Expression of PRAME gene in Egyptian adult acute myeloid leukaemia and its correlation with clinical response

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Abstract: Preferentially expressed antigen of melanoma (PRAME) is a cancer-testis antigen (CTA) belonging to the group of tumor associated antigens. Te PRAME gene expression is low or absent in almost all normal adult tissues. The PRAME transcript is highly expressed in acute myeloid leukemia patients and is usually associated with a favorable prognosis. The aim of this work is to assess the expression PRAME gene in Egyptian adult acute myeloid leukemia patients at diagnosis and to correlate its expression with the clinical response. PRAME transcript expression was studied in sixty adult acute myeloid leukemia patients using RT-PCR. PRAME m-RNA expression was detected in 33 (55%) of patients. No significant correlation was found between PRAME gene positivity and any of the clinical or hematological variables except for hepatomegaly. PRAME negative patients showed good response to treatment compared those who were PRAME positive. The rate of CR was 37.5% compared to 65.2% in PRAME positive and PRAME negative patients, respectively (p value = 0.043). It seems that there is an increase in the overall survival among the PRAME negative compared to the PRAME positive group although the difference was not significant (p value = 0.06). In conclusion, PRAME is an attractive tumor-associated antigen. Its expression was associated with poor prognosis. More studies should aim at detailed understanding of the mechanisms of PRAME action and its use in minimal residual disease detection and immunotherapy.

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1. Introduction

Acute myeloid leukemia represents a group of clonal hematopoietic stem cell disorders in which failure to differentiate and over proliferation in the stem cell compartment result in accumulation of non-functional myeloblasts. Preferentially expressed antigen of melanoma (PRAME) is a cancer-testis antigen (CTA) belonging to the group of tumor associated antigens. The PRAME gene maps on chromosome 22 at 22q11. It was first detected in a case of malignant melanoma [(1)].

Te PRAME gene expression is low or absent in almost all normal adult tissues except for testis, adrenals, ovaries and endometrial tissues. This gene is expressed at a high level in a very large fraction of tumors, such as melanomas, non-small-cell lung carcinomas, sarcomas, head and neck tumors and renal carcinomas. But in contrast with most other tumor associated antigens, it is also expressed in leukemias [(2)]. In spite of the fact that the PRME antigen is recognized by autologous cytotoxic T cell-mediated immune responses, its expression is well retained. This suggests that expression of PRAME is addressed to be involved in the tumorigenic process [(3)].

The mRNA level of PRAME is used as a tumor marker due to its over expression in various malignancies. The PRAME transcript is highly expressed in AML patients and is usually associated with a favorable prognosis [(4-6)].

Retinoic acid (RA) induces proliferation arrest, differentiation, and apoptosis. Defects in retinoic acid receptor (RAR) signaling have been implicated in cancer. PRAME has been reported to function as a repressor of retinoic acid (RA) signaling through interactions with retinoic acid receptors (RARs) which was proposed as important contributory factor in AML disease progression [(3,7)].

The effect of PRAME on gene expression in leukemic cells remains controversial, while cell based reported the down-regulation of genes such as S100A4, RARb2, p21 and Hsp27 [(8)], another study reported lack of association between expression levels of these genes and PRAME expression in pediatric AML [(9)].

Quantification of PRAME transcript in acute myeloid leukemia could be used to monitor minimal residual disease for AML, patients with higher than normal levels and its increase over or persistently higher than normal range predict hematological relapse [(6,10)]. PRAME is also considered a good target for tumor immunotherapy [(5)].

The aim of this work is to assess the expression PRAME gene in Egyptian adult acute myeloid leukemia patients at diagnosis and to correlate its expression with the clinical response.

2. Material and Methods

Sixty adult acute myeloid leukemia patients attending the National Cancer Institute, Cairo

University between October 2008 and January 2010 and 10 healthy age and sex matched controls were included in this study. Patients' clinical characteristics are shown in Table (1). The study involved 33 females (55%) and 27 males (45%). The female to male ratio was 1.2: 1. The age of the studied patients ranged from 18-70 years with a median of 36 years and mean 37.7 years.

