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ORIGINAL ARTICLE

CIP2A expression in Bortezomib-treated multiple myeloma

Ahmed M. L. Bedewy, Shereen M. Elmaghraby

Hematology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt

Summary

Purpose: *This study aimed to investigate the expression and the prognostic value of CIP2A in multiple myeloma (MM).*

Methods: *The expression levels of CIP2A was measured in 33 newly diagnosed MM patients (at presentation and after 4 cycles of Bortezomib-Dexamethazone (BD) regimen) and 15 healthy controls by real time quantitative polymerase chain reaction (QRT-PCR).*

Results: *CIP2A expression was upregulated in MM patients compared to controls. There was a significant reduction in CIP2A expression after treatment with BD regimen. Patients with expression levels ≤ 16.45 EU (expression unit) were more likely to respond to BD regimen (23 patients out of 23) than those with expression level >16.45 (6 patients out of 10) (p=0.005). Lower progression-free survival (PFS) (16.7%) was observed among patients with high CIP2A expression levels compared to 50% PFS in patients with lower CIP2A expression levels (p=0.006).*

Conclusion: *CIP2A is upregulated in MM and bortezomib downregulated its expression. High CIP2A level is associated with shorter PFS and poor response to BD in MM. Therefore, beside its value as a poor prognostic indicator in MM, CIP2A suppression might be a fruitful future targeted therapeutic agent aiming to improve the outcome in MM.*

Key words: *multiple myeloma, CIP2A, bortezomib, real time-PCR*

Introduction

Multiple myeloma (MM) is a hematological malignancy of plasma cells. Different stages of differentiation of the neoplastic clone may cause heterogeneity in MM [1]. MM is relatively uncommon but can be fatal. The crude incidence rate of MM in Egypt among males was 0.6%, and 0.4% among females in the period between 2008 and 2011 [2]. The initial response to novel drugs in MM is satisfactory. However, patients eventually relapse and succumb to disease after several months or years [1,3]. Therefore, the need for exploration of new therapeutic targets and agents is deep to improve the survival of patients with MM.

Cancer inhibitor of protein phosphatase 2A (CIP2A) is a tumor associated antigen and has been demonstrated in patients with different types of cancer. CIP2A overexpression was reported in various solid and hematological tumors [4,5]. CIP2A is a novel human oncoprotein that can prevent c Myc dephosphorylation through inhibiting protein phosphatase 2 activity [6]. CIP2A stabilizes c-Myc protein by inhibiting its degradation mediated by PP2A in cancer cells [7]. In addition to inhibiting c-Myc degradation, CIP2A is regulated in a positive feedback loop with c-Myc by promoting each other's expression [8].

The present study aimed to evaluate the expression levels of CIP2A in patients with MM at presentation and after treatment with bortezomib-dexamethazone, and its relation to disease characteristics and treatment outcome.

Corresponding author: Ahmed M. L. Bedewy, MD. Medical Research Institute, Alexandria University
Abraj Al-Shaker, Zaky Ragab street, Smouha, Alexandria.
Tel: +201000040511, Fax: +2035222602, Email: dr_ahmed_bedewy@yahoo.com
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Methods

Patients

MM patients' samples were obtained after signing informed consents from September 2014 to October 2015. Thirty-three treatment-naïve MM patients and 15 healthy controls (age ranged 30-55 years, median 40), with no current or previous diagnosis of hematological malignancy, were enrolled. The diagnosis of MM patients was determined using the International Myeloma Working Group 2012 system [9]. Patients with Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2, or grade 2 or more peripheral neuropathy were excluded from the study [10,11]. All patients received bortezomib-dexamethasone (BD) regimen (four 3-week cycles of bortezomib1.3 mg/m^2 intravenous bolus days 1, 4, 8, and 11 plus dexamethasone 40 mg days 1 to 4 (all cycles) and days 9 to 12 (cycles 1 and 2) [12]. The response was evaluated after 4 cycles of BD [13]. Responders were followed up to evaluate PFS.

Methods

CD138+ cells from MM patients and controls were isolated from BM mononuclear cells using EasySep™ Human CD138 Positive Selection Kit II and EasySep™ magnet (STEMCELL Technologies, Vancouver, Canada).

