The prevalence of leukemia- associated antigen MPP11 m-RNA expression in Egyptian patients with CML

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Abstract

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disease with distinct biological and clinical features. For an effective specific immunotherapy in CML, the use of leukemia-associated antigens (LAAs) with an optimal expression pattern is required. Among these leukemia-associated antigens (LAAs) that induce a humoral immune response in CML patients is M-phase phosphoprotien 11 (MPP11)M-phase phosphoprotien 11 (MPP11) gene is one of the leukemia-associated antigens (LAA) identified in patients with myeloid leukemias. It plays a putative oncogenic role with a highly tumor specific expression level. In the present work we aimed at studying the frequency of expression of (MPP11) in Egyptian patients with CML, both in chronic phase and accelerated/blastic phase, as a potential target for cellular immunotherapies. Patients and methods: MPP11 expression was tested in peripheral blood mononuclear cells of 43 CML patients divided into 2 groups, group A: 32 chronic phase CML patients, group B: 11 accelerated/blastic phase patients as well as 15 healthy volunteers. RT-PCR technique was used to detect MPP11 m-RNA. Our results demonstrated that 90.6% of our chronic CML patients showed positive MPP11 expression similar to the accelerated/blastic phase patients. This highlighted the contribution of MPP11 expression with CML disease through out the disease course. Conclusion: Our work demonstrated a similar high incidence of MPP11 expression in both chronic and accelerated/blastic phase CML patients. This suggests the possible therapeutic value of MPP11 derived peptide vaccination with conventional CML therapy in chronic and accelerated phases, in order to achieve complete molecular remission for our patient.

Introduction

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disease with distinct biological and clinical features. It is characterized by the presence of the Philadelphia (Ph) chromosome, which results from a reciprocal translocation between the long arms of the chromosomes 9 and 22 t(9;22)(q34;q11). This translocation creates two new genes, BCR-ABL on the 22q- (Ph chromosome) and the reciprocal ABL-BCR on 9q. The BCR-ABL gene encodes for a 210-kD protein with deregulated tyrosine kinase (TK) activity, which is crucial for malignant transformation in CML [1].

The chronic phase (CP) of CML is characterized by excess numbers of myeloid cells that proliferate extensively. Between 90% and 95% of patients will be diagnosed in this phase of the disease. If untreated, within an average of 4 to 6 years, the disease transforms through an "accelerated phase" (AP) to an invariably fatal acute leukemia, also known as blast crisis. Disease progression is likely due to the accumulation of molecular abnormalities that lead to a progressive loss of the capacity for terminal differentiation of the leukemic clone. The current treatment tries to maintain remission and prevent progression of the disease to accelerated phase (AP) or blast phase (BP) while minimizing any therapy-related toxicity [2].

The first tyrosine kinase inhibitor (TKI), introduced into clinical practice in 1998, was imatinib mesylate. which is a small-molecule drug designed to interfere with BCR-ABL tyrosine kinase activation by competitive binding at the ATP-binding site, Imatinib became the first choice drug in chronic phase CML, because of its high efficacy, low toxicity and ability to maintain durable hematological and cytogenetic responses. However, approximately 20-25% of patients initially treated with imatinib needed alternative therapy, due to drug intolerance or drug resistance [3].

Specific immunotherapies for CML patients targeting T cell antigens might eliminate residual CML cells after chemotherapy, in combination with imatinib or other tyrosine kinase inhibitors, and might enhance a specific graft versus leukemia effect after allogeneic stem cell transplantation without aggravating the graft versus host disease. For an effective specific immunotherapy in CML, the use of leukemia-associated antigens (LAAs) with an optimal expression pattern is required [4]. Among these leukemia-associated antigens (LAAs) that induce a humoral immune response in CML patients is M-phase phosphoprotien 11 (MPP11) [4, 5].

The MPP11 gene is located on the chromosome 7q22-31.1. A highly tumor-specific expression level of MPP11 gene and increased chromosome 7 copy number was associated with malignancies. In primary head and neck squamous cell cancer, MPP11 was found to have a putative oncogenic role [7].

