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CD19 expression in acute leukemia is not restricted to the cytogenetically aberrant populations

Jawad Francis, MD^{1,*}, Avinash V. Dharmadhikari, MS^{1,*}, Sheila N.J. Sait, PhD², George Deeb, MD³, Paul K. Wallace, PhD⁴, James E. Thompson, MD¹, Eunice S. Wang, MD¹, Meir Wetzler, MD¹

¹Leukemia Section, Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY.

²Clinical Cytogenetics Laboratory, Roswell Park Cancer Institute, Buffalo, NY.

³Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY.

⁴Department of Flow & Image Cytometry Laboratory, Roswell Park Cancer Institute, Buffalo, NY.

Abstract

Aberrant expression of the B lymphoid marker, CD19, in acute myeloid leukemia (AML) has frequently been associated with t(8;21)(q22;q22). However, AML cases lacking t(8;21) may occasionally express CD19. We asked if CD19 expression is restricted to the karyotypically abnormal leukemic cells in primary leukemia samples. We compared, by fluorescence in situ hybridization, CD19-positive and CD19-negative cells from nine acute leukemia patients: three non-t(8;21) AML, three t(8;21) AML, and three acute lymphoblastic leukemia cases. There were no significant differences in the karyotypic pattern between the CD19-positive and CD19-negative leukemic cells, raising the concern that therapeutically targeting CD19 for acute leukemia may not eradicate all malignant clones.

Keywords

Acute myeloid leukemia; CD19; Flow cytometry; fluorescence in-situ hybridization

INTRODUCTION

The B-lymphocyte antigen CD19 has increasingly being utilized as a biological target of choice for novel immunotherapeutic approaches in leukemia therapy. Such approaches include blinatumumab, a bispecific single chain specific for CD19 and CD3 [1,2] and genetically modified T cells against CD19-positive tumor cells [3–5]. Expression of CD19 is

Address Correspondence to: Meir Wetzler, M.D., Leukemia Section, Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, Phone: (716) 845-8447, Fax: (716) 845-2343, meir.wetzler@roswellpark.org.

*Both authors contributed equally

CONTRIBUTION:

J. Francis identified the cases, A.V. Dharmadhikari performed the FISH analyses, S.N.J. Sait supervised the FISH analyses G. Deeb reviewed the pathology, P.K. Wallace supervised the cell sorting, J.E. Thompson provided cases, E.S. Wang provided cases, M. Wetzler oversaw the conduct of the study. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST:

The authors declare no competing financial interests.

also found in some acute myeloid leukemia (AML) subsets, specifically AML carrying t(8;21)(q22;q22) [6]. Since CD19 expression is sometimes not homogenously distributed by all leukemic cells from a patient, we asked whether blasts expressing CD19 have a different karyotype than those that do not express this lymphoid marker. To answer this question, we analyzed our database at Roswell Park Cancer Institute (RPCI) and found three AML cases with viable cryopreserved samples, whose cells had variable expression of CD19 along with cytogenetic aberrations other than t(8;21), three AML patients with t(8;21), and three acute lymphoblastic leukemia (ALL) patients whose blasts expressed CD19. The results of these studies may have significant implications for the further development of CD19-targeted therapies.

METHODS AND PATIENTS

Patients

A total of nine cases were analyzed (Table I). These studies were approved by RPCI's Institutional Review Board.

Immunophenotype and Cell Sorting

Cryopreserved cells were thawed and immunophenotype staining was conducted as previously described [7]. Bone marrow cells were sorted using the FACSARIA™ (BD Bioscience, San Jose, CA) according to the specific immunophenotype [8]. In the bivariate display of forward versus side scatter, a generous mononuclear sorting region was drawn to exclude debris and aggregates, and the leukemic cells were further selected as being CD45 negative to dim and expressing CD19 and CD34 (Figure 1). Dead cells were excluded with fixable violet live dead reagent (Invitrogen, Carlsbad, CA).

