Abstract. Background/Aim: Budding uninhibited by benzimidazole-related 1 (BUBR1) plays an important role in the spindle assembly checkpoint to prevent chromosome missegregation and aneuploidy during mitosis. We previously generated mutant mice that express BUBR1 at only 20% of the normal level (BubR1 L/L mice). Here, we examined the effect of low BUBR1 expression on oxidative stress-induced carcinogenesis in mice. Materials and Methods: We orally administered either a potassium bromate (KBrO₃) solution (2 g/l) or tap water to BubR1 L/L and wild-type (BubR1 +/+ ) mice for 16 weeks and examined the subsequent incidence of tumours. Results: KBrO₃-treated BubR1 L/L mice showed significantly higher mortality than the KBrO₃-treated BubR1 +/+ and control tap water-treated mice (p=0.0082). Histopathological and immunohistochemical analyses revealed that the spleens of surviving BubR1L/L mice were occupied by non-B-, non-T-cells with high proliferative potential. Conclusion: Our results indicate that low BUBR1 expression increases oxidative stress-induced mortality in mice, possibly caused by splenic neoplasms.

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Key Words: Oxidative stress, aneuploidy, KBrO₃, splenic neoplasms.

Budding uninhibited by benzimidazole-related 1 (BUBR1) is a core component of the spindle assembly checkpoint, which monitors whether or not sister chromatids are correctly separated during mitosis. Dysfunction of BUBR1 provokes chromosomal instability, thus leading to aneuploidy (1). Baker et al. generated mice with BubR1 gene mutation with various decreased expression levels of BubR1 due to combinations of wild-type, null, and hypomorphic BubR1 alleles (2-4). The mice that were homogeneous for the hypomorphic allele showed an early onset of aging phenotypes, such as short lifespan, cachectic dwarfism, lordokyphosis, cataracts, loss of subcutaneous fat, impaired wound healing, and decreased vascular wall elasticity (2,4). Chromosomal instability, such as aneuploidy, frequently occurred in the cells derived from hypomorphic BubR1 mice, but few mice spontaneously died of cancer under normal conditions (2). Notably, Dai et al. reported that mice with the wild-type allele and null allele were susceptible to carcinogen-induced adenocarcinoma in the lungs and intestines (5), suggesting that aneuploidy caused by the decreased expression of BUBR1 might enhance carcinogen-induced tumorigenesis in mammals.

We recently generated a new strain of hypomorphic BubR1 mice in which the expression of BUBR1 is reduced to 20% of the normal level. These low BUBR1-expressing mutant mice (termed BubR1 L/L mice) do not display apparent abnormalities, such as progeria, infertility, shortened lifespan, or structural anomaly in the tissue during growth and development, under normal conditions. Therefore, BubR1 L/L mice are particularly suitable for investigating the precise roles of BUBR1 in age-related diseases, including cancer. Using these mice, we previously demonstrated that decreased expression of BUBR1 completely inhibits intimal hyperplasia after carotid ligation by suppressing the
proliferation of vascular smooth muscle cells (6). We also demonstrated that BubR1L/L mice show a delayed liver regeneration after partial hepatectomy because of intercalated disc abnormality associated with increased hepatocyte necrosis and the reduced expression of desmocollin-1 (7).

