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Gene Expression Profiling of Acute Lymphoblastic Leukemia in Children with Very Early Relapse

Juan Carlos Núñez-Enríquez,[a] Diego Alberto Bárcenas-López,[b] Alfredo Hidalgo-Miranda,[b] Elva Jiménez-Hernández,[c] Vilma Carolina Bekker-Méndez,[d] Janet Flores-Lujano,[a] Karina Anastacia Solis-Labastida,[e] Gabriela Bibiana Martínez-Morales,[a] Fausto Sánchez-Muñoz,[f] Laura Eugenia Espinoza-Hernández,[c] Martha Margarita Velázquez-Aviña,[g] Laura Elizabeth Merino-Pasaye,[h] Alejandra Jimena García Velázquez,[i] María Luisa Pérez-Saldívar,[a] Raúl Mojica-Espinoza,[j] Julián Ramírez-Bello,[k] Silvia Jiménez-Morales,[b] Juan Manuel Mejía-Aranguré,[a,l] and MIGICCL[m]

[a]*Unidad de Investigación Médica en Epidemiología Clínica, UMAE Hospital de Pediatría, Centro Médico Nacional (CMN) "Siglo XXI", Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*

[b]*Laboratorio de Genómica del Cáncer, Instituto Nacional de Medicina Genómica (INMEGEN), Mexico City, Mexico*

[c]*Servicio de Hematología Pediátrica, Hospital General "Gaudencio González Garza", Centro Médico Nacional (CMN) "La Raza", IMSS, Mexico City, Mexico*

[d]*Unidad de Investigación Médica en Inmunología e Infectología, Hospital de Infectología "Dr. Daniel Méndez Hernández", "La Raza", IMSS, Mexico City, Mexico*

[e]*Servicio de Hematología Pediátrica, UMAE Hospital de Pediatría, Centro Médico Nacional (CMN) "Siglo XXI", Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*

[f]*Departamento de Inmunología, Instituto Nacional de Cardiología "Ignacio Chávez" (INCICh), Mexico City, Mexico*

[g]*Servicio de Onco-Pediatria, Hospital Juárez de México, Secretaria de Salud (SSa), Mexico City, Mexico*

[h]*Servicio de Hematología Pediátrica, Centro Médico Nacional (CMN) "20 de Noviembre", Instituto de Seguridad Social al Servicio de los Trabajadores del Estado (ISSSTE), Mexico City, Mexico*

[i]*Servicio de Oncología, Hospital Pediátrico de Moctezuma, Secretaría de Salud del D.F., Mexico City, Mexico*

[j]*Unidad de Genotipificación y Análisis de Expresión, Instituto Nacional de Medicina Genómica, Mexico City, Mexico*

[k]*Unidad de Investigación de Enfermedades Metabólicas y Endócrinas, Hospital Juárez de México, Mexico City, Mexico*

[l]*Coordinación de Investigación en Salud, Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*

[m]*Mexican Inter-Institutional Group for the Identification of the Causes of Childhood Leukaemia, Instituto Mexicano del Seguro Social, Instituto de Seguridad Social al Servicio de los Trabajadores del Estado, Secretaría de Salud, Secretaría de Salud del Gobierno del Distrito Federal, Mexico City, México*

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Background and Aims. Acute lymphoblastic leukemia (ALL) is the most common childhood cancer worldwide. Mexican patients have high mortality rates, low frequency of good prognosis biomarkers (i.e., *ETV6-RUNX1*) and a high proportion is classified at the time of diagnosis with a high risk to relapse according to clinical features. In addition, very early relapses are more frequently observed than in other populations. The aim of the study was to identify new potential biomarkers associated with very early relapse in Mexican ALL children through transcriptome analysis.

Methods. Microarray gene expression profiling on bone marrow samples of 54 pediatric ALL patients, collected at time of diagnosis and/or at relapse, was performed. Eleven patients presented relapse within the first 18 months after diagnosis. Affymetrix Human Transcriptome Array 2.0 (HTA 2.0) was used to perform gene expression analysis.

Address reprint request to: Dr. Silvia Jiménez-Morales, Laboratorio de Genómica del Cáncer, Instituto Nacional de Medicina Genómica, Periférico Sur 4809, Arenal Tepepan, 14610 Ciudad de México, México. Tel. 53501900 - 1115, Fax: 53501999; E-mail: sjimenez@inmegen.gob.mx; Dr. Juan Manuel Mejía-Aranguré, Unidad de Investigación Médica en Epidemiología Clínica, UMAE Hospital de Pediatría, Centro Médico Nacional (CMN) "Siglo XXI", Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico and Coordinación de Investigación en Salud, Instituto Mexicano del Seguro Social (IMSS), Torre Academia Nacional de Medicina 4to piso, Av. Cuauhtémoc 330, 06720 México, DF, México. Phone: +52155 56276900 - 21294; FAX: +52 155 57610841; E-mail: juan.mejiaa@imss.gob.mx, arangurejm@hotmail.com.

