RESEARCH ARTICLE

The Meningioma 1 (MN1) Gene is an Independent Poor Prognostic Factor in Adult Egyptian Acute Myeloid Leukemia Patients

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Abstract

Aim: To determine the prognostic importance of meningioma 1 (MN1) gene expression levels in the context of other predictive markers for acute myeloid leukemia (AML) cases. **Methods:** MN1 expression was measured in 85 newly diagnosed adults younger than 60 years by real-time reverse-transcriptase polymerase chain reaction. **Results:** At diagnosis 67.4% of cases had elevated MN1 expression, this being associated with a worse prognosis, higher incidence of lymphadenopathy and CD34 transcript expression (p=0.02 and <0.001, respectively). No other molecular or clinical characteristics were significantly associated with MN1expression. Patients with high MN1 expression had lower complete response rate at day 15 compared to patients with low MN1 expression (p=0.09) and a significantly higher relapse rate (21.1% versus 7.7%, respectively, p=0.04). Patients with high MN1 expression had shorter TTP compared to those with low expression, p= 0.07. **Conclusion:** MN1 expression may predict outcome in AML patients. The MN1 gene and micro RNA expression suggest a biological feature that could be used as therapeutic targets.

Keywords: MN1 gene- AML- prognostic factors

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Introduction

AML in adults is a leading cause of leukemia-related deaths, and is characterized by uncontrolled proliferation and impaired differentiation of hematopoietic cells that results in accumulated myeloid blasts in the bone marrow and periphery (Rosenbauer et al., 2007). Tight control of the balance between proliferation and differentiation is essential for the maintenance of normal hematopoeisis. In various AML subtypes, deregulation of different or sometimes overlapping genes disrupts this balance and causes AML. These genes mostly control the survival, proliferation and differentiation programs of the hematopoietic stem/progenitor cells (HSPC) (Gilliland et al., 2001).

The meningioma 1 (MN1) gene is located at chromosome band 22q12 and encodes a protein of 136KDa which is unique as it does not show homology to any known proteins (Meester-smoor et al., 2005). MN1 is involved in AML either as a partner of the t (12;22) (p12;q12), creating an MN1-TEL fusion protein, (Buijs et al., 1995) or as an overexpressed gene (Valk et al., 2004, Ross et al., 2004 and Carella et al., 2007).

About half of patients with AML carry leukemic cells with a normal karyotype in which elevated MN1 expression correlates with poor prognosis (Baldus et al., 2007, Heuser et al., 2006). In addition, increased expression of MN1 cooperates with CBFβ-SMMHC),NUP98-HOXD13 and MLL-ENL (fusion proteins to induce leukemia, suggesting that deregulation of MN1 expression contributes to leukemogenesis. (Carella et al., 2007, Slape et al., 2007, Liu et al., 2010)

Several studies show that ectopic expression of MN1 in mouse HSPC (Hematopoietic Stem-Progenitor Cells) causes myeloid leukemia (Carella et al., 2007, Heuser et al., 2007) and MN1 induces proliferation and inhibits myeloid differentiation of both mouse and human HSPC. The differentiation inhibitory and proliferative effects of MN1 can be prevented by re-introduction of CEBPA (Kandilici et al., 2009).

The molecular mechanisms by which MN1 exerts its effects are largely unknown. Several experiments have shown that MN1 functions as a transcriptional co-activator and appears not to bind DNA directly. Thus, its output is relayed via other transcription factors, which may be contacted directly or indirectly.

MN1 activates transcription of the MSV-LTR through the nuclear receptor dimers RAR-RXR binding to direct repeat sequences (DR5) in the LTR; it interacts with RAR-RXR via the protein intermediates p300 and RAC3 (also known as nuclear receptor co-activator 3, NCOA3) (Van et al.,2003).

Co-expression of MN1 with p300 or RAC3 synergistically activates the transcriptional activity of

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RAR-RXR dimers in the presence of retinoic acid (Van et al.,2003).

MN1's coactivation activity is not restricted to the RAR-RXR nuclear receptor only but it also inhibits proliferation of an osteoblast cell line through coactivation of the vitamin D receptor (Sutton et al., 2005). Inhibition of growth of epithelial cell proliferation, is also associated with induction of MN1 expression (Chen et al., 2001, Kang et al., 2003).

MN1 can also bind to a transcription factor, which recognizes the CACCCAC sequence which together with MN1 transactivates transcription of the IGFBP5 promoter (Meester-smoor et al., 2007).

At the same time it was shown that TEL, an ETS transcription factor, was fused to MN1 in patients with myeloid leukemia or myelodysplastic syndrome containing the translocation t (12; 22) (p13;q11) (Buijs et al.,1995). The fusion protein MN1-TEL has transforming activity on NIH 3T3 cells and most likely acts as a disregulated transcription factor (Buijs et al., 2000).

