ELSEVIER

Cancer Genetics and Cytogenetics 163 (2005) 62–67

Cancer Genetics and Cytogenetics

Short communication

# CD79a expression in acute myeloid leukemia t(8;21) and the importance of cytogenetics in the diagnosis of leukemias with immunophenotypic ambiguity

Igor Kozlov[a], Kevin Beason[a], Cheng Yu[b], Michael Hughson[a,*]

[a]*Department of Pathology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505*
[b]*Department of Preventive Medicine, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216*

Received 29 March 2005; received in revised form 1 June 2005; accepted 2 June 2005

**Abstract** Acute leukemias that express antigens associated with more than one lineage have been classified as acute lymphocytic leukemia with myeloid markers, acute myeloid leukemia with lymphoid markers, or biphenotypic acute leukemia (BAL). Antibody to cytoplasmic CD79a has been recently introduced to flow cytometry. CD79a functions in and has a high degree of specificity for B-cell differentiation. It has only recently begun to be reported in biphenotypic acute leukemias. Cases of acute leukemia submitted to the flow cytometry laboratory were retrospectively reviewed beginning from the time analysis for cytoplasmic CD79a was added to leukemia and lymphoma panels. Among 89 cases of AML, 2 showed strong coexpression of CD79a. Both cases were differentiated FAB AML-M2 and demonstrated the t(8;21) with cytogenetics and the AML1/ETO rearrangement with fluorescence in situ hybridization (FISH). These are recurring abnormalities in FAB AML-M2. The immunophenotyping met proposed scoring criteria for a diagnosis of BAL. Nevertheless, the cytogenetic and FISH findings indicate that CD79a, despite its specificity for B-cell differentiation, represented the aberrant presence of a B-cell antigen in leukemias of distinct myeloid linage. It is doubtful that, in this setting, CD79a expression should be considered a manifestation of lineage ambiguity. 

## 1. Introduction

Leukemias are classified using a combination of morphologic, cytochemical, immunophenotypic, and cytogenetic studies. Flow cytometric analysis of leukemias with panels of monoclonal antibodies now provides 98% accuracy for distinguishing acute leukemias of myeloid and lymphoid origin. In rare cases, both myeloid and lymphoid antigens are expressed, creating ambiguity for lineage assignment and difficulties in establishing whether a leukemia represents a distinct clinical or biological entity. This category of acute leukemia has been termed biphenotypic leukemia and is included in the WHO classification of hematopoietic malignancies as acute leukemia of ambiguous lineage [1].

Biphenotypic acute leukemias (BALs) are reported to account for 4 to 8% of acute leukemias [1,2]. Conventional morphologic evaluation of BAL is usually of limited value, because the majority of cases display blasts with little cytologic differentiation. Currently, biphenotypic acute leukemia is diagnosed with immunophenotyping [3]. To clarify the definition of BAL, the European Group for the Immunological Classification of Acute Leukemia (EGIL) proposed a scoring system based on the number and degree of specificity of the lymphoid and myeloid markers expressed by leukemic cells (Table 1) [3]. According to this scoring system, a case is considered biphenotypic when the score from two separate lineages is $>2$.

The coexpression of B-lymphoid and myeloid antigens is the most common combination of markers, being found in approximately 70% of BAL [2,4]. Among the different B-cell markers, CD79a has the highest linage-specificity for B-cell differentiation, with a specificity of 88% and a sensitivity of 100% [5]. It is a cell-surface molecule having a cytoplasmic domain that is associated physically with membrane immunoglobulins [6]. CD79a is needed for B-cell differentiation and is expressed in the early and late stages of B-cell development. Approximately eight to nine cases of BAL with coexpressed CD79a have been

* Corresponding author. Tel.: (601) 984-1540; fax: (601) 984-1531.
*E-mail address:* mhughson@pathology.umsmed.edu (M. Hughson).

0165-4608/05/$ – see front matter 
doi:10.1016/j.cancergencyto.2005.06.002

Table 1
Scoring system for markers proposed by the European Group for the Immunologic Classification of Leukemia (EGIL)

| Score | B-lymphoid | T-lymphoid | Myeloid |
|---|---|---|---|
| 2 | CytCD79a[a] | CD3(m/cyt) | MPO[a] |
| 2 | Cyt IgM | anti-TCR | |
| 2 | CytCD22 | | |
| 1 | CD19[a] | CD2 | CD117[a] |
| 1 | CD20 | CD5 | CD13[a] |
| 1 | CD10 | CD8 | CD33[a] |
| 1 | | CD10 | CD65 |
| 0.5 | TdT | TdT | CD14 |
| 0.5 | CD24 | CD7 | CD15 |
| 0.5 | | CD1a | CD64 |

The EGIL proposals [3] are adapted from the WHO classification of tumors [1].

