Deletion of Genetic Material from a Poly(ADP-Ribose) Polymerase-like Gene on Chromosome 13 Occurs Frequently in Patients with Monoclonal Gammopathies

Jin Cao, Charlie H. Hong, Lee Rosen, Robert A. Vescio, Mark Smulson, Alan K. Lichtenstein, and James R. Berenson[1]

Division of Hematology/Oncology, Department of Veterans Affairs, West Los Angeles, Los Angeles, California 90073 [J. C., C. H. H., L. R., R. A. V., A. K. L., J. R. B.]; Department of Medicine, University of California at Los Angeles School of Medicine and Jonsson Cancer Center, Los Angeles, California 90024 [L. R., R. A. V., A. K. L., J. R. B.]; and Department of Biochemistry and Molecular Biology, Georgetown University School of Medicine, Washington, D.C. 20007 [M. S.]

Abstract

Recently, by using a probe for the nuclear DNA repair enzyme poly(ADP-ribose) polymerase gene, a pseudogene was found on the long arm of chromosome 13. RFLP analysis demonstrates the presence of a common "A" allele and a rare "B" allele, which has a deletion of approximately 200 bp. This deletion occurs more frequently in blacks than in whites in the United States. In two B-cell malignancies, Burkitt's and follicular lymphomas, there is a marked increased frequency of the expression of the B allele. Thus, we have analyzed the frequency of this allele in another B-cell malignancy, multiple myeloma (MM), which is also more frequently observed in blacks. We studied 97 patients with MM (41 black and 56 white patients) and 30 patients with the related disorder monoclonal gammopathy of undetermined significance (MGUS; 13 black and 17 white patients). The results demonstrate that the overall frequency of B allele expression (37%) is higher than in a noncancer control population (23%; $P < 0.01$). This difference is mainly due to the much higher frequency of B expression in black patients (62 *versus* 35% in black controls; $P < 0.01$), whereas there is no significant difference in white patients (18 *versus* 14% in white controls). Overall, B allelic frequency is similar in patients with MM and MGUS. Matched germline and tumor DNA show identical patterns of expression of these alleles. These results suggest germline B allelic expression predisposes one to MM and MGUS.

Introduction

Deletion of specific genetic material has been shown to occur frequently in several human cancers (1–7). In some cases, deletion of genetic material has led to the identification of tumor suppressor genes. Specifically, the retinoblastoma suppressor gene has been localized to the long arm of chromosome 13 at band q14 (8, 9), and recently has been shown to be frequently missing or altered in the B-cell malignancy, chronic lymphocytic leukemia (1).

PADPRP[2] is a DNA-binding protein that covalently modifies the chromatin structure adjacent to regions of DNA replication, recombination, and repair (10–16). Recently, the human PADPRP gene has been cloned. Although the active gene has been localized to chromosome 1, PADPRP-hybridizing sequences have also been localized to chromosomes 13 and 14. Structural alteration of the PADPRP gene has been observed in murine strains susceptible to the development of plasma cell tumors (17). At the site of the human PADPRP pseudogene on chromosome 13 at q33-qter, RFLP analysis has demonstrated the presence of two alleles, the common A allele and the less common B allele, which were identified as 2.8- or 2.6-kb *Hind*III fragments after hybridization to full-length human PADPRP cDNA. These fragments were originally thought to derive either from a PADPRP-processed pseudogene or from a gene with extensive identity to PADPRP. However, no functional protein product has ever been associated with the PADPRP-hybridizing sequences on chromosome 13. These sequences are distinct from the gene encoding the authentic protein on chromosome 1q or the pseudogene on chromosome 14 (1). A preliminary characterization of the two allelic polymorphisms on chromosome 13 shows that they always cosegregate together and differ by 200 bp between the respective A and B alleles. Recently, a 193-bp conserved, duplicated region within the A allele was precisely detected as the source of the polymorphism (2). Collectively, these RFLP studies suggested a deletion or insertion of this DNA fragment adjacent to or within PADPRP-like sequences.

Recently, the increased frequency of this deleted B allele has been found in tumor DNA from patients with Burkitt's and B-cell follicular lymphomas when compared with germline DNA in a noncancer population (1). In the noncancer population, a marked difference in the frequency of the B allele was also observed in germline DNA from black (35%) and white (14%) individuals (1). In certain malignancies more frequently found in blacks, a higher rate of B allelic expression has been observed in the black cancer patients, including the B-cell tumor Burkitt's lymphoma (1).

