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ORIGINAL ARTICLE

Expression of CD13/aminopeptidase N in precursor B-cell leukemia: role in growth regulation of B cells

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Abstract Expression of cell surface CD13 in acute B-cell leukemia (ALL-B) is often viewed, as an aberrant expression of a myeloid lineage marker. Here, we attempted to study the stage specific expression of CD13 on ALL-B blasts and understand its role in leukemogenesis as pertaining to stage of B-cell ontogeny. A total of 355 cases of different hematological malignancies were diagnosed by immunophenotyping. Among 68 cases of early B-cell ALL, 22 cases with distinct immunophenotype was identified as immature B-cell ALL. Blasts from these ALL-B patients demonstrated prominent expression of CD10, CD19, CD22, but neither cytoplasmic nor surface IgM receptors. This strongly indicates leukemogenesis at an early stage of B-cell development. We also identified, the existence of a subpopulation of cells with remarkably similar phenotype in non-leukemic marrow from healthy subjects (expressing CD10, CD19, CD22, CD24, Tdt together with the co-expression of CD13). This sub-population of B cells concomitantly expressing CD13 appeared to be a highly proliferating group. By blocking their cell surface CD13 in leukemic blasts with monoclonal antibody we were able to inhibit their proliferation. We hypothesized that neoplastic transformation at this stage may be facilitated by CD13. CD13 may thus be an important target for novel molecular therapy of early stage acute B-cell leukemia.

Keywords Acute lymphoblastic leukemia · B cell · CD13 · Ectopeptidase · Proliferation · Neoplastic transformation

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Introduction

Lympho-hematopoiesis is a complex biological process governed by multiple, complex sequential changes in gene expression and external cues emanating from the lympho-hematopoietic microenvironment in the bone marrow. B-cell development is a tightly regulated process and is characterized by stage specific expression of several regulatory genes, cell surface molecules, and somatic gene rearrangements leading ultimately to the production of immunoglobulin (Ig)-secreting plasma B cells. Different phases of B-cell development are controlled by contact dependent cellular interaction, cross-talk with hematopoietic growth factors present in the microenvironment and signaling through the Pre-B (precursor B) and B-cell antigen receptors. These receptors regulate at least two key steps during B-lineage development: (i) the Pre-B to immature B cell and (ii) naïve to activated B-cell transitions [1]. Pre-B and B-cell antigen receptor-dependent signals are essential for Ig chain allelic exclusion and proliferation of pre B cells. The current models of human B-lymphocyte ontogeny and the developmental hierarchy of immunoglobulin gene rearrangement and expression of cell differentiation antigens are largely based on studies performed on leukemic B-cell precursors from B-lineage acute lymphoblastic leukemia (B-ALL) patients. B lineage ALLs supposedly originate

due to anomalies of normal development of B-cell precursors during an early phase of ontogeny, leading to maturational arrest along with leukemic transformation at discrete stages of B lineage lymphopoiesis. Dysregulation of these signals can lead to humoral immune-deficiencies, autoimmunity and hematologic malignancy [2, 3].

Cell surface molecules are known to play an important role in regulating the growth and development of hematopoietic cells. These molecules are generally cytokines and signaling receptors. Evidences have been accumulating to support the hypothesis that cellular proteases or ectopeptidases play important role in activation, proliferation and communication among the cells of the immune system [4–6]. Ectopeptidases are integral membrane proteins, oriented asymmetrically with the peptidase domain exposed to the extracellular surface and mediates a wide range of physiological and pathological functions, such as signal transduction, cell adhesion and migration. Ectopeptidases play a pivotal role in protein turnover and peptide truncation. There is an increasing body of evidence that ectopeptidases also participate in the pathology of carcinomas. Their role in cancer progression and invasion is evidenced by the ability to influence proliferation, angiogenesis, tumor cell migration, and metastatic behavior [7–10]. The four leukocyte differentiation antigens, CD10 (neprilysin), CD13 (aminopeptidase N), CD26 (dipeptidylpeptidase IV) and CD143 (angiotensin-1 converting enzyme), have already been linked in various ways to the pathology of carcinogenesis, and all four peptidases are involved in the terminal degradation of small peptides and amino acids [11–13]. Association of these ectopeptidases with malignant cells intrigued us to look into the role of CD13 in ALL-B.

Among the ectopeptidases, CD10 and CD13 are most well studied cell surface antigens especially in context of acute lymphoblastic leukemia. CD10 (CALLA) is a marker strongly associated with precursor B-ALL. CD13 is present predominantly on the cells of myeloid origin but have also been found to be associated with ALL of T and B-cell origin. The expressions of myeloid markers on acute B-cell leukemia have often been observed, although their clinical significance remains poorly understood [14, 15]. CD13 positive B cells ALL are often characterized as bi-phenotypic or as an aberrant expression, but the possible role(s) that CD13 may play as an ectopeptidase in normal lymphohematopoiesis and leukemic transformation at various stages of B-cell development, still remains elusive.

ALL-B is one of the most widely studied entities in the field of hematological malignancies. However, studies on bi-phenotypic single cell level expression of myeloid antigens on ALL-B are inconclusive. In the present analysis, we investigated the surface phenotypes of ALL-B to elucidate the stage specific expression of ectopeptidase CD13 (aminopeptidase N).

