of CLL patients and $csCD150^+$ CLL cells are more sensitive to chemotherapeutic drugs [36] upregulation of CD150 cell surface expression have a good perspectives in optimization of CLL treatment. Signaling *via* CD180 may be one of the possible approaches to induce CD150 cell surface expression in $CD150^-$ CLL cells that will result in modulation of CLL B-cell pathobiology.

Taking together, our study showed that CLL cells are characterised by decreased PU.1 and elevated SPIB mRNA levels compared to normal B-cell counterparts. Protein expression of PU.1 and its direct target CD20 positively correlated with cell surface CD150 expression in CLL cells. Ligation of CD150 and CD180 alone or in combination upregulated IRF8 and PU.1 while downregulated of IRF4 mRNA expression. Signaling *via* CD150 or CD180 elevated the level of BCL6 mRNA. We found that in CLL cells CD150 is a negative regulator of SPIB whereas CD180 is involved in upregulation of EBF1 expression level. Moreover, CD180-mediated signaling leaded to increased CD150 mRNA level in CLL cells and could be considered as a potential positive regulator of CD150 expression level in malignant CLL cells. Thus, CD150 and CD180 receptors may modulate transcriptional program in CLL cells by regulating the TFs expression levels.

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