

Clinical features of Bloom syndrome and function of the causative gene, BLM helicase

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Bloom syndrome is a rare autosomal recessive genetic disorder characterized by growth deficiency, unusual facies, sun-sensitive telangiectatic erythema, immunodeficiency and predisposition to cancer. The causative gene for Bloom syndrome is *BLM*, which encodes the BLM RecQ helicase homolog protein. The first part of this review describes a long-term follow-up study of two Bloom syndrome siblings. Subsequently, the focus is placed on the functional domains of BLM. Laboratory diagnosis of Bloom syndrome by detecting mutations in *BLM* is laborious and impractical, unless there are common mutations in a population. Immunoblot and immunohistochemical analyses for the detection of the BLM protein using a polyclonal BLM antibody, which are useful approaches for clinical diagnosis of Bloom syndrome, are also described. In addition, a useful adjunct for the diagnosis of Bloom syndrome in terms of the BLM function is investigated, since disease cells must have the defective BLM helicase function. This review also discusses the nuclear localization signal of BLM, the proteins that interact with BLM and tumors originating from Bloom syndrome.

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The RecQ DNA helicase family includes five helicases: BLM, WRN, RecQ1, RecQ4 and RecQ5. They are the causative genes for many types of cancer, such as Bloom syndrome (BS), Werner syndrome and Rothmund–Thomson syndrome (*BLM*, *WRN* and *RecQ4*, respectively). These genes appear to maintain genomic stability [1–6]. Helicases are molecular motors that convert double-stranded nucleic acids into single-stranded molecules. To catalyze the disruption of hydrogen bonds that hold duplexes together, helicases use energy released from the hydrolysis of nucleotide 5'-triphosphates, usually ATP, to move along a strand of nucleic acid and unwind the duplex. The discovery of *recQ* in *Escherichia coli* in a screen for thymine-deficient, death-resistant mutants marked the identification of the first member of a large class of RecQ-related DNA helicases [7]. Several lines of study suggest that RecQ can suppress illegitimate recombination, thus helping to maintain genomic stability by preventing the formation of duplexes between imperfectly homologous DNA sequences. Therefore, the impairment of the RecQ helicase

results in genomic instability and the development of various types of cancer. This paper describes the clinical features of BS and the function of the BLM gene.

Bloom syndrome: a long-term patient study

A brother and sister in BS Registry were identified as HiOk and AsOk, respectively (TABLE 1) [8]. Clinical features included recurrent and prolonged middle ear and upper respiratory tract infections from the age of 2 years. This occurred several times per year until about the age of 14 years for HiOk and 12 for AsOk. The severity and frequency of infections were lower in AsOk than in HiOk and as they grew older, the recurrence of such infections gradually decreased. When HiOk began responding to therapy after the age of 16 years, the frequency of his middle ear infections decreased to once or twice per year at approximately 18 years of age.

Serum concentrations of the three major classes of immunoglobulin (Ig), IgM, IgG and IgA, were low at age 11 years in HiOk and at 9 years for AsOk, compared with age-matched

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control means. However, the magnitude of reduction for each class of Ig varied. In both patients, serum concentrations of IgM were markedly low but those of IgG and IgA only mildly decreased. Neither patient has ever been administered γ -globulin therapy during the past 14 years. The small decrease in serum concentrations of IgG and IgA increased significantly with age, whereas the IgM levels remained low. Neither patient had a significantly reduced percentage of circulating CD3⁺, CD4⁺, CD8⁺ or CD19⁺ cells when compared with controls [9]. As already described, these patients showed the typical phenotype of BS.

Lymphoma

B-cell lymphoma

A prominent characteristic of BS is the high predisposition to various types of cancer [10]. After 1 month of treatment with exogenous insulin, HiOk developed B-cell non-Hodgkin's lymphoma, which responded to radiation (30 Gy) in the nasopharyngeal portion. However, after 5 months of treatment with radiation the lymphoma relapsed, with widespread abdominal disease that resisted chemotherapy. He died 24 months after the onset of the lymphoma from hepatic metastases [11,12].

AsOk complained of severe abdominal pain at 25 years of age and was diagnosed as having acute abdominal lymphoma. A colon fiberoscope revealed neoplastic changes of the epithelium around the ileum end and surgical treatment was subsequently performed. The tumor was located on the oral side, 30 cm from Bauhin's valve, and obstructed the intestinal cavity.