MPP11 is the homologue to the murine Id-associated 1 gene (MIDA1) which consists of a Zuotin homology region and tryptophan-mediated sequences similar to the c-myc oncoprotien. MIDA1 associates with the helix-loop-helix protein of the conserved DNAJ motif of the Id protein involved in cell

type –specific transcription and cell linage commitment, so MPP11/MIDA1 is multifunctional protein involved in transcriptional control through interaction with multiple factors [6].

In rats MIDA1 was identified to induce humoral immune responses in glioma. Moreover, immunization with a plasmid- encoding MIDA1 resulted in a significant suppression of tumor growth in animals immunized with MIDA1 plasmid [8].

In **Greiner et al 2003** [8], four of 10 CML patients in chronic phase , specific immune response (developed IgG antibody responce) was detected after immunization against MPP11 derived epitope [8].

In view of the high cost of imitanib therapy, most of Egyptian CML patients are treated by alternative older generation therapies. Moreover there are no available data about the prevalence of MPP11 expression in our patients.

The aim of our work is to determine the incidence of MPP11 expression among Egyptian patients with CML, both in chronic phase and accelerated/blastic phase. Such a study could be of a potential value to adopt targeted therapy for patients, particularly who fails standard protocols.

Patients and methods

This study was carried out on 43 chronic myeloid leukemia patients, as well as 15 age and sex matched healthy volunteers as a control group. The patients were randomly chosen from outpatient clinic or in patients of the new Kasr el Aini teaching hospital, Cairo University. An informed consent was signed by both patients and control individuals.

Patients were stratified into 2 distinct groups: Group A: 32 patients in chronic phase and Group B: 11 patients in accelerated/blastic crisis phase.

In group A, patient's age ranged between 27 and 60 years. They were 20 females and 23 males.

In group B, patient's age ranged between 28 and 58 years. They were 10 female and 6 male.

All Patients and controls were examined for relevant clinical and laboratory findings, including: .full history taking, thorough clinical examination, complete panel of routine laboratory work up including LAP score. Detection of Philadelphia chromosome by conventional cytogenetic study, detection of BCR-ABL translocation, BCR-ABL/ABL ratio and MPP11m-RNA detection by RT-PCR.

Detection of MPP11 m-RNA expression by RT-PCR:

Five ml of blood were withdrawn from every patient as well as the healthy volunteers in a sterile EDTA vaccutainer. The mononuclear cells are separated and preserved at - 70 °c. Total cellular RNA was extracted from the monnuclear cells using the QIA amp RNA blood Mini kit (QIAGEN, Catalogue number. 52304), followed by c-DNA preparation using Revert AidTM First strand cDNA synthesis kit (Fermentas, K1621). A volume of 5 µl cDNA was added to a final PCR reaction mixture of 25 µl containing 12.5 µl Master Mix (Fermentas K0171 which contains TaqDNA polymerase in reaction buffer, MgCl2 and dNTPs), 1 µl of 10 µM of each of the forward and reverse MPP11 specific primers and 1 µl of 10 µM of each of the forward and reverse primers of β-actin. For standardization, expression of MPP11 was correlated with the expression of the house keeping gene β-actin. The primers forMPP11: forward primer: 5'-AAG ATC ATT ATG CAG TTC TTGG AC-3', reverse primer: 5'-CCA ATA ACA TCT TTG GCA GTT CT -3'. For β-actin: forward primer: 5'-GCA TCG TGA TGG ACT CCG-3', reverse primer: 5'-GCT GGA AGG TGG ACA GCG A-3' (Fermentas™ – Germany). PCR protocol for MPP11 was performed as described by **Greiner et al 2004** [9]. The following thermocycler program was performed: initial denaturation at 95^0 C for 1 minute, annealing at 56^0 C for 1 minute, and extension at 72^0 C for 1 minute. This was repeated for 35 cycles. The amplified products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide. The electrophoretic pattern was visualized under UV light then photographed using a Polaroid camera with a red orange filter. The sample was considered positive when a clear sharp band was observed at the specific molecular weight; 1243bp for MPP11 and 661 bp for β-Actin (Figure1and 2).

