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Differential expression of *AURKA* and *AURKB* genes in bone marrow stromal mesenchymal cells of myelodysplastic syndrome: correlation with *G-banding* analysis and FISH

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It has been demonstrated that genomic alterations of cells in the hematopoietic microenvironment could induce myelodysplastic syndromes (MDS) with ineffective hematopoiesis and dysmorphic hematopoietic cells, and subsequent transformation to acute myeloid leukemia. This investigation is the first attempt to correlate the gene expression profile of *AURKA* and *AURKB* in a cytogenetically stratified population of mesenchymal stem cells (MSCs) from MDS patients. We found that *AURKA* messenger RNA was expressed at significantly higher levels in MSCs even with normal/altered karyotype when compared with hematopoietic cells and healthy donors. In addition, we found that the presence of chromosomal abnormalities (mainly aneuploidy) in hematopoietic cells/MSCs was also associated with higher levels of *AURKA*. Different from previous investigations, our findings, regarding *AURKA* expression support the hypothesis that the presence of chromosomal abnormalities in MSCs from MDS is not a consequence of the method used for chromosome preparation. They may reflect the genomic instability present in the bone marrow microenvironment of MDS patients. This information is also supported by differences observed in the growth kinetics between MSCs from healthy donors (normal karyotype) and from MDS patients with abnormal karyotype. In summary, our results may not be considered evidence that MDS and MSCs are originated from a single neoplastic clone. In fact, both cells (hematopoietic and MSCs) may probably be altered in response to damage-inducing factors, and the presence of genomic abnormalities in MSCs suggests that an unstable bone marrow microenvironment may facilitate the expansion of MDS/leukemic cells. **© 2013 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.**

According to a multistep pathogenesis model proposed for leukemias, after the initial damage of the hematopoietic stem cell, additional genomic abnormalities may affect these cells, providing them a proliferative advantage [1,2]. In addition to the genomic instability, the hematopoietic microenvironment is involved in the pathophysiology of myelodysplastic syndrome (MDS) [3,4]. Disruption of the architecture of hematopoiesis is a common finding in MDS. It involves both altered localization of hematopoietic elements within the bone marrow and alterations in components that compose the microenvironment. In this way, all hematopoietic lineages in MDS may be affected [4–6].

Another important element of the hematopoietic microenvironment is the bone marrow stromal mesenchymal cells (MSCs). These cells have an important role supporting and regulating the proliferation and differentiation of hematopoietic stem cells; they also have an immunoregulatory function [7,8]. Whether abnormalities associated with MSCs contribute to pathogenesis of MDS and leukemias, and subsequently disease progression, is still not totally clear. However, some differences have been observed between the MSCs of leukemia patients and those of healthy donors [9,10]. It has been demonstrated that genomic alterations of cells in the hematopoietic microenvironment could induce MDS with ineffective

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hematopoiesis and dysmorphic hematopoietic cells, and subsequent transformation to acute myeloid leukemia (AML) [11]. However, the presence of cytogenetic abnormalities in the MSCs of patients with hematologic disorders is considered a controversial finding by some groups [12–15]. Classic cytogenetic and fluorescence in situ hybridization (FISH) aim to determine whether the MSCs of leukemia patients harbor genomic abnormalities that may act on the fate of hematopoietic stem cells [12–15].

Aurora kinases are mitotic kinases with an important role regulating the G2/M phase of the cell cycle and various mitotic events, including centrosome duplication, mitotic spindle assembly, chromosome segregation, and cytokinesis at the end of telophase [16]. A correlation between overexpression of the *AURKA* gene and clinical aggressiveness has been described in esophageal cancer, gastric cancer, bladder cancer, and hepatocellular carcinoma [17–20]. Recently, our group demonstrated a significant association between high expression of *AURKA* and cytogenetic profile in AML. According to our findings, *AURKA* expression was independently associated with high white blood cell counts, and the majority of AML patients who overexpressed *AURKA* and *AURKB* exhibited unfavorable cytogenetic abnormalities [21].

In the present study, we compared the expression profile of *AURKA* and *AURKB* in hematopoietic cells and MSCs of 60 MDS patients and 20 healthy donors. We also performed *G-banding*, spectral karyotype (SKY) analysis, and FISH on MSCs by using the most typical panel of FISH probes for MDS [inv(3q)(q21;q26.2), t(3;3)(q21;q26.2), −5/del(5q), −7/del(7q), +8 and del(20q)] and AURKA/B. Our results showed that significant differences were observed between the levels of expression of aurora *AURKA* and *AURKB* in MSCs compared with hematopoietic cells, healthy donors, and cytogenetic analysis.

Methods

Bone marrow samples

Bone marrow samples of 60 untreated MDS patients were selected for this study (22 men, 38 women; median age of 54 years; range, 28–85 years old). All samples were collected between May 2009 and June 2012. The patients selected for this study had no previous history of any other hematologic disorder and had a confirmed diagnosis of MDS by clinical and laboratory parameters. According to the World Health Organization classification [22], the majority of patients studied were classified as refractory cytopenia with multilineage dysplasia 34/60 (56%), followed by refractory anemia 11/60 (18%), refractory anemia with excess blasts I 11/60 (18%), and four cases of 5q syndrome (8%). The clinical and biological characteristics of the 60 MDS patients included in the present investigation are presented in Table 1. Normal bone marrow samples were obtained from 20 healthy donors (10 men, 10 women) with a median age of 52 years (Table 2). The MDS and healthy donor samples have been obtained from the University Hospital, Medical School of Ribeirão Preto, University of São Paulo, Brazil, and from other hematologic centers in the State of São Paulo, Brazil. The study was approved by the University Hospital Ethics Committee, and all individuals gave their written, informed consent before entering the study.

