International Blood Research & Reviews
2(6): 299-310, 2014, Article no. IBRR.2014.6.005

SCIENCEDOMAIN *international*
www.sciencedomain.org

An Immunohistochemical Study of PRAME Expression in Non-hodgkin's Lymphoma

Semra Paydas[1*], Arbil Avci Acikalin[2], Melek Ergin[2], Berna Bozkurt Duman[1] and Gulsah Seydaoglu[3]

[1]Departments of Oncology, Cukurova University, Faculty of Medicine, Adana, 01330, Turkey.
[2]Departments of Pathology, Cukurova University, Faculty of Medicine, Adana, 01330, Turkey.
[3]Departments of Biostatistics, Faculty of Medicine, Cukurova University, Adana, 01330, Turkey.

Authors' contributions

This work was carried out in collaboration between all authors. Author SP designed the study, wrote the protocol, managed the literature searches and wrote the manuscript. Authors AAA and ME were performed immunehistochemistry and photographed the tissue sections, author BBD reviewed the patients' data, author GS made the statistical analyses and tables. All authors read and approved the final manuscript.

Original Research Article

Received 31st December 2013
Accepted 28th June 2014
Published 26th July 2014

ABSTRACT

Aim: Preferentially expressed antigen of melanoma (PRAME) is a cancer-testis antigen with very low/no expression in normal tissues. PRAME is an important target for tumor immunotherapy. Prognostic and in some tumors predictive importance of this expression have been shown in some solid tumors. The aim of this study is to detect the prognostic and/or predictive value of PRAME expression in non-Hodgkin's lymphomas (NHL).
Study Design: Retrospective clinico-pathological study.
Methodology: PRAME expression was determined by immunohistochemistry (IHC) in 62 cases with NHL. However statistical analysis was performed in 54 cases [33 with diffuse large B cell lymphoma (DLBCL) and 21 with indolent lymphoma (IL)] due to the low number of the other subtypes.
Results: PRAME expression was detected in 20 of the total 62 cases (32.3%). Nine of 33 cases with DLBCL, 7 of 21 cases with IL, 4 of 6 cases with T-NHL and both of the 2 cases

**Corresponding author: E-mail: sepay@cu.edu.tr;*

of mantle cell lymphoma (MCL). Clinical variables including gender, stage, age, extranodal involvement and response to chemotherapy were not different in PRAME (+) and PRAME (-) cases. However PRAME (+) cases had longer PFS and OS than the PRAME (-) cases, however, no significant difference was found between groups in total. Furthermore, lymphoma subtype data indicated that while PRAME positivity was significantly associated with longer OS in cases with IL (p=0.049) but not in DLBCL cases (p=0.881). Multivariate Cox regression analysis showed that while response to chemotherapy was an independent risk factor, PRAME and NHL subtype were found not to be significant independent risk factors associated with the OS rate.

Conclusion: PRAME expression was found in one third of the cases with NHL and there was no difference in PRAME expression in indolent lymphomas and DLBCL. Although we did not find the prognostic importance of PRAME with NHL overall, lymphoma subtype data indicated that PRAME positivity was associated with OS. This may be due to the relatively low number of the cases and also lack of comparison with RT-PCR which is the most frequently used method in detection of PRAME expression.

Keywords: PRAME; lymphoma; prognosis; response to chemotherapy.

1. INTRODUCTION

Preferentially expressed antigen of melanoma (PRAME) has been isolated from a patient with malignant melanoma fifteen years ago [1,2]. Although strict PRAME expression has been shown in normal testis tissue, it is expressed only in low levels in endometrium, adrenal glands and ovaries. Microarray and PCR studies have been performed many times and showed that PRAME is absent in normal hematopoietic tissues including bone marrow, CD34 (+) stem cells in bone marrow, and B and T cells in peripheral blood [3,4]. For this reason PRAME is an important target for tumor immunotherapy [1,2]. This tumor associated antigen has been found to be expressed in hemopoietic neoplasias as well as solid tumors. In these tumors, biologic function of PRAME is not clear. In some tumors, PRAME has been found to be a poor prognostic indicator, while other studies found it to be associated with good clinical outcomes [1,2,5-7]. PRAME expression was found to be an important target in malignant tumors and has been explored in almost all of the neoplastic disorders. Among hemopoietic neoplasias, the most frequently studied entities are chronic myelocytic leukemia (CML) and acute myeloblastic leukemia (AML). However, data about the PRAME expression in lymphomas is relatively limited [8-20].

The aim of this study was to detect the PRAME expression in NHLs using IHC technique and determine its prognostic/predictive value in lymphoma cases.

2. PATIENTS AND METHODS

Lymphoma samples taken from 62 cases were used in this study. Samples were collected from pathology archives. Patients were diagnosed by expert hematopathologists (AA, ME). Staging was made according to the Ann Arbor staging and patients were treated by standard chemotherapy (R-CHOP for DLBCL and R-CVP for indolent lymphoma).