It was diagnosed as B-cell non-Hodgkin's lymphoma. The patient received a half dose of the acute lymphoblastic leukemia protocol, which is used by the children's cancer study group (9104 standard risk protocol in the Tokai Pediatrics Oncology Study Group). The patient was also administered the following drugs:

- Vincristine 0.75 mg/m² on days 1, 8, 15, 22, 29, 71, 85, 99, 113, 127, 134 and 141
- Dexamethasone 6 mg/m² on days 1-7 and 127-133
- Prednisolone 30 mg/m² on days 8-14 and 134-140
- Prednisolone 15mg/m² on days 15-28
- L-asparaginase 5000 u/m² on days 15, 18, 21, 24, 27, 30, 87, 101 and 115
- Methotrexate 6 mg/m², cytarabine 15 mg/m² and hydrocortisone 10 mg/m² intrathecally on days 22, 29, 36, 43, 72, 86, 100 and 114
- Daunorubicin 15 mg/m² on days 43, 50 and 57
- Cytarabine 35 mg/m² on days 44-47, 51-54 and 58-61
- Mercaptopurine 25 mg/m² on days 36-63
- Methotrexate 1500 mg/m² on days 85, 99 and 113 with leucovorin rescue
- Cyclophosphamide 300 mg/m² on days 87, 101 and 115 and pirarubicine 15 mg/m² on days 127, 134 and 141
- Enocitabine 50 mg/m² on days 128-131, 135-138 and 142-145

Table 1. Clinical features of two siblings with Bloom syndrome.

Features	HiOk	AsOk
Weight at birth	2100 g	2250 g
Height	143.1 cm (21 years old)	144.5 cm (19 years old)
Skin change	Telangiectatic erythema Brownish pigmentation with interspersed hypopigmented area Cafe-au-lait spots Atopic dermatitis	Cafe-au-lait spots Hypopigmented area
Facies	Malar hypoplasia Mandibular hypoplasia	Malar hypoplasia Mandibular hypoplasia
Intelligence	Within the normal range	Within the normal range
Immune system	Decreased IgM and IgA Increased IgE B-cell dysfunction	Decreased IgM and IgA Increased IgE B-cell dysfunction
Chromosome	Breakage of chromosome Increased SCEs	Breakage of chromosome Increased SCEs
Other findings	High-pitched voice Mild hepatic dysfunction Diabetes mellitus Small testes	Allergic rhinitis Early menopause

Ig: Immunoglobulin; SCE: Sister chromatid exchange.

The patient continues complete remission and is free of treatment complications 5 years after diagnosis. It should be noted that BS displays chromosomal instability when chemotherapy for malignancy is performed.

Phenotype of B-cell lymphoma originating from HiOk

Pathological findings showed diffuse, large cell lymphoma. Surface markers showed typical B-cell lineage without the expression of Ig chains. Also, Bcl-2 expression was not detected. The C μ chain and overexpression of *c-myc* and *p53* genes were not observed. Translocation of *c-myc* and Epstein-Barr virus (EBV) integration were not detected by Southern blotting.

p53 mutation of B-cell lymphoma

p53 mutations are often observed in B-cell lymphoma. The hot spots of *p53* mutations from exons 5-9 were investigated. No cases showed *p53* mutations in the genomic DNA extracted from neutrophils and lymphoma tissues from the original site. However, a metastatic lymphoma originating from the cecum of HiOk, 5 months after radiation therapy, showed a reduced length of the *p53* exon 7. DNA sequence analysis revealed a 9-bp deletion in exon 7 (TABLE 2) [11].

Replication error

The BLM gene encodes a RecQ helicase homolog and the RecQ helicase is reportedly involved in some aspect of DNA replication. Therefore, dinucleotide and trinucleotide repeat replications on the X chromosome have been investigated. Lymphoma DNA from both the original and metastatic sites showed a reduced length of repetitive DNA, as determined by polyacrylamide gel electrophoresis. One EBV cell derived from HiOk's B-cells also showed a reduced length of repetitive DNA. DNA sequence analysis of lymphoma in a recurrent site revealed a 6- or 3-bp deletion in a region of microsatellite DNA, HUMARA, and a 12- or 14-bp deletion in another region, DXS1113 (TABLE 2).

B-cell lymphoma and an EBV-transformed cell line derived from AsOk's B-cells did not show any changes in the length of repetitive DNA. Microsatellite instability has been detected in a variety of sporadic human tumors. This suggests that a mismatch repair deficiency could strongly accelerate malignant transformation of rapidly expanding cell populations. The function of the RecQ helicase, a homolog of BLM, is unclear, although it seems most likely to be one of the factors contributing to postreplication recombinational repair. Although there is no direct evidence that BLM participates in the mismatch repair system, the presence of microsatellite instability in lymphoma cells seems to suggest the direct or indirect involvement of BLM in that system.