Mononuclear cells: cell culture and characterization

Mononuclear cells (MNCs) were isolated from bone marrow samples using Ficoll-Paque Plus (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) during initial diagnosis. Eight MNCs were cultured in α-MEM (Invitrogen, Carlsbad, CA, USA) supplemented with 15% heat-inactivated standard fetal bovine serum (HyClone, Logan, UT, USA), L-glutamine (2 mmol/L; Invitrogen), and 1% penicillin-streptomycin (Invitrogen). All MSCs (MDS and healthy donors) were collected after a third passage for subsequent cytogenetic analyses, FISH studies, and RNA isolation. The following monoclonal antibodies were used to characterize both MSCs: CD105-phycoerythrin (PE) (Serothec, Oxford, UK), CD73-PE, CD31-fluorescein isothiocyanate (FITC), CD45-FITC, CD14-PE, CD34-PE, HLA-Dr-FITC, CD90-PE, CD13-PE, CD140-PE, and CD146-PE (Becton Dickinson, San Jose, CA, USA).

Differentiation assay

Adipogenic and osteogenic differentiation was evaluated as described previously [23]. MSCs were cultured at $3 \times 10^4/cm^2$ in either osteogenic or adipogenic medium (Dulbecco modified Eagle medium [DMEM] with 10 mmol/LM β-glycerophosphate, 10^{-7} mol/L dexamethasone, and 0.2 mmol/L ascorbic acid and DMEM with 10% FCS, 10^{-6} mol/L dexamethasone, 50 μg/mL ascorbic acid, and 100 μg/mL 1-methyl-3-isobutyl-xanthine, respectively) for up to 3 weeks. After 22 days, differentiation to osteoblasts was observed by Von Kossa stain for calcium phosphate. Evidence of adipocytes differentiation was shown by oil-red staining for lipid.

Growth kinetics

The doubling time of MSCs from healthy donors ($n = 6$) was compared with those of aneuploidy MSCs from MDS ($n = 14$). Doubling time was calculated at each passage as described previously [24].

Cytogenetic analysis and FISH

Cytogenetic analysis of hematopoietic cells and MSCs was performed as described in standard protocols. MNCs were cultured for 72 hours in α-MEM (Invitrogen) supplemented with 15% heat-inactivated standard fetal bovine serum (HyClone, Logan, UT, USA) supplemented with 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin. The karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2009) [25]. The metaphase images were acquired by using an Axio Imager M2 microscope (Zeiss, Jena, Germany) equipped with the BandView software, version 5.5 (ASI, Carlsbad, CA, USA).

To confirm chromosomal abnormalities identified by *G-banding* in both hematopoietic cells and MSCs, SKY analysis was performed. We also applied a panel of “MDS FISH probe set” [inv(3q)(q21;q26.2), t(3;3)(q21;q26.2), −5/del(5q), −7/del(7q), +8 and del(20q)] on MSCs to search for specific abnormalities of MDS. The hybridization spots were evaluated using an Axio Imager M2 microscope (Zeiss) equipped with the FISHView software, version 5.5 (ASI). The cutoff levels for t(3;3)/inv(3q)

Table 1. Clinical and cytogenetic characteristics of MDS and MSC MDS-derived samples

	MDS				MXC						
							MSC - FISH analysis				
	WHO classification	Age/Sex	Hematopoietic cells: *G-banding* analysis	IPSS	MSC: *G-banding* analysis	MSC: SKY analysis	t(3;3)/inv(3q)	del(5q)	del(7q)	trissomy 8	del(20q)
1	RA	67/M	46,XY[20]	Low	44,XY,−9[5],−12[6]/46,XY[13]	44,XY,−9[3],−12[3]/46,XY[7]	neg	neg	neg	neg	neg
2		58/F	46,XX[20]	Low	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
3		57/F	46,XX[20]	Low	49,XX,+7[4],+15[6],+17[4]/ 46,XX[14]	49,XX,+7[2],+15[3],+17[4]/ 46,XX[5]	neg	neg	neg	neg	neg
4		43/F	46,XX[20]	Low	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
5		47/F	46,XX,del(7)(q22q36)[3]/46,XX[17]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
6		80/F	47,XX,+15[4]/46,XX[16]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
7		45/F	46,XX[20]	Low	45,XX,−5[4]/46,XX[16]	45,XX,−5[3]/46,XX[7]	neg	pos	neg	neg	neg
8		71/M	46,XY[20]	Low	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
9		67/M	46,XY[20]	Low	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
10		54/M	47,XY,+8[6]/46,XY[14]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
11		64/M	46,XY[20]	Low	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
12	RCMD	66/F	46,XX,inv(3)(q21q26)[7]/46,XX[13]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
13		77/F	46,XX,del(20)(q11)[7]/46,XX[13]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
14		85/F	46,XX,del(20)(q11)[6]/46,XX[14]	INT-1	45,X,−X[3]/46,XX[17]	45,X,−X[3]/46,XX[7]	neg	neg	neg	neg	neg
15		53/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
16		59/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
17		57/F	47,XX,+8[20]	INT-1	43,XX,−13,−19,−22[6]/ 46,XX[14]	43,XX,−13,−19,−22[4]/46,XX[6]	neg	neg	neg	neg	neg
18		72/F	46,XX,dup(1)(q32q21)[5]/46,XX[15]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
19		59/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
20		55/F	46,XX,del(20)(q11)[8]/46,XX[12]	INT-1	45,XX,−7[4]/46,XX[16]	45,XX,−7[4]/46,XX[6]	neg	neg	pos	neg	neg
21		52/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
22		61/F	46,XX[20]	INT-1	47,XX,+15[2]/46,XX[18]	47,XX,+15[2]/46,XX[8]	neg	neg	neg	neg	neg
23		71/F	46,XX[20]	INT-1	44,XX,−6[3],−19[3]/46,XX[14]	44,XX,−6[2],−19[3]/46,XX[5]	neg	neg	neg	neg	neg
24		59/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
25		66/F	46,XX,del(7)(q22)[8]/46,XX[12]	INT-2	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
26		53/F	46,XX[20]	Low	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
27		57/F	47,XX,+i(17)(q10)[6]/46,XX[14]	INT-2	47,XX,+12,t(14;15)(q24; q22)[7]/46,XX[13]	47,XX,+12,t(14;15)(q24; q22)[2]/46,XX[8]	neg	neg	neg	neg	neg
28		52/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
29		65/F	44,XX,−4[3],del(7)(p15)[2],del(12) (p12)[3]/46,XX[10]	INT-2	45,XX,−6[5]/46,XX[15]	45,XX,−6[3]/46,XX[7]	neg	neg	neg	neg	neg
30		71/M	46,XY,del(20)(q11)[6]/46,XY[14]	INT-1	44,XY,−4[6],−5[7]/46,XY[7]	44,XY,−4[3],−5[2]/46,XY[5]	neg	pos	neg	neg	neg
31		72/M	45–46,X,−Y[6],del(17)(p12)[4]/ 46,XY[16]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
32		69/M	46,XY[20]	INT-1	46,XY,i(12)(q10)[3]/46,XY[17]	46,XY,i(12)(q10)[3]/46,XY[7]	neg	neg	neg	neg	neg
33		72/M	47,XY,+8[7]/46,XY[13]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
34		67/M	46–47,XY,add(17)(p12)[4],+8[6]/ 46,XY[14]	INT-2	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
35		67/M	47,XY,+7[8]/46,XY[12]	INT-2	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
36		62/M	46,XY,del(13)(q12q14)[9]/46,XY[9]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
37		65/M	46,XY[20]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg

38		61/M	47,XY,+8[8]/46,XY[11]	INT-1	46,XY,del(13)(q24)[6]/46,XY[14]	46,XY,del(13)(q24)[3]/46,XY[7]	neg	neg	neg	neg	neg
39		62/M	47–48,XY,+5[3],+del(8)(q21)[4]/46,XY[16]	INT-2	44,X,−Y[4],−19[5]/46,XY[12]	44,X,−Y[3],−19[6]/46,XY[6]	neg	neg	neg	neg	neg
40		44/M	47,XY,+8[20]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
41		39/M	46,XY[20]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
42		27/M	48,XY,+8,+9[14]/46,XY[6]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
43		33/M	46,XY[20]	INT-1	45,XY−6[5]/46,XY[15]	45,XY−6[2]/46,XY[8]	neg	neg	neg	neg	neg
44		42/F	46,XX,del(20)(q10)[8]/46,XX[12]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
45		36/F	47,XX,+8[12]/46,XX[8]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
46	RAEB I	62/F	47,XX,+9[6]/46,XX[14]	INT-2	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
47		83/F	47,XX,+4[5]/46,XY[15]	INT-2	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
48		47/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
49		65/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
50		59/F	46,XX[20]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
51		74/M	45,X,−Y[8]/46,XY[12]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
52		58/M	46,XY[20]	INT-1	46,XY[20]	46,XY[10]	neg	neg	neg	neg	neg
53		59/M	46,XY,dup(1)(q21q32)[9]/46,XY[11]	INT-2	45,XY,−Y[4]/46,XY[16]	45,XY,−Y[3]/46,XY[7]	neg	neg	neg	neg	neg
54		61/F	47,XX,+8,del(20)(q13q13)[14]/46,XX[6]	INT-2	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
55		47/F	46,XX,del(7)(q11)[6]/46,XX[14]	INT-2	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
56		28/F	47,XX,inv(3)(p21q25),+21[20]	INT-2	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
57	5q Syndrome	58/F	46,XX,del(5)(q31)[4]/46,XX[16]	Low	47,XX,+9[6]/46,XX[14]	47,XX,+9[2]/46,XX[8]	neg	neg	neg	neg	neg
58		34/F	46,X,i(X)(p10),del(5)(q13q33)[16]/46,XX[4]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
59		28/F	46,XX,del(5)(q13q33)[20]	Low	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg
60		45/F	47,XX,del(5)(q22q33),+8[15]/46,XX[5]	INT-1	46,XX[20]	46,XX[10]	neg	neg	neg	neg	neg

FISH = fluorescence in situ hybridization; IPSS = International Prognostic Score System; MSC = mesenchymal stem cell; RA = refractory anemia; RAEB I = refractory anemia with excess blasts-1; RCMD = refractory cytopenia with multilineage dysplasia; SKY = spectral karyotype; WHO = World Health Organization.

Table 2. Cytogenetic characteristics of health donors (control group)

Health donors	Age (years)	Sex	Hematopoietic cells: *G-banding* analysis	MSC: *G-banding* analysis
1	57	F	46,XX[20]	46,XX[20]
2	58	F	46,XX[20]	46,XX[20]
3	47	F	46,XX[20]	46,XX[20]
4	43	F	46,XX[20]	46,XX[20]
5	57	F	46,XX[20]	46,XX[20]
6	50	F	46,XX[20]	46,XX[20]
7	45	F	46,XX[20]	46,XX[20]
8	61	F	46,XX[20]	46,XX[20]
9	57	F	46,XX[20]	46,XX[20]
10	44	F	46,XX[20]	46,XX[20]
11	54	M	46,XY[20]	46,XY[20]
12	56	M	46,XY[20]	46,XY[20]
13	47	M	46,XY[20]	46,XY[20]
14	45	M	46,XY[20]	46,XY[20]
15	53	M	46,XY[20]	46,XY[20]
16	49	M	46,XY[20]	46,XY[20]
17	37	M	46,XY[20]	46,XY[20]
18	52	M	46,XY[20]	46,XY[20]
19	59	M	46,XY[20]	46,XY[20]
20	55	M	46,XY[20]	46,XY[20]

F = Female; M = male.