Immunohistochemistry: Five µm slices were cut from blocks of formaldehyde-fixed and paraffin-embedded tissues and were placed on slides for H&E and IHC staining. PRAME (ab32185, abcam, USA) antibody was performed to polylysine slides by Strept Avidin Biotin

complex method. Antigen retrieval treatment was performed for 20 minutes in 0.01 M sodium citrat buffer solution (Ph 6.0), using a microwave oven. Immune complexes were detected by Strept Avidin Biotin complex (DAKO, K0690, North America) method and visualized by AEC (3-Amino-9-ethylcarbazole). Slides were counterstained with Mayer's hematoxyline and mounted. Testis tissue was used as positive control and negative controls were obtained by omitting the primary antibody. Stained slides were evaluated according to intensity of cytoplasmic and/or nuclear positivity as follows; 0 (no staining), 1+ (weak or equivocal staining), 2+ (moderate staining), or 3+ (strong staining). Tumor positivity was considered when more than 5% tumor population was stained 2+ or 3+ intensity.

In total, 62 cases were evaluated for PRAME expression, however statistical analyses were performed on 54 cases (33 with DLBCL and 21 with IL) due to the low number of the other subtypes. The categorical variables between the groups were analyzed by using the Chi square test or Fisher's exact test. The predictors of survival were analyzed by the Kaplan-Meier method and compared by the Mantel log-rank test. Cox proportional-hazard regression model applied to identify multivariate predictors (forward procedure, Wald method). The results were reported as mean±SD, median, number (n) and percent (%) and p value <0.05 was considered as significant. Statistical analyses were performed using the statistical package SPSS v 18.0.

3. RESULTS

PRAME expression was determined in 62 cases with NHL. Ages ranged between 18-87 and the male/female ratio was 36/26. Mean age was 52.48±15.86 for whole group, 52.54±15.42 for females and 52.44±16.38 for males. Thirty three of the cases had DLBCL, 21 had ILincluding follicular lymphoma, marginal zone lymphoma and small lymphocytic lymphoma. Six cases had T-NHL and 2 had MCL. Four of 6 cases with T-NHL had PRAME expression while none of the 2 cases with MCL showed PRAME expression.

Figs. 1 and 2 show the positive and negative expression for PRAME by IHC. Fig. 3 shows the PRAME expression in isotype control (testis tissue).

PRAME expression was detected in 20 of the total 62 cases. PRAME expression was detected in 16 (29.6%) of the study group (n=54). Seven of 21 cases with IL, and 9 of 33 cases with DLBCL had PRAME expression. PRAME expression was not found to be different in cases with DLBCL and IL(p 0.634). Clinical variables including age, gender, stage, extranodal involvement-except central nervous system, resistance to initial chemotherapy were not different in cases with DLBCL and indolent lymphoma. The distribution of prame positivity was not found to be different according to these clinical variables (Table 1).

When we looked at PFS and OS times, patients with advanced stage disease and cases with resistant disease to the first line chemotherapy had shorter survival times as expected. In the whole group however PRAME (+) cases had longer PFS and OS than the PRAME (-) cases, but no significant difference was found between groups (Table 2).

Comparisons of PRAME expression according to NHL subtype and response to chemotherapy are shown in Table 3. PRAME positivity was not found to be associated with PFS and OS in cases with DLBCL. PRAME positivity was associated with longer OS in cases with IL (p=0.049). Although OS and PFS were shorter in cases with chemoresistant disease, while significant difference was found between chemoresistant and chemosensitive

groups in PRAME (-) cases (p=0.011 for OS and p=0.0001 for PFS), no significant difference was found between the groups in PRAME (-) cases.

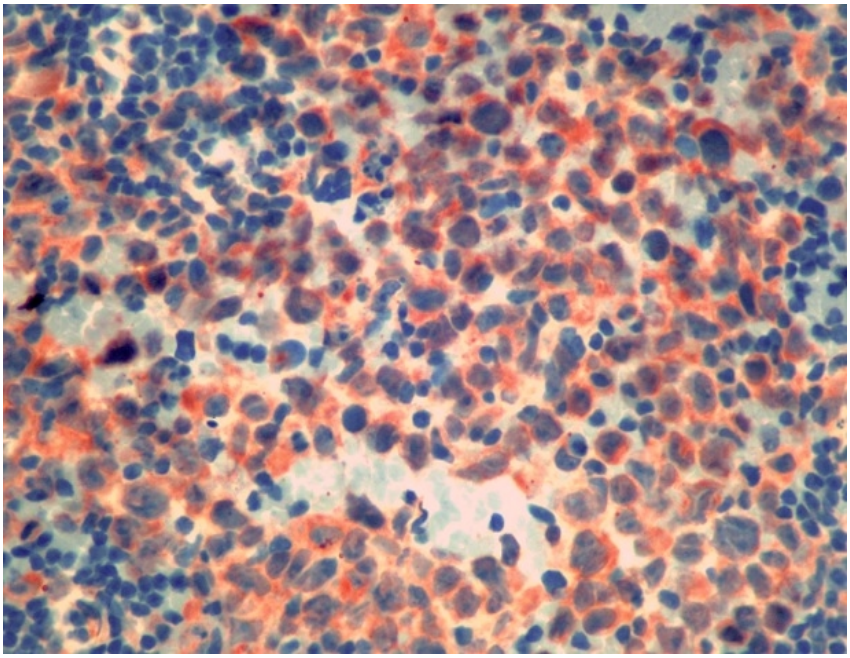

Fig. 1. Positive PRAME expression in lymphoma by immunohistochemistry (x400)