To investigate the role of MN1 overexpression in AML, we quantified MN1 expression in adult patients with AML and compared the prognostic relevance of MN1 with other prognostic factors.

Patients and methods

This study was carried on 85 consecutive newly diagnosed AML patients who presented to the Adult Medical Oncology Department, National Cancer Institute (NCI), Cairo University.

Diagnosis was established after clinical, morphological, cytochemical, flow cytometric and cytogenetic analysis. All the cases met the AML diagnosis standards.

A Written informed consent was approved by the Institutional Review board (IRB) ethical committee of the NCI which follows the rules of Helsinki IRB.

Inclusion criteria: i). Patients proven to have AML. ii). Newly diagnosed patients prior to any therapeutic intervention. iii). Either sex was eligible. vi). Age: 18-70 years. v). Egyptians patients.

Exclusion criteria: i). Treated acute myeloid leukemia patients. ii). Pediatric age group. iii). Non-Egyptians.

Sample collection and RNA extraction

Bone marrow samples (1ml) from patients and controls (donors of bone marrow were collected on EDTA from patients with AML. Bone marrow was treated with erythrocyte lysis solution; Leukocytes were collected and stored in buffer RLT (1x107leukocytes) at -80 °C till use for complete RNA extraction. Total RNA was extracted from bone marrow cells using QIAamp RNA extraction blood Mini kit (QIAGEN) following the standard procedures according to the manufacturer's instructions.

RNA was converted to complementary DNA (cDNA) using Applied Biosystems High Capacity cDNA Reverse Transcription Kit (Life Technologies) according to the manufacturer's instructions and stored at –20 °C till use.

Real-time RT-PCR

Quantitative assessment of gene expression levels was performed by TaqMan gene expression assay (Applied Biosystems, Foster City, CA, USA) as recommended by the manufacturer. The StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was used for real-time analysis. Relative expression of MN1gene was analyzed by the comparative Ct method ($2^{-\Delta\Delta Ct}$) (Livak and Schmittgen, 2001), using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control. Data were expressed as the fold change in MN1 gene expression in the patients normalized to the expression levels of the endogenous control and relative to the healthy controls.

Statistical method

Data management and analysis was performed using SPSS, version 20. Categorical data were summarized as percentages; numerical data were summarized using means and standard deviation or medians and ranges. Relation between MN1 and other variables was assessed using Chi-square test. Overall survival (OS) was defined as the time from diagnosis to the time of death from any cause. Patients who were alive on the date of last follow-up were censored on that date. Time to progression (TTP) is defined as the interval from CR achievement until documented progression. For patients without disease progression (DP) at the time of analysis, the date of last follow-up was considered right-censored. OS and TTP were estimated using the Kaplan-Meier analysis. Log rank test was used to compare survival curves. All tests of hypotheses were conducted at the alpha level of 0.05, with a 95% confidence interval (Dawson etal., 1994).

Results

Based on the median level of MN1 expression in control group which is 1 fold change, out of 85 AML patients, 57 (67.4%) patients had high MN1 expression. (Patients clinical and laboratory characteristics are listed in Table1).

MN1 expression and clinical and laboratory features

Higher MN1 expression was associated with higher incidence of lymphadenopathy, and CD34 transcript expression ($p=0.02$ &<0.001) respectively. No other molecular or clinical characteristics were significantly associated with MN1expression (Table 2).

The patients were classified according to their cytogenetics abnormalities into favorable, intermediate and high risk groups according to SWOG and MRC cytogenetic risk category (Dastugue et al., 1995). 75% of patients with high MN1 expression were intermediate risk $p=0.07$. MN1 and CD34 transcript expression were positively correlated ($p<0.001$).

MN1 expression and prognostic impact

The course of the disease in AML patients with high MN1 expression was unfavorable. Patients with high MN1 expression had lower CR rate at day 15 compared to patients with low MN1 expression, a difference which is nearly statistically significant ($p=0.09$).