(>2.5%), del(5q) (>3%), del(7q) (>2.4%), trisomy 8 (>2.5%), and del(20q) (>2.0%) were established according to the interphase FISH patterns observed in a group of 30 age- and sex-matched normal control peripheral blood samples studied with the same probes. The FISH probes used were purchased from Kreatech Diagnostics (Amsterdam, The Netherlands). For each sample, 300 interphase cells were viewed and counted. For SKY analysis, chromosome labeling was performed with the SKY fluorescent labeling kit (Applied Spectral Imaging, Migdal HaEmek, Israel) according to the manufacturer's protocol. A minimum of 20 metaphases were analyzed using the SkyView 5.5 software (ASI).

In addition, FISH for *AURKA* and *AURKB* amplifications was also performed according to the manufacturer's instructions, on interphase nuclei of both hematopoietic cells and MSCs using commercial probes (*AURKA*: on AURKA (20q13)/20q11; and *AURKB*: AURKB (17/p13)/SE17; Kreatech Diagnostics). AURKA and AURKB probes are designed as a dual-color assay to detect amplification at 20q13 and 17p13, respectively. Amplification involving these genes regions will show multiple red signals, whereas the controls (MPARE1 for AURKA and SE17 for AURKB), both located in the centromeric region of their chromosomes, will provide two green signals. The criteria used for *AURKA* and *AURKB* gene amplifications were based on the number of spots presented during analysis.

RNA extraction and analysis of AURKA and AURKB gene expression

Genomic RNA was isolated from both MNCs and MSCs using TRIzol reagent (Invitrogen) according to the manufacturer's recommendations. Complementary DNA (cDNA) was synthesized from ~1 μg of total RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) and following the manufacturer's instructions. For analysis of aurora kinase genes, primes and probes developed by Assay on Demand were used (*AURKA*: Hs00269212_m1; and *AURKB*: Hs00177782_m1; Applied Biosystems). The *AURKA* and *AURKB* genes and *GAPDH* messenger RNA, used as endogenous internal control for each sample, were analyzed in duplicate on the same MicroAmp optical 96-well plates using a 7500 Real-Time PCR System (Applied Biosystems).

Real-time quantitative polymerase chain reaction (PCR) assays were performed in a final reaction volume of 20 μL. The comparative cycle threshold (Ct) method was used to determine the relative expression level of *AURKA* and *AURKB* genes. On a comparative analysis of MDS, MSCs samples, and healthy donors (MNCs), *AURKA* and *AURKB* gene expression was calculated as a relative quantification to the *GAPDH* housekeeping gene. The gene expression of *AURKA* and *AURKB* from MDS and MSCs samples was calculated as relative quantification to normal controls ($\Delta\Delta Ct = \Delta Ct_{patient} - \Delta Ct_{healthy\ donors+}$) and expressed as $2^{-\Delta\Delta Ct}$.

Western blot analysis

MSCs from MDS-derived lysates and from healthy subjects were used for Western blot analysis to determine the AURKA and AURKB protein concentration. Protein electrophoresis was performed using 10% of sodium dodecyl sulfate polyacrylamide gel electrophoresis, and proteins were transferred to Hybond-P polyvinylidene diflouride membranes (Amersham Biosciences). The membranes were probed with rabbit anti-human Aurora-A antibody and Aurora-B antibody (Abcam, Cambridge, MA, USA; 1:500 dilution) and then probed again with anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal antibody (Ambion, Austin, TX, USA; 1:8000 dilution).

Statistics

Comparisons between different groups were made using the Student *t* test and the two sided exact Fisher test (dichotomous variables); $p < 0.05$ was considered significant.

Results

Cytogenetic and FISH in hematopoietic cell

Clonal chromosomal abnormalities were seen in 34 of 60 patients (57%), and normal karyotype was observed in 26 patients (43%). In all samples studied, the metaphase number was considered good, with more than 15 metaphases per slide (Table 1). Complex karyotype (≥3 abnormalities) was observed in only one patient (Fig. 1A, B), and most of the patients studied presented a single cytogenetic abnormality (24 of 60; 40%). Numerical abnormalities were seen in 18 of 60 (30%), with the extra copy of chromosome 8 being the most common abnormality. However, structural abnormalities were also present in 23 of 60 (38%) of the patients (Table 1). According to the ISCN criteria all abnormalities were clonal and confirmed by FISH.