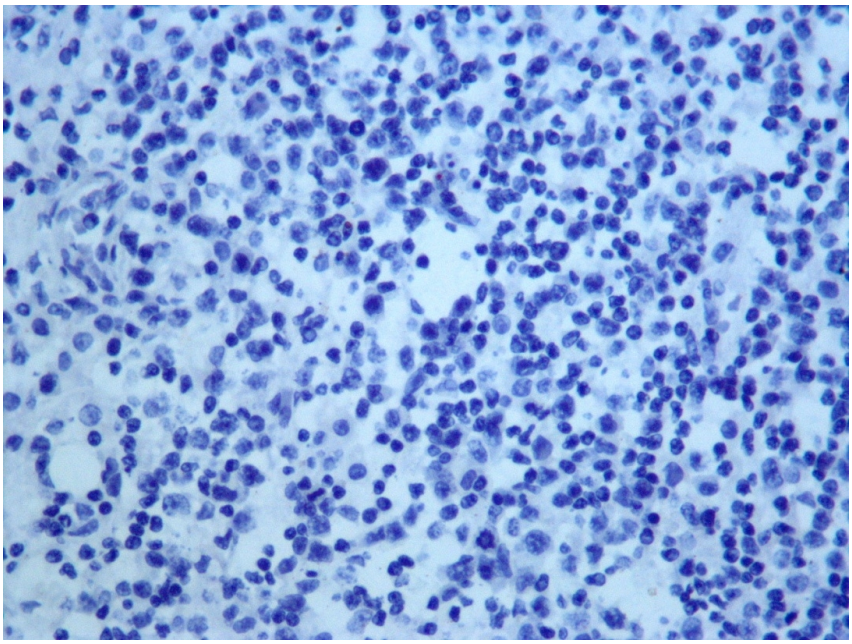

Fig. 2. Negative PRAME expression in lymphoma by immunohistochemistry (x400)

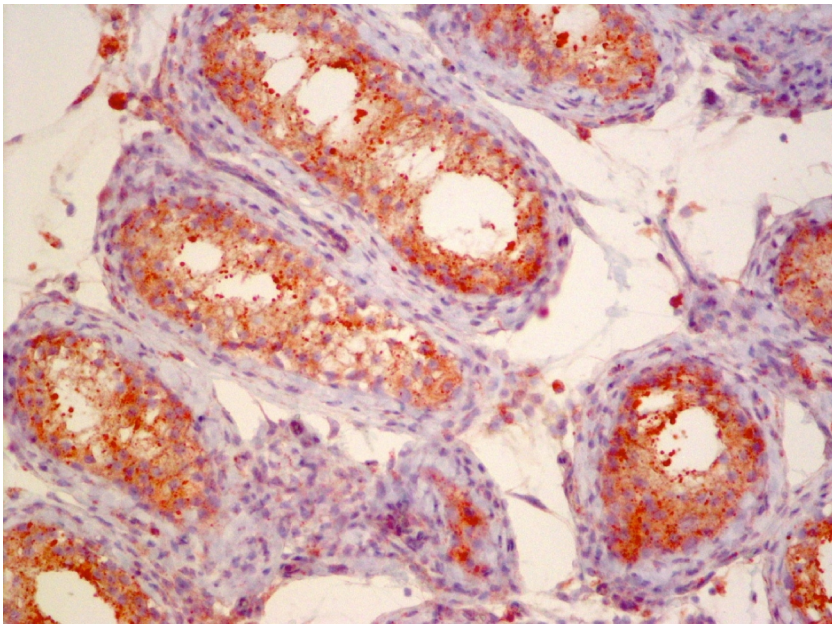

Fig. 3. PRAME expression in isotype control (testis) by immunohistochemistry

Table 1. Clinical variables according to the pathology groups and the distribution of PRAME positivity according to the clinical variables

