RERF TR 8-89
Research Project 14-85

Technical Report Series

Frequency of Variant Erythrocytes at the Glycophorin A Locus in Two Bloom's Syndrome Patients

Seishi Kyoizumi, Ph.D.; Nori Nakamura, Ph.D.; Hiraku Takebe, Ph.D.; Koichi Tatsumi, M.D.; James German, M.D.; Mitoshi Akiyama, M.D.

放影研 RERF
Radiation Effects Research Foundation
A Cooperative Japan–United States Research Organization

RERF Technical Report Series

Technical reports are the basic medium for reporting of original research carried out at the Radiation Effects Research Foundation. Reports in this series receive both internal and external peer review and may serve as the basis for publication in the open scientific literature, in part or in toto. Although they may be quoted and cited, these reports are considered to be internal publications of the Foundation. Copies are available upon request from: Editorial Office, RERF, 5-2 Hijiyama Park, Minami-ku, Hiroshima, 732 Japan.

Beginning in 1989, the RERF Technical Report Series is no longer being published in the traditional Japanese-English bilingual format. However, major reports continue to be available in both languages as separate publications. Selected reports of a highly specialized nature, for which there is presumably less general interest, are produced only in English with an extended Japanese summary.

In this way, the Foundation will be able to more expeditiously report recent findings on the late biological effects of exposure of man to ionizing radiation resulting from the atomic bombings of Hiroshima and Nagasaki.

1989年から，放射線影響研究所の業績報告書は，従来の日英両文を併記した方式では発行しない．主要な報告書については，今後も日英両文で印刷するが，それぞれ別に発行する．内容が高度に専門的であり，一般の関心が少ないと思われる報告書については英文のみとし，日本文の要約を添付する．

これにより，広島・長崎の原爆電離放射線被曝の人体に及ぼす晩発性生物学的影響に関する最近の知見を今までよりも速やかにお知らせできることと思う．

The Radiation Effects Research Foundation (formerly ABCC) was established in April 1975 as a private nonprofit Japanese Foundation, supported equally by the Government of Japan through the Ministry of Health and Welfare, and the Government of the United States through the National Academy of Sciences under contract with the Department of Energy.

財団法人　放射線影響研究所
業　績　報　告　書

RERF TR 8-89
研究課題　14-85

二人のブルーム症候群患者における グリコフォリンA遺伝子座の変異赤血球頻度§

Frequency of Variant Erythrocytes at the Glycophorin A Locus in Two Bloom's Syndrome Patients

京泉誠之[1]，中村　典[1]，武部　啓[2,3]，巽　紘一[3]，James German[4]，
秋山實利[1]

[1]放影研放射線生物学部；京都大学医学部[2]放射線基礎医学教室及び[3]分子腫瘍学教室；並びに
[4]New York 血液センター人類遺伝学研究室

要　約

MN血液型は赤血球表面に存在するグリコフォリンA(GPA)という糖タンパク質によって決定され，M型とN型の相違はアミノ末端領域における二つの異なったアミノ酸の存在による．GPAに特異的な2種の蛍光標識モノクローナル抗体を用いて，MNヘテロ接合型赤血球におけるGPA-M及びGPA-N対立遺伝子の体細胞突然変異をフローサイトメトリーにより定量的に測定することができる．我々は二人のブルーム症候群（BS）患者について検討した結果，変異体は一つの対立遺伝子の発現を欠損している（単純な遺伝子不活化あるいは欠損）か，又は一つの対立遺伝子について正常レベルの二倍量発現をしている（おそらく体細胞組み換え）か，のいずれかであり，それらは10^3個の赤血球当たり1～3個の頻度で生じていることがわかった．BS患者における赤血球のフローサイトメトリーでは，一つのGPA対立遺伝子の発現が中間的レベルを示す変異体を特徴とするsmear様のパターンを呈した．このことは実際の変異体頻度は測定値より大きいことを示している．一方，BS遺伝子をヘテロにもつ親は正常範囲内（$1 \sim 8 \times 10^{-5}$）の変異体頻度を示した．これらのデータは，BS患者の高発癌傾向は自然突然変異及び体細胞組み換え頻度の増加に起因しているという仮説を強く支持するものである．