There are also marked racial differences in the incidence of MM with blacks being twice as likely as whites to develop this malignancy (18). Thus, we have analyzed RFLP patterns of the human PADPRP-like gene on chromosome 13 in black and

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[1] To whom requests for reprints should be addressed, at 11-260 Factor Building, University of California at Los Angeles School of Medicine, 10833 Le Conte Avenue, Los Angeles, CA 90024.

[2] The abbreviations used are: PADPRP, poly(ADP-ribose) polymerase; MM, multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance; PBMC, peripheral blood mononuclear cells.

white patients with MM and in patients with another related B-cell disorder, MGUS.

Materials and Methods

Patients. Ninety-seven patients with MM and 30 patients with MGUS were studied. Multiple myeloma patients were identified as defined by the Myeloma Task Force (19). Median follow-up was 35 months from diagnosis. The diagnostic criteria for MGUS have been described elsewhere (20). The majority (80%) of patients with MGUS do not develop malignant or "serious" B-cell clonal disorders (20). All patients analyzed in this study were either white or black Americans. Data on the control group were taken from Bhatia *et al.* (1).

Isolation of Peripheral Blood Lymphocytes, Granulocytes, and Bone Marrow Plasma Cells. Peripheral venous blood and bone marrow samples were obtained from all the patients after informed consent and in accordance with the Human Subjects' Review Boards of the Veteran's Administration Wadsworth and University of California at Los Angeles Medical Center. The heparinized peripheral blood sample was centrifuged to separate the cells from the serum. The cells were exposed to Histopaque 1077 and 1119 (Sigma Chemical Co., St. Louis, MO) density gradient separation. This technique yields >99% pure granulocytes (as germline control) at the 1077/HBSS interface. The PBMC appear at the 1077/1119 interface. Bone marrow samples were exposed to Ficoll-Hypaque (Sigma) density gradient separation to yield enriched populations of plasma cells.

Southern Blot Analysis. Genomic high molecular weight DNA was prepared by proteinase K digestion and phenol-chloroform extraction (21) from PBMC, granulocytes, and bone marrow plasma cells from each patient. Ten μg of DNA were digested with either *Hind*III or *Eco*R I (GIBCO-BRL, Gaithersburg, MD) and separated by electrophoresis in a 0.8% agarose gel. The DNA was transferred to a nylon membrane by capillary diffusion. These filters were then hybridized with a PADPRP probe. To prepare this probe, a 3.7-kb *Xho*I-*Xho*I fragment was excised from pcD 12, an Okayama-Berg vector containing the full-length PADPRP cDNA (9). The probe was labeled with [^{32}P]dCTP by the Random Primed DNA-Labeling kit (Boehringer Mannheim, Indianapolis, IN). Hybridization and washing were performed as described elsewhere (1). After washing, the hybridized blots were autoradiographed by using Kodak XAR-5 film and intensifying screens at −70°C for 1 day.

Statistics. In the analysis of B allelic frequency among the different patient populations, we used the χ^2 method to determine their relative significance. In analysis of the MM patients' survival, we used both the Wilcoxon rank sum and Kaplan-Meier survival probability tests.