BUBR1 is involved in cell proliferation and DNA aneuploidy through stimulation of reactive oxygen species (ROS). Guntani et al. revealed that aging-related loss of BUBR1 and subsequent increases of ROS affect the reduction of proliferative capacity of aged smooth muscle cells (8). The expression of BUBR1 and the proliferative capacity of aged human aortic smooth muscle cells (hAoSMC) was reduced with a progression of passages. No such phenomena were observed in young hAoSMC. The production of ROS induced by angiotensin II was also higher in aged hAoSMC than in younger cells. Moreover, down-regulation of BubR1 via RNA interference reduced the proliferative activity and increased the ROS production in hAoSMC. These results strongly suggest that BUBR1 is involved in the proliferative potential and ROS production in these cells (8). Ikawa-Yoshida et al. demonstrated that BUBR1 contributes to oxidative stress-induced aneuploidy in p53-deficient cells (9). Oxidative stress activated the p53 signaling pathway and down-regulated both BubR1 and mitotic arrest deficient 2 (Mad2) expression in a p53-dependent. In cells made p53-deficient via RNA interference, treatment with potassium bromate (KBrO3) caused an increase of polyploidy and bi-nucleated cells. However, when the expressions of p53 and BubR1 were knocked-down simultaneously, there was not an increase in polyploidy or bi-nucleated cells (9). These results suggest that p53 might play a critical role in the suppression of oxidative stress-initiated mitotic errors through the down-regulation of spindle assembly check point factors. Additionally, cells lacking p53 function might have an increased ability to re-enter the cell cycle and initiate DNA replication even in cells with incorrect chromosomal numbers. Because insufficient BUBR1 leads to the inactivation of spindle assembly check point, resulting in an increased incidence of DNA aneuploidy (2), the co-down-regulation of p53 and BubR1 was expected to encourage an increase in polyploidy. However, contrary to this expectation, BubR1 down-regulation suppressed the emergence of polyploid cells that is caused by oxidative stress when p53 alone is suppressed. Although the underlying mechanism is unclear, these results suggest that other BUBR1 functions, such as its relationship to the p16INK4A-Rb signaling pathway, the ubiquitin-mediated proteasomal degradation of cell-division cycle protein 20 (CDC20) in the G0 phase, and the maintenance of anaphase-promoting complex/ cyclosome CDC20 homolog 1 activity, might contribute to the observed phenotypes (9).

ROS are inevitably produced in cells during the processes of aerobic metabolism, signaling, and biophylaxis. Formation of ROS is further enhanced by exogenous stimuli, such as ionizing radiation, ultraviolet light, and various chemical mutagens. ROS attack DNA, and the resulting DNA oxidation then causes either spontaneous mutagenesis or cell death, which is implicated in various age-related diseases, such as cancer and neurodegeneration. Among the various types of oxidative DNA damage, 8-oxoguanine (8-oxoG) is highly mutagenic. During DNA replication, 8-oxoG can form a pair with adenine or cytosine, thus causing a mutation in the next round of replication (10, 11). An oxidizing reagent, KBrO3, which is used as a food additive for wheat flour, is known to induce 8-oxoG in the genomes of rats and mice (12, 13). KBrO3 has also been recognized as a renal carcinogen in rats (14, 15). In mammals, three enzymes, mutT homolog 1 (MTH1), 8-oxoguanine glycosylase 1 (OGG1), and mutY DNA glycosylase (MUTYH), play important roles in preventing 8-oxoG-related mutagenesis (10). MTH1 hydrolyses 8-oxo-dGTP to its monophosphate form and pyrophosphates, thereby preventing the incorporation of the mutagenic substrate into DNA during replication. OGG1, an 8-oxoG DNA glycosylase, excises 8-oxoGs opposite cytosines in DNA, which minimizes the formation of the pre-mutagenic base pair, A:8-oxoG. MUTYH is a DNA glycosylase that excises adenines incorporated opposite 8-oxoG. Defects of these enzymes lead to an increased tumor incidence in mice and humans.

We previously developed an experimental system for oxidative DNA damage-induced tumorigenesis in the small intestine of mice (16). We showed that chronic oxidative stress experimentally induced by KBrO3 resulted in multiple tumour formation in the small intestines of Mutyh-null mice (16, 17). We also reported that mismatch repair-deficient mice show a susceptibility to KBrO3-induced intestinal carcinogenesis (18). In order to examine the effects of oxidative stress on mice with BUB1 insufficiency, we performed similar KBrO3-induced tumorigenesis experiments using BubR1L/L mice.

Materials and Methods

Experimental animals. Low-BUB1-expressing mutant (BubR1L/L) and wild-type (WT) littermates (BubR1+/-) with a mixed genetic background of C57BL/6J and 129/SvJ were generated in our laboratory as previously described (5). KBrO3-induced tumorigenesis experiments were performed using 6- to 8-week-old BubR1L/L and BubR1+/- mice. Mice with each genotype were divided into two groups: the control group and a group treated with KBrO3 (2 g/l; Nacalai Tesque, Kyoto, Japan) via drinking water. The dose of KBrO3 was selected on the basis of previous studies so as to be toxic without lethal effects (16, 18). At the end of the experimental period of 16 weeks, the mice were sacrificed by cervical decapitation. Blood and urine were collected and kept at 4°C for biochemical analyses, testes were frozen for use in western blotting, and spleens were fixed with formalin and embedded in paraffin for histopathological examination. The animals were treated...
according to the Guidelines for the Care and Use of Laboratory Animals of Kyushu University (Approval no. A28-153-0).