§本報告書にはこの要約以外に訳文はない．

承認1989年5月2日

印刷1989年9月

Frequency of Variant Erythrocytes at the Glycophorin A Locus in Two Bloom's Syndrome Patients§

Seishi Kyoizumi, Ph.D.[1]; Nori Nakamura, Ph.D.[1]; Hiraku Takebe, Ph.D.[2,3]; Koichi Tatsumi, M.D.[3]; James German, M.D.[4]; Mitoshi Akiyama, M.D.[1]

[1]RERF, Department of Radiobiology; Departments of [2]Experimental Radiology and [3]Molecular Oncology, Faculty of Medicine, Kyoto University; and [4]Laboratory of Human Genetics, the New York Blood Center

Summary

Blood type MN is determined by a glycoprotein called glycophorin A (GPA), which exists on the surface of erythrocytes and the difference between the M and N types is derived from the presence of two different amino acids at the amino terminal portion. Using a pair of fluorescence-labeled monoclonal antibodies specific to each GPA, somatic mutations in erythrocytes of MN heterozygotes at the GPA-M and -N alleles can be quantitatively determined by a flow sorter. Our results for two Bloom's syndrome (BS) patients showed that variants either lost expression of one allele (simple gene inactivation or loss) or expressed only one allele at twice the normal level (most probably somatic recombination), occurring at a frequency of about 1–3 per 10^3 erythrocytes. The flow cytometric patterns of erythrocytes from the BS patients showed a typical smear of variants bearing intermediate levels of expression of one GPA allele, indicating the real variant frequency is even greater than measured. On the other hand, the parents heterozygous for the BS gene showed variant frequencies which are within the normal range ($1–8 \times 10^{-5}$). These data strongly supported the hypothesis that cancer proneness of BS patients is due to their increased frequency of spontaneous mutations and somatic recombinations.

Introduction

Bloom's syndrome (BS), a kind of chromosome instability syndrome, is a cancer-prone hereditary disease and is characterized by a 10 times higher frequency of sister chromatid exchange (SCE) in cultured lymphocytes.[1] The frequent appearance of quadriradial configurations (Qr) of metaphase chromo-

§**The complete text of this report will not be available in Japanese.**
A paper based on this report has been accepted for publication in Mutation Research.

Approved 2 May 1989

Printed September 1989

somes had also been regarded as a diagnostic criterion before development of the SCE technique.[2] Qr is regarded as a consequence of somatic crossing-over between a pair of homologous chromosomes,[3] resulting in homozygosity of the distal part of the affected chromosomes. It is recognized that the rate of DNA chain elongation is retarded in cultured cells derived from BS patients[4] and recent biochemical examination revealed altered DNA ligase activity[5,6] or altered DNA topoisomerase II activity[7] in BS cells. Additionally, cells from BS patients show about a 10 times higher spontaneous mutation rate than from nonaffected people in skin fibroblasts and in peripheral lymphocytes as well.[8–10]

Recent developments in molecular oncology reveal the presence of possible anti-oncogenes.[11] In some hereditary cancer-prone diseases, specific cancer appears at specific sites with high frequency (e.g., retinoblastoma, Wilm's tumor, familial polyposis coli, etc.). The dominant mode of inheritance of such diseases is assumed to result from transmission of one mutant allele in germ cells and an additional mutation (one hit) in the remaining allele of somatic cells at the developmental stage, which leads to malignant transformation for the affected individuals. In nonaffected people, however, two independent mutations (two hit) at the homologous loci are required in the same somatic cells, and the probability must be much lower by a factor of 10^{-6} to 10^{-7}. Several causative mechanisms are shown in hereditary retinoblastoma cases, such as simple deletion of one homologous chromosome containing the normal allele, translocation, and somatic recombination—all these resulting in loss of function of the gene.[12] If we take into account these facts, viz., the high frequency of spontaneous mutation, somatic recombination, and the presence of anti-oncogenes, exceptional conditions under which BS cells transform in vivo easily can be visualized.

The erythrocyte mutation assay at the GPA locus (a determinant molecule of the MN blood type[13]) was originally developed by Langlois et al[14] and has been shown to detect an elevated variant frequency in patients who received chemo- or radiotherapy,[14] as well as in atomic bomb survivors.[15,16] The great advantage of the assay system is its ability to quantitatively detect not only hemizygous variants (Nϕ/ or Mϕ) but also homozygous recombinants (NN or MM) in MN heterozygous individuals. Since BS cells have a high mutation and recombination rate, we believed that they could provide an especially interesting study with the GPA assay.