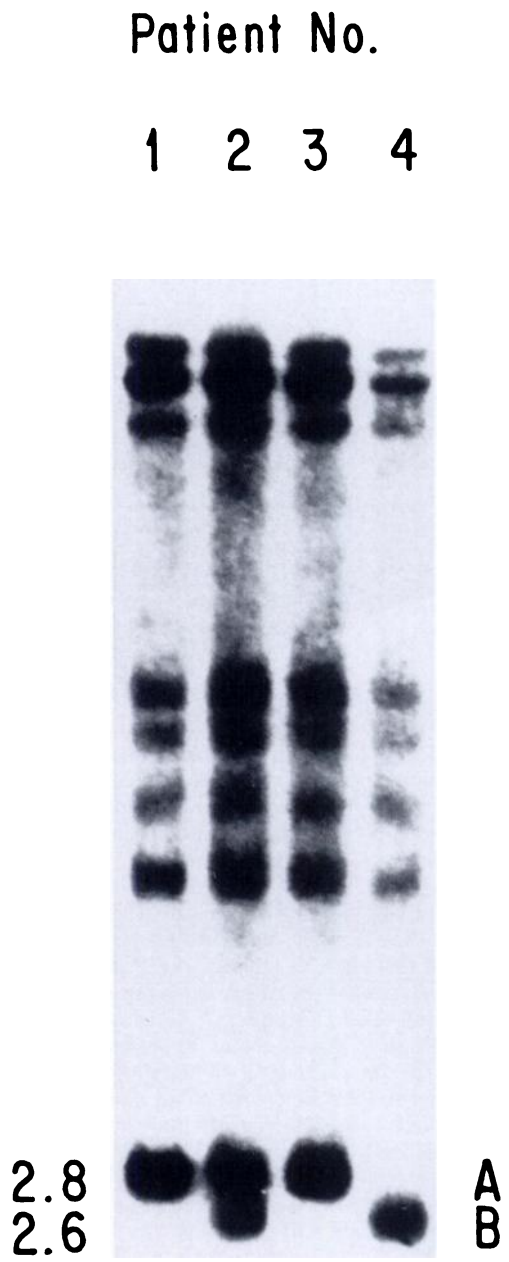

Fig. 1. Southern blot of bone marrow DNA from patients with MM or MGUS digested with *Hind*III and hybridized with the full-length PADPRP probe. *Lanes 1-3*, bone marrow DNA of three patients with MM; *Lane 4*, bone marrow DNA of one patient with MGUS. A and B alleles are at 2.8 and 2.6 kb, respectively.

Results

Characterization of the RFLP Analysis. The full-length PADPRP cDNA was used to analyze the RFLPs. Fig. 1 shows a representative Southern blot of *Hind*III-digested bone marrow DNA from 3 cases of MM and 1 MGUS patient. Hybridization under stringent conditions showed a strong signal intensity of the 2.8-kb (A allele) and 2.6-kb (B allele) *Hind*III fragments of genomic DNA from patients with MM or MGUS. This *Hind*III polymorphism represents a simple two-allele polymorphism of the PADPRP-like sequences on chromosome 13, and the three possible genotypes are seen in Fig. 1 (AA, BB, and AB). These RFLP studies suggest a deletion of around 200 bp adjacent to or within the polymerase-like sequences as reported previously in other studies of this pseudogene (1, 2).

Frequency of the PADPRP-like B Allele Patients with MM or with MGUS. Because the increased frequency of the B allele in patients with another B-cell malignancy, endemic Burkitt's lymphoma, has been reported recently (1), we analyzed bone marrow DNA from a large cohort of 97 patients with multiple myeloma. Overall B allelic expression in these patients was 35% *versus* a frequency of 23% in the control population adjusted for racial makeup of the myeloma cohort

Table 1 Frequency of the B allele in patients with MM or MGUS

	Total	Genotype (*n*)			B-allele frequency (%)	χ^2
		AA	AB	BB		
All patients	127	61	38	28	(37)	$P < 0.01^a$
Black	54	10	21	23	(62)	$P < 0.01^b$
White	73	51	17	5	(18)	NS[c]
Multiple myeloma	97	50	26	21	(35)	$P < 0.05^a$
Black	41	6	17	18	(65)	$P < 0.005^b$
White	56	44	9	3	(13)	NS[c]
MGUS	30	11	12	7	(43)	$P < 0.025^a$
Black	13	4	4	5	(54)	NS[b]
White	17	7	8	2	(35)	NS[c]
Noncancer population[d]	96	60	30	6	(23)	
Black	37	15	18	4	(35)	
White	59	45	12	2	(14)	

[a] Compared with the total noncancer population.
[b] Compared with the black noncancer population.
[c] NS, not significant; compared with the white noncancer population.
[d] Distribution of genotypes was taken from Bhatia *et al.* (1).

($P < 0.05$; Table 1). In 41 black patients with MM, the frequency of the PADPRP B allele was 65%, which was nearly double that observed in the noncancer black population (35%; $P < 0.005$). By contrast, the frequency of the B genotype among the 56 white patients was not significantly different from that of the control group (18 *versus* 14% in the white control group).

Because MGUS represents another form of monoclonal gammopathy with often a relatively benign course, we analyzed 30 patients with this clonal B-cell disorder. Similar to MM, the frequency of the B allele among the MGUS patients was nearly double (43 *versus* 23%) that of the noncancer control population matched for racial makeup of the MGUS patients analyzed ($P < 0.05$).