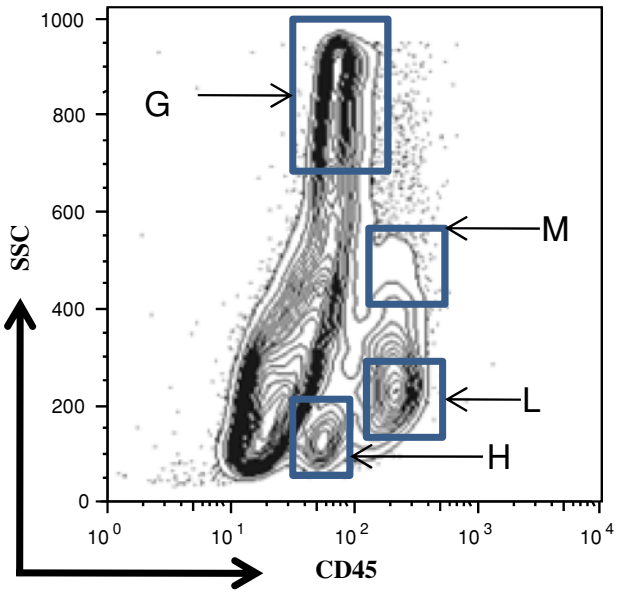

Fig. 1 CD45 versus side scatter (*SSC*) display of non-leukemic marrow specimen. Resolution of marrow cellularity into various components: lymphocytes (*L*), monocytes (*M*), granulocytes (*G*), normal B-cell precursors, hematogones (*H*)

We have also attempted to understand the possible role of ectopeptidase CD13, in stage specific growth regulation of B cells during ontogeny and/or in leukemogenesis. Our results unravel a unique association of CD13 with a particular stage of B-cell development. Here, we show the stage specific expression of CD13 on a relatively small fraction of B lineage cells in non-leukemic marrow, while it is completely absent on mature B cells. This population has earlier been defined as B lymphocyte precursors or hematogones (Fig. 1), originally recognized by their morphological feature in bone marrow smears [16, 17] rather than immunophenotypic characteristics. We provide elaborate immunophenotypic profile of this cells and evidence for the putative role of CD13 on the proliferative potential. Expression of CD13 during an early and specific stage of B-cell development highlights its role in B-cell ontogeny (Fig. 2). We also describe stage specific co-expression of CD13 on the blasts of a subset of ALL-B patients and the critical role that CD13 plays in the proliferation of B cells at this stage. Our finding brings mechanistic insights into the role of CD13 in B-cell development and leukemogenesis.

Materials and methods

Subjects

In total, 355 patients were immunophenotyped which comprised of patients suffering from acute leukemia, non-Hodgkin's lymphoma, Biphenotypic, bilineage and chronic

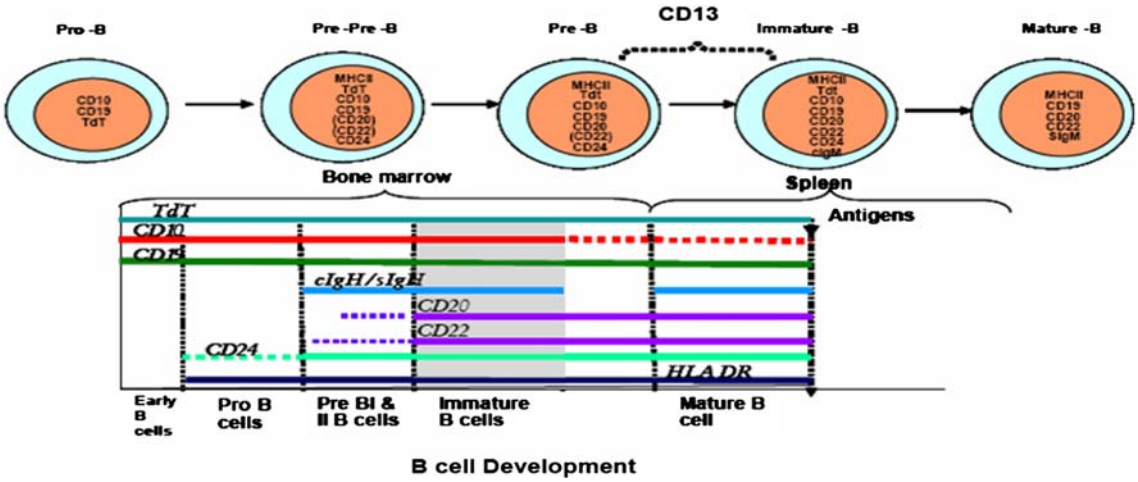

Fig. 2 Diagrammatic representation of B-cell ontogeny showing different stages of B-cell development and association of CD13 with Pre-B and immature B-cell stages. CD19/CD10 and CD22 are expressed from Pre-Pre B-cell stage to immature B-cell stage. Expression of CD10 ceases the B-cell immature stage. Other early stage antigens like TdT, HLA-DR, and CD24 are also expressed during early phase of development. Our findings suggest possible expression of CD13 during transitions of B cell from Pre-B cell stage to immature B-cell stage

lymphoproliferative disorders. The sources of sample were mainly bone marrow and peripheral blood. Between December 2005 and 2007 consecutive patients ($n = 355$) were referred to our laboratory for diagnostic flow cytometry based immunophenotyping (Table 1). Informed consent was obtained from them. Samples were mainly collected from our hospital as a part of leukemia phenotyping patient service carried out in Department of Transplant Immunology and Immunogenetics, AIIMS, New Delhi. Final diagnosis was based on the clinical presentation, morphology and FACS based immunophenotyping. Non-leukemic controls were patients undergoing bone marrow evaluation for the diagnosis of hematological conditions such as ITP, anaemias. They were subjected to morphologic evaluation and immunophenotyping and found not to have any hematological malignancies.