RNA extraction was done using QIAamp RNA blood Mini Kit. Reverse transcription was performed in 20ul reaction using random hexamer according to manufacturer's instructions (High capacity cDNA reverse transcription kit) (Applied Biosystems). PRAME and β-actin amplification were performed in two separate PCR reactions containing 1ug RNA, 1X buffer, 10 pmole of each primer: β-actin (5'-GTGGGGCGCCCCAGGCACCA-3') (5'-GTCCTT AAT GTC ACG CAC GAT TTC -3') [11] and PRAME: (5' -CTGTACTCATTTCCAGAGCCA-GA-3') (5'-TATTGAGAGAGGGTTTCCAAGGGGTT-3')[12], 1.5 mM Magnesium Chloride, 0.8 mM dNTPs (200 µM of each dNTP), 1.25 U Go Taq DNA Polymerase (Promega). The PCR conditions for B actin consisted of initial denaturation at 94ºC for 5 min, followed by 34 cycles of denaturation at 94ºC for 1min, annealing at 63ºC for 2min and extension at 72ºC for 3min. For PRAME, it consisted of Initial denaturation at 95ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 1min, annealing at 60ºC for 1min and extension at 72ºC for 2min. The PCR products were examined with gel electrophoresis using 1.5% agarose gel (Figures 1 and 2).

3. Results

PRAME m-RNA expression was detected in 33 (55%) of patients. None of the studied controls expressed PRAME m-RNA transcript. The difference between patients and controls was statistically significant (p=0.00).

No significant correlation was found between PRAME gene positivity and any of the clinical, hematological variables except for hepatomegaly (p=0.04) (Tables 1).

In our study out of 33 PRAME positive patients, fourteen patients were M2, seven were M1, five were M4, four were M3 and three were M5. PRAME expression was not correlated with immunophenotyping or stem cell marker CD34 expression on blast cells (Table 1).

Conventional cytogenetic study was done to 30 patients. Fifteen cases (50%) were of normal karyotype, five (16.6%) were positive to t(15:17), four patients (13.3%) were positive to inv(16) and two for t(8:21). While four cases show different cytogenetic abnormalities (-20, +21, del 11q23 and -14). Statistical analysis could not be done because of the small number of patients encountered in each cytogenetic abnormality.

In this study 10 out of 12 early death cases were PRAME positive. This group of patients shows a high leukocyte count and poor liver and kidney functions. In multivariate analysis the PRAME wasn't correlated with any of the laboratory or clinical criteria.

Complete remission was achieved in 27 out of 55 patients (49%). Five cases were missed in the follow up. Among those who were PRAME positive, 12/32 (37.5%) achieved CR compared to 15/23 (65.2%) of PRAME negative patients (Table 2). A statistical significant correlation was found between the response to treatment and PRAME gene expression with a (p value = 0.043). So PRAME negative patients showed a good response to treatment compared those who were PRAME positive.

After a follow up period of 16 months, the overall survival at one year was 37.9% with a median survival 4.2 month. In the group of patients with PRAME positive gene (32/55) the median survival was 1.5 month compared to 12.8 months in patients with PRAME negative gene (23/55). Although there is a bordered line statistical significant difference detected between the PRAME gene and the overall survival (p = 0.06), it seems that there is improvement in the overall survival among the PRAME negative compared to the PRAME positive group.

No statistical significant difference in median survival was found as regard age and sex with p =0.42 and 0.44 respectively. As regard the clinical findings, no statistical significant difference in median survival was found between patients with or without hepatomegaly, splenomegaly and lymphadenopathy with (p values = 0.91, 0.69, 0.99) respectively. As regard the hematological findings, no statistical significant difference in median survival was found regarding WBC and BM blasts.

Disease free survival (DFS) was 70% with a median duration of 13.1 months. No statistical significant difference in median survival was found between PRAME positive compared to PRAME negative group p value =0.4. No statistical significant difference in median survival was found as regard age, a statistical significant difference in median survival was found between males and females with p value =0.04. As regard the clinical findings, no statistical significant difference in median survival was found between patients with or without hepatomegaly and splenomegaly with a p = 0.81 and 0.64 respectively.

