Aberrations of BUBR1 and *TP53* Gene Mutually Associated with Chromosomal Instability in Human Colorectal Cancer

YAN ZHAO[1,2*], KOJI ANDO[1*], EIJI OKI[1], AYAE IKAWA-YOSHIDA[1,3], SATOSHI IDA[1], YASUE KIMURA[1], HIROSHI SAEKI[1], HIROYUKI KITAO[4], MASARU MORITA[1] and YOSHIHIKO MAEHARA[1]

Departments of [1]Surgery and Science and [4]Molecular Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]The Third Surgery Department, Liaoning Cancer Hospital and Institute, Shenyang, P.R. China; [3]Chugai Research Institute for Medical Science, Inc., Gotnemba, Japan

Abstract. *Background/Aim: Defects in mitotic checkpoint and p53-dependent pathways associate with chromosomal instability. In the present study, we investigated the interplay between BUBR1 and p53 and their association with genetic instability in colorectal cancer. Patients and Methods: 139 colorectal cases were examined for BUBR1, p53 and genetic instability indicators. BUBR1 expression was evaluated by immunohistochemistry and TP53 gene was directly sequenced. DNA ploidy was studied by laser scanning cytometry; MSI and TP53 loss of heterozygosity was also examined. Results: 64% of cases had high BUBR1 expression and were associated with the TP53 mutation. High BUBR1 expression and TP53 mutation associated with DNA aneuploidy and showed an inverse association with MSI. Cases with high BUBR1 expression and TP53 mutation had profound aneuploidy phenotypes and less frequent MSI compared to cases with one or neither aberration. Conclusion: Our findings indicated an interplay between BUBR1 and p53 in colorectal cancer. Altered expression of both molecules was associated with chromosomal instability.*

Genomic instability is a hallmark of most human malignancies (1). Many types of cancers, including colorectal cancer, display two independent types of genetic instabilities, namely microsatellite instability and chromosomal instability (2). Microsatellite instability occurs from defective DNA mismatch repair machinery and is found in approximately 15% of colorectal cancer cases. Chromosomal instability is reflected by gross chromosomal numerical and structural changes or DNA aneuploidy and considered as a possible driving force for tumorigenesis in the large majority of colorectal cancer cases. Chromosomal instability occurs as a result of altered mitotic divisions, duplication defects, aberrant centrosome segregation or impaired mitotic spindle checkpoint (3, 4).

The BUBR1 protein, encoded by the *BUB1B* gene, is one of the key elements of the mitotic checkpoint complex that monitors the mitotic spindle checkpoint by blocking the onset of anaphase until all chromosomes properly attach to spindles (5, 6). Recent studies revealed multiple roles for BUBR1 in the control of chromosome dynamics (7-9), suppression of centrosome amplification (10) and promotion of apoptosis (11). Hereditary defects in the *BUB1B* gene are etiological factors for the highly carcinogenic syndrome mosaic variegated aneuploidy, which is characterized by mosaic aneuploidies (12). Heterozygous mutations in *BUB1B* can result in premature chromatid separation, which is inherited as an autosomal dominant trait without phenotypic consequences. Somatic mutation of the *BUB1B* gene is uncommon in sporadic malignancies (12, 13), while elevated expression of BUBR1 has been observed in cancer cell lines with chromosomal instability phenotypes (14). However, studies into the relationship between aberrant BUBR1 expression and chromosomal instability phenotypes have revealed conflicting results in human malignancies (15-20).

The p53 protein maintains cellular homeostasis in response to various insults to genomic stability and functions in pathways involved in tumorigenesis and/or progression, such as cell-cycle control, apoptosis and angiogenesis (21-24). The *TP53* mutation is one of the most common genetic defects in human malignancies. Recent studies have shown that p53 regulates the transcription of BUBR1, while conversely BUBR1 mediates the activation of p53 during the

*These Authors contributed equally to this study.

Correspondence to: Dr. Eiji Oki, Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-Ku, Fukuoka, 812-8582, Japan. Tel: +81 926425466, Fax: +81 926425482, e-mail: okieiji@surg2.med.kyushu-u.ac.jp

Key Words: Chromosomal instability, *BUBR1, TP53* gene, colorectal cancer, microsatellite instability.

mitotic damage checkpoint (10, 25). Simultaneous defects in the mitotic checkpoint machinery and p53 result in increased chromosomal instability (26).

