Bol Med Hosp Infant Mex. 2017;74(3):181–192

Boletín Médico del
Hospital Infantil de México

www.elsevier.es/bmhim

RESEARCH ARTICLE

Proteomic changes in a childhood acute lymphoblastic leukemia cell line during the adaptation to vincristine

Ana Laura Guzmán-Ortiz[a,b], **Gerardo Aparicio-Ozores**[b], **Ricardo Valle-Rios**[a,c], **Oscar Medina-Contreras**[a], **Genaro Patiño-López**[a], **Héctor Quezada**[a,*]

[a] *Laboratorio de Investigación en Inmunología y Proteómica, Hospital Infantil de México Federico Gómez, Mexico City, Mexico*
[b] *Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico*
[c] *División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico*

Received 11 February 2017; accepted 28 March 2017
Available online 23 May 2017

KEYWORDS
Leukemia;
Chemoresistance;
Vincristine

Abstract
Introduction: Relapse occurs in approximately 20% of Mexican patients with childhood acute lymphoblastic leukemia (ALL). In this group, chemoresistance may be one of the biggest challenges. An overview of complex cellular processes like drug tolerance can be achieved with proteomic studies.
Methods: The B-lineage pediatric ALL cell line CCRF-SB was gradually exposed to the chemotherapeutic vincristine until proliferation was observed at 6 nM, control cells were cultured in the absence of vincristine. The proteome from each group was analyzed by nanoHPLC coupled to an ESI-ion trap mass spectrometer. The identified proteins were grouped into over-represented functional categories with the PANTHER classification system.
Results: We found 135 proteins exclusively expressed in the presence of vincristine. The most represented functional categories were: Toll receptor signaling pathway, Ras Pathway, B and T cell activation, CCKR signaling map, cytokine-mediated signaling pathway, and oxidative phosphorylation.
Conclusions: Our study indicates that signal transduction and mitochondrial ATP production are essential during adaptation of leukemic cells to vincristine, these processes represent potential therapeutic targets.

* Corresponding author.
E-mail address: hquezada@himfg.edu.mx (H. Quezada).

http://dx.doi.org/10.1016/j.bmhimx.2017.03.005

PALABRAS CLAVE
Leucemia;
Quimiorresistencia;
Vincristina

Cambios en el proteoma de una línea celular de leucemia linfoblástica aguda infantil durante la adaptación a vincristina

Resumen
Introducción: Aproximadamente el 20% de los pacientes mexicanos con leucemia linfoblástica aguda (LLA) infantil presentan recaídas. En este grupo, la quimiorresistencia es uno de los principales desafíos. Los estudios proteómicos pueden dar un panorama general de procesos celulares complejos como la tolerancia a fármacos.
Métodos: La línea celular de LLA de linaje B, CCRF-SB, fue expuesta de manera gradual al fármaco quimioterapéutico vincristina hasta observar proliferación celular en presencia de 6 nM, como control se cultivaron células en ausencia del fármaco. Se analizó el proteoma de cada grupo mediante nanoHPLC acoplado a un espectrómetro de masas de tipo trampa de iones. Las proteínas identificadas se agruparon en categorías funcionales sobre-representadas con el sistema de clasificación PANTHER.
Resultados: Encontramos 135 proteínas expresadas exclusivamente en presencia de vincristina. Las categorías funcionales más representadas fueron la señalización asociada a los receptores tipo Toll, señalización dependiente de Ras, activación de células B y T, mapa de señalización CCKR, señalización mediada por citoquinas y la fosforilación oxidativa.
Conclusiones: Nuestro estudio indica que la transducción de señales y la producción de ATP mitocondrial son procesos esenciales durante la adaptación de células leucémicas a vincristina por lo que estos procesos representan potenciales blancos terapéuticos.

1. Introduction

Childhood acute lymphoblastic leukemia (ALL) is the most common type of cancer and the second leading cause of mortality among Mexican children.[1] Overall cure rates of newly diagnosed ALL patients is around 80%, while chemoresistance is one of the main challenges in the relapsed population.[2–4] Although much progress has been achieved in the detailed characterization of the molecular processes directly involved in drug tolerance of cancer cells,[5,6] more research is needed to develop effective therapeutic strategies to resensitize chemoresistant cells.[6]

Vincristine is a vinca alkaloid, which interacts with tubulin disrupting microtubule polymerization and favoring the cell cycle arrest in the M phase which is followed by induction of apoptosis.[7] It is used in several stages of the ALL treatment.[3] It has been reported that the P-glycoprotein MDR1 actively pumps vincristine outside the cell reducing its therapeutic effect.[8] Inactivation of intracellular vincristine by the myeloperoxidase has also been reported to contribute to resistance in some types of leukemia.[9] Moreover, resistant leukemia, and other types of cancer cells, commonly show deregulated apoptosis[4,6,10] and signaling pathways involved in survival.[5,6,11] The complex state of resistance is the result of the concerted action of multiple interacting genes, proteins, and metabolites; and this scenario is well suited for characterization with the omic technologies. Proteomics studies provide an overview of the changes in the relative abundance of the proteins of a cell or tissue under different conditions.[12] The characterization of proteins, as the final executors of cellular activities, may lead to identification of putative therapeutic targets and a better understanding of the pathological states.[13] In the present work, we describe the changes in the proteome of a B-ALL cell line after adaptation to vincristine. Our results allowed the identification of targetable signaling and metabolic steps which may represent potential targets to resensitize leukemic cells to vincristine.

2. Methods

2.1. Growth conditions

The B-lineage pediatric ALL cell line CCRF-SB (ATCC CCL-120) was grown in RPMI-1640 with 10% (v/v) fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 1% sodium pyruvate at 37 °C under an atmosphere with 5% CO_2.

2.2. Vincristine exposition

Cell viability was estimated with the MTT assay in 96 well plates.[14] To determine the IC50, vincristine was added to 7 x10^4 cells in 100 μL of media per well at 0, 1, 2, 3, 4, 5, 6, 8, 10, 20 and 40 nM in triplicate for 48 hours (Figure 1A). Gradual exposition was made as follows: 3 x10^6 cells were cultured in 5 ml of media in the presence of 1 nM vincristine for five days, and then were exposed to 2 nM for the next five days, 3.5 nM for five days, 4.5 nM for three days, and 6 nM for four days (Figure 1B). In every step viable cells were enriched by centrifugation at 800 rpm 5 min, only the pelleted cells were transferred to the subsequent drug concentration. After 22 days of gradual exposition, cells proliferated in the presence of 6 nM vincristine, and cell viability was higher than 70%. Control cells were subjected to the same manipulations but cultured in the absence of vincristine.