Fluorescent In-Situ Hybridization (FISH)

FISH studies were carried out on the sorted samples as previously described [8]. Commercially available probes from Abbott Molecular, Inc (Des Plaines, IL) were used according to the manufacturer's instructions. Probes used were the Centromere enumeration probe -CEP X (SpectrumGreen) to detect monosomy X; the locus specific BCR (SpectrumGreen)/ABL1 (SpectrumOrange) dual color, dual fusion translocation probe was used to identify numerical changes of the BCR locus (22q11) and translocation (9;22); the locus specific D7S486 (7q31) SpectrumOrange/CEP 7 SpectrumGreen probe was used to identify monosomy 7; the centromere enumeration probe- CEP 7 SpectrumOrange probe was used to detect trisomy 7; the LSI RUNX1/RUNX1T1 dual color, dual fusion translocation probe was used to detect the t(8;21); the LSI MLL dual color break apart rearrangement probe was used to identify *MLL* rearrangements. Slides were visualized and analyzed on a Nikon Microscope using the CytoVision Program (Applied Imaging, Inc.). A total of 200/400 nuclei were assessed in each case

RESULTS

Table I represents the summary of our work. The first three cases were AML without t(8;21). In the first patient, by using the CEP X probe for chromosome X, there was equal number of

cells with loss of chromosome X (1 signal) between the CD19-positive and the CD19-negative subpopulations; similarly, there was equal distribution of cells without loss of chromosome X (2 signals) between CD19-positive and CD19-negative cells. The presence of three signals is most probably due to overlapping cells. In the second patient, we used the LSI *BCR/ABL1* probe to detect gain of chromosome 22. There was an equal number of cells with gain of chromosome 22 (3 signals) between CD19-positive and CD19-negative cells. Likewise, there were a similar number of cells without gain of chromosome 22 between CD19-positive and CD19-negative cells. Finally, for the third case, we used the LSI D7S486 and CEP 7 dual color probe, to detect loss of chromosome 7. All cells had one orange/one green signal pattern (monosomy 7), whether they expressed CD19 or not.

The next three cases had t(8;21) and as shown in Table I, there was a paucity of non-fusion signals in each of the cases whether we studied CD19-positive or CD19-negative cells.

The last three cases had ALL and interestingly all cases expressed the aberrant clone in both the CD19-positive and CD19-negative cells. A representative example of Case 9 is shown in Figure 2.

In summary, as shown in Table I and Figures 2, no significant differences were observed using FISH for the marker chromosomes in the karyotypic pattern between the CD19-positive and CD19-negative populations.

DISCUSSION

Our data demonstrate similar expression patterns of karyotypic abnormalities between the CD19-positive and -negative populations in three non-t(8;21) and three t(8;21)-positive AML cases as well as in three ALL diagnostic patient samples. Results presented here demonstrate that CD19-expression in acute leukemia samples is not restricted to different subpopulations of the malignant cells supporting a multi-clonal nature of disease. In the past, studies of the inactivation of the X chromosome in acute leukemia [9] have supported the monoclonal origin of acute leukemia. However, multiple unrelated immunophenotypic, karyotypic, or genomic abnormalities have clearly been identified in bulk primary ALL and AML samples at diagnosis, consistent with the presence of more than one disease clone [10,11]. The multi-clonal nature of leukemic disease is also supported by findings of immunophenotype [7] or karyotype [12] switch at disease relapse, suggesting that a dominant clone was eradicated over the course of treatment, allowing for the expansion of a second leukemic clone. We could not find similar analyses in the literature for either CD19-positive acute leukemias or other unique immunophenotypic markers in leukemia patients. An alternative interpretation of our data is that aberrant CD19 expression is acquired or lost by different karyotypic subpopulations of leukemia cells during clonal evolution. Additional studies are warranted to improve our understanding of the underlying processes governing expression of immunophenotypic markers in acute leukemia cells.

