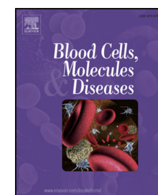
Blood Cells, Molecules and Diseases 59 (2016) 25–30

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd



The expression of Death Inducer-Obliterator (*DIDO*) variants in Myeloproliferative Neoplasms

Maria Gabriela Berzoti-Coelho [a,1], Aline Fernanda Ferreira [a,b,*,1], Natalia de Souza Nunes [a], Mariana Tomazini Pinto [a,b], Maurício Cristiano Rocha Júnior [a,b], Belinda Pinto Simões [b,c], Carlos Martínez-A [d], Elizabeth Xisto Souto [e], Rodrigo Alexandre Panepucci [b,c], Dimas Tadeu Covas [b,c], Simone Kashima [a,b], Fabíola Attié Castro [a,b]

[a] *Department of Clinical Analysis, Toxicology and Food Science, School of Pharmaceutical Sciences of Ribeirão Preto, USP, Brazil*
[b] *Center for Cell-Based Therapy, Ribeirão Preto, Brazil*
[c] *Department of Internal Medicine, Ribeirão Preto Medical School, USP, Brazil*
[d] *Department of Immunology and Oncology, Centro Nacional de Biotecnología, Universidad Autónoma, Campus de Cantoblanco, Madrid, Spain*
[e] *Hospital de Transplantes Euryclides de Jesus Zerbini, São Paulo, Brazil*

ARTICLE INFO

Article history:
Submitted 4 January 2016
Revised 10 March 2016
Accepted 26 March 2016
Available online 8 April 2016

Editor: Mohandas Narla

Keywords:
Myeloproliferative Neoplasms
DIDO
Apoptosis
JAK2^{V617F}
BCR-ABL1

ABSTRACT

Chronic Myeloid Leukemia (CML), Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) are Myeloproliferative Neoplasms (MPN) characterized by clonal myeloproliferation without cell maturation impairment. CML pathogenesis is associated with the Ph chromosome leading to BCR-ABL tyrosine-kinase constitutive expression. The Ph negative MPN (PV, ET and PMF) are characterized by the mutation JAK2^{V617F} of the JAK2 protein in the auto-inhibitory JH2 domain, which is found in most PV patients and in approximately half of ET and PMF patients. Considerable effort is being made to understand the role of JAK2^{V617F} at the MPN initiation and to clarify the pathogenesis and apoptosis resistance in CML, PV, ET and PMF patients. In the present investigation, we evaluated the Death Inducer-Obliterator (*DIDO*) (variants *DIDO 1*, *2* and *3*) levels in CML, PV, ET and PMF patients. Our data reported the *DIDO 1*, *2* and *3* differential expressions in Myeloproliferative Neoplasms.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Myeloproliferative Neoplasms (MPN) are a group of diseases associated with clonal expansion of the hematopoietic stem cell (HSC) characterized by hematopoietic progenitor independence from or hypersensitivity to cytokines [1]. The classification proposed by the World Health Organization (WHO) includes Chronic Myelogenous Leukemia (CML), Polycythemia Vera (PV), Essential Thrombocythemia (ET), Primary Myelofibrosis (PMF), Chronic Neutrophilic Leukemia (CNL), Chronic Eosinophilic Leukemia (CEL) (not otherwise categorized), Hypereosinophilic Syndrome (ES), Mast Cell Disease and unclassifiable MPNs [2].

Chronic Myelogenous Leukemia is Ph positive (CML) and Polycythemia Vera (PV), Primary Myelofibrosis (PMF) and Essential Thrombocythemia (ET) are Ph negative diseases [3]. CML is characterized by the translocation between chromosomes 9 and 22, which results in the Philadelphia chromosome (Ph). This alteration gives rise to the classic fusions, *B2A2* or *B3A2*, fusing exon 13 (*B2*) or exon 14 (*B3*) of *BCR*, respectively, to exon 2 (*A2*) of *ABL*. The type of *BCR-ABL1* transcripts is associated with different prognosis, as well as with different clinical procedures. This genetic event gives rise to the translation of a 210 KDa chimeric protein (p210), leading to enhanced tyrosine kinase activity and activation of leukemogenic pathways, as well as cell growth, and promoting apoptosis impairment [4,5].