Materials and Methods

Blood samples

Blood samples were obtained from five Japanese BS patients and three of their parents. A detailed description of the patients and their families is published elsewhere.[17] They were screened for MN blood type using rabbit typing sera (Ortho Diagnostic Systems, Raritan, NJ), and two of these BS patients and two of their parents were MN heterozygotes. Erythrocytes from these MN heterozygous donors were examined for GPA variant phenotypes, after fixation with formalin or dimethylsuberimidate for immunofluorescence staining, as described previously.[14,16]

Monoclonal antibodies and immunofluorescence

The following four monoclonal antibodies (MonAb) to GPA were used for this study[14,16]: the GPA(M)-specific MonAbs 6A7 and 9A3; the GPA(N)-specific MonAb NN3; and the GPA-specific MonAb 10F7 that binds equally well to both the M and N types of GPA. These MonAbs were labeled with appropriate fluorescent dyes, as described previously.[16] MonAbs 10F7 and 9A3 were directly conjugated with fluorescein (indicated by use of an F suffix on the antibody name), and MonAbs 6A7 and NN3 were conjugated with biotin followed by labeling with streptavidin-conjugated phycoerythrin (-B-AvPE, Becton Dickinson Immunocytometry Systems, Mountain View, Calif). Fixed erythrocytes were incubated for one hour at room temperature with a mixture of the primary MonAbs, were washed twice, and then were incubated for one hour with AvPE (10 μg/ml). Before flow cytometry, propidium iodide (Sigma Chemical Co., St. Louis, Mo) was added (10 μg/ml) to gate out the contaminating nucleated cells from the sorting windows, as described previously.[16]

Measurement of variant cell frequencies

The principle for the flow cytometric detection of variant erythrocytes is described elsewhere.[14] This method was originally developed using a dual beam cell sorter and was later modified to use a single-beam cell sorter (FACStar, Becton Dickinson).[16]

Using the modified method, four types of variants lacking the expression of one GPA allele were distinguished from normal MN heterozygous cells using a pair of MonAbs. By combining MonAbs 6A7-B-AvPE and 10F7-F, two variant cell types, hemizygous Nϕ and homozygous NN cells can be detected simultaneously using 1Way1 (1W1) assay. Normal MN cells were stained with both fluorescent antibodies, while Nϕ or NN cells were stained with MonAb 10F7-F only. Nϕ cells retained half the normal 10F7-F fluorescence and NN cells displayed normal 10F7-F fluorescence. By combining MonAbs 9A3-F and NN3-B-AvPE, hemizygous Mϕ and homozygous MM cells from MN heterozygous donors can be measured simultaneously using 2Way2 (2W2) assay. Mϕ and MM variant cells lacked expression of GPA(N) but expressed GPA(M) at a level equivalent to or twice as much as normal MN heterozygous cells, respectively.

Both fluorescent dyes, fluorescein (F) and phycoerythrin (PE), were excited with a 300 mW laser beam at 488 nm, and fluorescence was detected through a 530 nm band pass filter for F and through a 585 nm band pass filter for PE. The spectral overlap of F and PE fluorescence was electronically compensated for, and fluorescence window positions for sorting variant erythrocytes were defined using a standard erythrocyte mixture of the MM, MN, and NN type. Variant cells showing hemizygous or homozygous phenotype were sorted onto glass slides. Cells, having erythrocyte morphology and F and PE fluorescence matched for the variant phenotype, were counted under a fluorescence microscope. As shown in previous studies, the frequency of Nϕ, Mϕ, and MM cells for healthy normal individuals was approximately $1\text{–}2 \times 10^{-5}$. However, the reproducibility of NN

cell frequency was low, and this is probably caused by staining artifacts.[14–16] Consequently, samples from the same individuals at different times may show a large variation in NN cell frequency ($1–10 \times 10^{-5}$).