Our findings that CD19-negative karyotypically abnormal malignant cells are present alongside CD19-positive ones in the same patient also raise questions regarding the ability of CD19-targeted approaches to fully eradicate disease in such patients. For example,

blinatumumab [1,2], the bi-specific single chain antibody directed against both CD19 and CD3, is designed to recruit CD3 cytotoxic T cells to CD19-expressing leukemia blasts and induce cell kill. This agent was recently shown to induce molecular complete remissions in ALL patients who had persistent disease after previous chemotherapy [1,2]. Other therapeutic approaches targeting CD19 involve its introduction into genetically targeted autologous T cells [3–5] as a means of eradicating CD19-positive tumor cells in ALL and chronic lymphocytic leukemia patients [3–5]. The concern raised by our findings is that CD19-negative cancer cells present in primary acute leukemia samples will not be affected by novel CD19-targeted approaches, such as blinatumumab, and will ultimately result in recurrent disease.

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Figure 1.

Isolation of CD19- and CD19dim B acute lymphoblastic leukemia (B-ALL) cells. Patient bone marrow cells were stained with CD45, CD19 and CD34 then sorted on a FACSaria II using the selection criteria defined above. First a forward vs side scatter region was defined (R1) around the B-ALL cells, then a CD34 region (R2) was defined and finally the CD34+ cells were sorted into CD19- and CD19dim populations. In this example, the purity of the sorted cells was 93.1% for the CD34+CD19- population and 87.6% for the CD34+CD19dim population.

Figure 2. Representative example of fluorescent in-situ hybridization using the *BCR/ABL1* dual probe demonstrating dual fusion in CD19-positive (A) and negative (B) cells in case 9.
Figure a: FISH using the BCR/ABL1 dual color, dual fusion probe in CD19 positive cells in case 9.
Figure b: FISH using the BCR/ABL1 dual color, dual fusion probe in CD 19 negative cells in case 9.

Table 1:

Fluorescent in-situ hybridization results based on CD19 expression

Case #	Diagnosis	Karyotype	Probe	Signal Pattern	CD19-positive	CD19-negative
1	AML	45,X,-X[6]/46,XX[14]	CEP X SpectrumGreen Probe	1G Signal	20	30
				2G Signals	180	170
2	AML	46,XX,t(1;10)(q24;p15),del(7)(q31q36),inv(16)(p13q32)[1]/47,idem,+22[19]	LSI BCR/ABL1 Dual Color, Dual Fusion Probe (Count BCR (Green signals) for +22)	3G Signals	185	187
				2G Signals	15	13
3	AML	45,XY,t(2;3)(p22;q26),-7[18]/46,XY,t(2;3)(p22;q26),-7+18[2]	LSI CEP 7/D7S486Dual Color Probe	1G/1O Signal	195	196
				2G/2O Signals	5	4
4	AML	46,XX,t(8;21)(q22;q22)[20]	LSI RUNX1/RUNX1T1 Dual Color, Dual Fusion Probe	2G/2O/1F Signals	81	83
				2G/2O Signals	19	17
5	AML	46,XX,t(8;21)(q22;q22)[20]	LSI RUNX1/RUNX1T1 Dual Color, Dual Fusion Probe	1G/1O/2F Signals	67	99
				2G/2O Signals	2	1
6	AML	45,X,-X,t(8;20;21)(q22;p11;q22)[19]/46,XX[1]	LSI RUNX1/RUNX1T1 Dual Color, Dual Fusion Probe	2G/2O/1F Signals	95	96
				2G/2O Signals	2	7
7	ALL	48,XX,+7,+del(12)(q21q24),?(14;18)(q32;q21)[20]	CEP 7	3O Signals	73	65
				2O Signals	27	35
8	ALL	46,XY,t(4;11)(q21;q23)[20]	LSI MLL Dual Color,Breakapart Rearrangement Probe	Split Signal	100	97
				Intact signals	0	3
9	ALL	46,XY,t(9;22)(q34;q1.2)[20]	LSI BCR/ABL1 Dual Color, Dual Fusion Probe	2F/1O/1G signal	96	93
				2O/2GSsignals	4	7

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Gi, green signal; O, orange signal; F, fusion