To our knowledge, the interplay between BUBR1 and p53, as well as their association with chromosomal instability, has not been investigated in human malignancies. The aim of the present study was to examine the potential interplay between BUBR1 and p53, as well as their connection with genomic instability patterns in sporadic colorectal cancer. Our data indicated that aberrations in *BUBR1* and *p53* were cooperatively associated with the chromosomal instability phenotype in colorectal cancer.

Materials and Methods

Patient and tissue specimens. Cancerous tissues and the corresponding non-cancer mucosa were obtained from 139 Japanese patients diagnosed with sporadic colorectal cancer who underwent surgery in the Department of Surgery and Science, Kyushu University Hospital from 1998 to 2004. The patients' characteristics are shown in Table I. Written informed consent for the studies of the excised tissues was obtained from each patient. To exclude possible hereditary non-polyposis colorectal cancer cases, the Amsterdam Criteria were used (27). All cases received no neo-adjuvant chemotherapy. All specimens were obtained immediately after resection and placed in liquid nitrogen for analysis. The remaining specimens were routinely processed for histopathological analysis. A histological diagnosis of the specimens was performed by specialists of our Hospital. Frozen tissues were ground in liquid nitrogen and lysed in digestion buffer (10 mM Tris-Cl, pH 8.0, 0.1 M EDTA, pH 8.0, 0.5% SDS, 20 μg/ml pancreatic RNase; supplier, address). After treatment with proteinase K and extraction with phenol, DNA was precipitated with ethanol and dissolved in 1× TE (10 mM Tris-Cl, pH 7.5, 1 mM EDTA).

Immunohistochemical staining. Formalin-fixed, paraffin-embedded tissue specimens were used for immunohistochemical staining. Blocks containing both cancerous and adjacent non-cancerous tissues were selected for examination. Sections (5 μm) were de-paraffinized in xylene and rehydrated in a gradient series of ethanol. Immunohistochemical staining was performed as previously reported (15). Briefly, specimens were pre-treated in an autoclave for 15 min in 0.01 mol/l citrate-buffered saline (pH 6.0) for antigen retrieval. After quenching the endogenous peroxidase activity in methanol with 0.3% (v/v) hydrogen peroxidase for 10 minutes at room temperature, the sections were briefly washed with PBS and then incubated with 10% normal rabbit serum to block nonspecific binding of the immunoreagents. The sections were then incubated with monoclonal antibodies against BUBR1 (Clone 9, 1:100; BD Transduction Laboratories, San Jose, CA, USA) at 4˚C overnight. Streptavidin-biotin complex and horseradish peroxidase were applied and the reaction products were visualized using the Histofine SAB-PO (M) or (R) immunohistochemical staining kit (Nichirei, Tokyo, Japan), according to the manufacturer's instructions. For negative controls, primary antibodies were replaced with non-immune, normal serum. Two blinded observers (Y.Z. and K.A.) independently examined immunostained sections. An Olympus microscope (CKX41; address) was used for examination. Intensity of cytoplasmic staining of BUBR1 was scored according to a previous study on a three-point scale: 0, no staining; 1, minimal staining; 2, moderate to strong staining in at least 20% of cells; 3, strong staining in at least 50% of cells (20, 28). The lymph follicle always showed strong staining and thus was used as an internal control. Cells with strong to moderate expression in the mitotic phase were excluded from evaluation (19). An average score of at least three randomized field-of-views of cancerous regions was calculated. Final scores of 2 or less were defined as "low expression"; a score of 4 was defined as "high expression".

TP53 gene direct sequencing. Amplification of *TP53* from exon 5 to exon 9 including exon-intron junctions was performed as described elsewhere (29). Primers for p53 polymerase chain reaction (PCR) amplification were purchased from Nippon Gene (Tokyo, Japan) and TaKaRa Ex Taq, a Taq polymerase with 3' exonuclease activity, was purchased from TaKaRa Bio Inc. (Otsu, Japan). The PCR products were purified and later used as templates for cycle sequencing reactions using a BigDye terminator cycle sequencing kit Ver.1.0 (Applied Biosystems, Foster City, CA, USA). Mutations found in one PCR product were verified by reverse sequencing and confirmed in two independent PCR amplifications.

High-resolution fluorescent microsatellite analysis (HRFMA). HRFMA was used for microsatellite instability and TP53 loss of heterozygosity (LOH) analysis. HRFMA methods have been described in detail elsewhere (30, 31). In each case, a labeled size marker was simultaneously separated by electrophoresis to standardize the mobility of the sample. The data were processed using the ABI software package from GeneScan (Applied Biosystems). According to the guidelines established by the National Cancer Institute (NCI), microsatellite instability was classified based on the frequency of positive findings in a panel of reference markers (32) high-frequency microsatellite instability (MSI-H), over 40% of loci positive for microsatellite instability; low-frequency microsatellite instability (MSI-L), less than 40% positive loci; and microsatellite stable (MSS), no positive cases. MSI-H is the marker of a defective DNA mismatch repair system. In this study, we designated MSI-H as MSI(+) and grouped MSS and MSI-L as MSI(–).