It is possible that loss of helicase activity generates abnormal DNA structures during replication, which indirectly affects the activity of other DNA-binding proteins or activate repair mechanisms.

Ig gene rearrangement in BS lymphocytes

Almost all patients with BS have abnormally low concentrations of one or more of the plasma Igs and fail to show delayed hypersensitivity. The mechanisms of immunodeficiency remain to be elucidated. The involvement of BLM in DNA repair also remains to be determined. Werner syndrome is characterized by premature aging and its causative gene, WRN, is homologous to BLM. However, immunodeficiency is not a characteristic clinical feature of Werner syndrome. BLM is preferentially expressed in the thymus and testes while WRN is ubiquitously and strongly expressed in the testes [13,14]. The difference in the expression patterns of BLM and WRN might explain the clinical features of immunodeficiency in BS. BLM expression was detected in all of the examined hematopoietic cell lines, although in varying amounts. It was assumed that a strong or low expression of BLM would appear to correlate with a fast or slow cell growth rate in each cell line. BLM expression in myelomonocytes was detected.

Table 2. Mutations in B-cell lymphoma originating from Bloom syndrome.

Genomic locus	Lymphoma original site (HiOk)	Lymphoma recurrence site (HiOk)	EB line (HiOk)	Lymphoma original site (AsOk)	Lymphoma EB line (AsOk)
p53	WT	9-bp deletion in exon 7 CTCACCATC ⁵	WT	WT	ND
Dinucleotide repeat (DXS1113)	Deletion of one CT repeat and five CA repeats	Deletion of one CT repeat and five or six CA repeats	Deletion of one or two CT repeats and five or seven CA repeats	Germline ⁵⁵	Germline ⁵⁵
Trinucleotide repeat (HUMARA)	Deletion of two GCA repeats	Deletion of one or two GCA repeats	Deletion of two GCA repeats	Germline ⁵⁵	Germline ⁵⁵

⁵Mutation causes deletion of three amino acids (position 252-254).

⁵⁵Germline type was judged by detecting identical bands to neutrophil DNA.

ND: Not done; WT: Wild type.

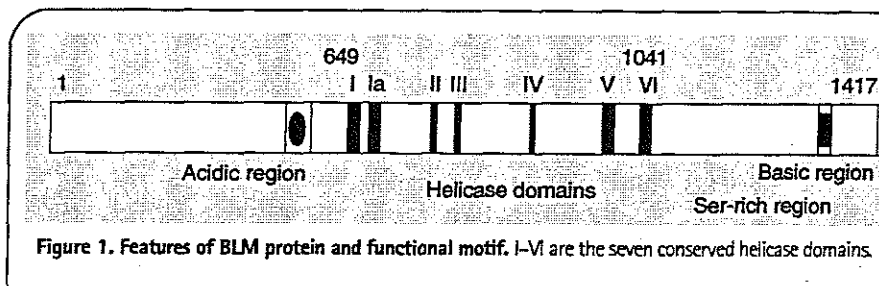


Figure 1. Features of BLM protein and functional motif. I–VI are the seven conserved helicase domains.

Although the function of myelomonocytes in BS has not yet been determined, these results may explain why some BS patients with normal serum Ig concentrations are also susceptible to infection.

An increased frequency of abnormal T-cell rearrangement was observed in the peripheral blood lymphocytes (PBL) of patients with BS compared with control individuals. However, the frequency of abnormal rearrangement in the PBL of patients with BS is lower than the PBL in ataxia-telangiectasia. Consistent with a previous report on peripheral B-cells from patients with BS, the sequences of the CDR3 region in the VDJ rearrangement were in-frame and insertion of the N region was also detected [15–17]. These results suggested that the DNA helicase function of BLM is not involved in VDJ recombination directly. Both T- and B-cells utilize the same machinery for VDJ recombination. BS cells have normal VDJ recombination in Ig, therefore it is not likely that the increased frequency of the abnormal T-cell receptor gene rearrangement in BS is caused by an abnormal catalytic function of the recombinase. The increased frequency of the abnormal T-cell rearrangement suggests that BLM is involved in the maintenance of DNA stability.