RNA preparation and real-time quantitative PCR

Total RNA was extracted from the isolated CD138+ cells using the Trizol reagent (Invitrogen, CA, USA). RNA was reverse transcribed to complementary DNA using a REVERTAid FIRST-STRAND cDNA Synthesis Kit (Thermo Fisher, CA, USA). RT qPCR was performed using SYBR Green Master mix (Applied Biosystems, CA, USA) on a StepOne Real-Time PCR system (Thermo Fisher, CA, USA). The thermocycling conditions were as follows: 40 cycles of 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec. For QPCR, we used CIP2A gene forward primer 5'-TGCGGCACTTGGAGGTAATTTC-3', CIP2A gene reverse primer5'-AGCTCTACAAGGCAACTCAAGC-3'; GAPDH forward primer 5'- TGTTGCCATCAATGACCCCTT-3', GAPDH reverse primer 5'- CTCCACGACGTACTCAGCG-3'(14). The expression of the CIP2A gene was examined by real-time quantitative PCR (QPCR) normalized to the expression of GAPDH. The experiments were performed in duplicate and gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1. CIP2A expression of the multiple myeloma patients at presentation in relation to the studied parameters

Parameters		*CIP2A expression*		*Test of significance*	*p*
		Below median (n=16)	*Above median (n=17)*		
Sex				x^2=0.308	0.728
Male, n (%)	19 (57.6)	10 (62.5)	9 (52.9)		
Female, n (%)	14 (42.4)	6 (37.5)	8 (47.1)		
Age (years)				t=0.104	0.918
Median (Min-Max)	63 (51-75)	64.5 (51-74)	62 (58-75)		
Mean ± SD	64.7 ± 6.1	64.8 ± 6.8	64.9 ± 5.5		
Hemoglobin level (g/dl)				U=125.50	0.705
Median (Min-Max)	8.9 (4.8-10.6)	8.5 (5.4-10.6)	9 (4.8-10.1)		
Mean ± SD	8.4 ± 1.4	8.3 ± 1.6	8.6 ± 1.3		
Plasma cell %				U=112.0	0.387
Median (Min-Max)	36 (12-81)	35 (12-78)	39 (17-81)		
Mean ± SD	39.8 ± 19.9	36.5 ± 19	42.8 ± 20.7		
Serum albumin (g/dl)				t=0.565	0.576
Median (Min-Max)	3.3 (2.7-4.1)	3.3 (2.7-4.1)	3.1 (2.7-4)		
Mean ± SD	3.3 ± 0.5	3.4 ± 0.5	3.3 ± 0.5		
Serum calcium (mg/dl)				t=0.424	0.675
Median (Min-Max)	10.1 (8.4-12.1)	10.3 (8.4-11)	10 (8.4-12.1)		
Mean ± SD	10.1 ± 0.8	10.2 ± 0.7	10 ± 0.9		
Serum calcium				0.762	0.311
Hypercalcemia	10	6	4		
No hypercalcemia	23	10	13		
Serum B_2M (μg/ml)				U=115.0	0.449
Median (Min-Max)	4.1 (0.9-8.2)	4.1 (0.9-8.2)	4.5 (0.9-6.2)		
Mean ± SD	3.9 ± 1.9	4.3 ± 2	3.6 ± 1.8		