For detection of *TP53* LOH, we used two microsatellite instability markers, D17S796 and D17S1353, which are close to the 5' and 3' ends of *TP53*, respectively. The experimental procedures for LOH detection have been described elsewhere (33). Briefly, the fluorescence of a peak decreases more than 30% of the normal control when LOH occurs in the amplified region of the genomic DNA from tumor tissue specimens. When the paternal and maternal alleles overlap, the case is not informative for LOH estimation.

Laser scanning cytometry (LSC). LSC (CompuCyte Corporation, Westwood, MA, USA) was used to indicate nuclear DNA index (DI), as described elsewhere (34). Briefly, cell nuclei were recovered from two samples of a 50 μm-thick slice from paraffin-embedded blocks. The blocks had been diagnosed by routine immunohistochemical staining and a tumor area of over 30% in dimension was chosen for LSC detection. Single-layered nuclei were spread on a slide glass and stained with propidium iodide with RNase ((PI)/RNase staining solution; Sigma, St. Louis, MO, USA) for 15 minutes at room temperature. The slide was then briefly washed with PBS and covered using mounting medium

Table I. *Characteristics of colorectal cancer patients of the study.*

Factors	n (%)
Age (years, mean±SD)	64.8±12.2
Gender	
Male	78 (56.1)
Female	61 (43.9)
Location of the tumor	
Ascending	34 (24.4)
Transverse	11 (7.9)
Descending	9 (6.4)
Sigmoid	29 (20.9)
Rectum	56 (40.3)
Stage	
I	18 (12.9)
IIA	39 (28.1)
IIB	10 (7.2)
IIIA	1 (0.7)
IIIB	29 (20.9)
IIIC	20 (14.4)
IV	21 (15.1)

with propidium iodide. A DNA content histogram was generated by LSC and DI was calculated as previously published (35). For each case, the nuclei were reviewed after scanning. The morphology of the nuclei was confirmed to exclude debris and attached nuclei from the analysis. The DI of lymph cell nuclei with dimensions of approximately 40 μm was used as a reference of DI=1.0. Tumors with a DI 1.0±0.2 were defined as diploid; DI ≥1.2 or samples with multi-DNA indexed were designated as aneuploidy (19, 28).

Statistical analysis. Statistical analyses were performed using the JMP 7.0 software (SAS Institute, Cary, NC, USA). The Chi-square test, Fisher's exact test and Student's *t*-test were employed as appropriate. For all statistical tests, $p<0.05$ was considered significant.

Results

Patterns of genetic instability in colorectal cancer. We examined patterns of genetic instability in colorectal cancer by analyzing the DNA index and microsatellite instability. A large portion (66.2%, 92/139) of colorectal cancer samples showed DNA aneuploidy, which was used as a marker for chromosomal instability. Microsatellite analysis revealed MSS, MSI-L and MSI-H in 71.9% (100/139), 13.7% (19/139) and 14.4% (20/139) cases, respectively. The vast majority of aneuploidy cases (87.0%, 80/92) were MSS (–), which included MSS and MSI-L. In comparison, 60% (12/20) of MSI (+) cases, including only MSI (+), were diploid. MSI (+) and aneuploidy co-existed in a small portion of cases (8.6%, 12/129), while the rest of the cases were diploid and MSI (–) (39/139, 28.1%).

Table II. *Relationship between BUBR1 expression, TP53 mutation and LOH in colorectal cancer.*

Factors	BUBR1 expression, n (%)		*p*-Value
	Low (n=49)	High (n=90)	
TP53 gene			
Wild	38 (77.6)	52 (57.8)	0.017
Mutant	11 (22.4)	38 (42.2)	
TP53 loci LOH			
ROH	15 (30.6)	19 (21.1)	0.078
LOH	28 (57.1)	67 (74.4)	
NI	6 (12.2)	4 (4.4)	

LOH: Loss of heterozygosity, ROH: retention of heterozygosity, NI: not informative. Underlined *p*-value is for *p*-value less than 0.05.