BLM gene & mutation

BLM was identified in 1995. It is 4437 bp long and encodes a 1417-amino acid peptide homologous to RecQ helicases, a subfamily of DExH box-containing DNA and RNA helicases (FIGURE 1) [18]. *BLM* is also highly homologous to the product of the yeast gene *SGS1* (slow-growth suppresser). It is assumed that the BLM protein plays an important role in the maintenance of genomic stability in somatic cells. In this syndrome, increased spontaneous sister chromatid exchanges (SCEs) have been observed and it is believed to be the most malignancy-prone chromosomal disorder. The underlying lesion might be caused by a deficiency in DNA damage repair.

Ellis and German reported 14 unique mutations in 20 out of 25 people examined with BS. Three of the mutations were putative missense substitutions, six were nonsense mutations, two were frameshift mutations, two were exon-skipping mutations and one was a gross deletion detectable by Southern blot analysis (TABLE 3) [19]. The relatively high frequency of *BLM* mutations in the Ashkenazim has been reported as *blmAsh*, which is a 6-bp deletion/7-bp insertion at nucleotide 2281 in the open reading frame of *BLM*. This results in a frameshift and a stop codon at nucleotide 2292 [20,21]. Li and coworkers

have reported that the *blmAsh* mutation is present in one out of 107 of this particular Ashkenazi Jewish population, a carrier frequency of 0.0093. However, a common mutation of *BLM* has not yet been reported in the other population. Furthermore, it is sometimes difficult to distinguish between BS and other DNA instability syndromes, such as the Fanconi or Rothmund–Thomson syndromes,

based on clinical manifestations.

The mobility of the PCR-amplified *BLM* fragments from HiOk differed from that of healthy control DNA and was similar to that of AsOk DNA in acrylamide gel electrophoresis studies (FIGURE 2) [22]. PCR-amplified *BLM* fragments from their father and mother's DNA showed two bands, one with the same mobility as healthy control DNA and the other with the same mobility as HiOk and AsOk. A 3 bp deletion was detected in the *BLM* sequence from HiOk and AsOk DNA. This deletion caused the generation of a stop codon at amino acid 186. Both deleted and normal-sized *BLM* sequences were obtained from the father and mother's DNA.

Recent studies have identified a small increase in the risk of colorectal cancer developing in individuals who are heterozygous for the *blmAsh* allele [23]. Consistent with these studies, mice that are heterozygous for *BLM* mutations show enhanced tumorigenesis when infected with the murine leukemia virus or when crossed with mice that are heterozygous for the adenomatous polyposis of the colon (*APC*) tumor-suppressor gene [24]. This data indicates that *BLM* heterozygotes, other than those with the *blmAsh* allele, may be similarly cancer prone.

Functional domains of BLM

Nuclear localization signal in BLM

Based on previously published manuscripts whereby many *BLM* mutations truncate the protein upstream of the helicase domain, *WRN* mutations truncate the protein beyond the helicase domain. These *WRN* mutants probably retain DNA helicase activity. Lu and coworkers reported that site-directed mutations that eliminate the helicase activity of yeast *SGS1* can still complement certain *SGS1* mutants [25]. It is assumed that different disease symptoms caused by *BLM* and *WRN* mutations may be attributed to a loss of a function other than the helicase activity [26].

By searching for *BLM* amino acid sequences, putative bipartite nuclear localization signals (NLS) were found in the C-terminal domain (FIGURE 3) [27]. The fragments P-9, P-10 and P-11 were produced to correspond to the mutations previously reported in BS Registry designations 97, 112 and 93, respectively. The predicted peptides of the frameshift mutation observed in registry numbers 15, 42, 107 and NR2 were 739 amino acid residues and located between the fragments of P-7 and P-8. A green fluorescent protein (GFP) vector inserted with a full coding sequence of *BLM* was transfected into HeLa

Table 3. BLM mutations identified in Bloom syndrome patients [19].