Results

Table 1 shows the frequencies of variant erythrocytes lacking the expression of either the M or N allele at the GPA locus in two BS patients (homozygous [bl/bl]) who were heterozygous for the GPA locus (that is, MN type). For patient A.O., examination by 2W2 assay was not achievable due to partial hemolysis of the blood sample and due to subsequent severe aggregation after antibody staining. Nevertheless, the present results clearly showed that variant cell frequency was greatly elevated not only for gene function loss (i.e., hemizygous Mϕ and Nϕ), but also for homozygous phenotypes (MM and NN) which have most probably resulted from somatic crossing-over at a frequency of about 1–3 per 10^3. On the other hand, the frequencies for parents of BS patients (heterozygous [bl/+] for the BS gene) were within the normal range ($1–8 \times 10^{-5}$).

Table 1. Variant erythrocyte frequencies in Bloom's syndrome homozygotes (bl/bl), heterozygotes (bl/+), and normal (+/+) individuals

Donors	Genotype	Blood type	Variant frequencies ($\times 10^{-6}$)			
			Nϕ	Mϕ	NN	MM
Family I						
Mother (M.O.)	bl/+	MN	42	2	66	12
Father (S.O.)	bl/+	MN	56	26	52	32
Daughter (A.O., #97[a])	bl/bl	MN	2940	ND[b]	1960	ND
Son (H.O., #96)	bl/bl	MM	-	-	-	-
Family II						
Son (A.S., #78)	bl/bl	MN	1399	546	887	1772
Control[c] I	+/+	MN	32	16	88	8
II	+/+	MN	7	10	4	20
Normal[d]	+/+	MN	22±10	10±9	70±86	12±12

[a] An identification number in the Bloom's Syndrome Registry.[17]

[b] Test "not done" due to heavy aggregation.

[c] Variant frequencies for two normal donors who were examined at the same time as BS samples were examined.

[d] Mean and standard deviation of variant frequencies of normal donors from a previous study.[16]

Figure 1 shows the flow distributions of the 1W1 mutation assay for 2×10^5 erythrocytes from the BS patient A.O. (bl/bl), her heterozygous parent S.O. (bl/+), and an A-bomb survivor, whose Nϕ variant frequency has been reported to be exceptionally high.[16] The contour graphs show that, for the BS patient, several hundred events are seen in each of the sorting windows for Nϕ and NN variant