Table III. *Relationship between TP53 gene mutation and TP53 LOH.*

	TP53 gene status, n (%)		*p*-Value
	Wild (n=90)	Mutant (n=49)	
TP53 loci LOH			
ROH	33 (36.7)	1 (2.0)	<0.0001
LOH	47 (52.2)	48 (98.0)	
NI	10 (11.1)	0 (0)	

LOH: Loss of heterozygosity, ROH: retention of heterozygosity, NI: not informative.

BUBR1 expression in colorectal cancer. Previous studies have reported weakly expressed or undetectable BUBR1 in the non-cancerous mucosa of colorectum, except for cells in mitotic phases (19, 20). p53 staining was negative in non-cancerous tissues. Among the 139 colorectal cancer cases, high BUBR1 expression was detected in 64.7% of the cases (90/139) (Table II). Figure 1 shows two representative cases of BUBR1 expression.

TP53 gene alternations in colorectal cancer. We also examined *TP53* gene genetic alternations (mutation and LOH) and studied their associations with BUBR1. Mutations in the *TP53* gene were observed in 35.2% (49/139) of colorectal cases. *TP53* LOH was informative in 129 cases and LOH was observed in 73.6% (95/129) of cases. High BUBR1 expression correlated with *TP53* gene mutation ($p=0.017$) and tended to associate with *TP53* LOH ($p=0.07$) (Table II). A significant relationship was found between *TP53* gene mutation and LOH (Table III).

Genetic instability and BUBR1 and p53 expression. High BUBR1 expression staining was significantly associated with DNA aneuploidy ($p=0.05$) and tended to associate with MSI

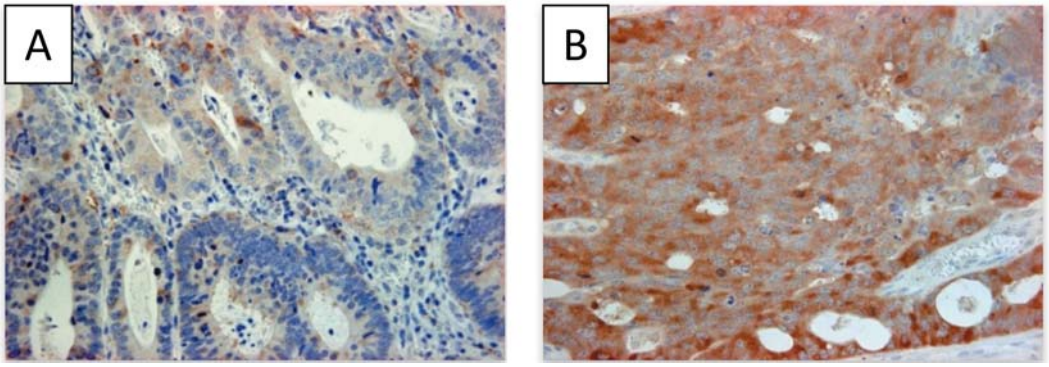

Figure 1. *Immunohistochemical analysis of BUBR1 expression in colorectal cancer. A, Representative image of a case with low BUBR1 expression; B, Representative image of a case with high BUBR1 expression. Photographs were taken at ×200 magnification.*

(–) (p=0.09) (Table IV). *TP53* gene mutation was significantly associated with DNA aneuploidy and MSI (–) (p=0.01, p=0.0005, respectively) (Table V).

Because of the important roles of both BUBR1 and *TP53* gene in maintaining chromosomal homeostasis, we examined potential correlations of both BUBR1 and *TP53* gene with genetic instability. Cases were categorized into three groups according to the status of BUBR1 and *TP53* gene. Both high BUBR1 expression and *TP53* gene mutation were found in 28.1% (39/139) cases; either BUBR1 or TP53 gene aberration was confirmed in 25.2% (35/139); while the remaining 46.8% (65/139) showed neither of the aberrations. These three groups were accordingly named "Both", "Either", and "Neither". DNA ploidy and microsatellite status were compared among the three groups (Figure 2). The "Both" group had a more profound chromosomal instability phenotype revealed by DNA aneuploidy, compared to the "Neither" group (p=0.005). The "Either" group had a moderate aneuploidy ratio. The "Neither" group showed a higher MSI (+) ratio (26.1%, 17/65) than the "Either" (5.7%, 2/35) or "Both" group (2.5%, 1/39) (p=0.027 and p<0.001, respectively).

Table IV. *Patterns of genomic instability and BURB1 expression in colorectal cancer.*

Factors	BUBR1 expression, n (%)		*p*-Value
	Low (n=49)	High (n=90)	
DNA ploidy			
Diploid	21 (42.9)	26 (28.9)	0.05
Aneuploid	28 (57.1)	64 (71.1)	
MSI status			
MSI (–)	38 (77.6)	81 (90.0)	0.09
MSI (+)	11 (22.4)	9 (10.0)	

Underlined *p*-value is for *p*-value less than 0.05.