Mutations	Identification	Ancestry	Zygoty of the mutation	Nucleotide change	Protein change
Missense mutations	139(ViKre)	Mixed European	Heterozygous	A2089G	Q627R
	31 (CaDe)	Dutch	Heterozygous	A2089G	Q627R
	40(DoRoe)	Mixed European	Heterozygous	G2776A	C901Y
	113(DaDem)	Italian	Homozygous	G3238C	C10558
Nonsense mutations	96(HiOk)	Japanese	Homozygous	631delCAA	S186X
	97(AsOk)	Japanese	Homozygous	631delCAA	S186X
	112(NaSch)	Mixed European	Heterozygous	A888T	K272X
	98(RoMo)	Mixed European	Heterozygous	A1164	R364X
	81 (MaGrou)	French Canadian	Homozygous	C1858A	S595X
	11 (IaTh)	Mixed European	Heterozygous	C2007T	Q645X
	61 (DoHop)	Mixed European	Homozygous	C2007T	Q645X
	NRI(ErBor)	Mixed European	Heterozygous	C2007T	Q645X
	NR8(KeSol)	Mixed European	Heterozygous	C2007T	Q645X
	51 (KeMc)	Mixed European	Homozygous	C2172T	Q700X
Frame shift mutations	93 (YoYa)	Japanese	Homozygous	1610insA	514-1-X
	15(MaRo)	Ashkenazi Jewish	Homozygous	2281delATCTGAinsTA GATTC	735-4-X
	IQ 42(RAFR)	Ashkenazi Jewish	Homozygous	2281delATCTGAinsTA GATTC	735-4-X
	107(MyAsa)	Ashkenazi Jewish	Homozygous	2281defATCTGAinsTA GATTC	735-4-X
	NR2(CrSpe)	Ashkenazi Jewish	Homozygous	2281delATCTGAinsTA GATTC	735-4-X
	(BrNa)	Ashkenazi Jewish	Heterozygous	2281de1ATCTGAinsTA GATTC	735-4-X
Exon-skipping mutations	IC 126(BrNa)	Ashkenazi/ Sepharadic	Heterozygous	Skip exon 2	
	112(NaSch)	Mixed European	Heterozygous	Skip exon 6	
	92(VaBia)	Italian	Homozygous	Skip exons 11 and 12	

cell lines, which express BLM abundantly, and GFP was found localized in the nucleus. A truncated form of BLM (by 1341 amino acids), containing only the proximal arm of basic amino acids, was not transported to the nucleus but instead remained in the cytoplasm. Another truncated form (by 1356 amino acids), containing two arms, was transported to the nucleus. The GFP vector inserted with DNA fragments with two arms of basic amino acids in the C-terminal, X-1, was also transported to the nucleus. The fragments with only the distal arm, X-2, were transported to the nucleus. These results suggest that the distal arm of the basic residues in the BLM protein is essential for nuclear localization. Truncated BLM proteins

corresponding to previously reported BLM mutations were retained in the cytoplasm or both the cytoplasm and the nucleus. The distribution of both the cytoplasm and nucleus observed in the short BLM fragments, which were P-9 to P-11, might reflect the characteristics of GFP itself. In these cases, even if such short BLM fragments are transported to the nucleus, they could not have a function due to the defective helicase domain.

Full BLM and WRN proteins differ in length by only 15 residues and share a highly conserved helicase domain. Both the N- and C-terminal domains are unique to BLM and WRN, respectively, and these unique domains play a role in