[a] Antigens expressed in >20% of the blast population from the reported cases.

reported—although it must be noted that, in the larger series, most of the leukemias were not tested for the antigen, and several of these series report the same group of patients [2,4,7–10].

We report two cases of AML with differentiation (FAB AML-M2) that coexpressed CD79a. Both cases cytogenetically demonstrated a t(8;21) translocation, and under fluorescence in situ hybridization (FISH) both showed the AML1/ETO rearrangement, recurring cytogenetic and molecular genetic abnormalities associated only with AML. (Note that the genes involved, *AML1* and *ETO*, have since been reclassified and renamed as *RUNX1* and *RUNX1T1*, respectively; the older terminology will be used here, for convenience.) The cases are presented to emphasize the primary role that cytogenetics or molecular genetics should play in the diagnosis of acute leukemia.

## 2. Materials and methods

### 2.1. Case selection

Leukemias submitted for flow cytometry at the University of Mississippi Medical Center were retrospectively reviewed for the period since the laboratory began analyzing specimens for cytoplasmic CD79a. The cases were classified on the basis of conventional morphology, cytochemistry, and flow cytometric phenotype. During a 34-month period, 89 cases of AML were diagnosed; of these, 2 cases showed coexpression of CD79a and myeloid antigens.

### 2.2. Flow cytometry

Immunophenotyping was performed with flow cytometric analysis of peripheral blood and bone marrow aspirates collected in sodium heparin anticoagulant using an FC500 or Epics XL flow cytometer (Beckman Coulter, Miami, FL). Samples were washed with 30% fetal bovine serum RPMI medium. The portions of the sample to be surface-labeled were incubated for 30 minutes at room temperature with conjugated monoclonal antibodies and then were processed into a cell button that was resuspended with 0.5 mL of paraformaldehyde. The cytoplasmic labeled portion of the washed sample was surface-stained with CD45. Then, the surfaces of the cells were fixed using IntraPrep fixation reagent (Beckman Coulter) 1 for 15 minutes and after washing was treated with IntraPrep permeabilization reagent 2. The cytoplasmic conjugated monoclonal antibodies were added, and following a 30-minute incubation, the cytoplasmic stained cells were washed, and the final cell button was resuspended in 0.5 mL of paraformaldehyde.

Abnormal cell populations were gated using right-angle side-scatter versus CD45. Five-color analysis was performed with the use of monoclonal antibodies labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE/RD1), phycoerythrin–TexasRed–x (ECD), phycoerythrin–cyanin 5.1 (PC5/PE-Cy5), and phycoerythrin–cyanin 7 (PC7). All samples were stained with an acute leukemia panel of conjugated monoclonal antibodies purchased from Immunotech (Beckman Coulter) or Oncomark (BD Biosciences, San Jose, CA). Myeloid markers consisted of CD13, D15, CD33, and cytoplasmic myeloperoxidase (Immunotech clone CLB-MPO-1). B-lymphocyte markers were CD19, CD20, cytoplasmic CD22, surface and cytoplasmic anti-κ and anti-λ, and cytoplasmic CD79a (Immunotech clone HM47). Monocyte markers were CD4, CD14, and CD64. Markers for stem cells consisted of CD34 and CD117. A threshold of 20% of labeled blasts was set as the positive cutoff for each marker.

### 2.3. Conventional cytogenetic analysis

Cytogenetic analysis of metaphase cells was performed on bone marrow specimens using standard techniques. Chromosomes of cultured bone marrow cells from 24-hour cultures were analyzed by applying the GTG banding technique to identify the individual chromosomes. At least 15–20 metaphase cells were counted and examined for each case.

### 2.4. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed on cultured bone marrow cells obtained for cytogenetic analysis. Cell were dropped on slides and fixed with a 3:1 ethanol acetic acid solution. Dual-color DNA probes (Vysis, Downers Grove, IL) for *AML1* at 21q22 (SpectrumGreen) and *MTG8* (now renamed *RUNX1T1*), the *ETO* gene, at 8q22 (SpectrumOrange) were used to detect the AML1/ETO translocation. In normal interphase nuclei or metaphase chromosomes, there will be two separate red and green signals. In cells containing the t(8;21), there will one red and one green signal and two fusion signals indicating the translocation between *AML1* and *ETO* on the derivative chromosomes der(8) and der(21).