Table V. *Patterns of genomic instability and TP53 gene status in colorectal cancer.*

Factors	*TP53* gene status, n (%)		*p*-Value
	Wild (n=90)	Mutant (n=49)	
DNA ploidy			
Diploid	37 (42.9)	10 (28.9)	0.011
Aneuploid	53 (57.1)	39 (71.1)	
MSI status			
MSI (–)	71 (78.9)	48 (98.0)	0.0005
MSI (+)	19 (21.1)	1 (2.0)	

Underlined *p*-value is for *p*-value less than 0.05.

Discussion

BUBR1 and p53 are both essential in maintaining chromosomal homeostasis and have been reported to interact through multiple mechanisms (10, 25, 26, 36). In the present study, we analyzed chromosomal instability and microsatellite instability to elucidate patterns of genetic instability, based on the hypothesis that these are two distinct pathways in the tumorigenesis of colorectal cancer, as well as for many malignancies (37).

High expression of *BUB1B* mRNA was previously described in colorectal cancer (18), as well as for other malignancies (16, 19). The association between BUBR1 expression and chromosomal instability in sporadic malignancies is still largely controversial. A previous study detected elevated *BUB1B* mRNA transcription in a large majority of colorectal cancer cases. However, a small portion of colorectal cancer cases had decreased *BUB1B* mRNA

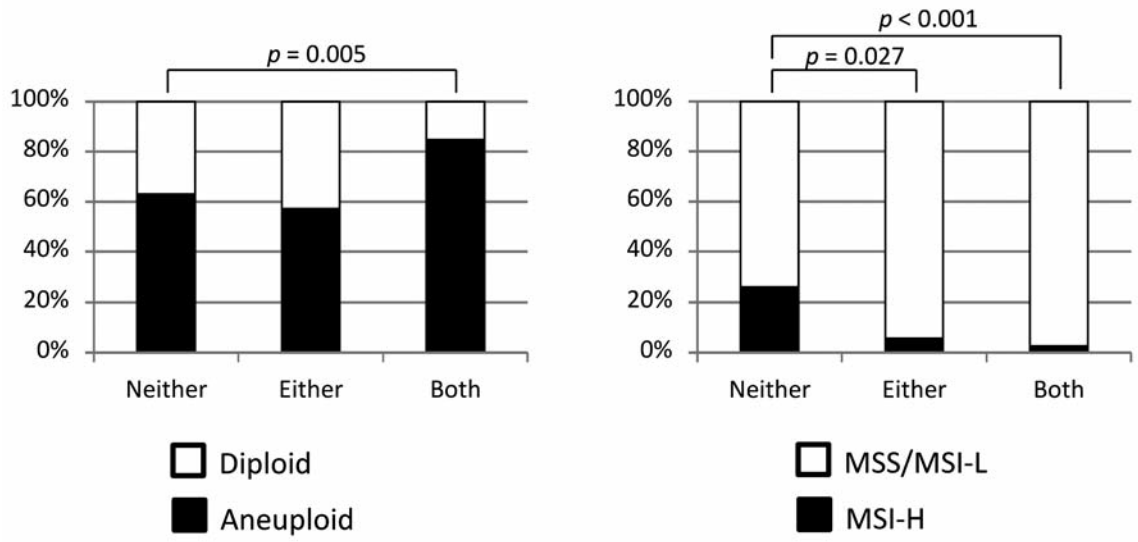

Figure 2. *BUBR1 expression/TP53 gene status and genomic instability reflected by DNA ploidy and microsatellite instability status. DNA ploidy (left) and microsatellite status (right) in three groups classified based on BUBR1 and TP53 status. "Both" indicates cases with high BUBR1 expression and TP53 gene mutation; "Either" includes either high BUBR1 expression or TP53 gene mutation; "Neither" indicates cases of low BUBR1 expression and wild-type TP53 gene.*

levels, which was considered to originate from epigenetic silencing and correlated with worse prognosis (18). In our study, we observed a strong tendency for association between high expression of BUBR1 in colorectal cancer and DNA aneuploidy, the outcome of the chromosomal instability phenotype (38). In addition, high BUBR1 expression was less frequently observed in MSI(+) tumors, the category of genetic instability independent of chromosomal instability.