		Pathology group		p	PRAME expression group		p
		DLBCL (n=33) n (%)	Indolent lymphoma (n=21) n (%)		Negative (n=38) n	Positive (n=16) n (%)	
Gender	Female	17 (51.5)	7 (33.3)		17	7 (29.2)	
	Male	16 (48.5)	14 (66.7)	0.190	21	9 (30.0)	0.947
Extranodal	No	5 (15.2)	6 (28.6)		6	5 (45.5)	
involvement	Yes	28 (84.8)	15 (71.4)	0.233	32	11 (25.6)	0.198
Bone	No	29 (87.9)	14 (66.7)		31	12 (27.9)	
	Yes	4 (12.1)	7 (33.3)	0.059	7	4 (36.4)	0.584
Bone marrow	No	29 (87.9)	21 (100.0)		34	16 (32.0)	
	Yes	4 (12.1)	0 (0.0)	0.097	4	0 (0.0)	0,177
Gastrointestinal	No	26 (78.8)	20(95.2)		32	14 (30.4)	
	Yes	7 (21.2)	1 (4.8)	0.097	6	2 (25.0)	0.756
Central Nervous	No	27 (81.8)	21(100.0)		33	15 (31.2)	
System	Yes	6 (18.2)	0 (0.0)	0.043	5	1 (9.1)	0.461
Head-neck	No	26 (78.8)	17 (81.0)		28	15 (34.9)	
	Yes	7 (21.2)	4 (19.0)	0.847	10	1 (9.1)	0.095
Stage	Early	20 (60.6)	9 (42.9)		21	8 (27.6)	
	Advanced	13 (39.4)	12 (57.1)	0.202	17	8 (32.0)	0.723
Relapse	No	23 (69.7)	16 (76.2)		26	13 (33.3)	
	Yes	10 (30.3)	5 (23.8)	0.604	12	3 (20.0)	0.337
Chemotherapy	Sensitive	28 (84.8)	18 (85.7)		32	14(30.4)	
Response	Resistant	5 (15.2)	3 (14.3)	0.930	6	2(25.0)	0.756
PRAME	Negative	24 (72.7)	14 (66.7)				
	Positive	9 (27.3)	7 (33.3)	0.634			

Abbreviations: DLBCL: Diffuse large B cell lymphoma

Table 2. Three years of overall survival and progression free survival

		OS (%)	p*	PFS (%)	p*
Pathology group	DLBCL	56		52	
	Indolent Lymphoma	68	0.397	59	0.705
Stage	Early	81		72	
	Advanced	38	0.009	34	0.004
Chemotherapy response	Sensitive	69		62	
	Resistant	25	0.050	14	0.0001
Extranodal involvement	No	75		75	
	Yes	57	0.370	50	0.302
PRAME	Negative	54		48	
	Positive	77	0.143	68	0.296
Total		61		55	

**Log Rank test, Abbreviations: DLBCL: Diffuse large B cell lymphoma, OS (%): Overall survival and PFS (%): Progression free survival results at 3 year*

Table 3. Three years of overall survival and progression free survival according to the pathologic subgroup and PRAME expression

Groups		Subgroups	OS (%)	p*	PFS (%)	p*
	DLBCL	PRAME (-)	56		49	
Pathologic subgroup		PRAME(+)	57	0.881	57	0.999
		Overall	56		52	
	IL	PRAME (-)	50		45	
		PRAME(+)	100	0.049	80	0.130
		Overall	68		59	
PRAME expression	Negative	CT Sensitive	63		59	
		CT Resistant	17	0.011	0	0.0001
		Overall	54		48	
	Positive	CT Sensitive	82		72	
		CT Resistant	50	0.987	50	0.449
		Overall	77		68	

**Log Rank test, Abbreviations: DLBCL: Diffuse large B cell lymphoma, IL: Indolent lymphomas, CT: chemotherapy, OS (%): Overall survival and PFS (%): Progression free survival results at 3 year*

Fig. 4 shows the comparisons of the different subgroups for OS curves according to the pathologic subgroup, PRAME expression and response to chemotherapy.

Multivariate Cox regression analysis showed that while response to chemotherapy was found to be an independent risk factor, PRAME and NHL subtype were not found to be independent risk factors significantly related with the OS rate (Table 4).

Table 4. Results of multivariate cox regression analyses

	B	SE	OR (95% CI)	p
PRAME (negative)	0.672	0.697	1.9 (0.5-7.7)	0.335
Pathology (IL)	0.761	0.669	2.1 (0.5-7.9)	0.255
Chemotherapy (Resistance)	1.410	0.594	4.1 (1.3-13.1)	0.018
Age	0.060	0.021	1.1 (1.0-1.1)	0.004

IL: Indolent lymphomas, SE: Standard error, B: Regression coefficient, OR: Odds ratio, CI: confidence intervals