Cytogenetic and FISH in MSCs

Among the MSC samples, 42 of 60 (70%) presented a normal karyotype, and 18 patients (30%) had

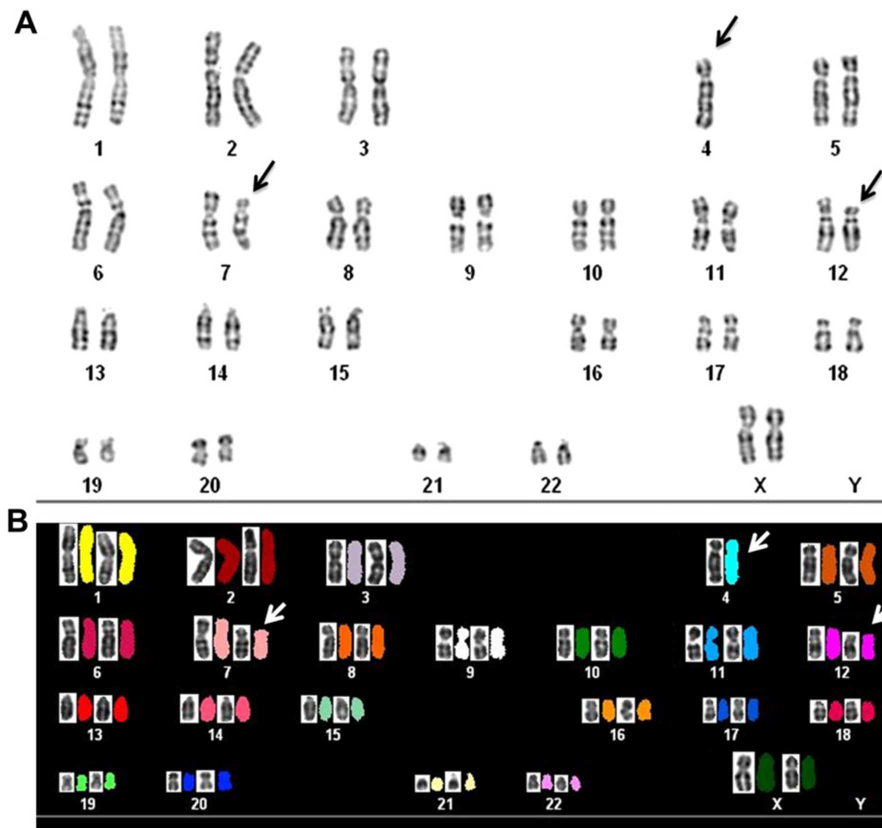

Figure 1. (**A**) *G-banding* analysis from bone marrow cells of a patient with MDS and complex karyotype. Cytogenetic analysis identified loss of chromosome 4, and deletions of 7p15 and 12p12, respectively (arrows). (**B**) Spectral karyotype (SKY) analysis of the same patient. For each chromosome, inverted DAPI (4,6-diamino-2-phenylindole; left profile) and classified image (right profile) are shown. SKY confirmed the same abnormalities as previously seen by *G-banding* (arrows).

chromosomal abnormalities (Table 1). As also seen in hematopoietic cells, the metaphase number in MSCs was considered satisfactory, with >10 metaphases per slide. Twenty metaphase cells were analyzed for each patient, with an estimated resolution of 450 bands per haploid set. Most of the chromosomal abnormalities seen in MSCs samples were numeric (14 of 18; 78%). In three patients, we found structural chromosomal abnormalities (Table 1). SKY analysis confirmed all alterations detected in MSCs (Table 1; Fig. 2). In addition, FISH analysis using a panel of probes for common abnormalities seen in MDS patients showed loss of chromosome 7 in only one MSC sample (Table 1). Considering the MSC group with abnormal karyotype, previous chromosomal abnormalities were seen in hematopoietic cells of 10 patients. However, the profile of cytogenetic abnormalities found was different. On the other hand, the six MDS patients with normal karyotype presented chromosomal abnormalities in MSCs (Table 1).

Cytogenetic analysis of MSCs of healthy donors

Classic cytogenetic analysis (*G-banding*) was performed in 20 MSC samples of healthy donors (median age, 52 years). The median number of passages was three, and all karyotypes were described according to the ISCN (2009) [25]. For each patient, 20 metaphase cells were analyzed, at an average resolution of 450 bands per haploid set. All samples displayed a normal karyotype (Table 2).

Growth kinetics

The average doubling times for MSCs from healthy donors (n = 6) and aneuploidy MSCs from MDS (n = 7) groups were 47.6 and 78.6 hours, respectively.

Expression of aurora kinase AURKA and AURKB

We compared the expression profile of *AURKA* and *AURKB* in hematopoietic cells, MSCs, and healthy donors. We stratified the patients according to the karyotype presentation (normal vs. abnormal), for each group (hematopoietic cells and MSCs), significant differences were observed (*AURKA* [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 3.6 ± 0.1 vs. 4.6 ± 0.2 [$p < 0.001$] in healthy donors vs. normal karyotype hematopoietic cells; *AURKA* [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 3.6 ± 0.1 vs. 7.9 ± 0.2 [$p < 0.001$] in healthy donors vs. normal karyotype MSCs; *AURKA* [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 4.6 ± 0.2 vs. 14.7 ± 0.5 [$p < 0.001$] in normal karyotype hematopoietic cells vs.