Overall, the frequency of the B allelic expression in blacks with MM and MGUS was 62% (noncancer blacks = 35%; $P < 0.01$). Although the expression of the B allele in whites with MM and MGUS was somewhat higher than in the noncancer white population (18 *versus* 14%), this difference was not significant ($P > 0.05$).

MM Patient Outcome. Because other studies have suggested that the prognosis for blacks with MM is worse than for whites (18), we examined the MM patients according to B allelic expression for survival from time of diagnosis with a median follow-up of 35 months. There were no significant differences in the outcome of patients with the three different genotypes (AA, AB, or BB). In addition, similar separate analyses of black and white MM patients also failed to reveal any survival differences based on A and B allelic expression.

High Frequency of the B Allele in Germline DNA from Patients with MM and MGUS. Matched normal (granulocyte), PBMC, and bone marrow DNA samples were collected from individual patients with MM or MGUS. Consistent with studies of this pseudogene in other tumor types (1), identical patterns of AA, AB, and BB genotypic expression were seen when DNA from these three tissues was analyzed in each patient. In Fig. 2, representative results from one patient are shown. Because the tumor (bone marrow) DNA was contaminated with nontumor material, it is possible that patients with AB genotypes (30%) may in some cases have contained

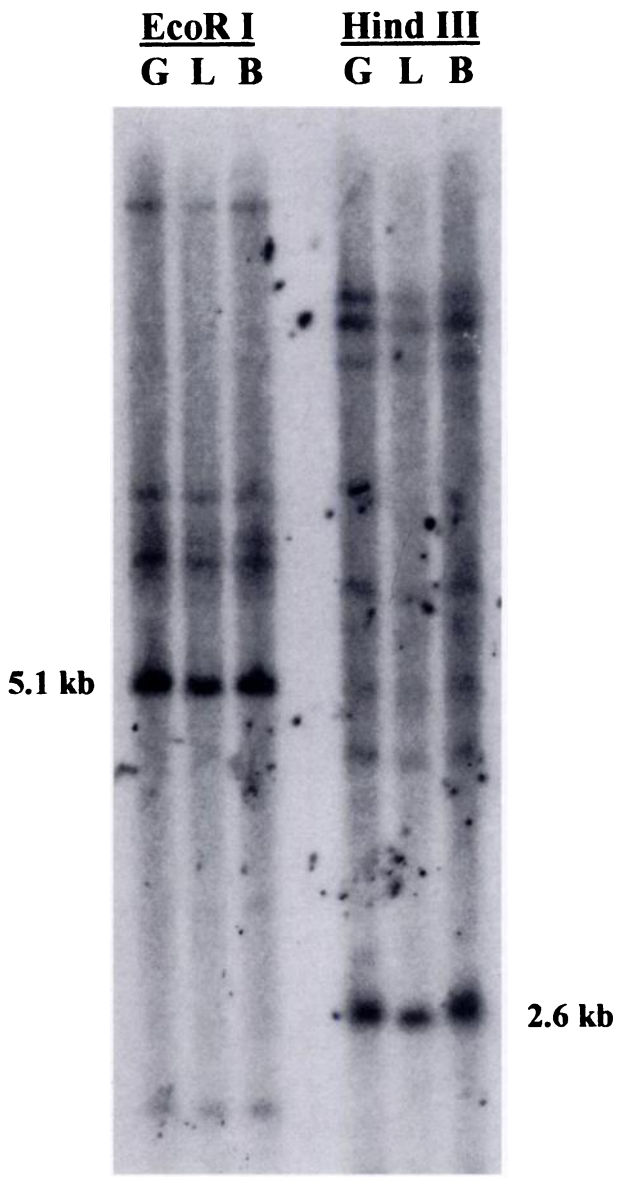

Fig. 2. Southern blot analysis of DNA from a myeloma patient's bone marrow plasma cells (*B*), PBMC (*L*), and granulocytes (*G*). DNA from each source was digested separately with two restriction enzymes (*Hind*III and *Eco*RI). The size of the B allele is 5.1 and 2.6 kb in *Eco*RI- and *Hind*III-digested DNA, respectively. With each restriction enzyme, identical hybridization patterns are seen with the three different samples in this patient with a BB genotype.

homozygous genotypes in their pure tumor. However, for patients with homozygous [AA (48%) or BB (22%)] genotypes, this analysis would demonstrate differences in allelic patterns, but this was never observed. Thus, the increased frequency of B allelic expression in MM and MGUS patients is present in the germline DNA and does not develop during the transformation process.

