Aurora-B expression may not contribute to disease progression: a reflection of the heterogeneous pathogenesis?

We read with great interest the paper by Yoshida *et al.* entitled "Marked upregulation of survivin and Aurora-B kinase are associated with disease progression in the myelodysplastic syndromes".[1]

Aurora kinases are key players in ensuring accurate chromosome segregation during the cell cycle, maintaining genetic integrity in cell division.[2] Over the last few years, much attention has been focused on the involvement of these proteins in the process of tumorigenesis. High Aurora-B and Aurora-A expression has been reported in various types of commonly occurring malignancy and, in some cases, correlated with aneuploidy and poor prognosis. Despite the many reports of high expression of Aurora-B and Aurora-A in solid tumors,[3,4] information regarding expression in hematologic malignancies is still limited,[5,6,7] especially in MDS.

Our study included 61 MDS patients, 31 male and 30 female (median age 66 years; range 15-91 years). According to WHO classification, there were 6 patients with refractory anemia (RA), 2 patients with refractory neutropenia (RN), 2 patients with isolated del(5q), 9 patients with ring sideroblasts (RARS), 28 patients with refractory cytopenia with multilineage dysplasia (RCMD), 3 patients with RA with excess of blasts 1 (RAEB-1), 5 patients with RA with excess of blasts 2 (RAEB-2), and 6 patients with therapy-related MDS. Four samples from healthy volunteers were used as controls. Among these patients, 85% were stratified as low risk and 15% as high risk according to IPSS.

Total RNA from MDS patients and donor bone marrow cells were isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA). To analyze aurora kinases genes, TaqMan Assays were used (Aurora-A: Hs00269212_m1 and Aurora-B: Hs00177782_m1; Applied Biosystems). β2-microglobulin (B2M: Hs99999907_m1) and Ubiquitin C (UBC: Hs00824723_m1) were chosen as endogeneous internal control for each sample. The comparative cycle threshold (Ct) method was used to determine the relative expression levels of Aurora-B and Aurora-A genes. Their

Table 1. Age, sex, WHO and IPSS classification, karyotype and gene expression of 61 MDS patients.

Case	Age/Sex	WHO	IPSS	Karyotype	Aurora-A gene expression ($2^{-\Delta\Delta Ct}$)	Aurora-B gene expression ($2^{-\Delta\Delta Ct}$)
1	84/F	RA	NA	No metaphases	0.05292	0.06832
2	29/M	RA	L	46,XY	0.02659	0.04126
3	72/F	RA	L	46,XX[6]	0.06416	0.06501
4	82/M	RA	NA	No metaphases	0.12341	0.16405
5	47/F	RA	L	46,XX[8]	0.02173	0.01533
6	64/F	RA	NA	No metaphases	0.00536	0.01268
7	15/M	RN	L	46,XY,del(5)(q22q33)[5]/46,XY[7]	0.01575	0.01626
8	23/F	RN	L	46,XX,del(5)(q15q33)[4]/46,XX[18]	0.21428	0.21872
9	69/M	5q-	I-1	46,XY,del(5)(q15q33)[7]/46,XY[11]	0.03151	0.07029
10	58/M	5q-	I-1	46,XY,del(5)(?q15q33)[8]/46,XY[12]	0.01459	0.01722
11	28/F	RARS	L	46,XX[20]	0.06732	0.16031
12	91/M	RARS	L	46,X-Y[4]/46,XY[16]	0.03619	0.04803
13	83/M	RARS	I-1	46,XY,del(5)(?q22q33)[3]/45,XY,del(5)(?q22q33),-18[3]/46,XY[14]	0.00682	0.00740
14	42/F	RARS	NA	No metaphases	0.04953	0.03923
15	66/F	RARS	NA	No metaphases	0.06127	0.04695
16	84/F	RARS	NA	No metaphases	0.01393	0.02615
17	81/F	RARS	L	46,XX[5]	0.05356	0.04556
18	73/F	RARS	L	46,XX[12]	0.02148	0.02973
19	74/M	RARS	L	46,XY[6]	0.13400	0.14854
20	24/M	CRDM	I-1	46,XY[9]	0.00924	0.00481
21	41/F	CRDM	NA	No metaphases	0.01315	0.00672
22	32/M	CRDM	I-1	46,XY,del(5)(q15q33),del(17)(p11.2)[7]/46,XY[13]	0.05764	0.05981
23	33/M	CRDM	I-1	46,XY,del(5)(?q15q33)[7]/46,XY[8]	NE	0.03880
24	74/M	CRDM	I-1	46,XY[5]	0.02756	0.03774
25	45/M	CRDM	I-1	47,XY,+mar[3]/46,XY,[17]	0.16567	0.10740
26	67/F	CRDM	I-1	46,XX[7]	0.03086	0.04387
27	85/M	CRDM	NA	No metaphases	0.04821	0.05046
28	40/M	CRDM	I-1	47,XY,+8[4]/46,XY[5]	0.02477	0.01236