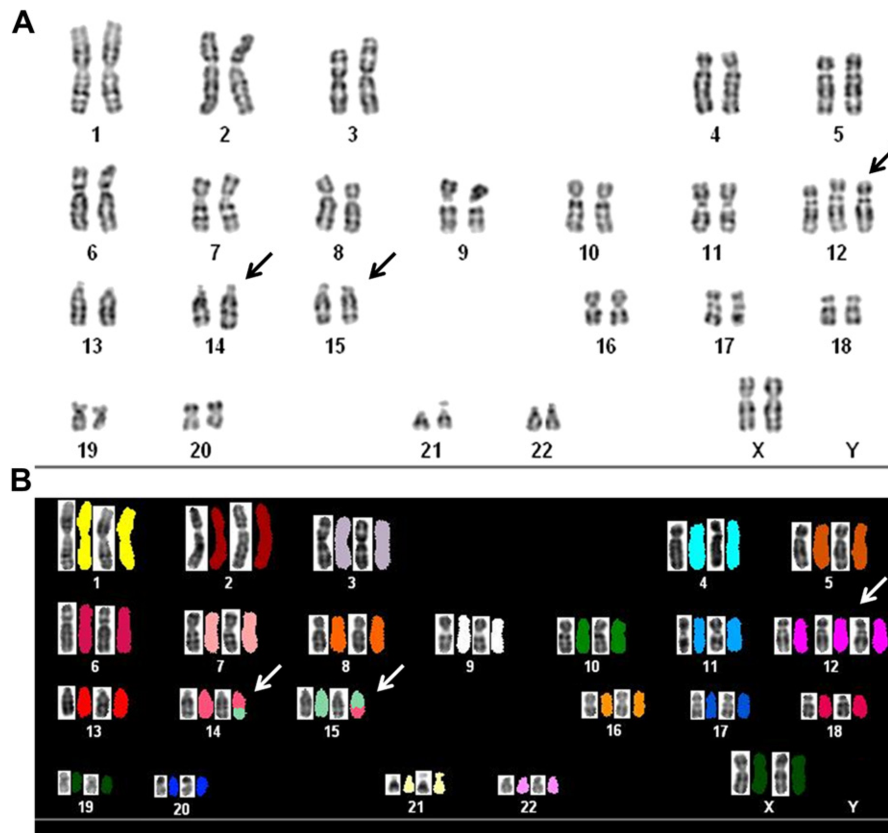

Figure 2. (**A**) *G-banding* analysis of a bone marrow stromal mesenchymal cells MDS derived. Cytogenetic analysis identified an extra copy of chromosome 12 and a balanced translocation involving chromosomes 14 and 15 (arrows). (**B**) Spectral karyotype (SKY) analysis of the same patient. For each chromosome, inverted DAPI (4,6-diamino-2-phenylindole; left profile) and classified image (right profile) is shown. SKY analysis confirmed the same abnormalities as seen previously by *G-banding* analysis (arrows).

abnormal karyotype hematopoietic cells; *AURKA* [mean value of $2^{-\Delta\Delta Ct}$ $\pm$ SD]: 7.9 $\pm$ 0.2 vs. 11.7 $\pm$ 0.49 [$p < 0.001$] in normal karyotype MSCs vs. abnormal karyotype MSCs; Fig. 3A). The higher expression of *AURKA* in hematopoietic cells and MSCs with altered karyotype versus normal karyotype in the same population was confirmed by FISH (Fig. 3B). Using the same stratification criteria for the patients as previously shown (*AURKA*), we found significant differences in healthy donors versus normal karyotype hematopoietic cells and normal karyotype MSCs for *AURKB*, respectively (*AURKB* [mean value of $2^{-\Delta\Delta Ct}$ $\pm$ SD]: 1.6 $\pm$ 0.05 vs. 2.8 $\pm$ 0.11 [$p < 0.001$] and vs. 3.08 $\pm$ 0.08 [$p < 0.001$]). However, when we compared normal versus altered karyotype for hematopoietic cells and MSCs, we found no difference between the groups (*AURKB* [mean value of $2^{-\Delta\Delta Ct}$ $\pm$ SD]: 2.83 $\pm$ 0.11 vs. 2.67 $\pm$ 0.07 [$p = 0.245$] in normal karyotype hematopoietic cells vs. abnormal karyotype hematopoietic cells and [mean value of $2^{-\Delta\Delta Ct}$ $\pm$ SD]: 3.08 $\pm$ 0.08 vs. 3.3 $\pm$ 0.08 [$p = 0.129$] in normal karyotype MSCs vs. abnormal karyotype MSCs; Fig. 4A). The results obtained were also confirmed by FISH (Fig. 4B).

Western blot analysis

Western blot analysis showed that AURKA and AURKB were expressed in all 18 MSCs (MDS derived), with abnormal karyotype and in 10 hematologically healthy donors. K562 leukemia cells were used as a positive control and the *GAPDH* gene was used as loading control. The intensities ratios of AURKA and AURKB, in regard to GAPDH expression, were analyzed in MSCs MDS-derived and MSCs-derived healthy donors. We observed that all MSCs MDS-derived with abnormal karyotype had higher levels of AURKA and AURKB expression compared with the control group ($p < 0.001$; Fig. 5).

Discussion

The presence of cytogenetic abnormalities in the MSCs of patients with hematologic diseases is controversial [12–15], and only few investigations have shown the presence of chromosomal abnormalities in MSCs of leukemia patients [12–15]. However, previous data have shown a distinctive gene-expression profile of MSCs of MDS and AML patients compared with MSCs of healthy donors using