Table 1 Diagnostic details of ALL patients in total $N = 355$ patients

Demographic parameters	
Gender (female/male)	68.4/31.6
Age (median, range)	23 (1–77)
Diagnostic parameters	
WBC count (median, range)	13,690/dl (3,500–900,000)
Peripheral blast (median, range)	68% (36–85%)
Bone marrow blast (median, range)	30% (15–63%)
ALL-B	127
ALL-T	54
AML	59
Biphenotypic	4
CLL	43
CML	10
NHL	19
Without conclusion	49
Mediatinal involvement	24
Lymphadenopathy	42
Hepatomegaly	40
Splenomegaly	47

Peripheral blood mononuclear cell isolation

Peripheral blood samples and bone marrow aspirate from patients were collected after informed consent. Patient's blood or bone marrow samples were collected in heparinized vials and processed for mononuclear cell isolation within 24 h after sample collection. The peripheral blood mononuclear cells were isolated by Ficoll hypaque density gradient centrifugation method as described earlier. The isolated cells were then washed twice with complete RPMI-1640 media comprising 10% fetal calf serum (FCS), counted with the help of a hemocytometer and stored at 4°C in complete RPMI-1640 media.

Immunofluorescence staining and flow cytometery

Flow cytometery was used to detect the expression of various cell surface antigens for leukemia immunophenotyping. Cells suspended in staining buffer (phosphate-buffered saline with 0.1% Bovine serum albumin, 0.05% NaN_3) for cell-surface staining were incubated with antibodies designated for cell surface markers for 30 min on ice, washed with staining buffer twice and then fixed with 4% formaldehyde. For cytoplasmic staining, cells were suspended in staining buffer containing 0.05% saponin (Sigma Chemical Co, St Louis, MO, USA) and 10 mmol/l HEPES, pH 7.3,

and stained as for cell-surface staining. Throughout the staining and washing procedures, 0.05% of saponin was kept in buffer. Stained cells were applied to the flow cytometer FACS-Calibur (Becton Dickinson, Mountain View, CA, USA). Cells present in the lymphocyte gate defined by light scatter were analyzed.

Monoclonal antibodies against human CD19, CD22, CD79b, CD24, CD13, CD22, CD45, HLA-DR, CD10, bromodeoxyuridine (BrdU) were purchased from Pharmingen (San Diego, CA, USA). Anti-TdT and anti-IgM were procured from caltag, USA and Diatech, UK, respectively. All of these antibodies were conjugated to FITC, PE or Cy5PE fluorochromes. Purified antibody against CD13 (clone WM15) and CD22 (clone-2D6) was obtained from e-biosciences, San Diego, CA, USA.

Cell proliferation assay and CD13 blocking experiment

Mononuclear cells were isolated from peripheral blood and bone marrow aspirate of patients, confirmed for CD19+/CD13+ dual positive ALL-B, showing early B lineage markers. Mononuclear cells (1×10^6) isolated were incubated with anti human CD13 mAb (10 μg/ml) for 48 h at 37°C, 5% CO_2 (BrDU) was added to the culture 18 h before the termination of reaction. Cells were harvested and stained with anti human CD19mAb and anti bromodeoxyuridine (BrDU) antibodies. In another set of experiment mononuclear cells were cultured for 48 h and proliferation was detected with BrDU staining as mentioned earlier. Purified Mab against human CD22 was used as a control to rule out non-specific inhibition of proliferation. Cells were assessed for the positivity of CD22. On confirmation they were treated with mAb CD22 along with anti human CD13 for similar duration of time.

Statistical analysis

Statistical analyses were performed using a software package (SPSS for Windows, version 11.5, SPSS Inc., Chicago, IL, USA). Normally distributed continuous variables are presented as mean ± SD and were compared between leukemic cells positive for CD13 and cultured with and without anti-CD13 monoclonal antibody. Both the groups were compared using independent *t* test. Tests performed were two sided and $P < 0.05$ was considered significant.

Results

CD19+/CD13+ ALL-B frequency in Indian population

A total of 355 cases of different forms of hematological malignancies were diagnosed by flowcytometry based immuno-phenotyping. Dead cell exclusion in blasts (CD45 dim SSC low) [18] and lymphocytes (CD45 bright) was done by staining cells with 7-AAD. Among these patients 127 cases were found to be of ALL-B. A total of 68 cases were found to be positive for early B-cell markers, CD10, CD19 and CD22. However, the extended imunophenotypic panels to study various B-cell stage associated antigens was performed in 22 cases, the extended panels comprised of CD10, CD19, CD22, CD24, CD13 and IgM. In this study, we have found that the frequency of CD10+/CD19+/CD22+ B-cell leukemia was 54% (68 cases out of 127) out of which only 22 cases could have been diagnosed with the help of extended panel, which includes both early Immature stage cytoplasmic immunoglobulin M (cIgM−) as well as late immature (cIgM+) as discussed earlier. We were interested to see if there was any preferential expression of CD13, stemming either from the early or late phase of B-cell development. We observed selective expression of CD13 on cIgM− stage of precursor ALL-B blasts (Fig. 3a, b). Significantly high expression of CD13 was found to be restricted only to the blasts of early immature B-cell ALL (cIgM−), in comparison to the blasts of late stage immature B ALL (cIgM+) (Fig. 3c, d, $P = 0.01$). Gated blasts primarily comprised of B cells in immature stages as defined by presence of CD10, CD19, CD22, CD24, TdT and HLA-DR on the blasts. Expression of early immature markers defined with the help of above mentioned antigens was found to be dominantly associated with blast of CD19+ALL-B expressing CD13 (Fig. 4a). ALL-B patients were broadly divided into two stages on the basis of cIgM. Out of our 22 ALL-B cases, eight were of early immature stage (IgM−) and rests were IgM+ with no expression of CD13. Expression of different markers described above was not fount to be significantly different in the two groups except CD13 which was found to be high in cIgM−/CD13+ group ($P < 0.0001$, Fig. 4b, c). While the former (IgM−) may be involved in a prolonged maintenance of basal level of immature B-cell repertoire the later (IgM+) represents an antigen specific pool of naïve B cells. This later population is involved in a rapid production of mature B lymphocytes during an antigen driven B-cell response. Our findings suggest selective expression of CD13 on cIgM− stage of precursor ALL-B blasts. It also indicates loss of CD13 as precursor B cells begin to express cIgM. These results hint towards a possible functional role of CD13 on the B cell and their leukemic transformation at an early developmental stage.