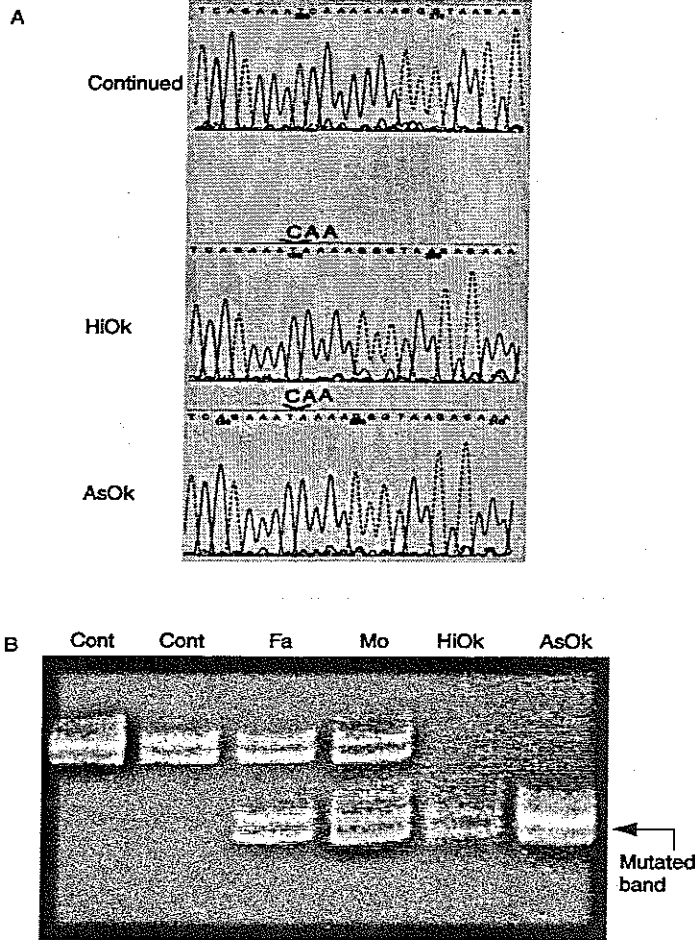


Figure 2. Mutation of BLM gene. BLM (residues 514–741) was amplified by PCR and electrophoresed in 12% acrylamide gel for 30 h at 400 V. Lane: 1,2: healthy control; Lane 3: father; Lane 4: mother; Lane 5: HIOk; Lane 6: AsOk. 514–741 reveals the position of nucleotides identified in in the BLM cDNA H1–5' [18].

expressing disease symptoms. The nuclear localization signal of BLM was identified and, in addition, WRN also has the functional NLS in its C-terminus. These results were acceptable, considering that helicases catalyze the unwinding of double-stranded DNA to provide single-strand templates in the nucleus.

In the BLM protein, the C-terminal region plays a crucial role in the proper function of BLM. In contrast to WRN, reported BLM mutations are assumed to lose their helicase activity. This, in part, is affected by the information based on the small number of BLM mutations in BS patients. Observations have showed that correct BLM function requires both intact helicase activity and an intact NLS in the C-terminus. Therefore, the mutation of NLS could cause BS as well as the mutations, which abolish the helicase activity of BLM.

Expression of BLM gene in peripheral blood mononuclear cells & fetal tissues

Since the BLM protein is relatively large, identification of the gene mutation in the BLM gene for the diagnosis of BS is laborious. Therefore, for screening BS patients, the expression of the BLM protein and nuclear dot formation were investigated. This was performed in EBV-transformed lymphoblastoid cell lines and phytohemagglutinin (PHA)-induced lymphoblastoid cells originating from control and BS patients [28]. In order to search for the source of BLM protein expression, BLM gene expression was investigated. As already described, BLM was expressed in freshly prepared peripheral blood mononuclear cells (PBMCs) and fetal tissues, although the EBV-transformed B-cell line strongly expressed BLM. PBMCs stimulated with PHA showed slight induction of BLM expression. BLM expression in human fetal tissues was investigated by northern blotting. The fetal kidney, heart and liver scarcely expressed BLM. BLM expression was strongly detected in the 7-week-old brain in contrast to the adult brain. As fetal neurons proliferated, BLM might be expressed. In a whole embryo, BLM is strongly expressed at 6 weeks.

Expression of BLM protein in hematopoietic cell lines & PHA-stimulated PBMCs

BLM expression in various hematopoietic cell lines was investigated using polyclonal BLM antibodies. A 160-kDa protein was detected in control EBV-transformed cell lines. By immunoblotting, the 160-kDa band was strongly detected in hematopoietic leukemic cell lines including B-cells, T-cells and myelomonocytes. Ten additional control cell lines were examined and the BLM protein was clearly detected in all of these cell lines when 30 µg of protein was loaded. However, in EBV cell lines obtained from BS patients (3403F and H9152 BS cell lines purchased from the American Type Culture Collection [ATCC]), BLM was not detected. In freshly isolated PBMCs, BLM was scarcely detected. As the amount of BLM protein was very low in quiescent PBMCs, it was predicted that this might also be partly reflected in BLM messenger RNA (mRNA) levels. However, in PHA-stimulated lymphoblasts, expression of BLM protein was significantly induced. The induction of BLM protein started at day 2 after stimulation and continued until day 5. However, PHA-stimulated lymphoblasts from AsOk showed no induction of BLM protein.

Immunohistochemical analysis of the BLM protein was then performed (FIGURE 4). In the control EBV cell line, nuclear dot formation was observed. When stimulated with PHA, some PBMCs exhibited nuclear dots in the nucleus, while others did not, suggesting that nuclear dot formation depends on the cell cycle of each cell. However, in PHA-stimulated PBMCs obtained from BS, no dot formation was observed. PBMCs from AsOk showed levels of ³H-thymidine incorporation (51337cpm) comparable

