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Stathmin 1 is involved in the highly proliferative phenotype of high-risk myelodysplastic syndromes and acute leukemia cells

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ABSTRACT

Stathmin 1 is an important cytoplasmic microtubule-destabilizing protein that plays critical roles in proliferation and accurate chromosome segregation through regulation of microtubule dynamics. High levels of Stathmin 1 expression have been reported in leukemia and solid tumors. However, Stathmin 1 has not been studied in myelodysplastic syndrome cells. We, herein, report that significantly higher Stathmin 1 levels were observed in proliferating hematopoietic cells, in high-risk MDS and acute leukemia cells. In addition, Stathmin 1 silencing in U937 and Namalwa leukemia cells reduced cell proliferation and clonogenicity. Our data suggest that Stathmin 1 expression may be related to the highly proliferative phenotype of hematopoietic cells and add new insights into the participation of Stathmin 1 in hematological malignancies.

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1. Introduction

Myelodysplastic syndrome (MDS) encompasses a heterogeneous group of clonal hematopoietic stem cell disorders, characterized by ineffective hematopoiesis and a tendency to progress toward acute myeloid leukemia (AML) [1]. The occurrence of acute leukemia results from a combination of changes in cellular signaling, which confer the ability of proliferation, defects in cell differentiation and apoptosis [2].

Stathmin 1, also known as Oncoprotein 18 (OP18) or Leukemia-associated phosphoprotein p18 (LAP18), is an important cytoplasmic microtubule-destabilizing protein that plays a critical role in the process of mitosis, proliferation and accurate chromosome segregation through regulation of microtubule dynamics [3,4]. Stathmin 1 was initially characterized as a peptide of 18 kDa overexpressed in acute leukemia and proliferating lymphocytes [5,6]. High levels of Stathmin 1 have been reported in solid tumors and have been associated with poor prognosis in various types of cancers [4].

To learn more about Stathmin 1 in hematological malignancies, we evaluated Stathmin 1 expression in proliferating and non-proliferating hematopoietic cells, in bone marrow cells from healthy donors and from patients with myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL). We also evaluated the effects of Stathmin 1 silencing in a myeloid (U937 cells) and in a lymphoid (Namalwa cells) leukemia cell line regarding cell proliferation, clonogenicity and apoptosis.

2. Materials and methods

2.1. Bone marrow samples

Bone marrow samples collected from patients with *de novo* MDS ($n = 65$), AML ($n = 60$), ALL ($n = 19$) and healthy donors ($n = 30$) were analyzed. MDS, AML and ALL patients included in the study were untreated at the time of sample collection. Patients' characteristics are described in Table 1. During the course of the study, 5

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Table 1
Patients' characteristics.

Patients	Number
MDS	**65**
Gender	
Male/female	33/32
Age (years), median (range)	69 (16–90)
WHO 2008 classification	
Low-risk (RA/RARS/del(5q)/RCMD)	4/5/1/36
High-risk (RAEB-1/RAEB-2)	9/10
Number of cytopenia	
0/1	8/23
2/3	20/14
Karyotype	
Normal karyotype	51
−Y	1
Monosomy 7	1
Trisomy 8	2
del(11)(q23)	1
inv(9), del(16)	1
del(5q)	1
inv(9)	1
+mar	1
Not available	3
Complex	2
BM blast (%)	
<5%	46
≥5% and <10%	9
≥10% and <20%	10
AML	**60**
de novo AML/AML–MRC	47/13
Gender	
Male/female	29/31
Age (years), median (range):	60 (18–93)
BM blasts (%), median (range)	60 (20–98)
Karyotype	
Normal karyotype	23
t(8;21)	3
inv (16)	1
inv (12)	1
Trisomy 8	4
Monosomy 7	4
−X	1
−Y, del 5(q)	1
del 5(q)	1
Trisomy 13	1
Trisomy 14	1
Complex	9
Not available	11
ALL	**19**
Gender	
Male/female	7/12
Age (years), median (range)	33 (19–79)
BM blasts (%), median (range)	89 (54–99)
Karyotype	
Normal karyotype	8
t(9;22)	1
t(4;11)	1
Complex	1
Not available	8

Abbreviations: MDS, myelodysplastic syndromes; WHO, World Health Organization; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; del(5q), MDS with isolated del(5q); RCMD, refractory cytopenia with multilineage dysplasia; RAEB-1, refractory anemia with excess blast-1; RAEB-2, refractory anemia with excess blast-2; BM, bone marrow; AML, acute myeloid leukemia; AML–MRC, acute leukemia with myelodysplasia-related changes; ALL, acute lymphoid leukemia.