CD13 expression on B cells in non-leukemic marrow

Intrigued with definitive expression of CD13 only on the early cIgM− B cell blasts, we intended to see if cells with similar phenotype existed in the normal healthy bone marrow. Their existence would signify a distinct function of CD13 expression on the precursor B cells during the ontogeny.

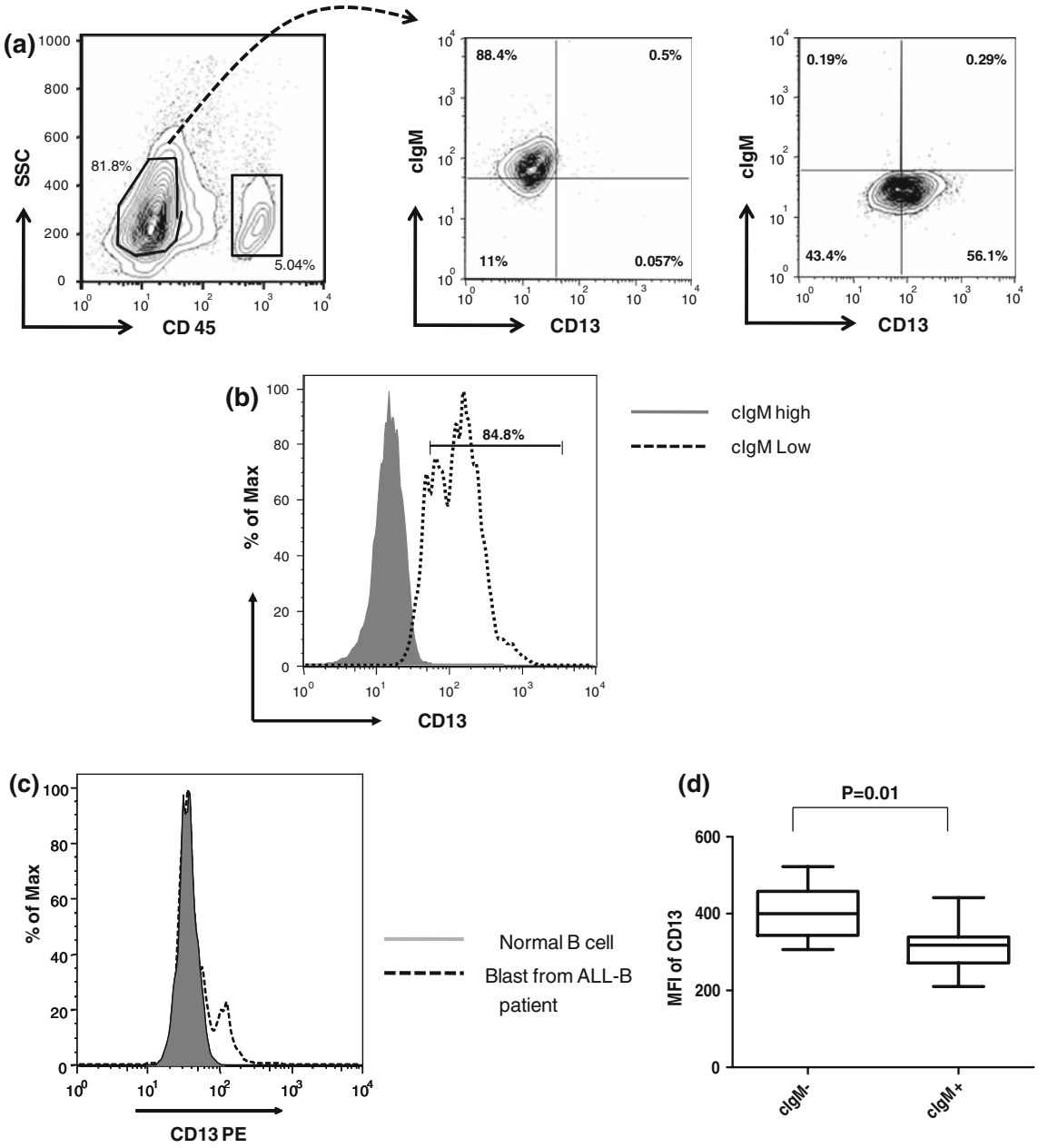

Fig. 3 Expression of CD13 and cIgM (single cell level) on the blasts. **a** Gated CD45 dim SSC low population was evaluated for simultaneous expression of CD13 and cIgM. FACS plot showing reciprocal expression CD13 and cIgM. **b** Histogram overlay of CD13 expression on cIgM high (*solid*) and cIgM low (*dotted*) population of B-ALL blasts. **c** Histogram overlay FACS plot showing CD13 expression on blasts of CD13+ B-ALL (*dotted*) and autologous normal B cells (*shaded*). **d** Expression of CD13 on early (cIgM−) and late (cIgM+) immature ALL-B blasts. Significantly higher expression of CD13 was observed on the early blast

Bone marrow aspirate obtained from patients other than leukemia (requiring a diagnostic bone marrow aspirate) were characterized by FACS based immuno-phenotyping. Mononuclear cells obtained as such were stained with cell surface and intracellular markers associated with various B-cell developmental stages (CD45, CD10, CD19, IgM, HLA-DR, CD20, CD22 and terminal deoxynucleotidyl transferase (TdT)). A distinct population showing simultaneous expression of CD19 and CD13 at single cell level together with CD10 and other early B-cell developmental markers such as TdT, HLA-DR, CD24, and CD22 was observed (Fig. 5a, b). However, this population did not have any detectable cIgM (Fig. 5a left plot). Among healthy bone marrows (non-leukemic) this population ranged between 2 and 5% (Fig. 5c). Thus, it indicates the presence of a stage of B-cell development with definitive expression of CD13. Detection of blasts with remarkably similar phenotype among a subset of patients with ALL-B