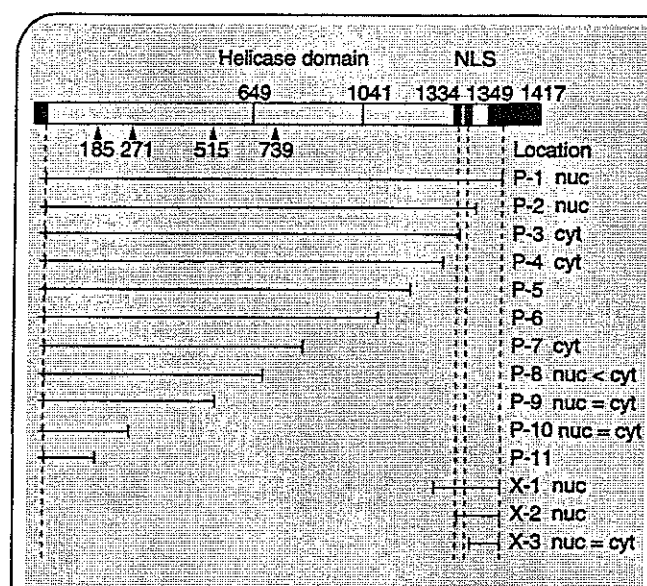


Figure 3. Summary of the localization of GFP-BLM fusion proteins and the DNA fragments of BLM used in these experiments. The numbers show the position of amino acid residues in BLM protein. Arrowheads reveal the position of non-sense mutations in a previously reported manuscript. In those cases where the subcellular location was not exclusively nuclear or cytoplasmic, the results are given by nuc > cyt, nuc = cyt, or nuc < cyt to indicate the predominant pattern of staining observed in transfected cells. cyt: Cytoplasmic localization of GFP in all cells observed; GFP: Green fluorescent protein; nuc: Nuclear localization of GFP in all cells observed; NLS: Nuclear localization signal.

with PBMCs from healthy control (37700–62400 cpm), indicating normal activation of peripheral T-cells in the BS patient.

The combined analysis of immunoblotting and immunohistochemistry is thus useful for laboratory diagnosis of BS.

Proteins associated with BLM

BLM physically and functionally interacts with an array of proteins whose primary role is the maintenance of genome integrity, such as p53, topoisomerase II α and SUMO-1 (FIGURE 5) [29–33]. It was found that BLM formed dots/rod-like structures associated with SUMO-1 in the nucleus, for which the region from amino acids 238–586 of BLM is required. The biological relevance of the formation of these dot-like structures, which are associated with SUMO-1, is unclear at present. Meetei and coworkers reported the purification and analysis of proteins in three BLM-associated multiprotein complexes from HeLa cells [34]. Interestingly, one of these complexes includes five Fanconi anemia core complex proteins, which suggests a functional connection between the pathways disturbed in these genomic instability syndromes.

BLM interacts directly with ataxia-telangiectasia-mutated protein

The BS phenotype suggests that BLM plays a role in recognizing abnormal structures in DNA and suppressing recombinational events that lead to genomic instability. To understand the role of BLM in maintaining genomic stability more fully,

the yeast two-hybrid system was initially employed to identify proteins that interact with and influence the function of BLM. Sequence analysis of two strongly positive clones identified a 5.5 kb fragment from the C-terminus of the ataxia-telangiectasia-mutated protein (ATM) cDNA and BLM cDNA. Self-interaction is in keeping with other results, which demonstrate that BLM forms hexameric ring structures [35].

BLM bound to glutathione S-transferase (GST)-ATM-1 (amino acid residues 1–257), GST-ATM-10 (residues 2427–2641) and GST-ATM-12 (residues 2682–3012) in reactions without ethidium bromide. However, when this was included, GST-ATM-1 and GST-ATM-12 were found to bind equally well to BLM but there was reduced interaction with GST-ATM-10. Binding of BLM to GST-ATM-10, which contains the kinase domain of ATM, is consistent with this protein being a substrate for ATM kinase. Binding studies with ^{35}S -labeled BLM revealed that the smallest region of overlap was a sequence of roughly 24 nucleotides, which corresponds to amino acids 82–89. Mapping the region of interaction in BLM was carried out using four overlapping GST fusions (GST 1–4, numbered from the N-terminus). Binding of GST-BLM-3 (amino acid residues 636–1074) to ^{35}S -labeled full-length ATM was observed. This data suggests that ATM and BLM function together to recognize abnormal DNA structures by direct interaction.

Knockout mice

Several laboratories have reported the establishment of BLM knockout mice [36,37]. The features of the knockout mice showed striking differences, since the disruption was achieved using different strategies.

However, unlike human BS, three of the knockout mice showed embryonic lethality and only one developed to maturity. Chester and coworkers reported that mice which are homozygous for a targeted mutation in murine BLM are developmentally delayed and die by embryonic day 13.5. Cultured murine BLM $^{-}$ fibroblasts showed high numbers of SCEs. The growth retardation in mutant embryos can be accounted for by a wave of increased apoptosis. The mutant embryos do not survive past day 13.5 and exhibit severe anemia at that time.