The relapse rate for patients with high MN1 expression was significantly higher compared to patients with low MN1 expression (21.1% versus 7.7%, respectively,

Table 1. AML Patients Clinical and Laboratory Characteristics

Patients characteristics	N=85	(%)
	Mean	±SD
Age	38.73	±1.45
Sex		
Male	39	(45.9)
Female	46	(54.1)
Extramedullary infiltrartion	47	(55.5)
Splenomegaly	30	(35.3)
Hepatomegaly	36	(42.4)
Lymphadenopathy	19	(22.4)
Laboratory finding	Mean	+/- SD
TLC	49.96	+/-6.91
HB	8.03	+/-0.19
Platelet	57.47	+/-7.99
Peripheral blood blasts%	52.8	+/-2.89
Bone marrow blasts%	63.2	+/-2.20
Bone marrow cellularity	N	(%)
Normo-cellular	14	(16.5)
Hyper-cellular	71	(84.9)
FLT status	N=72	(%)
Wild	58	(80.6)
Mutant	14	(19.4)
FAB		
M0	1	(1.2)
M1	15	(17.6)
M2	27	(31.8)
M3	7	(8.2)
M4	21	(24.7)
M5	4	(4.7)
M7	2	(2.4)
Secondary leukemia	8	(9.4)
Cytogenetics	74	(%)
Favorable risk	14	(18.9)
Intermediate risk	52	(70.3)
High risk	8	(10.8)
Meningioma gene	N=85	
overexpressed	57	(67.1)
underexpressed	28	(32.9)

p=0.04)

Patients with high MN1 expression showed shorter TTP compared to those with low expression, median TTP for patients with high MN1 expression was 17.81 (95% confidence interval 11.67-23.95) months and was not reached for patients with low MN1 expression, a difference which is nearly statistically significant p= 0.07. (Figure 1)

Median OS for all patients was 8.05 months (95% confidence interval 3.32-12.77), patients with under-expressed and over-expressed MN1 had a median OS of 10.87 months (95% confidence interval 2.15-19.59), and 7.12 (95% confidence interval 2.82- 11.42) respectively, a

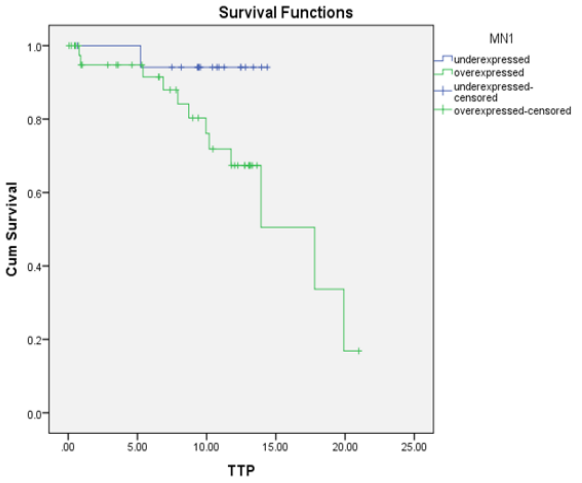

Figure 1. Time to Progression (TTP) in AML Patients According to MN1 Expression

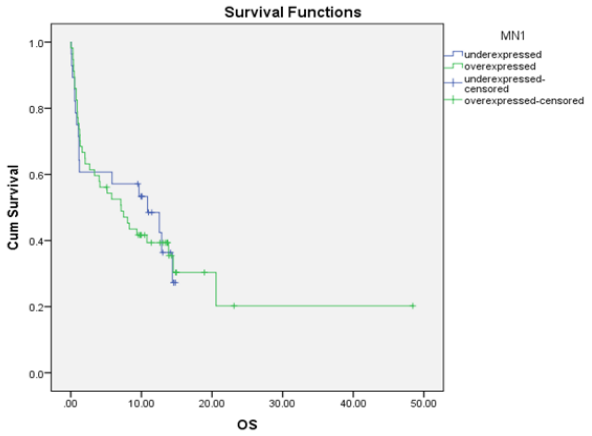

Figure 2. Overall Survival (OS) in AML Patients According to MN1 Expression

difference that didn't reach statistical significance (p=0.87) (Figure 2).

The insignificant difference in survival can be attributed to the fact that more patients in the MN1 overexpressed group underwent bone marrow transplant: 9 patients were transplanted, 7 were over expressed and 2 under expressed (14% versus 7.1% respectively) and also to the small study population.

Discussion

In the present study we evaluated the prognostic significance of MN1 mRNA expression levels in 85 adult patients with AML. High MN1 expression was detected in 67% of the patients. Nearly similar finding was detected by Heuser et al., (2007) who found that 50% of AML patients had high MN1 expression and they reported that MN1 is a potent oncogene in hematopoiesis that enhances proliferation, self-renewal, and also blocks differentiation by repressing transcription of differentiation-associated genes.