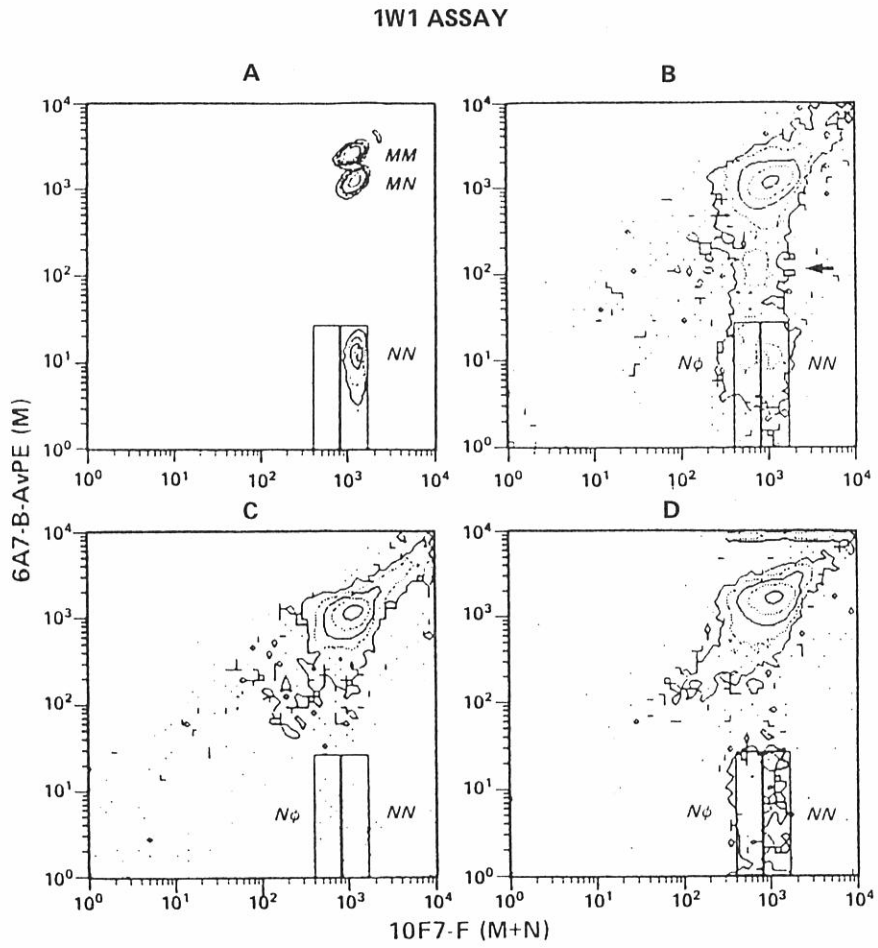

Figure 1. *Fluorescence distributions of 1W1 assay on a standard erythrocyte mixture of MM, MN, and NN types (A); and 2×10^5 erythrocytes from the BS patient, A.O. (B); the heterozygote, S.O. (C); and the A-bomb survivor with a high Nϕ cell frequency (1331×10^{-6})[16] (D). Contour plots in B, C, and D differ by a factor of 10 in events per channel with the lowest contour representing one event per channel. The windows labeled Nϕ and NN correspond to the hemizygous or homozygous variant which completely lost expression of the GPA(M) allele. The window positions for variants were defined using a standard erythrocyte mixture (A), as described previously.[16] The arrow in B indicates a discrete cluster of partial-loss variants.*

cells (Figure 1B), while only 5–10 corresponding variants were detected for the heterozygous parent (Figure 1C). The contour plot for the A-bomb survivor also represents a cluster of several hundred Nϕ cells, but NN variant frequency is lower (Figure 1D). A characteristic of flow patterns for erythrocytes from the BS patient is the presence of a considerable number of cells bearing intermediate levels of GPA, shown by continuous distribution in a region between the normal MN population and the variant cell window (Figure 1B), whereas Nϕ variants in the A-bomb survivor form a discrete cluster in the mutant window. A similar

broad distribution of erythrocytes was also observed in another BS patient, A.S. (data not shown). The 2W2 analysis of erythrocytes (5×10^5) from BS patient A.S., heterozygous S.O., and the A-bomb survivor showed similar results (Figure 2). In this assay, the broad distributions of GPA expression in erythrocytes from the BS patient can be clearly seen for both alleles (Figure 2B). The small peak positions shown by the arrows in Figures 1B and 2B correspond to 8%–13% of fluorescence intensity for normal erythrocytes. It should be noted that the frequencies of hemizygous and homozygous phenotypes listed in Table 1 were obtained by calculation using the number of complete-loss mutants sorted from each variant window, and they do not include such cells of intermediate levels.