The BCR-ABL-negative MPNs are characterized by the recurrent acquired mutation of the JAK2 protein leading to a valine-to-phenylalanine substitution (JAK2^{V617F}) in the auto-inhibitory JH2 domain, which is found in most patients with PV and in approximately half of the patients suffering from PMF or ET [6]. Although PV, ET, and PMF exhibit similar phenotype and clinical evolution, it remains unclear how a unique mutation can be involved in the pathogenesis of three different diseases [7]. Considerable effort has been made to understand the role of JAK2^{V617F} at the MPNs initiation and to clarify the pathogenesis and apoptosis resistance in CML, PV, ET and PMF patients [8,9,10,11].

* Corresponding author at: School of Pharmaceutical Sciences of Ribeirão Preto, USP, Brazil.
E-mail address: alineferreira591@gmail.com (A.F. Ferreira).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bcmd.2016.03.008
1079-9796/© 2016 Elsevier Inc. All rights reserved.

Despite all the knowledge about the PV, ET and PMF pathogenesis, there is no treatment leading to disease staging or cure. In CML, the tyrosine kinase inhibitors (TKIs) have been largely used in the clinical practice. Although TKIs induce to hematological and cytogenetic response, some patients are refractory or resistant to all available TKIs [12]. Based on Death Inducer-Obliterator (*DIDO* or *DIO*) roles associated with apoptosis regulation [13,14], we evaluated *DIDO 1*, *2* and *3* levels in CML, PV, ET and PMF.

The *DIDO* gene locus was mapped in human chromosome 20q13.33 and encodes for three different transcripts, *DIDO 1*, *DIDO 2* and *DIDO 3*. *DIDO 1* is the smallest while *DIDO 3* is the largest and most widely expressed isoform [14,15]. García-Domingo et al. [13,16] showed that *DIDO 1* translocates to the nucleus when is overexpressed and activates apoptosis *in vitro* by the upregulation of procaspase 3 and 9. The deletion of *DIDO* in mice caused a disease suggestive of Myelodysplastic Syndrome/MPN as observed by Fütterer and cols [14]. They also reported the reduced expression of *DIDO 2* and *3* in Myelodysplastic Syndrome and in MPN patients.

Therefore, our hypothesis is that the *DIDO* alterations may contribute to pathogenesis and apoptosis resistance in Myeloproliferative Neoplasms. Thus, our results might contribute to the search for new therapeutic targets for these hematological disorders.

2. Subjects, material and methods

2.1. Patients and controls

Peripheral blood (PB) samples were obtained from 60 CML patients, 34 patients were from the Clinical Hospital of the Ribeirão Preto Medical School — USP and 26, from the Hospital de Transplantes Euryclides de Jesus Zerbini (São Paulo, SP — Brazil). Thirty-five patients were male and 25 were female, 55 patients were Caucasian, while four were non-Caucasian and one was Asiatic. The median age of the group was 45, range 19 to 77 years.

Regarding CML phase, 41 patients were in chronic phase (CP), 10 were in accelerated phase (AP) and nine were in blast phase (BP). The control group consisted of 57 healthy subjects, 35 were male and 22 were female, 55 were Caucasian, one non-Caucasian and one Asiatic. The median age of the group was 45 years, range 20 to 80 years.

Twenty-four patients composed the ET group; 22 donated bone marrow (BM) and peripheral blood (PB), one donated only PB and one donated only BM. Seventeen were female and seven were male, 20 were Caucasian, four were non- Caucasian and the median age was 59.5 years (range 40 to 81). Twelve patients were positive for $JAK2^{V617F}$ mutation.

The PV group was composed of 14 patients; 11 donated BM and PB and three donated only BM. Twelve were Caucasian, one was non-Caucasian and one was Asiatic. Seven were female and seven were male. The median age was 59 years (range 39–80). Eight patients were positive for $JAK2^{V617F}$ mutation.

Nine patients composed the PMF group, six of them donated PB and BM, and three donated only BM. All of them were Caucasian, three were female and six were male. The median age was 69 years, range 59 to 79 years. Four patients were positive for $JAK2^{V617F}$ mutation.

The control group for the PB samples was composed of 32 healthy individuals, 13 were male and 19 were female. Two of them were non-Caucasian and 30 were Caucasian. The median age was 57.5, range 31 to 80 years. Eight healthy individuals, three females and five males, composed the control group for BM samples. All of them were Caucasian. The median of age was 41 years, range 16 to 54.