The promoter of *BUB1B* has not been well-studied and the mechanisms of *BUB1B* regulation remain largely unknown. Elevated *BUBR1* expression in highly proliferating cells is consistent with its role as a conserved mitotic surveillance system, which is required in cycling cells (16). Overexpression of BUBR1 as well as other *BUB* genes might also be compensation as a response to an abnormal mitotic checkpoint, perhaps through less well-understood feedback mechanisms. In addition, inappropriately high levels of BUBR1 protein may have other functional consequences, due to BUBR1 functions in cellular pathways other than the spindle assembly checkpoint.

A previous study showed that mouse embryonic cells with reduced BUB1B expression exhibit a more profound chromosomal instability phenotype. In this mouse model, reduction of BUB1B expression was associated with early onsets of age associated phenotypes and birth defects, although no elevated tumor incidence was detected (39). Thus, a reduced mitotic checkpoint might be a causative factor for chromosomal instability. However, both decreased and increased expression of mitotic checkpoint proteins have been related to chromosomal instability. A study of BUB1, another key molecule of the spindle assembly checkpoint, indicated that inappropriately high, as well as low expression significantly altered mitotic checkpoint function (40). Some evidence has shown that, rather than gaining a selective advantage, cells lacking the spindle assembly checkpoint undergo cell death (41), which may be due to the loss of genes essential for survival that accompanies the development of the chromosomal instability phenotype.

In addition to BUBR1, molecules involved in maintaining chromosomal stability, such as the centrosome cycle and cytokinesis components, as well as their connections with chromosomal instability should be investigated to further our understanding on chromosomal instability machineries in human malignancies (42). Genetic and epigenetic aberrations of several genes involving the mitotic spindle checkpoint machineries have also been suggested to be oncogenic, such as *PLK1, AURORA1, AURORA2, BUB1* (43-45).

In this study, we used LSC to define the cellular ploidy status. A high throughput approach is still not useful for assessment of chromosomal instability phenotypes because of the technological complexity and high cost, which restricts practical usage. Previous reports have suggested that DNA ploidy, as it results from LSC, is strongly associated with findings using fluorescence *in situ* hybridization (46), thus we used this approach and considered aberrations in DNA index as a marker for chromosomal instability. LSC allowed us to confirm the morphology of each cell, thus reducing any contamination

from debris and adhesive cells. However, this methodology may give false-negative results due to pseudo-euploidy, a special type of chromosomal instability that does not show any obvious alternation in DNA content. Such a bias should not be ignored. These two pathways are independent but not necessarily exclusive. The simplified tumorigenesis model employed in this study may not correctly elucidate the carcinogenesis background in all cases.

In conclusion, the colorectal cases which show both high expression of BUBR1 and abnormalities of the *TP53* gene have a profound chromosomal instability phenotype. Together our findings indicate that aberrations of these two key molecules were associated with the chromosomal instability phenotype in a cooperative manner in sporadic colorectal cancer.

Conflicts of Interest

The Authors have no conflicts to disclose.

Acknowledgements

The Authors thank Ms. Yuuko Kubota for preparing the immunohistochemistry samples.