As regard the hematological findings, no statistical significant difference in median survival was found regarding WBC and BM blasts. Also no statistical significant difference in median survival was found regarding FAB subtype or immunophenotyping.

Table (1): Correlation of clinical and haematologic criteria with PRAME expression in AML patients

	Total	PRAME positive N(%)	PRAME negative N(%)	*p*- value
Age (y)				
<45		23(57.5)	17(42.5)	
≥45		10(50.0)	10(50.0)	0.58
Sex				
Female		17(51.5)	16(48.5)	0.55
Male		16(59.3)	11(40.7)	
Signs				
Hepatomegaly		20(69.0)	9(31.0)	0.04
Splenomegaly		13(72.2)	5(27.8)	0.08
Lymphadenopathy		3(60)	2(40)	1.0
Haematologic				
WBCs ($X10^9L$) ≥ 50		11(55)	9(45)	1.0
Immunophenotype				
Myeloid with monocytic		8(61.5)	5(38.5)	0.59
CD34 positive		15(53.6)	13(46.45)	0.84

Table (2): Complete remission in PRAME positive and PRAME negative groups

	PRAME positive N(%)	PRAME negative N(%)	*p*-value
Complete remission	12 (37.5)	15 (65.2)	0.04
No complete remission	20 (62.5)	8 (34.8)	

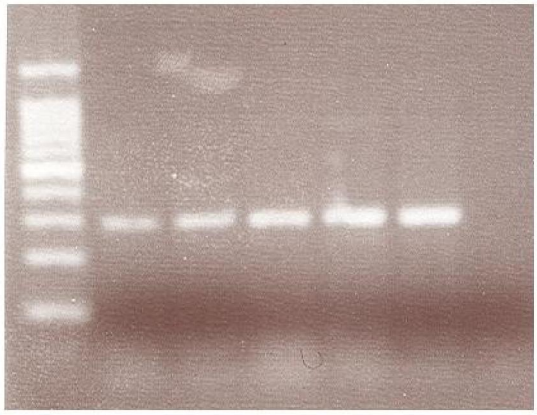

Figure (1): RT-PCR analysis showing β-actin expression
Lane 1: Molecular weight marker 1 kb (Fermentas).
Lanes 1- 5: β-actin positive cases.

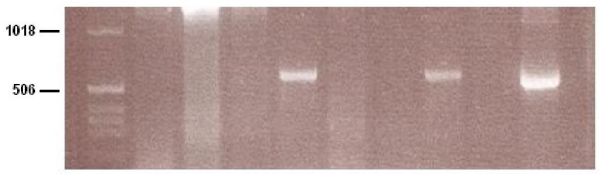

Figure (2): RT-PCR analysis showing PRAME expression.
Lane 1: Molecular weight marker 1 kb (Invitrogen).
Lanes 5,8,10: PRAME positive cases (556bp).
Lanes 2,3,4,6,7,9: PRAME negative cases.

4. Discussion

PRAME gene is highly expressed in AML patients and is usually associated with a favorable response to chemotherapy and prolonged survival [4,5]. It has been reported to function as a repressor of retinoic acid (RA) signaling through interactions with retinoic acid receptors (RARs). Expression of PRAME gene was assessed in adult acute myeloid leukemia patients at diagnosis and correlated with clinical outcome. Of sixty patients examined, thirty three patients (55%) expressed PRAME mRNA. This finding is approximately similar to that reported by Qin *et al* [13] in which PRAME was detected in 55.4% of AML patients and slightly lower than 64% reported by Greiner *et al.,* and Zhu *et al.,* [14,15]. On the other hand, PRAME expression was higher than was reported by Greiner *et al*, Zhou *et al.,* Tajeddine *et al.,* and Paydas *et al.,* [16,17,18,19] (47%, 42.9%, 40% and 30%, respectively). Ortmann *et al.,* [20] reported that PRAME expression has been observed in 30-64% of acute myeloid leukemia cases. In our study, PRAM mRNA was not detected in normal bone marrow cells. The difference between patients and control was statistically significant (*p*=0.00). Similar observation was demonstrated by Zhu *et al.,* and Zhou *et al.,* [15,17].