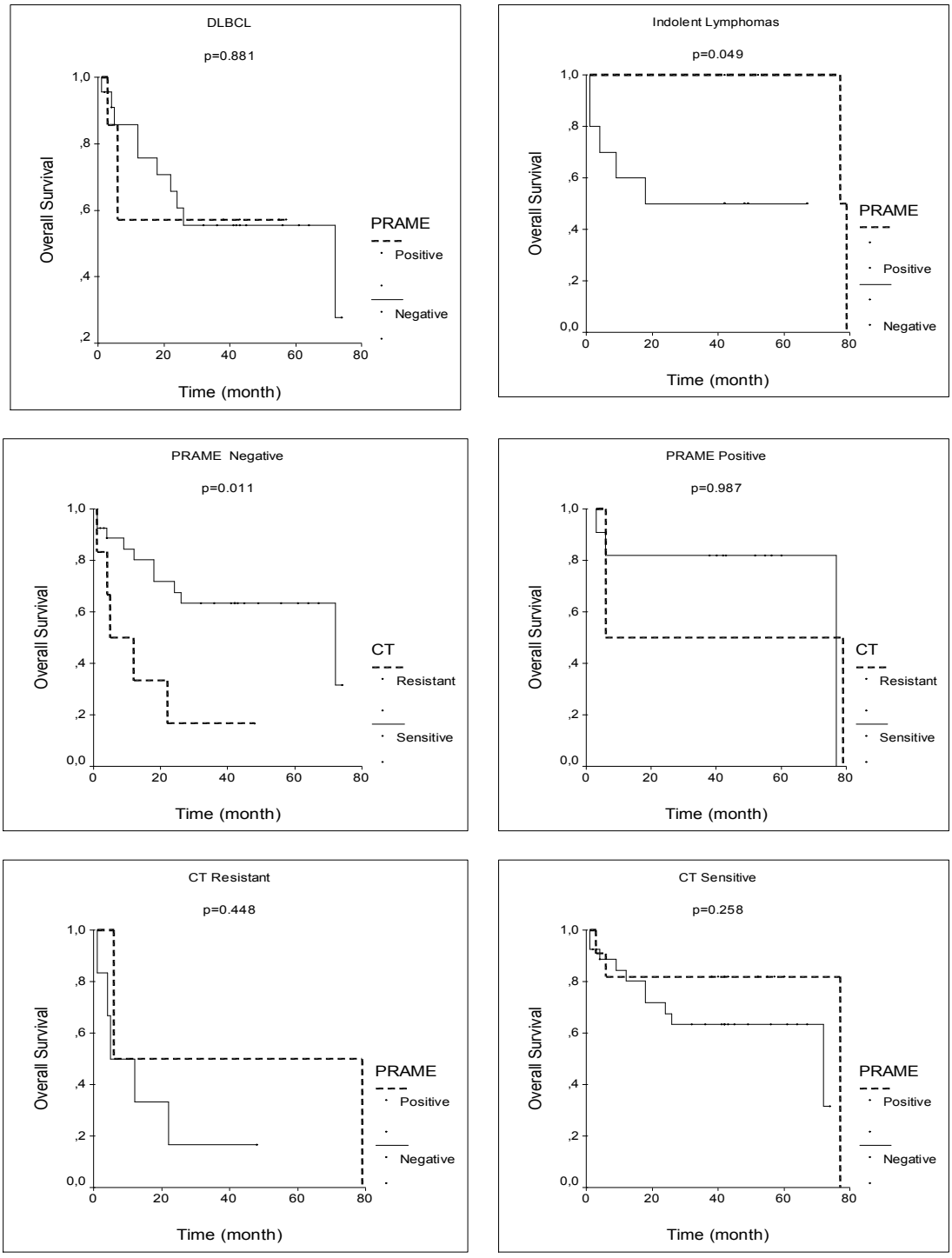

Fig. 4. Overall Survival curves according to the NHL subtype, PRAME expression and response to chemotherapy (CT)

4. DISCUSSION

Since the discovery of PRAME, studies about its significane are increasing with some promising results. NHLs are highly heterogeneous neoplastic disorders with variable responses to treatments and outcome. Besides the morphologic subtype, biologic properties are very important. The prognostic significance of PRAME has been shown in a variety of malignant tumors and, in limited studies, in lymphoma as well. There is relatively limited data about PRAME expression in lymphomas. In a study (n= 108) covering various hemopoietic neoplasias PRAME has been found in 3 of 30 cases with NHL and 1 of 7 cases with Hodgkin's lymphoma (HL) [5]. In another study PRAME expression was found in 7 of 16 cases with MCL [21]. In our study group there were only 2 cases with MCL and none of these 2 cases showed PRAME expression. Gene-expression profile was analyzed in HL cell lines and PRAME expression was found in resistant cell lines .It has been suggested that aberrantly expressed PRAME may be a target for immunotherapy in HL [22-24].