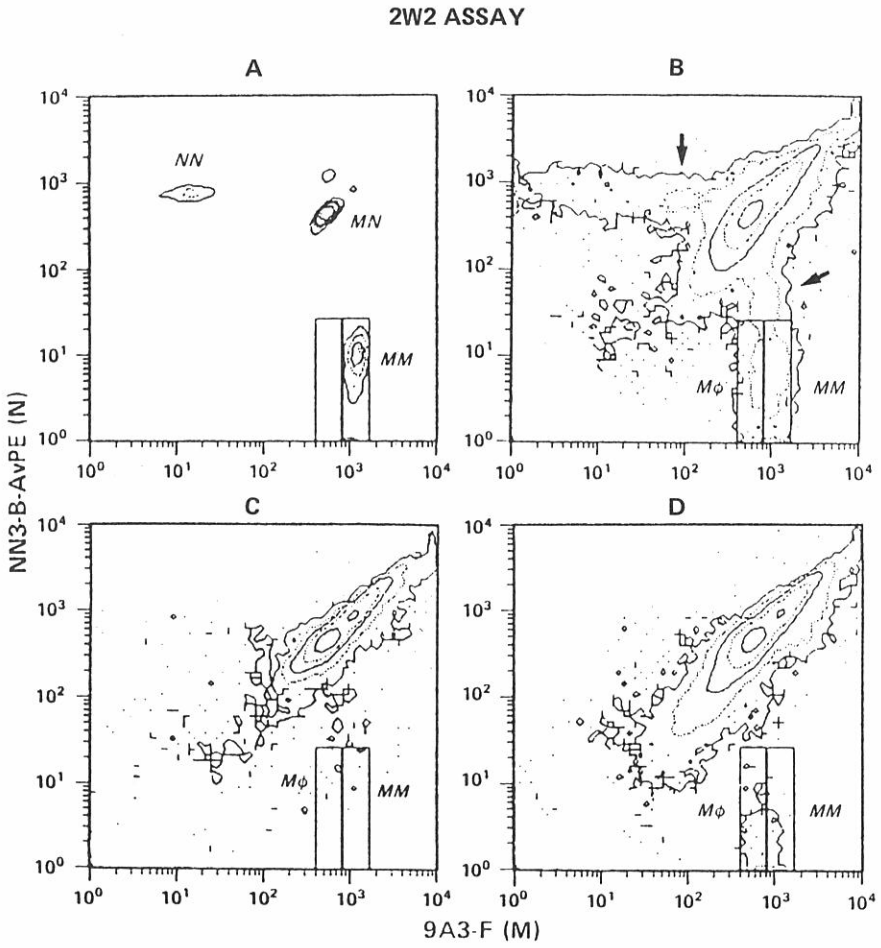

Figure 2. *Flow cytometric patterns of 2W2 assay on a standard erythrocyte mixture (A); and of 5×10^5 erythrocytes from the BS patient, A.S. (B); the heterozygote, S.O. (C); and the A-bomb survivor with a high Mϕ cell frequency (343×10^{-6})[16] (D). Contours in B, C, and D are expressed in the same way as in Figure 1. The windows labeled Mϕ and MM correspond to the hemizygous or homozygous variant, respectively, which lacks expression of GPA(N). The arrows in B indicate subpopulations partially lacking expression of the M or N GPA allele.*

Discussion

Studies of mutations in cultured skin fibroblasts and peripheral lymphocytes from BS patients show about a 10 times higher frequency than in nonaffected cases.[8–10] Unexpectedly, the present results demonstrated that the mutant frequency of the GPA locus in erythrocytes of BS patients is enormously high, e.g., 20–100 times greater than that for normal people. Why is the mutation frequency at the GPA locus of erythrocytes so high compared to that at other loci? We believe there are two main reasons.

Firstly, erythrocytes are nondividing in nature, whereas lymphocytes or fibroblasts do divide. Mutant cells bearing large structural alterations, such as chromosome breaks or chromosome loss, may be lethal for the dividing cells, and therefore their detection by colony formation assay is not expected. In contrast, such alterations in erythroblasts would still be detected as aberrant erythrocytes. For example, the micronucleus test in erythrocytes has been used in rodents for detection of clastogenic agents in vivo,[18] which demonstrates the existence of aneuploid products in these cells that would have led to cell death in other normally nucleated cell types.

Secondly, those mutation studies in skin fibroblasts or peripheral lymphocytes mainly used inactivation of the X chromosomal hypoxanthine phosphoribosyltransferase gene (*hprt*) as a marker. Since only one X chromosome is active in mammalian somatic cells, large deletions including *hprt* and adjacent loci would severely affect growth potential or be eventually lethal for the mutant cells. However, the GPA locus is autosomally located and a pair of genes is active in the cells. Therefore, the same kind of deletion would only cause a 50% decrease of gene activity and would be less harmful than a deletion in the X chromosome. In addition, autosomes may undergo homologous recombination, resulting in homozygosity if the exchange occurs at the appropriate position, or partial deletion and duplication if the exchange occurs at slightly different positions between the homologues. Such recombinations cannot be expected for the single X chromosome in male cells and are unlikely events in female cells since one X chromosome replicates quite late in the S phase. In fact, variant cell frequencies at the GPA locus in erythrocytes are three to five times higher than those at the *hprt* locus in peripheral T lymphocytes of healthy persons[19] and this may explain, at least partly, the excessively high variant frequency in erythrocytes of BS patients.

Flow cytometric distributions of erythrocytes from BS patients show an unusual pattern that is quite different from that of A-bomb survivors. Radiation-induced mutations currently detectable in erythrocytes of A-bomb survivors must have occurred in long-lived hemopoietic stem cells,[15,16] since the life span of erythrocytes in peripheral blood is only about 120 days.[20] Mature erythrocytes derived from such mutant stem cells are expected to completely lack GPA expression, as is indeed shown in Figures 1D and 2D.