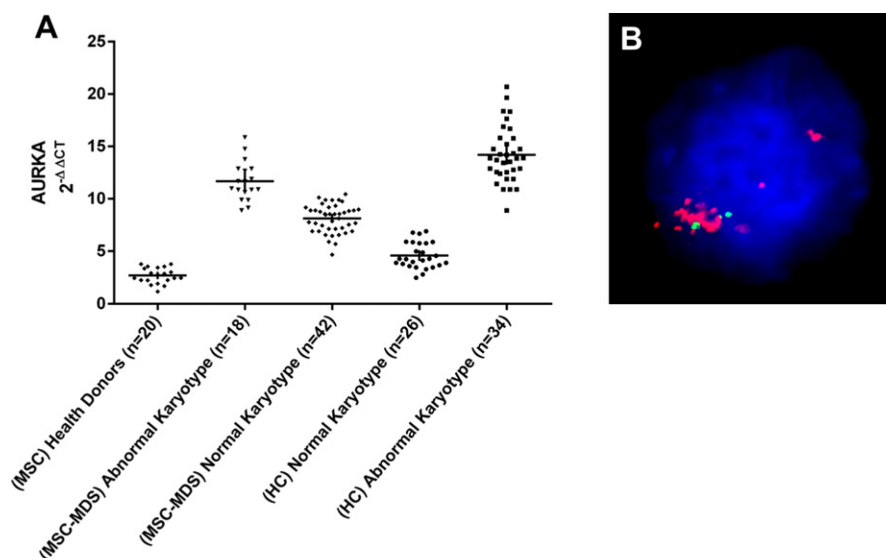

Figure 3. *AURKA* expression in mesenchymal stem cells (MSCs), healthy donor–derived MSCs, MDS-derived, and hematopoietic stem cells of myelodysplastic syndrome (MDS; HC), according to cytogenetic profile (normal vs. abnormal karyotype) and FISH analysis of representative samples. (**A**) *AURKA* gene expression by real-time quantitative polymerase chain reaction. The horizontal bars represent the mean value of gene expression relative to the *GAPDH* housekeeping gene for MDS/MSCs samples from adult healthy donors. The values of gene expression were presented as fold change ($2^{-\Delta\Delta Ct}$) using the relative expression of *AURKA* gene of bone marrow cells of heath donors as reference. (**B**) Interphase FISH analysis of *AURKA* gene demonstrating elevated DNA copy number in an MSC-MDS sample with abnormal karyotype (spots in red, control in green).

microarray technology [26]. In order to have more insight into this field, we investigated the expression profile of aurora kinase genes (*AURKA* and *AURKB*) and cytogenetic characterization of MSCs and hematopoietic cells of 60 MDS patients. We explored a differential gene expression profile in MSCs and compared these findings with abnormalities in hematopoietic cells and controls. MSCs of 20 healthy donors have been analyzed.

Aurora kinases (*AURKA*, *AURKB*, and *AURKC*) regulate mitosis and meiosis in all eukaryotes, and increasing evidence links these kinases to oncogenesis [27]. Our study showed deregulated *AURKA* and *AURKB* expression in MSCs from patients with MDS. We found that *AURKA* messenger RNA was expressed at significantly higher levels in MSCs, even with normal or altered karyotype, when compared with hematopoietic cells and healthy donors. In

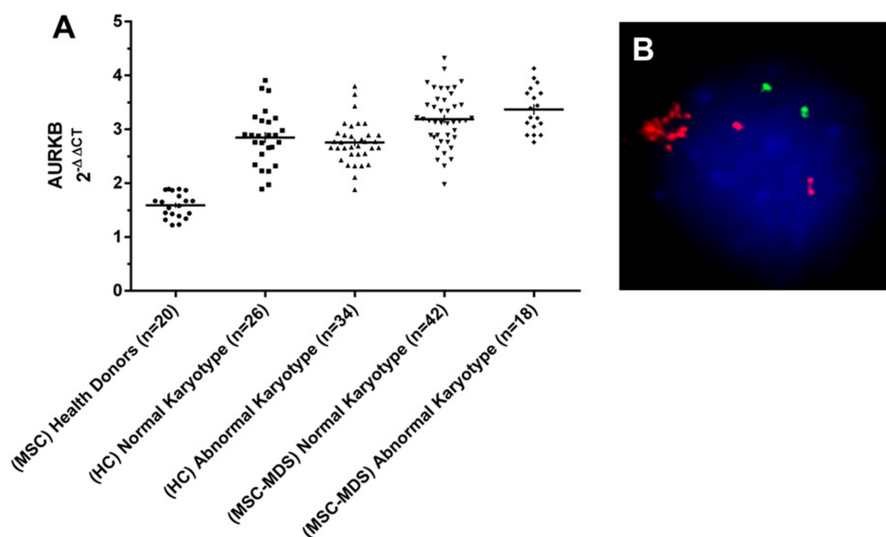

Figure 4. *AURKB* expression in mesenchymal stem cells (MSCs), healthy donor–derived hematopoietic stem cells of myelodysplastic syndrome (MDS; HC), and MSC MDS-derived samples according to cytogenetic profile (normal vs. abnormal karyotype) and FISH analysis of representative samples. (**A**) *AURKB* gene expression by real-time quantitative polymerase chain reaction. The horizontal bars represent the mean value of gene expression relative to *GAPDH* housekeeping gene for MDS/MSC samples and for adult healthy donors. The values of gene expression were presented as fold change ($2^{-\Delta\Delta Ct}$) using the relative expression of the *AURKB* gene in bone marrow cells of heath donors as reference. (**B**) Interphase FISH analysis of *AURKB* gene demonstrating elevated DNA copy number in an MSC-MDS sample with abnormal karyotype (spots in red, control in green).

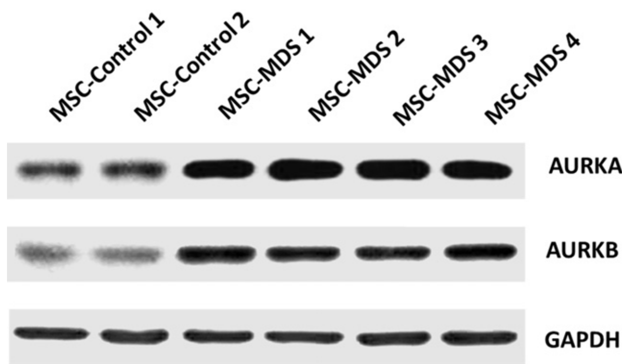

Figure 5. Analysis of AURKA and AURKB expression by Western blot analysis. Mesenchymal stem cells (MSC) MDS-derived lysates and MSCs samples of healthy subjects were used to determine the AURKA and AURKB protein concentration. The results show the distribution of patients and controls in terms of AURKA and AURKB levels expressed as the ratio to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