Quantitative analysis of urine 8-oxo-2'-deoxyguanosine (8-OHdG). To assess the level of oxidative stress, urinary 8-OHdG was measured. Urine collected from BubR1/L/L and BubR1/+/+ mice after administration of KBrO₃ or tap water was used in these tests. 8-OHdG was detected by performing New 8-OHdG check ELISAs (Nikken SEIL, Fukuroi, Japan) according to the manufacturer’s instructions. 8-OHdG was standardized to urine creatinine levels, which were measured by LabAssay Creatinine (Wako Pure Chemical Industries, Osaka, Japan).

Cell culture. In order to compare the proliferative ability and sensitivity to the toxic effect of KBrO₃, hematopoietic cells were obtained from femoral bone marrow of untreated BubR1/L/L and BubR1/+/+ mice. The hematopoietic progenitor cells were collected by using an EasySep Mouse Hematopoietic Progenitor Cell Enrichment Kit (STEMCELL Technology Inc., Vancouver, B.C. Canada) according to the manufacturer’s instructions. Collected progenitor cells were counted and cultured at a density of 2x10⁵/ml in RPMI-1640 (Wako Pure Chemical Industries) with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich Corp, St. Louis, MO, USA), 100 U/ml penicillin and 100 μg/ml streptomycin (Thermo Fisher Scientific Inc., Waltham, MA, USA) in low-cell-binding plastic disposable tissue culture 24-well dishes at 37°C in 5% CO₂/95% air incubator. Cells were treated with or without 10 mM KBrO₃. Cultured cells were washed with phosphate-buffered saline and harvested with 1 ml of 0.05% trypsin-EDTA solution (Thermo Fisher Scientific Inc.) and counted when cells became subconfluent (n=3).

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue specimens were used for immunohistochemical analysis. Samples were de-paraffinised in xylene, rehydrated with graded ethanol, and washed in distilled water. The sections were pre-treated for 20 min at 120°C in 0.01 M citrate buffer, pH 6.0, for antigen retrieval. After a blocking procedure with 3% skim milk at room temperature for 10 min, the sections were incubated with a primary antibody against cluster of differentiation 3 (CD3) (rabbit monoclonal, ab5690; 1:100; Abcam), cluster of differentiation 56 (CD56) (rabbit polyclonal, ab81289; 1:250; Abcam), myeloperoxidase (MPO) (rabbit polyclonal, ab139748; 1:1000; Abcam), cluster of differentiation 45R (CD45R) (rat monoclonal, ab6410; 1:100; Abcam), myeloperoxidase (MPO) (rabbit polyclonal, ab139748; 1:1000; Abcam), cluster of differentiation 34 (CD34) (rabbit monoclonal, ab81289; 1:250; Abcam), cluster of differentiation 56 (CD56) (rabbit polyclonal, ab95153; 1:500; Abcam), or Ki-67 (rabbit monoclonal, #12202; 1:400; Cell Signaling Technology, Danvers, MA, USA) in a humidifying chamber at 4°C overnight. Endogenous peroxidase activity was blocked by incubating tissue sections in 0.3% H₂O₂. After incubation with an horse radish peroxidase-conjugated secondary antibody, the signals were visualized using 3,3′-diaminobenzidine. Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue specimens were used for immunohistochemical analysis. Samples were de-paraffinised in xylene, rehydrated with graded ethanol, and washed in distilled water. The sections were pre-treated for 20 min at 120°C in 0.01 M citrate buffer, pH 6.0, for antigen retrieval. After a blocking procedure with 3% skim milk at room temperature for 10 min, the sections were incubated with a primary antibody against cluster of differentiation 3 (CD3) (rabbit monoclonal, ab5690; 1:100; Abcam), cluster of differentiation 56 (CD56) (rabbit polyclonal, ab81289; 1:250; Abcam), myeloperoxidase (MPO) (rabbit polyclonal, ab139748; 1:1000; Abcam), cluster of differentiation 45R (CD45R) (rat monoclonal, ab6410; 1:100; Abcam), myeloperoxidase (MPO) (rabbit polyclonal, ab139748; 1:1000; Abcam), cluster of differentiation 34 (CD34) (rabbit monoclonal, ab81289; 1:250; Abcam), cluster of differentiation 56 (CD56) (rabbit polyclonal, ab95153; 1:500; Abcam), or Ki-67 (rabbit monoclonal, #12202; 1:400; Cell Signaling Technology, Danvers, MA, USA) in a humidifying chamber at 4°C overnight. Endogenous peroxidase activity was blocked by incubating tissue sections in 0.3% H₂O₂. After incubation with an horse radish peroxidase-conjugated secondary antibody, the signals were visualized using 3,3′-diaminobenzidine.