In summary, overall frequency of B allelic expression in MM and MGUS was increased compared to a noncancer population; furthermore, this difference was most evident in black patients with these B-cell disorders. On the other hand, the distribution of the B genotype did not differ significantly

among the white MM patients compared with the noncancer white population. Moreover, this overexpression is present in the germline DNA from these patients.

Discussion

Multiple myeloma is a B-cell malignancy characterized by the accumulation of a clonal population of plasma cells in the bone marrow that secrete a monoclonal immunoglobulin protein. Abnormal karyotypes have been found in bone marrow cells in one-third of these patients with complex chromosomal abnormalities with numerous modal and structural anomalies (22–27). However, specific numerical anomalies were observed most frequently involving chromosome 11 and structural aberrations in chromosomes 1, 11, 13, and 14 (22).

Certain cancers are more common in the American black population, including carcinoma of the prostate and colon and the B-cell malignancies, multiple myeloma and Burkitt's lymphoma. Although this higher cancer rate in blacks may be due to socioeconomic and lifestyle factors, underlying genetic factors may also contribute to this difference.

It is interesting that a polymorphism demonstrating deletion of 193 bp of PADPRP-like sequences on the long arm of chromosome 13 (B allele) is much more frequently expressed in blacks than whites. Furthermore, previous laboratory studies show that in certain cancers that more commonly affect blacks, a much higher expression of this B allele is seen compared with a normal control population (1).

Because myeloma is more frequently found in blacks and chromosome 13 abnormalities are frequently observed in myeloma karyotypes, we studied this polymorphism in patients with this B-cell malignancy and the related entity MGUS. The increased frequency of the B allele observed in patients with MM and MGUS compared to a noncancer population suggests that this PADPRP polymorphism may represent a valid marker for a predisposition to these B-cell disorders. This increased B allelic expression was most evident in blacks with monoclonal gammopathies similar to findings reported in another related B-cell malignancy, Burkitt's lymphoma (1). However, black lung cancer patients do not show this association (2), and the higher frequency of this cancer in blacks may reflect the higher smoking rates rather than a genetic predisposition (28, 29). The correlation between the increased B allele frequency and malignancies does not occur in the white population, either in previous studies (1, 2) or in our study of multiple myeloma. Thus, the higher frequency of the B allele in patients with cancer seems to be largely restricted to the black population.

The PADPRP-like sequences at the q33-qter region on chromosome 13 that are associated with a predisposition to cancer were recently cloned and characterized. A 193-bp duplication within the PADPRP sequences was found to be responsible for the differences between the A and B polymorphisms (2). Also Lyn *et al.* (2) used PCR to confirm that the RFLP A/B polymorphism was a 193-bp duplication. More studies of PADPRP and its associated loci may help further our overall understanding of the genetic predisposition to cancer. For example, a rat liver PADPRP activity was depleted during the promotion step in the experimental models (3). Kubo *et al.* (30) reported that PADPRP activity might have some role in regulating the cytotoxicity of anticancer agents selectively through modification of nuclear enzymes such as topoisomerase II.

In the patients with MM, the expression of the B allele was not related to patient outcome. Given that the higher expression of the B allele was also observed in patients with the relatively benign MGUS, this result is not surprising. These findings demonstrate that the expression of the B allele is associated with the increased likelihood of developing a clonal plasma cell disorder rather than in determining prognosis once the disease has been diagnosed.

In summary, we have demonstrated an increased frequency of the PADPRP-like B allele on the long arm of chromosome 13 in patients with monoclonal gammopathies. These differences were largely a reflection of the higher allelic expression in black patients than black controls. No significant differences in the distribution of this allele in patients with MM compared to its clonal but relatively benign counterpart, MGUS, were observed. Identical patterns of A and B allelic distribution have been found in matched germline and tumor samples in these patients. Thus, the frequent loss of genetic material at this chromosomal site is an abnormality present in the germline DNA of patients with monoclonal gammopathies.

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