In our study, patients with high MN1 expression have higher incidence of lymphadenopathy and low platelets

Table 2. AML Patients Characteristics According to MN1expression Levels

	meningioma		P- value
	Under expressed N=28 (%)	Over expressed n =57 (%)	
Sex			0.27
Male	11 (39.3)	28 (49.1)	
Female	17 (60.7)	29 (50.9)	
Age			0.65
≤60	26 (92.9)	53 (93)	
>60	2 (7.1)	4 (7)	
TLC			0.31
≤100	25 (89.3)	47 (82.5)	
>100	3 (10.7)	10 (17.5)	
Hb level			0.49
≤8	19 (71.4)	39 (68.4)	
>8	8 (28.6)	18 (31.6)	
Platelets			0.03
≤100,000	28 (100%)	47 (82.5%)	
≥100,000	0 (0%)	10 (17.1%)	
FAB classification		|	Cannot be calculated
M0	0 (0)	1 (1.8)	
M1	1 (3.6)	14 (24.6)	
M2	6 (21.4)	21 (36.8)	
M3	6 (21.4)	1 (1.8)	
M4	9 (32.1)	12 (21.1)	
M5	4 (14.3)	0 (0)	
M7	1 (3.6)	1 (1.8)	
Secondary ML	1 (3.6)	7 (9.4)	
Extramedullary infitration			0.08
absent	16 (57.1)	22 (38.6)	
present	12 (42.9)	35 (61.4)	
Lymph nodes			0.02
Negative	26 (92.86)	40 (70.2)	
positive	2 (7.14)	17 (29.8)	
spleen			0.25
Negative	20 (71.4)	35 (61.4)	
positive	8 (28.6)	22 (38.6)	
liver			0.26
Negative	18 (64.3)	31 (54.4)	
positive	10 (35.7)	26 (45.6)	
Cytogenetic			P=0.07
Favourable risk	8 (32)	6 (12.2)	
Intermediate risk	15 (60)	37 (75.5)	
High risk	2 (8)	6 (12.2)	
FLT			0.53
wild	19 (79.2)	39 (81.2)	
mutant	5 (20.8)	9 (18.8)	
CD34			<0.001
Absent	24 (85.7)	16 (28.1)	
present	4 (14.3)	41 (71.9)	

Table 3. Response to Induction Therapy According to MN1 Expression Levels in AML Patients Studied

Response	Underexpressed N (%)	Overexpressed N (%)	P value
			P=0.09
CR	18 (64.3)	35 (61.4)	
Bad prognosis	10 (35.7)	22 (38.6)	
Resistant	0 (0)	8 (14)	
Death	10 (35.7)	14 (24.6)	

count, a finding which isn't consistent with findings from other studies. No significant correlations were found as regard age, sex , other clinical and laboratory characteristics between high and low MN1 expression groups. Similar findings were observed by (Heuser etal., 2016; Aref et al., 2013).

CD34 expression has been suggested as a poor prognostic marker in AML. In our study, the MN1 expression and CD34 were positively correlated. Similar finding was detected by Heuser etal., (2016) who reported that these two genes were significantly correlated.

Interestingly, our work revealed that 75% of patients with higher MN1 expression were intermediate risk, which was expressed in our work in the form of cytogenetically normal AML patients. Such finding is consistent with what was reported by Heuser etal., 2016 and Langer et al., 2009 who found over expression of MN1 in cytogenetically normal AML patients.

Patients with high MN1expression showed poor treatment outcome. They had a nearly statistically significant lower CR rate at D 15 compared to those with low MN1 expression (p=0.09). This finding is consistent with data reported by Heuser etal., (2016) and Aref et al (2013).

Grosveld et al., (2007) showed that the levels of MN1 expression directly correlated with the risk of failing remission induction chemotherapy. Also Langer et al., (2009) found that higher MN1 expression was associated with a lower CR rate.

In our series patients with high MN1 expression were at a high risk for relapse compared with those with low expression (p=0.04), a finding which is consistent with Aref et al 2013 who reported frequent relapse with MN1 over expression. Patients with high MN1 expression who achieved CR had shorter TTP compared to patients with low MN1 expression (p=0.07), a finding which is consistent with what was reported by Heuser etal., (2016). This finding may be applied to stratify patients who achieved good response to induction chemotherapy based on their relapse risk at this early time point in order to improve their treatment outcome by allocating them to experimental strategies.

Previous studies, reported that patients with high MN1 expression show shorter OS (Heuser etal., 2016, Aref et al 2013, Grosveld et al., 2007 and Langer et al., 2009). However in our study, there was no significant difference in survival between the two groups, this can be explained by the fact that more patients in the MN1 overexpressed group underwent bone marrow transplantation.

In conclusion, MN1 overexpression is a new prognostic

marker in AML, especially for the cytogenetically normal AMLs which is associated with poor response to induction therapy, higher relapse rate and shorter disease free survival. A marker which can predicts poor clinical outcome. This will lead to improving risk stratification of this heterogeneous group of patients with AML.

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