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Parameters		*CIP2A expression*		*Test of significance*	*p*
		Below median (n=16)	*Above median (n=17)*		
International staging system (ISS), n (%)				x^2=2.072	MCp=0.363
I	15 (45.5)	7 (43.8)	8 (47.1)		
II	11 (33.3)	4 (25)	7 (41.2)		
III	7 (21.2)	5 (31.3)	2 (11.8)		
Revised ISS, n (%)				x^2=1.594	MCp=0.507
I	12 (36.4)	7 (43.8)	5 (29.4)		
II	18 (54.5)	7 (43.8)	11 (64.7)		
III	3 (9.1)	2 (12.5)	1 (5.9)		
Serum LDH, n (%)				x^2=0.510	FEp=0.688
Normal	25 (75.8)	13 (81.3)	12 (70.6)		
High	8 (24.2)	3 (18.8)	5 (29.4)		
High risk cytogenetics (del 17p, t(4;14) or t(14,16)), n (%)				x^2=0.113	FEp=1.000
No	26 (78.8)	13 (81.3)	13 (76.5)		
Yes	7 (21.2)	3 (18.8)	4 (23.5)		
M band type, n (%)				x^2=1.962	MCp=0.517
IgG	24 (72.7)	13 (81.3)	11 (64.7)		
IgA	7 (21.2)	3 (18.8)	4 (23.5)		
Light chain	2 (6.1)	0 (0)	2 (11.8)		
Involved Light chain type, n (%)				x^2=0.061	0.805
Kappa	22 (66.7)	11 (68.8)	11 (64.7)		
Lambda	11 (33.3)	5 (31.3)	6 (35.3)		
Response to BD				x^2=4.284	FEp=0.103
No response	4 (12.1)	0 (0)	4 (23.5)		
Response	29 (87.9)	16 (100)	13 (76.5)		
CR	10 (30.3)	5 (31.3)	5 (29.4)		
PR	19 (57.6)	11 (68.8)	8 (47.1)		
NR	4 (12.1)	0 (0)	4 (23.5)		
CIP2A expression after BD, n (%)				x^2=4.192	MCp=0.137
Below median	16 (48.5)	11 (68.8)	5 (29.4)		
Above median	17 (51.5)	5 (31.3)	12 (70.6)		
Min-Max	1.4 (0-247.4)	1 (0-14.3)	10 (0-247.4)	U=55.500*	0.004*
Mean ± SD	24.4 ± 59.3	2.1 ± 3.7	45.5 ± 77.8		

x^2: chi square test, MC: Monte Carlo test, FE: Fisher's Exact test, U: Mann Whitney U test, t: Student t-test *Statistically significant at p ≤ 0.05

Table 2. CIP2A expression of the studied multiple myeloma patients at presentation in relation to the response to bortezomib-dexamethazone regimen

		CIP2A expression		*Total*	*p*
		≤16.45 EU n	*> 16.45 EU n*		
Response to BD	CR	5	5	10	<0.001*
	PR	18	1	19	
	NR	0	4	4	
	Total	23	10	33	
Response to BD	Response	23	6	29	0.005*
	No response	0	4	4	
	Total	23	10	33	

*Fisher's exact test, CR: complete response, PR: partial response, NR: no response, *statistically significant

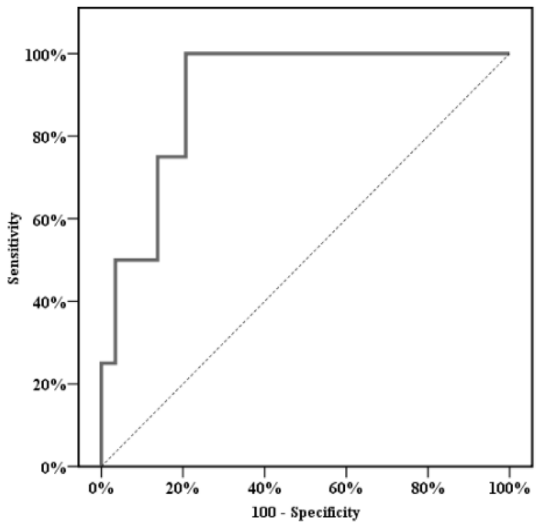

Figure 1. ROC curve for pretreatment CIP2A expression ≤16.45 EU in relation to achievement of response to bortezomib-dexamethazone regimen (area under the curve=0.905, p=0.010).

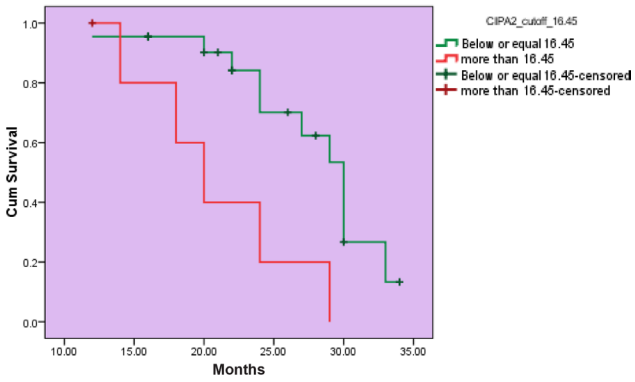

Figure 2. Progression-free survival of 28 multiple myeloma patients according to CIP2A expression (p=0.006).