Biochemical analyses. Plasma levels of blood urea nitrogen (BUN), creatinine (Cr), aspartate:2-oxoglutarate aminotransferase (AST) and alanine:2-oxoglutarate aminotransferase (ALT) were analyzed by Nagashima Life Sciences Laboratory (Shiga, Japan).

Protein preparation and western blot analysis. Mouse testes were minced, and the proteins were extracted with CelLytic MT Cell Lysis Reagent (Sigma-Aldrich) supplemented with Halt Protease Inhibitor Cocktail 100x (Thermo Fisher Scientific) according to the manufacturer’s instructions. The protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. The blots were probed with a primary antibody for BUBR1 (NBPI-19555; 1:1000; Nobus Biological, Littleton, CO, USA). Equal loading was confirmed using an antibody against tubulin (ab4074; 1 μg/ml; Abcam).

Statistical analysis. Data are presented as the mean±standard error (SE). The Student’s t-test and the log-rank test were used where appropriate with JMP pro (version 11.0.0; SAS Institute Inc., Cary, NC, USA). Significance was assumed at p<0.05.

Results

BubR1/L/L and BubR1/+/+ mice suffer equally from KBrO₃-induced oxidative stress. In order to compare the effects of KBrO₃-induced oxidative stress on mice with low or normal levels of BUBR1, we prepared seven BubR1/L/L and 10 BubR1/+/+ mice with similar body weights (20.0±1.5 vs. 20.9±4.0, respectively; p=0.47) for the experiments. The insufficiency of BUBR1 in BubR1/L/L mice was confirmed by western blotting assays (Figure 1a). According to the chronological body weights of surviving mice, both BubR1/L/L and BubR1/+/+ mice given KBrO₃ gradually lost weight (Figure 1b). There were no significant differences in weight loss between the two groups treated with KBrO₃.

Urinary 8-OHdG is a marker for oxidative stress, therefore after administration of KBrO₃, urine was collected and 8-OHdG levels were measured. The amounts of 8-OHdG were normalized using urine creatinine content. Urine 8-OHdG was elevated by the administration of KBrO₃, but the levels of urine 8-OHdG were not significantly different in BubR1/L/L and BubR1/+/+ mice following KBrO₃ treatment (Figure 1c). These results indicate that BubR1/L/L and BubR1/+/+ mice suffered equally from the effects of oxidation caused by KBrO₃.

The administration of KBrO₃ increases the mortality of BubR1/L/L mice. Over the period of the 16-week administration of KBrO₃, the mortality rate of BubR1/L/L mice was significantly higher than that of BubR1/+/+ mice (Kaplan–Meier analysis; p<0.05) (Figure 2a). Three BubR1/L/L mice died in the early phase (in the first eight weeks) and two died in the second eight-week period, while only one BubR1/+/+ mouse died over the entire period. During necropsy of these mice, we observed dark red intestinal fluids suggestive of gastrointestinal hemorrhage in the two mice that died in the later phase but saw no apparent lesions in the three BubR1/L/L mice that died in the early phase (Figure 2b). None of the mice given tap water in either the BubR1/L/L or BubR1/+/+ groups died during the entire period.

Surviving BubR1/L/L mice exhibited an elevated BUN level. After the administration of KBrO₃, blood was collected from
the surviving mice, and biochemical analyses of the blood were performed. The results show that the surviving KBrO$_3$-treated $BubR1^{L/L}$ mice had a significantly increased level of BUN compared with the untreated mice, but AST and ALT levels were not significantly elevated by KBrO$_3$ treatment (Figure 2c). No increase in the BUN level was observed in the KBrO$_3$-treated $BubR1^{+/-}$ mice. There was no difference in the creatinine levels between the animals with and without KBrO$_3$ treatment in either genotype group. These results suggest that the KBrO$_3$-treated $BubR1^{L/L}$ mice suffered from dehydration.