References

1 Loeb LA: A mutator phenotype in cancer. Cancer Res *61*: 3230-9, 2001.
2 Hanahan D and Weinberg RA: The hallmarks of cancer. Cell *100*: 57-70, 2000.
3 Kops GJ, Weaver BA and Cleveland DW: On the road to cancer: aneuploidy and the mitotic checkpoint. Nature Rev Cancer *5*: 773-785, 2005.
4 Malumbres M: Cyclins and related kinases in cancer cells. Journal of BUON: official journal of the Balkan Union of Oncology *12(Suppl 1)*: S45-52, 2007.
5 Chen RH: BubR1 is essential for kinetochore localization of other spindle checkpoint proteins and its phosphorylation requires Mad1. JCB *158*: 487-496, 2002.
6 Lampson MA and Kapoor TM: The human mitotic checkpoint protein BubR1 regulates chromosome-spindle attachments. NCB *7*: 93-98, 2005.
7 Harris L, Davenport J, Neale G and Goorha R: The mitotic checkpoint gene BubR1 has two distinct functions in mitosis. Exp. cell research *308*: 85-100, 2005.
8 Meraldi P and Sorger PK: A dual role for Bub1 in the spindle checkpoint and chromosome congression. The EMBO journal *24*: 1621-1633, 2005.
9 Warren CD, Brady DM, Johnston RC, Hanna JS, Hardwick KG and Spencer FA: Distinct chromosome segregation roles for spindle checkpoint proteins. MBC *13*: 3029-3041, 2002.
10 Oikawa T, Okuda M, Ma Z, Goorha R, Tsujimoto H, Inokuma H and Fukasawa K: Transcriptional control of BubR1 by p53 and suppression of centrosome amplification by BubR1. MCB *25*: 4046-4061, 2005.
11 Shin HJ, Baek KH, Jeon AH, Park MT, Lee SJ, Kang CM, Lee HS, Yoo SH, Chung DH, Sung YC, McKeon F and Lee CW: Dual roles of human BubR1, a mitotic checkpoint kinase, in the monitoring of chromosomal instability. Cancer cell *4*: 483-497, 2003.
12 Hanks S, Coleman K, Reid S, Plaja A, Firth H, Fitzpatrick D, Kidd A, Mehes K, Nash R, Robin N, Shannon N, Tolmie J, Swansbury J, Irrthum A, Douglas J and Rahman N: Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nature genetics *36*: 1159-1161, 2004.
13 Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, Edkins S, O'Meara S, Vastrik I, Schmidt EE, Avis T, Barthorpe S, Bhamra G, Buck G, Choudhury B, Clements J, Cole J, Dicks E, Forbes S, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jenkinson A, Jones D, Menzies A, Mironenko T, Perry J, Raine K, Richardson D, Shepherd R, Small A, Tofts C, Varian J, Webb T, West S, Widaa S, Yates A, Cahill DP, Louis DN, Goldstraw P, Nicholson AG, Brasseur F, Looijenga L, Weber BL, Chiew YE, DeFazio A, Greaves MF, Green AR, Campbell P, Birney E, Easton DF, Chenevix-Trench G, Tan MH, Khoo SK, Teh BT, Yuen ST, Leung SY, Wooster R, Futreal PA and Stratton MR: Patterns of somatic mutation in human cancer genomes. Nature *446*: 153-158, 2007.
14 Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW and Vogelstein B: Mutations of mitotic checkpoint genes in human cancers. Nature *392*: 300-303, 1998.
15 Burum-Auensen E, De Angelis PM, Schjolberg AR, Kravik KL, Aure M and Clausen OP: Subcellular localization of the spindle proteins Aurora A, Mad2, and BUBR1 assessed by immunohistochemistry. The journal of histochemistry and cytochemistry *55*: 477-486, 2007.
16 Grabsch H, Takeno S, Parsons WJ, Pomjanski N, Boecking A, Gabbert HE and Mueller W: Overexpression of the mitotic checkpoint genes BUB1, BUBR1, and BUB3 in gastric cancer–association with tumour cell proliferation. The Journal of pathology *200*: 16-22, 2003.
17 Pinto M, Vieira J, Ribeiro FR, Soares MJ, Henrique R, Oliveira J, Jeronimo C and Teixeira MR: Overexpression of the mitotic checkpoint genes BUB1 and BUBR1 is associated with genomic complexity in clear cell kidney carcinomas. Cellular oncology *30*: 389-395, 2008.
18 Shichiri M, Yoshinaga K, Hisatomi H, Sugihara K and Hirata Y: Genetic and epigenetic inactivation of mitotic checkpoint genes hBUB1 and hBUBR1 and their relationship to survival. Cancer Res *62*: 13-17, 2002.
19 Yamamoto Y, Matsuyama H, Chochi Y, Okuda M, Kawauchi S, Inoue R, Furuya T, Oga A, Naito K and Sasaki K: Overexpression of BUBR1 is associated with chromosomal instability in bladder cancer. Cancer genetics and cytogenetics *174*: 42-47, 2007.
20 Yuan B, Xu Y, Woo JH, Wang Y, Bae YK, Yoon DS, Wersto RP, Tully E, Wilsbach K and Gabrielson E: Increased expression of mitotic checkpoint genes in breast cancer cells with chromosomal instability. Clin. Cancer Res *12*: 405-410, 2006.
21 Attardi LD and DePinho RA: Conquering the complexity of p53. Nature genetics *36*: 7-8, 2004.
22 Harris SL and Levine AJ: The p53 pathway: positive and negative feedback loops. Oncogene *24*: 2899-2908, 2005.