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29	66/M	CRDM	I-1	47,XY,+mar[5],46,XY[11]	0.02797	0.02891
30	45/M	CRDM	I-1	46,XY,-?10,+mar[5]/46,XY[1]	0.34952	0.11750
31	73/M	CRDM	I-2	46,XY,del(7)(q32)[2]/46,XY[18]	0.03029	0.02722
32	77/M	CRDM	I-2	46,XY,add(13)(p11)[12]/46,XY,del(7)(q32),add(13)(p11)[4]/48,XY,add(13)(p11),+22,+mar[9]/48,XY,del(7)(q32),add(13)(p11),+22,+mar[3]/46,XY[2]	0.05956	0.10687
33	86/M	CRDM	NA	No metaphases	0.02842	0.02770
34	79/M	CRDM	I-1	89,XXY,-20,-22,-Y[4]/46,XY,del(16)(?q22)[5]/46,XY[11]	0.02157	0.04396
35	59/F	CRDM	NA	No metaphases	0.00183	0.00063
36	54/M	CRDM	NA	No metaphases	0.00078	0.00050
37	46/M	CRDM	I-1	46, XY[8]	0.09016	0.10810
38	57/F	CRDM	L	46,XX[20]	NE	0.00307
39	72/F	CRDM	I-1	46,XX[25]	0.02746	0.06693
40	81/F	CRDM	I-1	46,XX[20]	0.16853	0.20710
41	41/F	CRDM	I-1	46,XX,del(5)(q15q33)[9]/46,XX,del(5)(q15q33),del(11)(?q25)[7]/46,XX[4]	0.04916	0.04557
42	57/F	CRDM	I-1	46,XX[8]	0.08919	0.05943
43	41/F	CRDM	I-1	44,XX-13,-17[4]/46,XX[5]	0.00765	0.00947
44	55/F	CRDM	NA	No metaphases	0.00642	NE
45	74/M	CRDM	I-1	46,XY[11]	0.07445	0.03196
46	77/F	CRDM	NA	No metaphases	0.04174	0.03994
47	81/F	CRDM	I-1	47,XX,t(4;11)(q27;q32),+mar[4]/46,XX[16]	0.03511	0.05229
48	67/M	RAEB-1	I-1	46,XY[20]	NE	0.09912
49	89/M	RAEB-1	I-1	46,XY,del(5)(q31q35)[6]/46,XY[17]	0.02697	0.06070
50	66/M	RAEB-1	NA	No metaphases	0.06464	0.05335
51	63/M	RAEB-2	H	47,XY,+8[6]/47,XY,del(7)(q32),+8[7]/46,XY[2]	0.14447	0.12942
52	30/F	RAEB-2	H	90,XXXX,-6,-7,-8,11,+21,+22[5]/46,XX,del(7)(q23),del(20)(q13.1)[3]/45,XX,-7[5]/45~46,XX,-7,del(7)(q32),del(11)(q32),-17,del(17)(p11.2),del(20)(q13.1)[cp11]	0.00379	0.00416
53	49/M	RAEB-2	I-2	47,XY,+mar[6]/48,XY,+8,del(16)(?q22),+mar[4]/47~50,XY,del(4)(?q35),+8,+10,+11,del(16)(?q22),+21,+mar[cp8]	0.05414	0.05448
54	64/M	RAEB-2	I-2	37,X,-2,-3,-9,-11,-12,-15,-16,-18,-Y[8]/46,XY,del(5)(q15q33)[5]/46,XY[6]	0.08179	0.09465
55	72/M	RAEB-2	NA	No metaphases	0.05196	0.04295
56	65/F	MDS T-related	NA	46,XY[9]	0.02074	0.02837
57	74/F	MDS T-related	NA	No metaphases	0.02003	0.01317
58	18/F	MDS T-related	NA	46,XX[22]	0.08106	0.10104
59	27/F	MDS T-related	NA	46,XX,del(17)(p11.2)[3]/46,XX[4]	0.01318	0.01184
60	82/F	MDS T-related	NA	46,XX[11]	0.01649	0.02942
61	71/F	MDS T-related	NA	175,XXXXXXXX,-5,-6,-7,-8,-9,-11,-13,-14[4]/46,XX,del(5)(q15q33)[8]/46,XX[19]	0.03598	0.03653

WHO: World Health Organization; IPSS: International Prognosis Scoring System; F: female; M: male; L: low risk; I-1: intermediate risk 1; I-2: intermediate risk 2; H: high risk; RA: refractory anemia; RN: refractory neutropenia; 5q-: del(5q); RARS: refractory anemia with ring sideroblasts; RCDM: refractory cytopenia multilineage dysplasia; RAEB: refractory anemia with excess of blasts; MDS T-related: MDS therapy related (i.e. secondary to treatment); NA: not applied; NE: not evaluated.

expression was calculated as a relative quantification to the average value of B2M and UBC housekeeping genes. The Mann-Whitney and Kruskal-Wallis rank sum test was used for quantitative analysis comparing the expression level of aurora kinases in different groups.