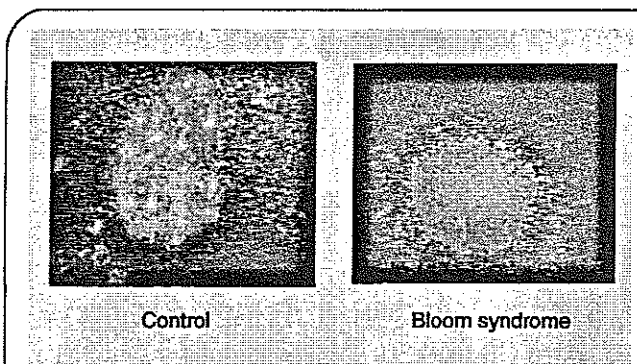
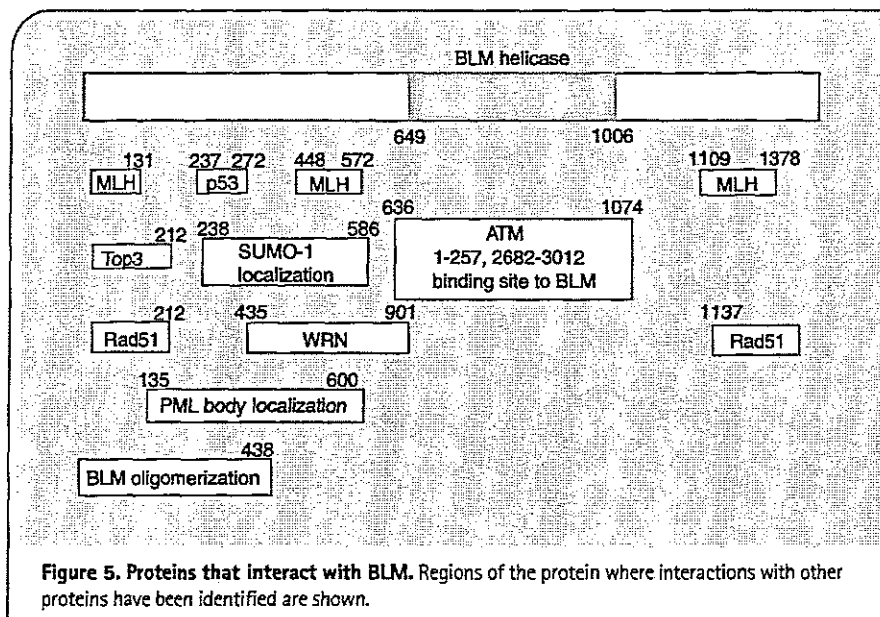


Figure 4. Nuclear dot formation of BLM in phytohemagglutinin-stimulated peripheral blood mononuclear cells from a control and Bloom syndrome patient (AsOk).



Luo and coworkers reported on mice homozygous for a BLM allele with a duplicated exon 3, which do not express detectable BLM protein. The cell lines from these mice show elevations in the rates of mitotic recombination. The mice are viable although by 20 months, nearly a third of them had developed cancer, mainly lymphomas and carcinomas. Luo then demonstrated that the increased rate of loss of heterozygosity resulting from mitotic recombination *in vivo* constitutes

the underlying mechanism of tumor susceptibility in those mice. This indicates that BLM is rate limiting for maintaining genomic instability and for the avoidance of tumors. These observations define a type of genetic instability that has significant implications for the evolution of cancer. The aim to elucidate the precise function of RecQ helicase in DNA repair and replication should not only be to improve our understanding of the molecular basis for tumorigenesis, but also to extend the range of potential therapeutic targets.

Key issues

- RecQ helicases are highly conserved from bacteria to humans. BLM, the causative gene for Bloom syndrome (BS), belongs to the RecQ helicase family.
- BS is uniquely associated with a predisposition to cancers of all types. BLM seems to maintain genomic stability by functioning at the interface between DNA replication and DNA repair.
- The BLM protein translocates into the nucleus. The distal arm of the bipartite basic residues in the C-terminus of the BLM protein is essential for targeting the nucleus.
- The combinational analysis of immunoblotting and immunohistochemistry is a useful approach for laboratory diagnosis of BS.
- BLM interacts with numerous binding partners. Ataxia-telangiectasia-mutated protein, the causative gene for Ataxia-telangiectasia, is one such associated protein.

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