On the other hand, the flow patterns of erythrocytes from BS patients showed an array of variant cells expressing different levels of GPA-M or -N. Such partial loss variants are expected, if we suppose function-loss mutations had occurred in proliferating erythroblasts which have already started GPA synthesis.[21] In these cells, further cell divisions will certainly result in dilution of the formerly produced GPA protein on the cell surface, and various amounts of GPA finally remain on mature erythrocytes depending on the residual number of cell divisions. According to this hypothesis, it can be predicted that variants of different levels of GPA expression will be equally generated unless the spontaneous mutation rate per cell generation varies during the erythroid maturation processes. Indeed, the results shown in Figures 1B and 2B support this model. However, it appears that the figures also indicate the presence of small peaks as shown by the arrows. Langlois et al[22] suggested that the peaks are caused by unequal recombinations either between sister chromatids or between homologues, resulting in production of hybrid molecules of GPA protein, e.g., a hybrid of GPA and glycophorin B.[23,24] Our recent data showed that such peaks were also observed for patients with Fanconi's anemia (FA) whose Mϕ or Nϕ mutant frequencies were around 10^{-3} (Kyoizumi S, unpublished). Since evidence for increased frequencies of somatic recombinations has not been demonstrated in FA patients, we are not certain whether the small peaks observed here are uniquely associated with BS patients. The reason the frequencies of partial and total loss variants of GPA products are similar remains speculative, since no confirmation at the DNA level is possible. The unequal exchange hypothesis of Langlois et al[22] assumes hot spots for erroneous exchanges which generate equal and unequal recombinations with similar frequencies. Instead, we suppose that erythroid differentiation is performed over n generations of cell division from long-lived stem cells to final enucleation, and GPA synthesis starts at the (n/2)th generation; therefore, mutations occurring before or after the (n/2)th generation will result in total and partial loss of GPA mutants, respectively, with a final mutant erythrocyte ratio of 1:1. Because of the paucity of our knowledge regarding the kinetics of erythroid cell differentiation, this hypothesis remains open for future examination.

Compared to the data for A-bomb survivors in previous studies,[15,16] the variant erythrocyte frequency of about 1 per 10^3 in BS patients approximates a radiation dose of more than 20 Gy. Needless to say, such doses are superlethal to humans, causing extensive stem cell death in the bone marrow and crypt. But if we neglect the killing effect of radiation and consider only mutagenic activity of radiation, it may be easily recognized that BS patients are continuously exposed to incredibly strong endogenous mutagenic stress. For example, among the A-bomb survivors, leukemia occurred in about 3% of people who received 2–3 Gy,[25] whereas in BS patients about 30%–40% developed various types of cancer by the age of 30.[26]

Some uncertainty remains in the etiology of BS. There is evidence that BS cells are defective in O_2 radical metabolism[27] other than DNA ligase[5,6] and topoisomerase II[7] activity. Although it has been speculated that O_2 radicals may

be an important promoter of cancer,[28] the present results for erythrocyte mutation at the GPA locus strongly suggest that irreversible changes, viz., initiation rather than promotion, take place at a very high frequency in the somatic cells of BS patients.

Acknowledgments

We wish to thank Hisayo Mochizuki for excellent technical support and Michiko Takagi for manuscript preparation. We also thank Sohei Kondoh, Ph.D., for valuable discussion and encouragement throughout these studies.

References

1. Arlett CF, Lehman AR: Human disorders showing increased sensitivity to the induction of genetic damage. Ann Rev Genet 12:95–115, 1978

2. Chaganti RSK, Schonberg S, German J: A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. Proc Natl Acad Sci USA 71:4508–12, 1974

3. German J: Cytological evidence for crossing-over in vitro in human lymphoid cells. Science 144:298–301, 1964

4. Hand R, German J: A retarded rate of DNA chain growth in Bloom's syndrome. Proc Natl Acad Sci USA 72:758–62, 1975

5. Willis AE, Lindahl T: DNA ligase I deficiency in Bloom's syndrome. Nature 325:355–7, 1987

6. Chan JYH, Becker FF, German J, Ray JH: Altered DNA ligase I activity in Bloom's syndrome cells. Nature 325:357–9, 1987