Non-B-, non-T-cells occupied the spleens of $BubR1^{L/L}$ mice treated with KBrO$_3$. In order to investigate the effects of KBrO$_3$ on the mice in more detail, the surviving mice were euthanized and dissected. An intestinal tumor with a 1-mm diameter was detected in one $BubR1^{+/-}$ mouse, but no such tumors were observed in $BubR1^{L/L}$ mice. However, splenomegaly was identified in two of the surviving $BubR1^{L/L}$ mice, while such lesions were not observed in $BubR1^{+/-}$ mice. The inspection of spleens stained by hematoxylin-eosin revealed that the pulp boundary was unclear in the spleens from two surviving $BubR1^{L/L}$ mice. Additionally, many lymphocyte-like cells had gathered in the red pulp of these mice, suggestive of lymphoma or invasive leukemia (Figure 3a). There were no such lesions in the $BubR1^{+/-}$ or $BubR1^{L/L}$ mice that were treated with tap water.

In order to identify the lineage of these cells, immunohistochemistry was performed. The cells did not express the B-lymphocyte marker CD45R nor the T-lymphocyte marker CD3. Moreover, the expressions of MPO, CD34, and CD56 were also negative. A subset of the cells was positive for Ki-67 expression (Figure 3b). These findings suggest that the cells might be non-B-, non-T-cell neoplasms with a high proliferative potential.

Sensitivity to oxidative stress was high in $BubR1^{L/L}$ mouse progenitor cells. The findings of splenic neoplasms similar to stem cell lymphoma prompted us to examine the proliferative potential of the hematopoietic progenitor cells derived from $BubR1^{L/L}$ mice as well as the effect of oxidative stress on these cells. The proliferative potential of the $BubR1^{L/L}$ progenitor cells was significantly lower than that of the $BubR1^{+/-}$ cells. The proliferative potential of the progenitor cells decreased following treatment with 10 μM KBrO$_3$. This effect was more profound in the $BubR1^{L/L}$ progenitor cells than it was in the $BubR1^{+/-}$ cells (Figure 4).

Discussion

Here, we reveal that $BubR1^{L/L}$ mice are hypersensitive to oxidative-stress compared to $BubR1^{+/-}$ mice. Moreover, the cells with immunophenotypic profile of CD3$^-$, CD45R$^-$,
MPO−, CD56−, and CD34− occupied the spleens of surviving BubR1 L/L, suggesting the development of stem cell lymphoma, a group of non-B-, non-T-cell neoplasms. Splenic neoplasms were recognized only in the BubR1 L/L mice treated with KBrO3. A single factor, either the insufficiency of BUBR1 or the administration of KBrO3, was unable to cause these phenomena, thus suggesting that the combination of low BUBR1 expression with increased oxidative stress may lead to a reduced tolerance to oxidative stress and the development of splenic lymphoma in mice.

KBrO3 is a well-known oxidizing agent which is used as a food additive. The toxicity of KBrO3 as a causative agent of oxidative stress has been well-studied (19). Here, the dosage of KBrO3 was selected by referring to previous studies so as not to administer a lethal dose (16, 18, 20, 21). The dosage of KBrO3 per body weight was very similar between the two