In this study, no significant correlation was found between PRAME expression and age, sex, white blood

cell count and the percentage of blasts in bone marrow at the diagnosis. These results are similar to that encountered by Paydas *et al.,* Zhou *et al.,* and Zhu *et al.,* [15,17,19].On the contrary, in a pediatric study done by Steinbach *et al.,* [4], he found that PRAME expression was negatively correlated to white blood cell count at diagnosis. PRAME expression was not correlated with immunophenotyping or stem cell marker CD34 expression on blast cells, this correspond to that reported by Paydas *et al.,* [19] who didn't find any important correlation between PRAME expression and cell surface antigens. PRAME expression mainly belongs to M2 FAB subtype but a valid significant statistics could not be done because of the small number of the cases included in different FAB groups. In a study carried Zhu *et al.,*[6], he reported that among the FAB subtypes, those with M1, M2, M3 and M4 had significantly higher level of PRAME transcripts than controls, however, those with M5 had similar level of PRAME transcripts as controls, also among cases with AML-M2, those with t(8;21) had significantly higher level of PRAME transcripts than those without[10].

As regard the clinical data, a significant correlation was found between PRAME expression and hepatomegaly (p=0.036), while no significant correlation was found with the splenomegaly and lymphadenopathy. This was different from what is reported by Paydas *et al.,* [19] who didn't find any correlation between PRAME expression and organomegaly or lymphadenopathy. However, our results can be explained by the high incidence of the hepatic bilharziasis in Egypt.

In this study a statistical significant correlation was detected between the response to therapy and PRAME expression where the number of CR is more in negative PRAME expression with a p value = 0.04. While, Zhou *et al.,* [17] and Zhu *et al.,* [6] found that PRAME is an indicator of favorable prognosis and can be a useful tool for monitoring minimal residual disease (MRD) in AML patients.

In this study 10 out of 12 early death cases were PRAME positive and this group of patients showed a high leukocyte count and poor liver and kidney functions which might lead to this result especially that in our multivariate analysis the PRAME wasn't correlated with any of the laboratory or clinical criteria.

Most studies on acute leukemia cases could not draw a clear association of PRAME expression as an independent prognostic [1,4]. Also in a study carried by Paydas *et al.,* [19], he didn't find any important correlation between PRAME expression and response to therapy. But various quantitative studies has shown that PRAME levels decrease in remission and increase in relapse suggesting its association to detect minimal residual disease [13,18,19].

On the contrary, high PRAME expression was found to be an independent prognostic marker of poor outcome in breast cancer and neuroblastoma [21,22]. Additionally, PRAME has been suggested as a predictive factor to determine the blastic phase in CML cases [23]. Also, it was found more frequently in mantle cell lymphoma as compared with other chronic lymphoproliferative disorders, thus making this antigen a marker for differentiation from other types of lymphoproliferative disorders [24].

As regard overall and disease free survival no statistical significant correlation was found with the PRAME expression. It seems that there is an increase in the overall survival among the PRAME negative compared to the PRAME positive group although the difference was not significant (p value = 0.06).This goes with Paydas *et al.,* [19] who didn't find correlation between PRAME expression and progression-free and overall survival. On the contrary, Greiner *et al.,* [5] found a significant correlation between high m-RNA levels of PRAME and longer overall survival. Also in a pediatric study, Steinbach *et al.,* [4], has found that the rates of overall and disease-free survival in the group of patients with high PRAME expression were higher than in patients with no or low expression.

In conclusion, PRAME is particularly an attractive tumor-associated antigen. Its expression was associated with poor prognosis. Its level of expression should be monitored during the course of the disease as a useful marker to predict remission or relapse. However, more studies should aim at detailed understanding of the mechanisms of PRAME actions and its use in minimal residual disease detection and in immunotherapy.

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1117