Successful cytogenetic analyses (by G-banding) were available for 45 patients (74%). Of these, 27 (60%) were classified as having good IPSS cytogenetic risk, 10 (22.2%) as having intermediate risk, and 8 (17.8%) as poor risk. Among these 45 patients, at least one clonal alteration was observed in 25 cases (55.6%) (Table 1).

Although many studies have reported high expression of Aurora-B and Aurora-A in solid and hematologic cancers, in our study we did not find any association between clinical or laboratory features with prognostic impact (number of cytopenias, cytogenetic abnormalities, transfusion dependency, WHO classification and IPSS group) and the expression of both Aurora-B and Aurora-A kinases.

Recently, Lucena-Araujo *et al.*[5] showed a significant association between high expression of Aurora-A and unfavorable cytogenetics in AML patients, but not with

Table 2. Comparison of MDS patients' gene expression according to prognostic groups.

	Aurora-A gene expression ($2^{-\Delta\Delta Ct}$)				Aurora-B gene expression ($2^{-\Delta\Delta Ct}$)			
	Minimum	Median	Maximum	*P*	Minimum	Median	Maximum	*P*
WHO groups:								
- Low risk (RA, RARS, RCMD)	0.00078	0.03511	0.34952	**0.84**	0.00050	0.04257	0.20710	**0.65**
- High risk (RAEB-1, RAEB-2)	0.00379	0.05413	0.14447		0.00416	0.05758	0.12941	
- MDS treatment-related	0.01318	0.02039	0.08106		0.01184	0.02890	0.10104	
- Control group	0.01169	0.02742	0.03374		0.01152	0.03094	0.04192	
Low risk groups:								
- Refractory anemia (RA)	0.00536	0.03975	0.12341	**0.93**	0.01268	0.05314	0.16405	**0.77**
- Refractory anemia with ring sideroblsts (RARS)	0.00682	0.04953	0.13400		0.00740	0.04556	0.16031	
- Refractory cytopenia with multilineage dysplasia (RCMD)	0.00078	0.03058	0.34952		0.00050	0.03994	0.20710	
IPSS groups:								
- Low risk (Low, Int-1)	0.00078	0.03299	0.34952	**0.97**	0.00050	0.04210	0.20710	**0.67**
- High risk (Int-2, High)	0.00379	0.05685	0.14447		0.00416	0.07456	0.12942	

Aurora-B. Furthermore, Ye *et al.*[6] analyzed 20 patients with MDS and did not detect any significant correlation of Aurora-A gene expression with bone marrow blast counts, viability of CD34+ blast cells, cytogenetic abnormalities or IPSS score.

Our study included a good representative number of lower risk patients and this may have contributed to the absence of any statistical difference among groups (Table 2). However, no difference was found when only the lower risk groups (RA, RARS, RCMD) were compared, in contrast to that reported by Yoshida *et al.*[1]

One possible explanation for these different results could be related to the genetic variation between study populations. Some studies showed that the difference in the incidence of chromosomal abnormalities in MDS patients varies according to the group under study (low *vs*. high risk), with possible geographical and ethnic influences.[8] According to the Brazilian National MDS Register,[9] Magalhaes observed significant differences between other American, European and Asian reports, and even between Brazilian geographical regions; indicating that racial miscegenation might play a role.

Despite the limited information regarding the expression of Aurora-B kinase in hematologic malignancies, our data suggest that Aurora-B expression may not have a prognostic significance in MDS patients, as shown in other tumors. This may reflect the heterogeneous presentation of MDS and the diversity of the mechanisms involved in its pathogenesis.

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Key words: survivin, Aurora-B kinase, myelodysplastic syndromes, heterogeneous.

Citation: Fernandez Heredia F, de Sousa JC, Carvalho AF, Meira Magalhaes SM, Pinheiro RF. Aurora-B expression may not contribute to disease progression: a reflection of the heterogeneous pathogenesis. Haematologica 2012;97(10):e37-39. doi:10.3324/haematol.2012.068296

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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