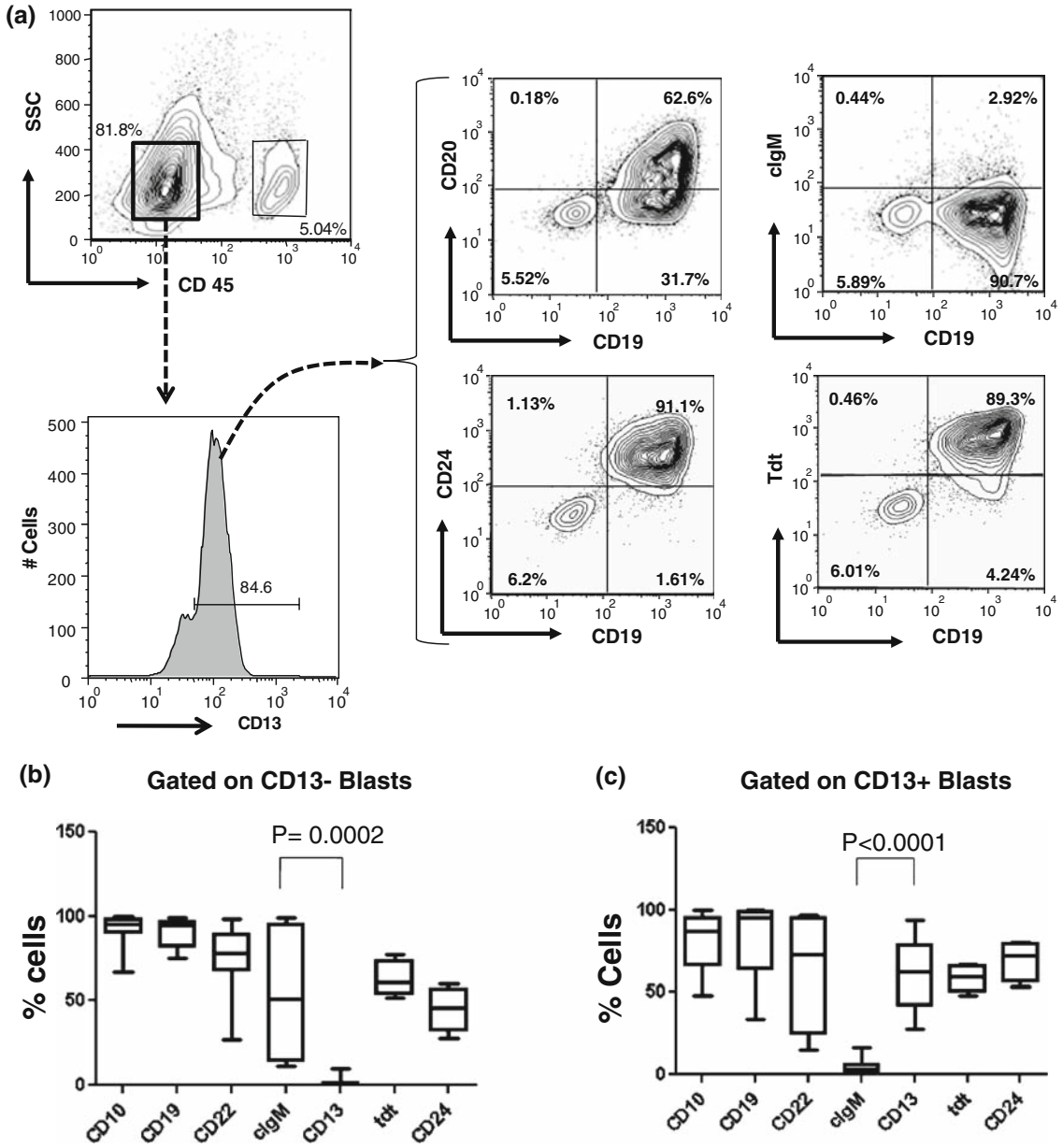

Fig. 4 FACS plot showing early immature stage of CD13+ blasts from ALL-B patients. **a** Expression of B-lineage markers (CD19, CD10, CD24, cIgM and TdT) on CD13+CD13+ B-cell blasts are in early immature B-cell stage as evident by TdT expression (90%) and lack of cIgM (3%). **b**, **c** Box plot depecting expression of various B-lineage markers on CD13+ ($n = 8$) and CD13− ($n = 15$) blasts derived from patients with ALL-B. Evidently CD13+ blasts were negative for cIgM suggesting there early differentiation stage

indicates a possible developmental arrest of precursor B cells at the stage of CD13 co-expression followed by neoplastic transformation. These findings suggest possible role of CD13 expressed during B-cell ontogeny and its role in cellular growth and leukemogenesis.

Spontaneous in vitro proliferation of CD19+CD13+ B cells in non-leukemic marrow and in leukemic blasts

Considering the reported function of ectopeptidase CD13 in the process of cellular proliferation as an activator of growth factors and/or deactivator of apoptotic factor(s) by cleavage of critical residues, we attempted to understand the role of CD13 on in vitro proliferative capability of the CD13+ immature B cells and blasts (CD19+CD13+). For this purpose we performed in vitro BrDU incorporation assay and flowcytometric phenotyping of the dual positive cells (CD19+CD13+). It was found that BrDU incorporation was primarily restricted to the CD19+ cells derived from non-leukemic marrow which co-expressed CD13 (2–5%) as well. Interestingly, CD13 negative immature cells (CD19+CD13−) failed to incorporate BrDU (Fig. 6a).