MDS patients presented disease progression (as detailed in Table 2) and bone marrow samples were collected at diagnosis and at MDS progression. All healthy controls and patients provided informed written consent and the study was approved by the ethics committee of the University of Campinas. Patients that attended the clinic between 2005 and 2013, and signed the consent for the study were included.

2.2. Leukemia cell lines

A panel of human leukemia cell lines that included myeloid (K562, KU812, NB4, HL60, P39, HEL, U937, KG1 and THP1) and lymphoid cells (Jurkat, MOLT4, Daudi, Raji, Namalwa and Karpas 422) was used. Cell lines were obtained from ATCC (Philadelphia, PA, USA) and cultured in appropriate medium containing 10% fetal bovine serum with addition of penicillin/streptomycin and amphotericin B, maintained at 37 °C, 5% CO_2. The P39 cell line was kindly provided by Prof. Dr. Eva Hellström-Lindberg (Department of Medicine, Division of Hematology, Karolinska University Hospital – Huddinge, Karolinska Institute, Stockholm, Sweden). For quantitative PCR and Western blot analysis, leukemia cells in exponential growth were used.

2.3. Peripheral blood mononuclear cells and peripheral blood lymphocytes activation

Non-proliferating hematopoietic cells were obtained by Ficoll gradient separation, from normal peripheral blood mononuclear cells (PBMC) [7]. Peripheral blood lymphocyte (PBL) activation was performed as previously described [7].

2.4. Bone marrow CD34$^+$ cells, peripheral blood lymphocytes, monocytes and granulocytes isolation

CD34$^+$ bone marrow cells were separated by MIDI-MACS immunoaffinity columns (Miltenyi Biotec, Auburn, CA, USA) using anti-CD34 monoclonal antibody from healthy donors ($n = 08$), and from MDS (low-risk: $n = 07$; high-risk: $n = 07$) and AML (*de novo* AML: $n = 05$; AML with myelodysplasia-related changes: $n = 03$) patients. PB lymphocytes and monocytes were separated by MIDI-MACS immunoaffinity columns using anti-CD3 and anti-CD14 monoclonal antibodies, respectively, from normal PBMC. Peripheral blood granulocytes were obtained from polymorphonuclear cell fraction using Ficoll gradient density centrifugation after removal of erythrocytes by hemolysis.

2.5. Quantitative PCR (qPCR) analysis

Total bone marrow cells obtained after removal of erythrocytes by hemolysis or CD34$^+$ purified cells were submitted to RNA extraction. Quantitative PCR (qPCR) was performed with an ABI 7500 Sequence Detector System (Applied Biosystems, Foster City, CA, USA) with specific primers for *Stathmin 1* (forward: AGCCCTCGGTCAAAAGAATC; reverse: TTCAAGACCTCAGCTTCATGGG) [8] and *HPRT* (*hypoxanthine phosphoribosyltransferase* 1; forward: GAACGTCTTGCTCGAGATGTGA; reverse: TCCAGCAGGTCAGCAAAGAAT). The relative gene expression was calculated using the equation, $2^{-\Delta\Delta CT}$ [9].

2.6. Western blot

Equal amounts of total cell extract proteins were separated by SDS-PAGE, and Western blot analysis with the indicated antibodies was carried out using the ECL^{TM} Western Blotting Analysis System (Amersham Pharmacia Biotech Ltd., Buckinghamshire, UK), as previously described [10]. Antibodies against Stathmin 1 (OP18; sc-55531) and Actin (sc-1616) were acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Blots were stripped and reprobed as necessary.

2.7. Transduction of lentivirus

U937 and Namalwa cells (a myeloid and a lymphoid leukemia cell line, respectively) were transduced with lentivirus-mediated

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