Thus, PRAME expression in NHL has not been decisive due to the small number of the cases studied. mRNA expression of 32 cancer testis antigens has been assessed by RT-PCR in 9 DLBCL cell lines and PRAME has been found in all of the 9 cell lines. Among these cell lines, 3 had ABC-like gene expression profile [25]. So far, the most comprehensive study about PRAME expression in NHL has been reported by Kawano et al. [26]. cDNA microarray analysis has been used by Kawano to identify the genes expressed in chemotherapy resistant and sensitive cases with DLBCLs (7 cases and 6 cases, respectively). Nine genes on the cDNA chip showed increased expression in anthracycline resistant patients and the highest expression belonged to PRAME. This group of investigators also studied PRAME by RT-PCR in 45 lymphoma samples. PRAME expression was found in 12 of 45 casesand most prominent in patients with anthracycline resistant disease. PFS was found to be shorter in PRAME (+) cases than PRAME (-) cases. Also progressive disease was higher in PRAME (+) cases as compared with PRAME (-) cases (50% vs 18%, respectively). Although the authors did not determine mechanisms involved in cases where there was no response to therapy in PRAME (+), the authors suggested that PRAME specific immunotherapy may be a clinical strategy in anthracycline resistant patients. In summary, prognostic and predictive value of PRAME expression has been shown clearly in Kawano's study [26]. In similar way, PRAME expression has been found in resistant HL cell lines and these studies also suggested an association between anthracycline resistance and PRAME expression [22]. We found PRAME expression in one third of the cases with NHL and one fourth of the cases with DLBCL. We did not find clear prognostic/predictive significance of this antigen, but we detected longer PFS in cases with IL and we could not define the cause of this result. PRAME was not found as an independent risk factor in multivariate analysis. Response to initial treatment was not different in our PRAME (+) and (-) cases while in Kawano's study PRAME positivity was found to be related with chemotherapy resistance and progressive disease. The difference may be due to the different methodologies used in these 2 studies. They used RT-PCR while we used IHC and we know that RT-PCR is the most commonly used method for detection of PRAME. However, recently IHC has been used in cases with osteosarcoma and prognostic significance was clearly shown [27]. IHC is an easy and usable technique in almost every laboratory and is useful method in daily clinical practice. Additionally we compared RT-PCR and IHC in HL and we found a correlation between these two methods (unpublished data). Another potential explanation for the different results may be related to the use of rituximab. Patients in Kawano's study did not receive rituximab, while our cases with DLBCL did . We know that rituximab containing regimen is standard for B cell NHL. As pointed out very well, rituximab induces apoptosis, promotes phagocytosis and cross priming cytotoxic T cells

(CTLs) [28]. It is possible that CTLs can be activated with rituximab use in PRAME (+) DLBCLs. So we can define the lack of difference for response in PRAME (+) and (-) cases in DLBCL cases receiving rituximab according to this scientific data. In fact there are many studies about the prognostic/predictive properties of the PRAME and also strategies for immunotherapeutic approach both in solid tumors and in hemopoietic neoplasias [29-41].

We found PRAME in 4 of the 5 cases with T-NHL. We did not find a previous study evaluating PRAME in T-NHL. Unfortunately, we could not compare the clinical significance due tothe very low number of the cases.

Our study has 2 limitations, first is low number of the cases and second is the lack of comparison of IHC with RT-PCR method. We have evidence, although not published, that IHC results correlate well with RT-PCR results in Hodgkin's lymphoma case. Similar confirmation came from a study with osteosarcoma as mentioned above. Thus our results should be considered preliminary and further studies using a larger sample size are still needed to allow us to better assess the prognostic and predictive value of PRAME expression in cases with lymphoma.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ikeda h, Lethe B, Lehmann F, van Baren N, Baurain JF, de Smet C, Chambost H, Vitale M, Moretta A, Boon T, Coulie PG. Characterization of an antigen that is recognized on a melanoma showing partial, HLA loss By CTL expressing an NK inhibitory receptor. Immunity. 1997;6:199-208.
2. Epping MT, Bernards R. A causal role for the human tumor antigen preferentially expressed antigen of melanoma in cancer. Cancer Res. 2006;66:10639-10642.
3. Epping MT, Hart AA, Glas AM, Krijgsman O, Bernards R. PRAME expression and clinical outcome of breast cancer. Br J Cancer. 2008;99:398-403.
4. Roman-Gomez J, Jimenez-Velasco A, Agirre X, Castillejo JA, Navarro G, Jose-Eneriz ES, Garate L, Cordeu L, Cervantes F, Prosper F, Heiniger A, Torres A. Epigenetic regulation of PRAME gene in chronic myeloid leukemia. Leuk Res. 2007;31:1521-1528.