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Annotation and functional enrichment analyses were carried out using Gene Ontology, KEGG pathway analysis and Ingenuity Pathway Analysis tools.

Results. *BLVRB, ZCCHC7, PAX5, EBF1, TMOD1* and *BLNK* were differentially expressed (fold-change >2.0 and p value <0.01) between relapsed and non-relapsed patients. Functional analysis of abnormally expressed genes revealed their important role in cellular processes related to the development of hematological diseases, cancer, cell death and survival and in cell-to-cell signaling interaction.

Conclusions. Our data support previous findings showing the relevance of *PAX5*, *EBF1* and *ZCCHC7* as potential biomarkers to identify a subgroup of ALL children in high risk to relapse.

Key Words: Acute lymphoblastic leukemia, Gene expression profiling, Children, Very early relapse, Biomarkers.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer worldwide (1). Mexico has one of the highest ALL incidence rates reported (2) and it is one of the few countries in which mortality has not been reduced despite of using the same chemotherapy regimens than in developed countries (3,4). In previous studies, we reported that at the time of diagnosis, almost half of ALL Mexican children are classified as having high risk of relapse according to clinical criteria (5,6) and $<20\%$ are identified as positive for one of the four most common gene rearrangements (*ETV6-RUNX1, TCF3-PBX1, BCR-ABL1* and *MLL* rearrangements) associated with ALL prognosis (5). On the other hand, in developed countries, only one third of patients are classified as having a high risk of relapse at the time of diagnosis (7) and in 32% one of the four most common gene rearrangements mentioned above is detected (8).

Moreover, relapse is one of the main obstacles for achieving better survival rates in our population. Relapses occur in 26.2% of Mexican ALL pediatric patients (9). Noteworthy, they frequently occur in patients of standard risk group (55%) and very early during treatment (9), highlighting the importance of improving current clinical and molecular prognostic stratification in Mexican children with ALL.

Global gene expression profiling has been used in other populations to identify new potential genetic biomarkers associated with relapse in ALL pediatric patients (10–12). This methodology has also revealed possible mechanisms involved in relapse (13,14). *FLT3, XIAP, CCNB2, IKBKG, LIMS1, TEGT, DEFA1-3, SH3, BP5, TOSO, survivin, TOP2A, cyclin B1*, etc. are some examples of genes that have been reported as abnormally expressed in relapsed ALL (15–17). The aim of this study was to perform a transcriptome analysis in very early relapsed ALL children in order to identify new potential biomarkers associated with this outcome in our population.

Materials and Methods

Patients

Mexican Inter-Institutional Group for the Identification of Causes of Childhood Leukemia (MIGICCL) conducted a multicenter cohort study of patients aged <17 years with newly diagnosed ALL between August 1, 2014 and July 30, 2016 treated in Mexico City public hospitals. Diagnosis of ALL was based on the morphologic and immunophenotypic features of leukemic cells. Bone marrow samples (BMS) at the time of diagnostic confirmation and/or at the time of very early relapse (VER) were gathered.

Clinical Data Collection

Information regarding gender, age at diagnosis, white blood cell count (WBC), percentage of leukemic blasts in bone marrow, immunophenotype, and dates of ALL diagnosis, treatment initiation, last visit, death, and relapse was collected from the patients' clinical charts.

Risk classification at the time of diagnosis was based on the National Cancer Institute (NCI) risk criteria. Patients between 1 and 10 years old and with a leukocyte count $<50 \times 10^9$/L were classified as NCI standard-risk, whereas those aged ≥ 10 years or with a leukocyte count $\geq 50 \times 10^9$/L were classified as NCI high-risk, as previously was described (6).

Very early bone marrow relapse was defined when a patient who reached first complete remission (CR) presented $\geq 25\%$ lymphoblasts in a bone marrow aspirate within the first 18 months after diagnostic confirmation. Very early central nervous system (CNS) relapse was characterized as the presence of morphologically identified lymphoblasts on smears of cerebrospinal fluid (CSF) cytocentrifuged preparations with a CSF mononuclear cell count $>5/\mu l$ or as the evidence of tumor infiltration in the CNS following the first CR during the first 18 months after ALL diagnosis (18).

Approval by the National Scientific Research and Ethics Committee was obtained with the number R-2013-785-068. Written informed consent was obtained from the child's parents and assent was obtained from patients ≥ 8 years of age.

RNA Isolation and Gene Rearrangement Detection

White blood cells from bone marrow were treated with TRizol reagent (Invitrogen Life Technologies) and stored at $-80°C$. Total RNA was extracted and purified using standard protocols. With a conventional RT-PCR, chimeric