7. Heartlein MW, Tsuji H, Latt SA: 5-Bromodeoxyuridine-dependent increase in sister chromatid exchange formation in Bloom's syndrome is associated with reduction in topoisomerase II activity. Exp Cell Res 169:245–54, 1987

8. Warren ST, Schultz RA, Chang CC, Wade MH, Trosko JE: Elevated spontaneous mutation rate in Bloom's syndrome fibroblasts. Proc Natl Acad Sci USA 78:3133–7, 1981

9. Gupta RS, Goldstein S: Diptheria toxin resistance in human fibroblast cell strains from normal and cancer-prone individuals. Mutat Res 73:331–8, 1980

10. Vijayalaxmi, Evans HJ, Ray JH, German J: Bloom's syndrome: Evidence for an increased mutation frequency in vivo. Science 221:851–3, 1983

11. Knudson AG Jr: Genetics of human cancer. Ann Rev Genet 20:231–51, 1986

12. Murphree AL, Benedict WF: Retinoblastoma: Clues to human oncogenesis. Science 223:1028–33, 1984

13. Furthmayer H: Structural comparison of glycophorin and immunochemical analysis of genetic variants. Nature 271:519–24, 1978

14. Langlois RG, Bigbee WL, Jensen RH: Measurements of the frequency of human erythrocytes with gene expression loss phenotypes at the glycophorin A locus. Hum Genet 74:353–62, 1986

15. Langlois RG, Bigbee WL, Kyoizumi S, Nakamura N, Bean MA, Akiyama M, Jensen RH: Evidence for increased somatic cell mutations at the glycophorin A locus in atomic bomb survivors. Science 236:445–8, 1987 (RERF TR 1-87)

16. Kyoizumi S, Nakamura N, Hakoda M, Awa AA, Bean MA, Jensen RH, Akiyama M: Detection of somatic mutations at the glycophorin A locus in erythrocytes of atomic bomb survivors using a single beam flow sorter. Cancer Res 49:581–8, 1989 (RERF TR 9-88)

17. German J, Takebe H: Bloom's syndrome XIV. The disorder in Japan. Clin Genet 35:93–110, 1989

18. Schlegel R, MacGregor JT: The persistence of micronuclei in peripheral blood erythrocytes: Detection of chronic chromosome breakage in mice. Mutat Res 104:367–9, 1982

19. Hakoda M, Akiyama M, Kyoizumi S, Awa AA, Yamakido M, Otake M: Increased somatic cell mutant frequency in atomic bomb exposed people. Mutat Res 201:39–48, 1988 (RERF TR 18-87)

20. Wintrobe MW, Lee RG, Boggs DR, Bithell TC, Foerster J, Athens JW, Lukens JN: Clinical Hematology, 8th Ed. Philadelphia, Lee and Febiger Inc., 1981. pp 171–5

21. Loken MR, Shah VO, Dattilio KL, Civin CI: Flow cytometric analysis of human bone marrow: I. Normal erythroid development. Blood 69:255–63, 1987

22. Langlois RG, Bigbee WL, Jensen RH, German J: Evidence for elevated in vivo mutation and somatic recombination in Bloom's syndrome. Proc Natl Acad Sci USA 86:670–4, 1989

23. Merry AH, Hodson C, Thomson E, Mallinson G, Anstee DJ: The use of monoclonal antibodies to quantify the levels of sialoglycoproteins α and δ and variant sialoglycoproteins in human erythrocyte membranes. Biochem J 233:93–8, 1986

24. Langlois RG, Bigbee WL, Jensen RH: Flow cytometric characterization of normal and variant cells with monoclonal antibodies specific for glycophorin A. J Immunol 134:4009–17, 1985

25. Beebe GW, Kato H, Land CE: Studies of the mortality of A-bomb survivors. 6. Mortality and radiation dose, 1950–1974. Radiat Res 75:138–201, 1978 (RERF TR 1-77)

26. German J, Passarge E: Bloom's syndrome XII. Report from the registry for 1987. Clin Genet 35:57–69, 1989

27. Emerit I, Cerutti P: Clastogenic activity from Bloom's syndrome fibroblast cultures. Proc Natl Acad Sci USA 78:1868–72, 1981

28. Copeland ES (Ed): A National Institutes of Health Workshop report. Free radicals in promotion—a chemical pathology study section workshop. Cancer Res 43:5631–7, 1983