5. Van Baren N, Chambost H, Ferrant A, Michaux L, Ikeda H, Millard I, Olive D, Boon T, Coulie PG.PRAME, a gene encoding an antigen recognized on a human melanoma by cytolytic T cells, is expressed in acute leukaemia cells. Br J Haematol. 1998;102:1376-1379.
6. Paydas S, Tanriverdi K, Yavuz S, Disel U, Baslamisli F, Burgut R. PRAME m RNA levels in cases with acute leukemia: clinical significance and future prospects. Am J Hematol. 2005;79:257-261.
7. Bankovic J, Stojsic J, Jovanovic D, Andjelkovic T, Milinkovic V, Ruzdijic S, Tanic N. Identification of genes associated with non-small cell lung cancer promotion and progression. Lung Cancer. 2010;67:151-159.
8. Costessi A, Mahrour N, Tijchon E, Stunnenberg R, Stoel MA, Jansen PW, Martin-Brown S, Washburn MP, Florens L, Guezennec XL, Conaway RC, Stunnenberg HG. The tumour antigen PRAME is a subunit of a Cul2 ubiquitin ligase and associates with active NFY promoters EMBO J. 2011;30(18):3786-98.
9. Tanaka N, Wang YH, Shiseki M, Takanashi M, Motoji T. Inhibition of PRAME expression causes cell cycle arrest and apoptosis in leukemic cells. Leuk Res. 2011;35:1219-1225.
10. Yin B. PRAME: From diagnostic marker and tumor antigen to promising target of RNA i therapy in leukemic cells. Leuk Res. 2011;35:1159-1160.
11. Weber JS, Vogelzang NJ, Ernstoff MS, Goodman OB, Cranmer LD, Marshall JL, Miles S, Rosario D, Diamond DC, Qiu Z, Obrocea M, Bot A. A phase 1 study of a vaccine targeting preferentially expressed antigen in melanoma and prostate-specific membrane antigen in patients with advanced solid tumors. J Immunother. 2011;34:556-567.
12. Radich JP, Dai H, Mao M Oehler V, Schelter J, Druker B, Sawyers C, Shah N, Stock W, Willman CL, Friend S, Linsley PS. Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci USA. 2006;103:2794-2799.
13. Steinbach D, Hermann J, Viehmann S, Zintl F, Gruhn B. Clinical implications of PRAME gene expression in childhood acute myeloid leukemia. Cancer Genet Cytogenet. 2002;133:118-123.
14. Paydas S, Tanriverdi K, Yavuz S, Seydaoglu G. PRAME m RNA levels in cases with chronic leukemia: clinical importance and review of the literature. Leuk Res. 2007;31:365-369.
15. Schenk T, Stengel S, Goellner S, Steinbach D, Saluz HP. Hypomethylation of PRAME is responsible for its aberrant overexpression in human malignancies. Genes Chromosomes Cancer. 2007;46:796-804.
16. Paydas S. Is everything known in all faces of iceberg in PRAME? Leuk Res. 2008;32:1356-1357.
17. Epping MT, Wang L, Edel MJ, Carlée L, Hernandez M, Bernards R. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. Cell. 2005;122:835-847.
18. Steinbeach D, Pfaffendorf N, Wittig S, Gruhn B. PRAME expression is not associated with down-regulation of retinoic acid signaling in primary acute myeloid leukemia. Cancer Genet Cytogenet. 2007;177:51-54.
19. Partheen K, Levan K, Osterberg L, Claesson I, Fallenius G, Sundfeldt K, Horvath G. Four potential biomarkers as prognostic factors in stage III serous ovarian adenocarcinomas. Int J Cancer. 2008;123:2130-2137.