The demographic data from patients and controls are described in the supplementary material (Tables 1, 2, 3, 4).

This study was approved by the Ethics Committee of the School of Pharmaceutical Sciences of Ribeirão Preto — USP and Clinical Hospital of the Ribeirão Preto Medical School — USP, N. 256 and 346940.

2.2. Material and methods

2.2.1. Cells isolation

Mononuclear cells from CML patients and controls were isolated by Ficoll Paque Plus according to the manufacturer's instructions (GE Healthcare, Sweden). Leukocytes from PB of PV, ET and PMF were isolated using the Haes-Steril method. This protocol uses whole blood mixed with Haes-Steril reagent in a proportion of 20 mL of blood for 8 parts of reagent. The mixture was homogenized and decanted for 90 min. The supernatant was collected, centrifuged (1810 $\times g$) and the red cells which were present in the precipitate were lysed using 2 mL of the ACK lyse buffer (8.3 g/L of NH_4Cl, 1 g/L of $KHCO_3$ and 0.036 g/L of EDTA) [9]. The $CD34^+$ hematopoietic stem cells (HSC) from BM of PV, ET and PMF patients were obtained, using an immunomagnetic isolation kit (MidiMacs; Miltenyi Biotec, Germany).

2.2.2. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted with TRIzol™ (Ambion, USA) as described by the manufacturer. cDNA was synthesized using High Capacity cDNA reverse transcription™ kit (Applied Biosystems™, USA). The oligonucleotide sequences, annealing temperature and amplicon size are described in Table 1. The analyses of *DIDO* variants and the *BCR-ABL1* (*B2A2* and *B3A2* transcripts) expression were performed by SYBR Green GoTaq qPCR Master Mix (Promega, USA). β-*ACTIN* mRNA was used for MPN Ph negative while β-*ACTIN* and *B2M* (Beta-2-Microglubulin) for CML samples normalization and analysis.

The threshold cycle values were measured using 7500 Sequence Detection System Software, version 2.0.5 (Applied Biosystems). The results are presented as relative expression units (REU), calculated by the formula: $REU = 10.000/2^{\Delta CT}$.

2.2.3. Detection of $JAK2^{V617F}$ mutation and $JAK2^{V617}$ allele burden calculation

The $JAK2^{V617F}$ mutation was detected by real-time allelic discrimination PCR assay as described by Tognon and cols [9]. The allele burden was estimated based on the normalized fluorescent signal of the mutant allele and wild-type allele of real-time PCR and reported as a percentage of $JAK2^{V617F}$ mutation or negative [9].

2.2.4. Statistical analysis

To compare the *DIDO 1*, *2* and *3* expressions among CML, PV, ET, PMF patients and controls and in CML patients in different phases of disease, the Mann–Whitney test was used. The correlation between *BCR-ABL1* levels and *DIDO 1*, *2* and *3* expressions, and between $JAK2^{V617F}$ allele burden and *DIDO 1*, *2* and *3* expressions was performed by Spearman statistical test. The analyses were performed using the software GraphPad Prism 6. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Control group and CML patients in advanced phases (accelerated phase and blast phase) or CML patients in chronic phase

DIDO 1 and *DIDO 2* showed higher expression (median (m) = 5.39; 2.92, respectively) in CML advanced phases in comparison with controls

Table 1
Real time PCR oligonucleotide sequences, annealing temperature and amplicon size.

Primer	Sequence (5′-3′)	At (°C)	Amplicon (bp)
DIDO 1 — F	AGG CGT TGG AGC GCG GAA AT	62	365
DIDO 1 — R	AGA GGC TGT TCC CGT GGA GT		
DIDO 2 — F	TCG GGG AAA TGG CTG CGA GA	62	331
DIDO 2 — R	AGA GGC TGT TCC CGT GGA GT		
DIDO 3 — F	GTC TTC CGA AAT GCG GTG CTC A	60	133
DIDO 3 — R	ATG GTG CAG CCG GTG TCT GT		

F: forward; R: reverse; AT: annealing temperature; bp: base pair.

Download English Version:

https://daneshyari.com/en/article/5913337

Download Persian Version:

https://daneshyari.com/article/5913337

Daneshyari.com