addition, it is clear that the presence of chromosomal abnormalities (mainly aneuploidy) in hematopoietic cells or MSCs was also associated with higher levels of *AURKA*. FISH analysis using an *AURKA* probe also confirmed the differential expression profile obtained in samples showing multiples copies of the gene labeled in red (Fig. 4). Previous studies have shown that *AURKA* overexpression is associated with the aneuploidy karyotypes, carcinogenesis, and drug resistance in many malignant tumors in humans [27–31]. However, the overexpression itself in primary cells is not sufficient to induce cellular transformation, indicating that *AURKA* requires additional oncogenic events, such as activated Ras-signaling to promote cell transformation [32].

Although some studies indicated that MSCs cells from MDS patients with normal karyotype are functional, other investigations presented evidence that chromosomal abnormalities in this specific group of MSCs are not random events [12–15]. Different from previous investigations, our findings regarding *AURKA* expression support the hypothesis that the presence of chromosomal abnormalities in MSCs MDS-derived is not a consequence of the method used for chromosome preparation. They may reflect the genomic instability present in the bone marrow microenvironment of these patients. This information is also supported by differences observed in the growth kinetics between MSCs from healthy donors (normal karyotype) and from MDS patients with abnormal karyotype in our investigation. Differential expression of *AURKA* leads to an increase in centrosome numbers, causes loss or gain of chromosomes, and results in either cell death or survival by malignant transformation [27,28]. According to Torres et al. [33], yeast strains that contain one or more additional chromosomes grow more slowly than their haploid counterparts. In addition, mouse cells modified to become trisomic for a specific chromosome exhibit a proliferation delay, compared with human fibroblasts derived from individuals with Down syndrome [34].

On the other hand, the role of *AURKB* in the neoplastic process is not clear. Different from *AURKA*, *AURKB* is located on a chromosomal region that has not been associated with amplification in human neoplasias (17p13.1) [27,28]; however, the overexpression of *AURKB* in certain tumor types has been described [35]. Our results showed no significant differences between hematopoietic cells with normal versus abnormal karyotype or between MSCs group (normal vs. abnormal karyotype). However, when comparisons were made between healthy donors and hematopoietic and MSCs cells, a small but statistical difference was noted. Currently, it is not clear whether the overexpression of *AURKB* is related to the high proliferative index of neoplastic cells or to the origin of the tumorigenesis process [27,28,35].

This investigation is the first attempt to correlate the gene expression profile of *AURKA* and *AURKB* in a cytogenetically stratified population of MSCs from MDS patients. Despite the differences observed among the gene expression obtained, some observations are important. In MSCs with chromosomal abnormalities, the karyotypes observed were totally different from those observed in their hematopoietic cells. We also performed FISH analysis on MSCs by using probes with the main abnormalities screened in MDS patients [inv(3q)(q21;q26.2), t(3;3)(q21;q26.2), −5/del(5q), −7/del(7q), +8 and del(20q)]; none of them were detected in MSCs. Unfortunately, there is no explanation for that. However, considering the fact that the chromosomal abnormalities seen in MSCs by *G-banding* analysis were also confirmed by SKY, two additional observations should be made: the chromosomal abnormalities showed in MSCs, MDS-derived are not random and the presence of abnormal karyotypes is an important evidence of genomic instability. In this study, MSCs from healthy donors presented a normal karyotype. Data obtained from other studies have been shown a normal karyotype in MSCs from healthy donors or patients with no hematologic disease [12,13,15]. Based on our findings, our results might not be considered evidence that MDS and MSCs are originated from a single neoplastic clone. However, it is possible to consider that hematopoietic stem cells and MSCs may share numerical or structural chromosomal abnormalities that are beyond the limited resolution of *G-banding* (∼10Mb), SKY analysis (∼2 Mb), and FISH analysis (∼500 Kb). In fact, both cells (hematopoietic and MSCs) are probably altered in response to damage-inducing factors, and the presence of genomic abnormalities in MSCs suggests that an unstable bone marrow microenvironment may facilitate the expansion of MDS or leukemic cells.

In summary, in our investigation we demonstrated a differential expression profile of *AURKA* and *AURKB* in a cytogenetically stratified group of hematopoietic cells and MSCs compared with healthy donors. Although we

observed a distinct distribution of chromosomal abnormalities between hematopoietic cells and MSCs, the clonal profile of abnormalities seen in the MSCs was confirmed by SKY analysis, assuming a nonrandom characteristic in this group. Additional studies using methodologies, such as arrayCGH, are needed to search for suitable common genomic abnormalities in MSCs and MDS that may predict a leukemic transformation pathway.

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Author contributions: F.M.O. set up and oversaw the project, defined the experimental design, performed the different manipulations, analyzed the data, and wrote the first draft of the manuscript; A.R.L-A. performed and analyzed the gene expression profile data; M.C.F. did the clinical management and provided samples and the clinical data of their patients; B.P.S. did the clinical management and provided samples and the clinical data of their patients; P.V.B.P. performed the immunophenotypic characterization of MSCs; E.M.R. did the clinical management and provided samples and the clinical data of their patients; R.P.F. is the supervisor of F.M.O., supervised the whole project, and reviewed and revised the manuscript; D.T.C. supervised the whole project and reviewed and revised the manuscript; A.M.F. supervised the whole project and reviewed and revised the manuscript.

Conflict of interest disclosure

No financial interest or relationships with financial interest relating to the topic of this article have been declared.

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