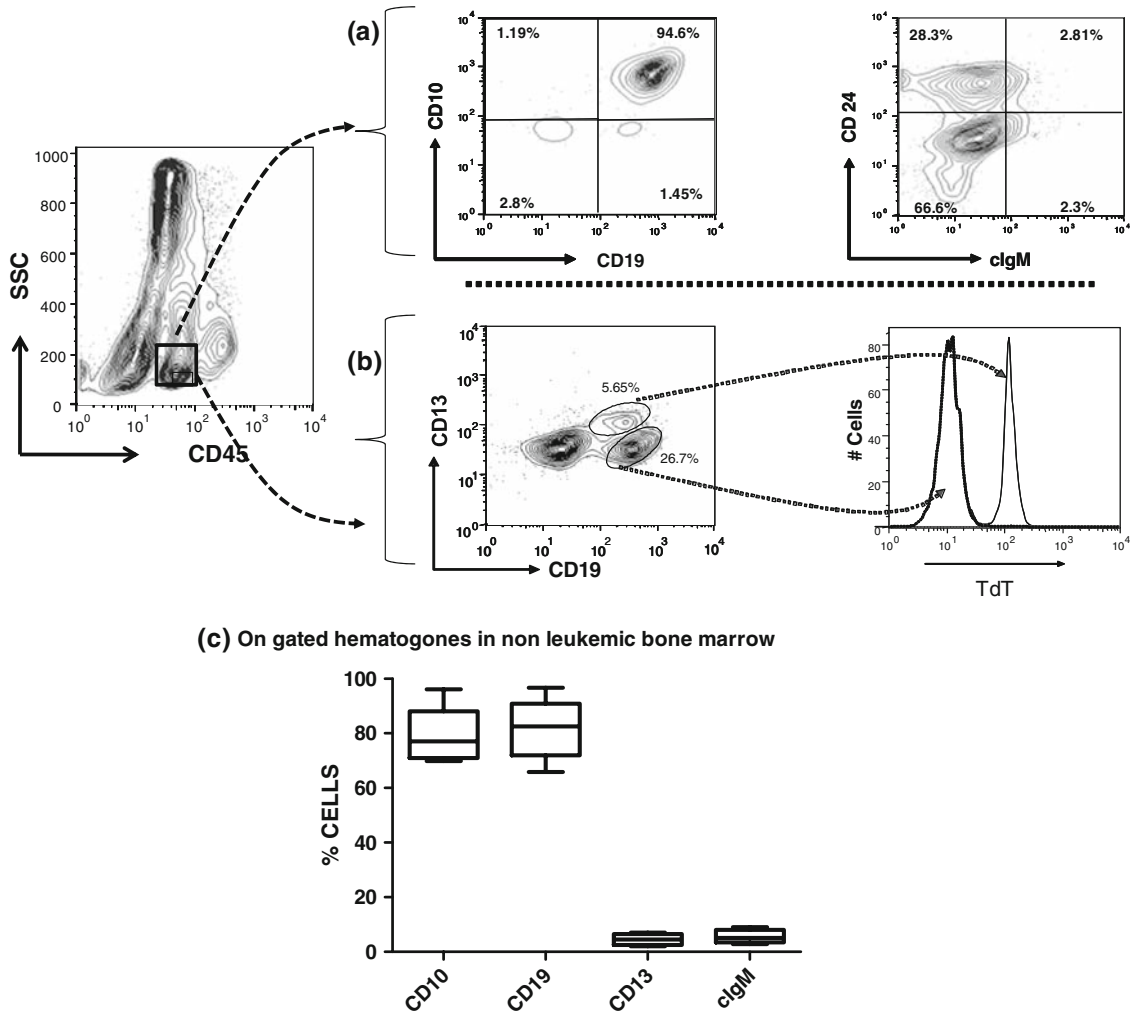

Fig. 5 Expression of B lineage markers on gated hematogones of non-leukemic marrow, hematogones were gated (CD45 dim SSC low). **a** Expression of B-Lineage markers (CD19, CD10 and CD24) including cIgM (defining early vs. late immature B-cell stage). **b** FACS plot showing the presence of CD19+CD13+ precursor B cell (*left*). Histogram overlay plot (*right*) showing TdT expression in 19+ mature B lymphocyte (*solid*) and CD19+CD13+ precursor B cell (*dotted*). TdT an associated marker of immature lymphocyte was found to be expressed only in CD19+CD13+ precursor B cells. **c** Box plot showing summary statistics of CD marker expression on hematogones of non-leukemic bone marrow

Co-expression of CD13 was found to be significantly high on gated CD19+/BrDU+ positive cells in comparison to CD19+/BrDU negative cells (Fig. 6b, c) as observed in five independent experiments performed with non-leukemic marrow cells ($n = 5$). These findings demonstrate a definitive association of spontaneous in vitro proliferation of CD19+ immature B cells co-expressing CD13 at the single cell level. This strongly suggests a possible role of CD13 on the cellular proliferation, particularly the cells co-expressing CD13/CD19.

To demonstrate the possible role of CD13 on the cellular proliferation of CD19+cIgM-CD13+ leukemic blasts of ALL-B patients and in CD19+ immature B cells in non-leukemic marrow, we performed similar in vitro bromodeoxyuridine (BrDU) uptake experiments with and without blocking CD13 by pure monoclonal antibody clone (WM15) against human CD13. It was noted that spontaneous in vitro proliferation of CD19+CD13+ blast cells (Fig. 6d) as well as CD19+ immature B cells from non-leukemic marrow, was significantly inhibited by anti-CD13 antibody. However, no effect was observed in case of monoclonal antihuman CD22 (as control antibody) or in untreated well (media alone), (Fig. 6e). Our results demonstrate a definitive role of CD13 in the cellular growth of B-cell blasts. We therefore concluded that CD13 expression on the immature B cells or blasts is not a mere associative phenomenon, rather CD13 plays an important role in the proliferative cycle of these cells and persistent presence on these subpopulation may be linked to the leukemogenesis of immature B cells.