Statistics

The data are presented as the mean ± standard deviation and analyzed using SPSS software 21.0 (SPSS Inc., Chicago, IL, USA). Student's t test was used to compare differences, and data were analyzed using the Pearson's x^2 test and Fisher's exact test. The significance of the relationships between CIP2A protein expression and clinicopathological parameters and CIP2A expression were evaluated using the x^2 test. DFS was calculated using the Kaplan-Meier method. A p value of ≤0.05 was considered statistically significant.

Results

CIP2A expression was upregulated in patients with newly diagnosed MM (56.38±107.5 EU) compared to healthy controls (4.93±5.62 EU; p=0.023). The relationship between CIP2A expression levels and the clinicopathological characteristics of the studied patients was analyzed. No significant association was observed between CIP2A expression and age, gender, type of myeloma, ISS stage, or percent of marrow plasma cells (Table 1).

There was a significant reduction in CIP2A expression after treatment with BD (Table 1). The prognostic value of CIP2A expression in MM patients was evaluated by comparing the patients with CIP2A expression levels equal to or below the calculated cutoff value versus CIP2A expression levels above it as regards the response to BD. Patients with expression levels ≤ 16.45 EU were more likely to respond to BD (23 patients out of 23) than those with expression level >16.45 (6 patients out of 10) (p=0.005; Table 2). According to reactive oxygen species (ROC) curve, pretreatment CIP2A expression ≤16.45 EU was indicative of achievement of response to BD protocol with 100% sensitivity and 79.31% specificity (Figure 1).

The 28 responsive patients were followed up. The follow up period was 12-34 months with a median of 22.5 months. PFS was studied as regards pretreatment CIP2A expression levels. Lower PFS (16.7%) was observed among patients with high CIP2A expression levels (>16.45 EU) compared to 50% PFS in patients with CIP2A expression levels ≤ 16.45 EU (p=0.006; Figure 2).

Discussion

CIP2A expression was studied by multiple researchers in solid malignancies and its overexpression was thought to be associated with failure of apoptosis, tumour growth, resistance to chemotherapy, and inferior prognosis in many human solid malignancies including head and neck, gastric, breast, tongue, ovarian, and lung cancers [15-20].

Barragán et al [22] showed that high CIP2A expression was a recurrent event in acute myeloid leukemia (AML), where it was a marker of reduced overall survival and poor prognosis. In addition, CIP2A depletion was shown to downregulate cell proliferation. Thus, they postulated CIP2A as a novel therapeutic target in AML. Furthermore, CIP2A was reported by Ventela et al as a marker of radioresistance and was suggested as a modulator of breast cancer cells sensitivity to bortezomib treatments [23]. CIP2A overexpression has been shown also in aggressive subtypes of B-cell lymphoma in association with clinical aggressiveness of these subtypes [24]. In chronic myeloid leukemia (CML), CIP2A expression was found to be a determinant of disease progression and was suggested as a biomarker of blastic crisis [25].

The present study investigated the role of CIP2A expression in MM. Our results were in agreement with Liu et al [26] and Zheng et al [27] who showed that CIP2A expression was increased

in MM patients compared to healthy controls. However, unlike of our findings, Liu et al demonstrated that higher CIP2A expression was significantly correlated with the international staging system stages and percent of marrow plasma cells. On the other hand, in agreement with our data, they found that overexpression of CIP2A was associated with lower survival in MM [26].

In our study, CIP2A expression levels in MM patients were significantly reduced after bortezomib treatment. This is in accordance with the findings reported by Liu et al [28] who confirmed that bortezomib-induced apoptosis in leukemia cells occurs via upregulation of protein phosphatase 2A (PP2A) activity. Subsequently, through inhibition of PP2A CIP2A possibly counteracts the apoptotic effect of bortezomib in leukemia cells. On the other hand, they reported bortezomib-mediated downregulation of CIP2A expression in sensitive leukemia cells but not in resistant ones through non-delineated machinery [28].

In conclusion, CIP2A was upregulated in MM and bortezomib downregulated its expression. High CIP2A level is associated with lower PFS and poor response to BD in MM. Therefore, beside its value as a poor prognostic indicator in MM, CIP2A suppression might be a fruitful future targeted therapeutic strategy aiming to improve the outcome in MM.

Conflict of interests

The authors declare no conflict of interests.

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