20. Pellat-Deceunynck C, Mellerin MP, Labarriere N, Jego G, Moreau-Aubry A, Harousseau JL, Jotereau F, Batallie R. The cancer germ-line genes MAGE-1, MAGE-3 and PRAME are commonly expressed by human myeloma cells. Eur J Immunol 2000;30:803-809.
21. Proto-Siqueira R, Falco RP, Souza CA, Ismael S, Zago MA. The expression of PRAME in chronic lymphoproliferative disorders. Leuk Res. 2003;27:393-396.
22. Steage MS, Banning-Eichenseer U, Weibflog G, Volkmer I, Burdach S, Richter G, Mauz-Körholz C, Föll J, Körholz D. Gene expression profiles of Hodgkin's lymphoma cell lines with different sensitivity to cytotoxic drugs. Exper Hematol. 2008;36:886-896.
23. Küppers R, Klein U, Schwering I, Distler V, Brauninger A, Cattoretti G, Tu Y, Stolovitzky GA, Califano A, Hansmann ML, Dalla-Favera R. Identification of Hodgkin and Reed-Sternberg cell-specific genes by gene expression profiling. J Clin Invest. 2003;111:529-537.
24. Willenbrock K, Küppers R, Renne C, Brune V, Eckerle S, Weidmann E, Brauninger A, Hansmann ML. Common features and differences in the transcriptome of large cell anaplastic lymphoma and classical Hodgkin's lymphoma. Haematologica. 2006;91:586-604.
25. Liggins AP, Lin AH, Soileux EJ, Pulford K, Banham AH. A panel of cancerütestis genes exhibiting broad-spectrum expression in haematological malignancies. Cancer Immunity. 2010;10:1-12.
26. Kawano R, Karube K, Kikuchi M, Takeshita M, Tamura K, Uike N, Eto T, Ohshima K, Suzumiya J. Oncogene associated c DNA microarray analysis shows PRAME gene expression is a marker for response to anthracycline containing chemotherapy in patients with diffuse large B cell lymphoma. J Clin Exp Hematop. 2009;49:1-7.
27. Tan P, Zou C, Yong B, Han J, Zhang L, Su Q, Yin J, Wang J, Huang G, Peng T, Shen J. Expression and prognostic relevance of PRAME in primary osteosarcoma. Biochem Biophys Res Comm. 2012;419:801-808.
28. Selenko N, Maidic O, Draxier S, Barer A, Jager U, Knapp W, Stöckl J. CD20 antibody (C2B8)-induced apoptosis of lymphoma cells promotes phagocytosis by dendritic cells and cross priming of CD8+ cytotoxic T cells. Leukemia. 2001;15:1619-1626.
29. Quintarelli C Dotti G, Hasan ST, De Angelis B, Hoyos V, Errichiello S, Mims M, Luciano L, Shafer J, Leen AM, Heslop HE, Rooney CM, Pane F, Brenner MK, Savoldo B. High-avidity cytotoxic T lymphocytes specific for a new PRAME-derived peptide can target leukemic and leukemic-precursor cells. Blood. 2011;117:3353-3362.
30. Oehler VG, Guthrie KA, Cummings CL, Sabo K, Wood BL, Gooley T, Yang T, Epping MT, Shou Y, Pogosova-Agadjanyan E, Ladne P, Stirewalt DL, Abkowitz JL, Radich JP. The preferentially expressed antigen in melanoma (PRAME) inhibits myeloid differentiation in normal hematopoietic and leukemic progenitor cells. Blood. 2009;114:3299-3308.
31. Winkler C, Steingrube DS, Altermann W, Schlaf G, Max D, Kewitz S, Emmer A, Kornhuber M, Banning-Eichenseer U, Staege MS. Hodgkin's lymphoma RNA-transfected dendritic cells induce cancer/testis antigen-specific immune responses. Cancer Immunology, Immunotherapy. 2012;61:1769-1779.
32. Vulcani-Freitas TM, Saba-Silva N, Cappellano A, Cavalheiro S, de Toledo SRC. PRAME gene expression profile in medulloblastoma [Perfil de expressão do gene PRAME em meduloblastoma Arquivos de Neuro-Psiquiatria. 2011;69:9-12.
33. Steinbach D, Hermann J, Viehmann S, Zintl F, Gruhn B. Clinical implications of PRAME gene expression in childhood acte myeloid leukemia. Cancer Genet Cytogenet. 2002;133:118-123.

34. Tajeddine N, Gala JL, Louis M, Van Schoor M, Tombal B, Gailly P. Tumor-associated antigen preferentially expressed antigen of melanoma (PRAME) induces caspase-independent cell death in vitro and reduces tumorigenicity in vivo. Cancer Res. 2005;65:7348-7355.
35. Toledo SRC , Zago M.A, Oliveira ID, Proto-Siqueira R, Okamoto OK, Severino P, Vêncio RZN, Gamba FT, Silva WA, Moreira-Filho CA, Torre CAD, Alves MTS, Garcia-Filho RJ, Simpson AJG, Petrilli AS. Insights on PRAME and osteosarcoma by means of gene expression profiling. Journal of Orthopaedic Science. 2011;16:458-466.
36. Oberthuer A, Hero B, Spitz R, Berthold F, Fischer M. The tumor-associated antigen PRAME is universally expressed in high stage neuroblastoma and associated with poor outcome. Clin Cancer Res. 2004;10:4307-4313.
37. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C,Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002;415:530-536.
38. Santamaría C, Chillón MC, García-Sanz R, Balanzategui A, Sarasquete ME, Alcoceba M, Ramos F, Bernal T, Queizán JA, Penarrubia MJ, Giraldo P, San Miquel JF, Gonzalez M. The prevalence of preferentially expressed antigen of melanoma (PRAME) as a marker of disease activity and prognosis in acute promyelocytic leukemia. Haematologica. 2008;93:1797-1805.
39. Santamaría CM, Chillón MC, García-Sanz R, Pérez C, Caballero MD, Ramos F, de Coca AG, Alonso JM, Giraldo P, Bernal T, Queizán JA, Rodriguez JN, Fernández-Abellán P, Bárez A, Peñarrubia MJ, Balanzategui A, Vidriales MB, Sarasquete ME, Alcoceba M, Díaz-Mediavilla J, San Miguel JF, Gonzalez M Molecular stratification model for prognosis in cytogenetically normal acute myeloid leukemia. Blood. 2009;114:148-152.
40. Zhu ZH, Qian J, Lin J, Yao DM, Qian Z, Wang YL, Chen Q, Han LX, Xiao G. Quantification of the PRAME transcripts in patients with acute myeloid leukemia. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2010;27:149-152.
41. Haqq C, Nosrati M, Sudilovsky D, Crothers J, Khodabakhsh D, Pulliam BL, Federman S, Miller JR 3rd, Allen RE, Singer MI, Leong SP, Ljung BM, Sagebiel RW, Kashani-Sabet M. The gene expression signatures of melanoma progression. Proc Natl Acad Sci USA. 2005;102:6092-6097.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=576&id=28&aid=5491