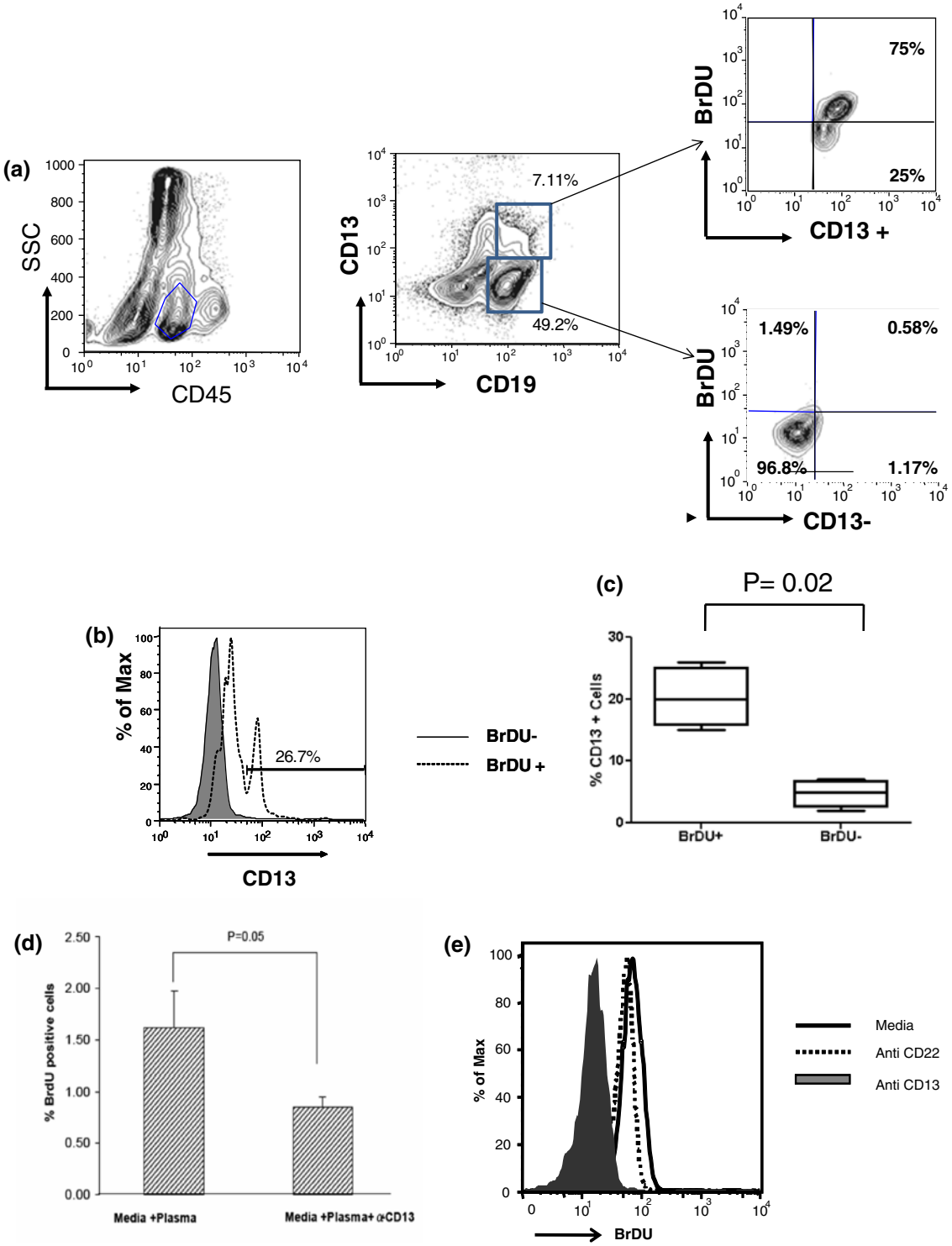

Fig. 6 FACS plot showing BrDU incorporation by CD19+CD13+ precursor B cells derived from non-leukemic marrow. **a** Spontaneous proliferation measured by BrDU incorporation was predominantly observed in CD13+ precursor B cells (CD19+) as opposed to CD13− B cells. **b** Histogram FACS overlay plot of CD13 expression by proliferating precursor B cells (gated on CD45 dim hematogones) in non-leukemic marrow. **c** Cumulative data of CD13 expression on proliferating precursor B cells in non-leukemic marrow is depicted. **d** Inhibition of BrDU uptake (proliferation) of gated blasts from CD13+ ALL-B patients by blocking of CD13 (with anti CD13 antibody) was found to be significant. **e** Spontaneous proliferation as measured by BrDU uptake of CD19+CD13+ hematogones (*solid*), in the presence of monoclonal antibody against CD22 (*dotted*) and CD13 (*shaded*). Anti CD13 clearly inhibited the proliferation, maximally as opposed to anti CD22

Discussion

Human B-cell development is a complex dynamic process involving various overlapping stages. However, specific stages can be distinguished on the basis of their phenotypes. B-cell precursors (BCPs) represent a rare population in normal human bone marrow, whereas clonal populations of leukemic BCPs are in abundance in the bone marrow and/or peripheral blood of ALL-B. Therefore, immunophenotypically distinct B-lineage ALLs, which reflect BCP phenotypes; corresponding to the sequential stages of B-lymphocyte ontogeny, provides opportunity for immunobiologic studies on rare and/or physiologically transient subpopulations of BCPs during B-cell ontogeny.