Figure 2. Mortality and abdominal findings following KBrO3 treatment in BubR1 L/L and BubR1 +/+ mice. BubR1 L/L (L/L) and BubR1 +/+ (+/+) mice were left untreated [KBrO3 (−)] or treated with KBrO3 [KBrO3 (+)]. a: The mortality of these mice over 16 weeks was assessed. A Kaplan–Meier analysis was performed to determine the significance of the differences between these groups [+/+ KBrO3 (−): n=5, +/+ KBrO3 (+): n=5, L/L KBrO3 (−): n=10, L/L KBrO3 (+): n=7, respectively]. *Log-rank p=0.0082. b: Image of the digestive tract of a BubR1 L/L mouse. Yellow arrows indicate hemorrhage. c: The results of biochemical analysis of plasma levels of blood urea nitrogen (BUN), creatinine (Cr), aspartate:2-oxoglutarate aminotransferase (AST), and alanine:2-oxoglutarate aminotransferase (ALT). The open and filled bars indicate KBrO3 (−) and KBrO3 (+), respectively. *Significantly different at p<0.05.
Figure 3. Splenic neoplasms after KBrO3 treatment. BubR1L/L and BubR1+/+ mice were left untreated (KBrO3 (−)) or treated with KBrO3 (KBrO3 (+)) for 16 weeks. Representative microscopy images of spleen sections stained with hematoxylin-eosin (a) and immunohistochemical staining of CD3, MPO, CD45R, CD34, CD56, and Ki-67 (b) are shown. Bars=25 μm, W: white pulp, R: red pulp.
groups because the BubR1<sup>L/L</sup> and BubR1<sup>+/+</sup> mice had almost the same body weights before the administration of KBrO<sub>3</sub>. The similarity of the KBrO<sub>3</sub> dosage per body weight is also supported by the finding that there was no significant difference between the amounts of 8-OHdG in the urine from the two groups of KBrO<sub>3</sub>-treated mice (Figure 1C). Despite undergoing equivalent levels of oxidative stress and resulting DNA damage, BubR1<sup>L/L</sup> mice had an extremely high mortality rate during the KBrO<sub>3</sub> treatment compared with BubR1<sup>+/+</sup> mice. It is possible that the poor proliferative potential of cells that is caused by BUBR1 insufficiency might lead to a reduced tolerance to oxidative stress in mice.

In this study, KBrO<sub>3</sub>-treated BubR1<sup>L/L</sup> mice had a high mortality rate. In order to investigate the cause of death in these mice, necropsies were performed on all the mice that died during the experiment. However, most of the mice did not exhibit any specific features indicating the cause of death, such as tumorigenesis. The results of biochemical analyses of the surviving BubR1<sup>L/L</sup> mice and necropsies of the non-survivors suggest that the clinical cause of death in the BubR1<sup>L/L</sup> mice was probably dehydration or gastrointestinal hemorrhage. The decline in cell proliferation due to BUBR1 insufficiency is likely one of the reasons that the BubR1<sup>L/L</sup> mice had a high mortality following KBrO<sub>3</sub> treatment. The BubR1<sup>+/+</sup> mice were able to recover from the damage induced by KBrO<sub>3</sub>, while the BubR1<sup>L/L</sup> mice were unable to overcome the toxicity of KBrO<sub>3</sub> because their lower proliferative capacity limited their recuperative abilities. The accumulated and unhealed damage induced by KBrO<sub>3</sub> injured the intestinal mucosa and might have resulted in the malabsorption of water and gastrointestinal hemorrhage.

In contrast, when the surviving BubR1<sup>L/L</sup> mice were sacrificed, the necropsies performed on these animals showed that their spleens were occupied by non-B-, non-T-cells with a high proliferative potential, similar to that of leukemia or lymphoma cells. Previous studies have shown that the oral ingestion of KBrO<sub>3</sub> causes intestinal tumors in animals with BubR1<sup>L/L</sup> mice and necropsies of the non-survivors indicated the they are likely to be immature cells, such as in lymphocytic cell surface markers CD3 and CD45R (22).

There are few reports indicating the direct relationship between the BUBR1 expression and leukemia. Dominik et al. identified that a low expression of BUBR1 affected the sensitivity to antimitotic therapy of many acute myeloid leukemia cell lines (23). However, the relationship between the expression level of BUBR1 and the occurrence of leukemia remains unclear. It is well-known that a genetic defect plays a role in leukemia. Micronuclei are a hallmark of DNA damage and markers of exposure to genotoxic agents (24). Using BubR1<sup>L/L</sup>- and wild-type mouse embryonic fibroblasts cells, Dai et al. showed that the number of micronuclei was greatly increased in the low BUBR1 condition (5). KBrO<sub>3</sub> also causes micronuclei in a dose-dependent manner (25). These double stimulations, such as chromosomal instability and genotoxicity, might accelerate the accumulation of DNA damage. Recently, one study reported that such accumulation of massive DNA damage in micronuclei may cause chromothripsis to occur (24). Chromothripsis explains massive local DNA fragmentation producing complex rearrangements restricted to only one or a few chromosomes. This phenomenon could be one possible mechanism of tumorigenesis.