Bcr-abl/abl ratio;

Bcr-abl/abl ratio was done by RT- PCR followed by densitometric analysis of the products and ratio calculation. The density of the amplified product of different samples was assessed by densitometric documentation program (version 0.3). Primer sequence, cycles and procedure were modified after Hagop et al [10].

Statistical analysis

Data were summarized and presented in the form of mean, range and standard deviation as descriptive statistics. Descriptive statistics and statistical comparison were performed using the statistical software program SPSS (version 14). Group comparison was done using analysis of variance (ANOVA test). For all of the above mentioned statistical tests done, the threshold of significance of difference is fixed at 5% level (p-value). Probability value (p-value) of more than 0.05 was considered non-significant.

Results

The current study included 43 CML patients divided into 2 distinct groups according to criteria described by **Richard et al.,** 1999 [11] the clinical and laboratory data of CML patients involved in the study is summarized in table 1.

Group A: 32 chronic CML patients, group B: 11 accelerated / blastic crisis patients as well as 15 healthy age and sex matched individuals as control group.

In group A: 29 /32 patients (90.6%) showed positive MPP11 expression by conventional RT PCR, while no MPP11 expression was detected in the peripheral blood mononuclear cells of the control group.

In group B (accelerated/blastic crisis phase) 10 /11(90.9% of cases) showed positive MPP11 expression by conventional RT-PCR.

Table (1) showed the comparison of the two studied groups regarding clinical and laboratory data .There was no statistical significant difference between group A and group B regarding MPP11 expression.

Discussion

The advent of tyrosine kinase inhibitors (TKIs) started a new era in the management of chronic myeloid leukemia (CML). Imatinib, the first TKI to be approved for the treatment of CML and the current standard first-line therapy, has significantly improved the prognosis of patients with CML. Nevertheless, a minority of patients in chronic-phase CML and even more patients with advanced-phase disease demonstrate resistance to imatinib or develop resistance

during treatment leading to inevitable disease progression. Consequently, researchers have developed novel, strategies that can overcome not only Bcr-Abl-dependent mechanisms of resistance, but also those that are Bcr-Abl-independent, as targeted immunotherapy [12].

Targeted immunotherapies require the identification and characterization of appropriate antigen structures. Initially, T-cell based cancer vaccines were designed for patients with solid tumors after the definition of suitable tumor-associated antigens. Several immunological and even clinical responses prompted researchers and clinicians to extend the spectrum of cancer vaccines towards hematologic malignancies such as acute and chronic myeloid leukemia. The graft-versus-leukemia (GVL) effect observed after allogeneic stem cell transplantation and donor lymphocyte infusions strongly suggests that T lymphocytes play a major role in the rejection of leukemic cells. Therefore, immunotherapy directed against leukemia associated antigens might elicit specific immune responses that could eliminate minimal residual disease after chemotherapy, or enhance the GVL effect after hematopoietic stem cell transplantation [13].

Peptide-based vaccines are able to induce cytotoxic T-lymphocyte responses that kill leukaemia cells. Based on this, pilot clinical trials have been initiated in chronic and acute myeloid leukaemia and other haematological malignancies, which include vaccination of patients with synthetic peptides derived from these LAAs. Results from these trials show that peptide vaccines are able to induce immune responses that are associated with clinical benefit. These early clinical results are promising and provide valuable information for future improvement of the vaccines [14].

An ideal LAA that qualifies as a potential target for immunotherapies should be expressed preferentially in leukemic blasts, but neither on haematopoietic stem cells nor on normal tissues [5]. Several leukemia-associated antigens (LAA) have been identified in patients with chronic myeloid leukemia as PRAME, proteinase 3, RHAMM, and MPP11 [15]. MPP11 is an antigen eliciting humoral immune responses in patients with AML, CML, and in patients with gastric and breast cancer [8].