Stages of B-cell ontogeny are broadly defined as progenitor (Pro)-B, Pre-B, Immature B-cell stages, etc. Pro-B cells express cell surface CD19 and CD10 but not cytoplasmic or cell surface μ heavy chains (μHCs). Pre-B cells express cytoplasmic μHCs in addition to CD10, CD19 and variably express cell-surface μHCs associated with surrogate light chain (ψLCs)—constituting the Pre-B cell receptor (pre-BCR). Immature B cells express cell-surface CD19 and cell-surface μHCs which is formed by the replacement of the surrogate light chain with κ or λ LCs, i.e., the B-cell receptor (BCR). B-cell precursor includes all B-lineages prior to immature B cells which express the functional BCR [17]. Expression of markers other than B-cell lineage on the blasts of ALL-B patients is well reported and generally viewed as aberrant or bi-phenotypic expression of non-committal marker(s). Expression of T lineage markers like CD7, CD3 and CD5 occurs less frequently than that of the myeloid marker(s) like CD13, CD33 on the ALL-B blasts [19]. High incidence of CD13 expression on ALL-B is intriguing and its role is still poorly understood [20]. In our study, about 32% of the patients with immuno-phenotypically defined immature ALL-B showed simultaneous expression of CD13. Interestingly, we observed that CD13 co-expression was primarily restricted on the cIgM− B-cell blasts, with no CD13 expression on cIgM+ blasts. This indicates a possible role that CD13 may play in leukemogenesis at early stage(s) of B-cell development as phenotypically defined leukemic blasts represents concomitant maturational arrest and neoplastic transformation at specific stage(s) of lymphocyte development. Moreover, we identified the existence of a small population of cIgM− B cells co-expressing CD13 in the non-leukemic marrow delineating an early stage of normal B-cell ontogeny with definitive expression of CD13. CD13+ precursor B cell in healthy bone marrow, called hematogones [21] is also known. However, CD13 co-expression on hematogones in stage specific manner of B-cell ontogeny and to the development of B-cell leukemia is not known. Here, we demonstrate, perhaps for the first time, that CD13 expression on the early IgM− stage of precursor B cells and blasts (CD19, CD10, CD20, IgM, CD24, TdT, Fig. 2). Therefore, CD13 expression on ALL-B should not be viewed as a simple aberrant, associative co-expression, rather it may have a distinct role to play in an early and transitory stage of B-cell development and in leukemogenesis of early IgM− stage.

CD13 is a myelomonocytic marker [21, 22], belonging to the family of ectopeptidases involved in cleavage of proteins, peptides, metastasis, activation and regulation of hematopoiesis [23–26]. Ectopeptidases can either activate or inactivate growth/apoptosis related peptide(s) depending on their nature (proliferative or apoptotic) [3]. Various cytokines, peptides and growth/survival related factors present in bone marrow can be cleaved by ectopetidases like CD13 to yield active product(s), resulting in enhancement of cellular growth. Cell surface CD13 has been demonstrated to inhibit the endothelial cell mediated interleukin-8 (IL-8) dependent apoptosis in leukemic cell lines. Cells expressing high level of CD13 such as promyelocytic cell line NB4, has been found to escape this endothelial cell secreted IL-8 dependent apoptosis [15]. This suggests that high expression of CD13 on neoplastic cells may be one of the causative factors resulting in malignancy.

In our study we have observed significantly high expression of CD13 on the blast cells of immature stages of B-cell ALL. Interestingly, we found 2–5% of immature B-cell population expressing CD13 exist in non-leukemic marrow. This population may represent an important fraction of cells at a particular phase of early B-cell ontogeny. Thus, these cells can be looked as house keeping B cells which are maintained in a very small number and are capable of replenishing the aging B-cell repertoire as and when required. Phase of CD13 expression seems to be a check point in normal hematopoiesis either from pre B cells to immature or from immature stage to committed B-cell stages. High incidence of this particular phenotype of immature B-cell ALL in hematological malignancies and their presence in non-leukemic marrow indicates towards a putative role of CD13 in the B-cell ontogeny as well as leukemic transformation.

Here we attempted to investigate the growth/proliferative potential of the precursor B cells relating to CD13 expression. It was interesting to note that precursor B cells co-expressing CD13 derived from non-leukemic marrow as well as ALL-B blasts showed higher in vitro proliferation compared to that of CD13-CD19+ B cells. This strongly indicates the inherent growth potential of the B cells or leukemic B cells blasts expressing CD13. Transient stage specific expression of CD13 on early B cells possibly confers the potential of proliferation and its dysregulated persistence may lead to the development of leukemia. This is supported by our result showing abrogation of spontaneous

in vitro proliferation of CD13+ ALL-B blasts in presence of blocking antibody against CD13. Thus, we concluded that B cells/blasts expressing CD13 are in an active phase of proliferation and CD13 being an ectopeptidase, probably helps in utilization of some growth factor(s) leading to accelerated growth.

Over all, our findings highlight certain important aspects of CD13 co-expression on acute leukemic B-cell blasts. Based on our results, we propose that the phenomenon of CD13 co-expression requires to be viewed from the perspective of its ectopeptidase function, rather than seeing it just as an associative phenomenon. Moreover, the presence of a relatively small fraction of precursor/immature CD13+ B cells in non-leukemic marrow strongly indicates an important role of CD13, in B-cell development. This probably reflects an essential stage through which B cells develop and neoplastic transformation at this phase may occur due to persistent dysregulated function of CD13 resulting in generation of blasts cells with this distinct phenotype. Our results also demonstrate a definitive contribution of CD13 on the growth of immature B-cell blasts simultaneously expressing CD13 at single cell.

CD13 appears to be an important ectopeptidase required during early B-cell development for a very brief period of time and plausibly help their growth and differentiation. After the cells cross this stage, CD13 expression is lost and the cells pass to the later stage. In B-cell leukemia, expression of CD13 probably indicates that the cells may have got arrested during this particular stage of development and because of either excessive utilization of certain growth protein(s) and/or failure of apoptosis inducing machinery, these cells proliferate in an uncontrolled fashion leading to leukemogenesis. CD13 and its ligand(s) should be further investigated as target(s) for developing adjunct therapeutic modalities and clinical management for this particular form of acute leukemia of B-cell origin.

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