23 Sengupta S and Harris CC: p53: traffic cop at the crossroads of DNA repair and recombination. Nature reviews Molecular cell biology *6*: 44-55, 2005.
24 Vogelstein B, Lane D and Levine AJ: Surfing the p53 network. Nature *408*: 307-310, 2000.
25 Ha GH, Baek KH, Kim HS, Jeong SJ, Kim CM, McKeon F and Lee CW: p53 activation in response to mitotic spindle damage requires signaling *via* BubR1-mediated phosphorylation. Cancer Res *67*: 7155-7164, 2007.
26 Baek KH, Shin HJ, Yoo JK, Cho JH, Choi YH, Sung YC, McKeon F and Lee CW: p53 deficiency and defective mitotic checkpoint in proliferating T lymphocytes increase chromosomal instability through aberrant exit from mitotic arrest. Journal of leukocyte biology *73*: 850-861, 2003.
27 Vasen HF, Watson P, Mecklin JP and Lynch HT: New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology *116*: 1453-1456, 1999.
28 Ando K, Kakeji Y, Kitao H, Iimori M, Zhao Y, Yoshida R, Oki E, Yoshinaga K, Matumoto T, Morita M, Sakaguchi Y and Maehara Y: High expression of BUBR1 is one of the factors for inducing DNA aneuploidy and progression in gastric cancer. Cancer science *101*: 639-645, 2010.
29 Egashira A, Morita M, Kakeji Y, Sadanaga N, Oki E, Honbo T, Ohta M and Maehara Y: p53 gene mutations in esophageal squamous cell carcinoma and their relevance to etiology and pathogenesis: results in Japan and comparisons with other countries. Cancer science *98*: 1152-1156, 2007.
30 Oda S, Oki E, Maehara Y and Sugimachi K: Precise assessment of microsatellite instability using high resolution fluorescent microsatellite analysis. Nucleic acids research *25*: 3415-3420, 1997.
31 Oki E, Oda S, Maehara Y and Sugimachi K: Mutated gene-specific phenotypes of dinucleotide repeat instability in human colorectal carcinoma cell lines deficient in DNA mismatch repair. Oncogene *18*: 2143-2147, 1999.
32 Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN and Srivastava S: A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res *58*: 5248-257, 1998.
33 Yoshino I, Osoegawa A, Yohena T, Kameyama T, Oki E, Oda S and Maehara Y: Loss of heterozygosity (LOH) in non-small cell lung cancer: difference between adenocarcinoma and squamous cell carcinoma. Respiratory medicine *99*: 308-312, 2005.
34 Sasaki A, Nagashima M, Shiseki M, Katai H, Maruyama K, Iwanaga R, Akiyama Y, Yuasa Y and Yokota J: Microsatellite instability in gastric cancer prone families. Cancer lett *99*: 169-75, 1996.
35 Hiddemann W, Schumann J, Andreef M, Barlogie B, Herman CJ, Leif RC, Mayall BH, Murphy RF and Sandberg AA: Convention on nomenclature for DNA cytometry. Committee on Nomenclature, Society for Analytical Cytology. Cancer genetics and cytogenetics 13:181-3, 1984
36 Fang Y, Liu T, Wang X, Yang YM, Deng H, Kunicki J, Traganos F, Darzynkiewicz Z, Lu L and Dai W: BubR1 is involved in regulation of DNA damage responses. Oncogene 25:3598-605, 2006
37 Lengauer C, Kinzler KW and Vogelstein B: Genetic instabilities in human cancers. Nature *396*: 643-649, 1998.
38 Bharadwaj R and Yu H: The spindle checkpoint, aneuploidy, and cancer. Oncogene *23*: 2016-2027, 2004.
39 Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja 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. Nature genetics *36*: 744-749, 2004.
40 Taylor SS and McKeon F: Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. Cell *89*: 727-735, 1997.
41 Kops GJ, Foltz DR and Cleveland DW: Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. PNAS *101*: 8699-8704, 2004.
42 Chi YH and Jeang KT: Aneuploidy and cancer. JCB *102*: 531-538, 2007.
43 Bischoff JR, Anderson L, Zhu Y, Mossie K, Ng L, Souza B, Schryver B, Flanagan P, Clairvoyant F, Ginther C, Chan CS, Novotny M, Slamon DJ and Plowman GD: A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancers. The EMBO J *17*: 3052-3065, 1998.
44 Knecht R, Elez R, Oechler M, Solbach C, von Ilberg C and Strebhardt K: Prognostic significance of polo-like kinase (PLK) expression in squamous cell carcinomas of the head and neck. Cancer Res *59*: 2794-2797, 1999.
45 Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR and Sen S: Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nature genetics *20*: 189-193, 1998.
46 Miyazaki M, Furuya T, Shiraki A, Sato T, Oga A and Sasaki K: The relationship of DNA ploidy to chromosomal instability in primary human colorectal cancers. Cancer Res *59*: 5283-5285, 1999.

Received June 23, 2014
Revised July 21, 2014
Accepted July 23, 2014