In the present work we studyied the MPP11 expression by conventional RT-PCR in peripheral blood mononuclear cells of 32 chronic myeloid leukemia patients and 11 blastic/acceleratic phase patients as well as 15 healthy normal volunteers as a control group.

All the control subjects were negative forMPP11. This is in agreement with previous studies by Greiner et al [8] and Schmitt et al [15] who reported that no m-RNA expression of MPP11 was detectable in peripheral blood samples of healthy volunteers involved in the study.

In the chronic phase patients (group A) , MPP11 expression was detected in 29/32 patients (90.6%) this is in agreement with Schmitt et al [15] and Greiner and Schmitt [5] who found 90% MPP11 expression in CML patients by RT-PCR . However Greiner et al [8] reported 7/10 (70%) MPP11 expression in chronic CML patients..

As regards the accelerated/blastic phase 9/11 (90.9%) showed positive MPP11 expression ,this was in accordance with Schmitt et al [15] who demonstrated that m-RNA expression of the MPP11 was expressed at high frequency in chronic and accelerated/blastic phase (91% vs. 92%) .

This demonstrates, in a way, a similarity in the prevalence of MPP11 expression between our CML Egyptian patients and CML patients studied by Greiner and Schmitt [5].

It is noteworthy that patient expressing LAA on their leukemic blasts had better therapy outcome and expression of theses LAA on lekeumic progenitors is associated with better post-tranplantation outcome in CML patients [16].

For sufficient presentation of peptides inducing Specific T Cells, MHC molecules and costimulatory molecules are necessary. CD34+CML expressed HLA-ABC and HA- DR but they lack all the costimulatory molecules, this might be essential for the process of tumor escape of CML cells especially the progenitor CML cells [16].

Many of the identified LAAs are now used for vaccinations, in many clinical trials worldwide whether as peptide derived antigens or for vaccination using dendretic cells to induce specific immune response against LAAs in CML and other myeloid leukemias [17]. Immunologic and clinical response had proved to be very promising, in reaching complete remission, maintaining major genetic remission, or at least prevention of disease progression [5].

Vaccination strategies inducing effector T cells against BCR-ABL, MPP11 and other LAAs expressed in CML are promising approaches to enhance specific immune responses against CML [7].

Because of the tissue – restricted m-RNA expression, high frequency of MPP11 expression in AML and CML and the differential humoral immune responses in different tumors but not in healthy volunteers MPP11 seems to be candidate for further vaccination studies. Experimental data in the animal model with its murine homologue MIDA are encouraging [8].

In conclusion our work demonstrated a high prevalence of MPP11 expression in Egyptian CML patients. This may open the door for subsequent studies suggesting the concomitant use of MPP11 derived peptide vaccination with conventional CML therapy in both chronic and accelerated phases, in order to achieve complete molecular remission for our patients.

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Tables

Table 1: Clinical and laboratory data of CML patients

	cases (n=43)	
	Range (median)	**Mean ±SD**
Age (years)	27-60(42)	43.46±9.78
Hb g/dl	7.3-13.5(11.5)	11.12± 1.75
WBCsX10³/dl	21.43-280.5(125.7)	116.97±77.02
Basophil %	1-10(5)	4.88±2.33
Esinophil %	0-5(1)	1.42±1.16
Blast cells%	9-17(4)	5.86±4.92
Promyelocytes %	2-14(5)	5.56±3.23
Myelocytes %	7-24(16)	16.32±4.02
Juvenil%	2-23(13)	11.93±5.07
Staff %	3-31(19)	18.39±5.97
Segmented %	10-45(26)	27.16±9.35
Lymphocytes%	1-12(5)	5.58±2.99
Monocytes%	0-9(3)	2.91±2.27
PlateletX10³/dl	81-448(199)	218.39±110.16

Table 2: Comparison of the two studied groups regarding clinical and laboratory data