In summary, mice with BUBR1 insufficiency show hypersensitivity to oxidative stress. The combination of BUBR1 insufficiency and administration of KBrO<sub>3</sub> worsens the proliferative capacity compared with the impaired proliferation induced by BUBR1 insufficiency alone; thus, this may lead to a reduced tolerance to oxidative stress. Treatment
with KBrO$_3$ resulted to high mortality in BubR1$^{+/+}$ mice, possibly due to dehydration or gastrointestinal hemorrhage. Furthermore, the combination of BUBR1 insufficiency and KBrO$_3$ treatment also produced non-B- and non-T-cell splenic tumours. This work provides new insights into the role of BUBR1 in oxidative stress, which may explain one mechanism of hypersensitivity to oxidative stress.

**Conflicts of Interest**

None of the Authors have any conflicts of interest in regard to this study.

**References**

1. Kapanidou M, Lee S and Bolanos-Garcia VM: BUBR1 kinase: protection against aneuploidy and premature aging. Trends Mol Med 21: 364-372, 2015.

2. Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneca S, Kopecka A, Kumar R, Jenkins RB, de Groen PC, Roche P and van Deursen JM: BUBR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat Genet 36: 744-749, 2004.

3. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, Nakatsu Y, Tsuzuki T and Nakatsu Y: Mutagenesis and carcinogenesis caused by the oxidative stress-induced aneuploidy in p53-deficient cells. Cancer Med 35: 440-445, 2004.

4. Kuwahara K, Ohshima K, Tsuchiya T, Yamaguchi T, Suefuji H, Nomura M and Maehara Y: BUBR1 Insufficiency impairs liver repair deficient mice show susceptibility to oxidative stress-induced intestinal carcinogenesis. Int J Biol Sci 10: 73-79, 2013.

5. Kurokawa Y, Maekawa A, Takahasi M and Hayashi Y: Toxicity and carcinogenicity of potassium bromate – a new renal carcinogen. Environ Health Perspect 69: 221-235, 1986.

6. Nakabeppu Y and Tsuzuki T: Abnormality in Wnt signaling is causatively associated with oxidative stress-induced intestinal tumorigenesis in Mutyh-null mice. Int J Biol Sci 10: 940-947, 2014.

7. Piao J, Nakatsu Y, Ohno M, Taguchi K and Tsuzuki T: Mismatch repair deficient mice show susceptibility to oxidative stress-induced intestinal carcinogenesis. Int J Biol Sci 10: 73-79, 2013.

8. Kurokawa Y, Maekawa A, Takahasi M and Hayashi Y: Toxicity and carcinogenicity of potassium bromate – a new renal carcinogen. Environ Health Perspect 87: 309-335, 1990.

9. Arai T, Kelly VP, Minowa O, Noda T and Nishimura S: The role of $Ogg1$ knockout mice exposed to potassium bromate under cell-free conditions and in mammalian cells. Carcinogenesis 35: 309-335, 1990.

10. Engelhardt M and Wäsch R: BUBR1 is frequently repressed in acute myeloid leukemia and its re-expression sensitizes cells to antimitotic therapy. Haematologica 98: 1886-1895, 2013.

11. Schnerch D, Schmidt A, Follo M, Udi J, Felthaus J, Pfeifer D, Engelhardt M and Wäsch R: BUBR1 is frequently repressed in acute myeloid leukemia and its re-expression sensitizes cells to antimitotic therapy. Haematologica 98: 1886-1895, 2013.

12. Ballmaier D and Epe B: Oxidative DNA damage induced by potassium bromate under cell-free conditions and in mammalian cells. Carcinogenesis 16: 335-342, 1995.

13. Kasi H1, Nishimura S, Kurokawa Y and Hayashi Y: Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA. Carcinogenesis 8: 1959-1961, 1987.

14. DeAngelo AB, George MH, Kilburn SR, Moore TM and Wolf DC: Carcinogenicity of potassium bromate administered in the drinking water to male B6C3F1 mice and F344/N rats. Toxicol Pathol 26: 587-594, 1998.

15. Kurokawa Y, Takayama S, Konishi Y, Hiasa Y, Asahina S, Takahashi M, Maekawa A and Hayashi Y: Long-term in vivo carcinogenicity tests of potassium bromate, sodium hypochlorite, and sodium chlorite conducted in Japan. Environ Health Perspect 69: 221-235, 1986.

16. Nakabeppu Y, Sakumi K, Sakamoto K, Tsuchimoto D, Tsuzuki T and Nakatsu Y: Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids. Biol Chem 387: 373-379, 2006.