	Blastic cases	% (median)	Chronic cases	% (median)	p value
Sex					1.0
Male	6	54.5	17	53.1	
Female	5	45.5	15	46.9	
Age (year)					0.9
mean±SD	43.64±9.87	30-60(42)	43.41±9.91	27-58(42)	
Spleno-megally					0.078
Mild to moderate	8	72.7	12	37.5	
Huge	3	27.3	20	62.5	
Hb g/dl					< 0.0001
mean±SD	9.17±1.37	7.3-11.2(8.8)	11.79±1.32	8.6-13.5(12.1)	
WBCsX10³/dl		21.43-227.9(137.7)		24.1-280.5(118.35)	0.83
mean±SD	121.14±76.47		115.54±78.37		
Basophil %					0.008
mean±SD	6.45±2.66	2-10(6)	4.34±1.98	1-8(4)	
Esinophil %					0.019
mean±SD	0.73±0.79	0-2(1)	1.66±1.18	0-5(2)	
Blast cells%					< 0.0001
mean±SD	13.27±1.74	11-17(13)	3.31±2.38	0-9(3)	
Promyelo-cytes %					0.29
mean±SD	6.45±4.03	2-14(5)	5.25±2.92	2-13(5)	
Myelocytes %					0.10
mean±SD	14.64±4.34	7-20(15)	16.91±3.8	10-24(17)	
Juvenil%					0.15
mean±SD	10±3.41	5-15(10)	12.59±5.42	2-23(14)	
Staff %					0.065
mean±SD	15.55±3.45	10-20(16)	19.38±6.37	3-31(19.5)	

Figures

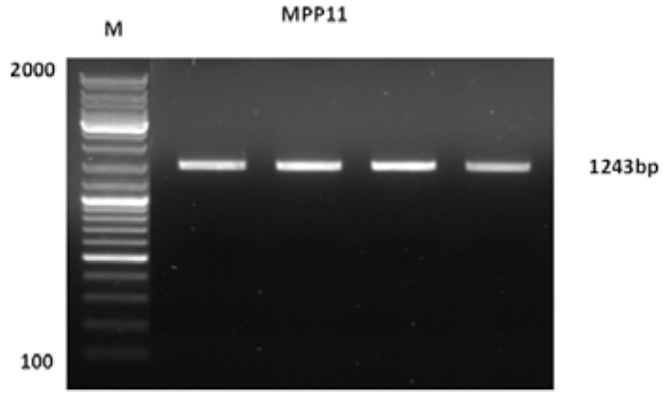

Fig. 1: Agarose Gel Electrophoresis Analysis of MPP11 m-RNA
M: PCR marker (100-200-300-400-500-600-700-800-900-1000-1500-2000bp).
MPP11 m-RNA was expressed in cases 90% of the cases.

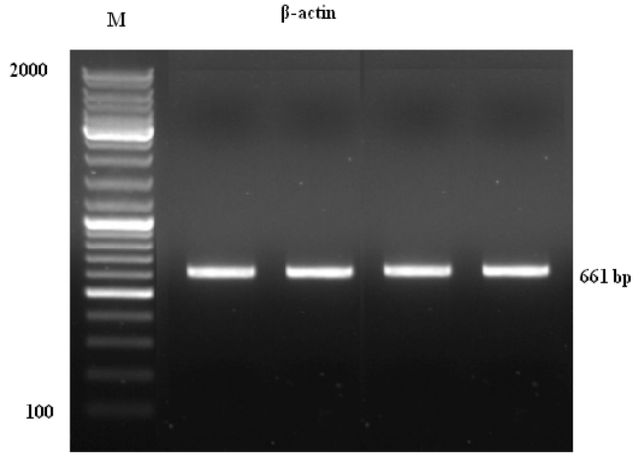

Fig. 2: Agarose Gel Electrophoresis Analysis of β-actin.
M: PCR marker (100-200-300-400-500-600-700-800-900-1000-1500-2000bp).