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Minireview

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How Chromosome Mis-segregation Leads to Cancer: Lessons from *BubR1* Mouse Models

Hyunsook Lee*

Alteration in chromosome numbers and structures instigate and foster rapid and massive genetic instability. As Boveri has seen a hundred years ago (Boveri, 1914; 2008), aneuploidy, chromosome number instability, is the hallmark of many cancers. However, whether aneuploidy is the cause or the result of cancer is still at debate. The molecular mechanism behind aneuploidy includes the chromosome mis-segregation in mitosis by the compromise of spindle assembly checkpoint (SAC). SAC is an elaborate network of proteins, which monitor that all chromosomes are bipolarly attached with the spindles. Therefore, the weakening of the SAC is the major reason for chromosome number instability; complete compromise of SAC results in detrimental death, exemplified in natural abortion in embryonic stage. Here, I will review on the recent progress on the understanding of chromosome mis-segregation and cancer based on the comparison of different mouse models of BubR1, the core component of SAC.

THE ROLE OF BubR1 IN MITOSIS

BubR1 is an orthologue of yeast Mad3 that binds and inhibits the multisubunit E3 ligase APC/C upon SAC activation (Chao et al., 2012; Taylor et al., 1998). As illustrated in Figure 1, human BubR1 is ~125 kDa protein, composed of 1,050 amino acids. Yeast Mad3 and mammalian BubR1 share homology at the N-terminus: TPR (tetratricopeptide repeat) domain is required for recruitment to kinetochore by binding to Blinkin/KNL1 (Kiyomitsu et al., 2007; Krenn et al., 2012). KNL1 is a core component of KMN network, essential for kinetochore-microtubule interaction for chromosome alignment (Takeuchi and Fukagawa, 2012; Varma and Salmon, 2012); GLEBS (Gle-2-binding sequence) motif is for Bub3 binding(Larsen et al., 2007); KEN boxes and D-box found in mammalian BubR1 are the degrons required for binding to Cdc20 and APC/C (Burton and Solomon, 2007; Lara-Gonzalez et al., 2011; Tian et al., 2012).

Unlike yeast Mad3, mammalian BubR1 has kinase domain similar to Bub1 at the C-terminus (Fig. 1). For a number of years, significant efforts were made to reveal the substrates of BubR1 kinase. An interesting idea was that CENP-E, the plus end-directed microtubule motor, binds to BubR1 and activates the kinase activity for BubR1 (Mao et al., 2003; 2005). However, numerous following works failed to confirm the kinase activity of BubR1. Recently, it was suggested that the C-terminal kinase domain of BubR1 is a pseudo-kinase domain, a resultant of amplification from Bub1, and thus BubR1 does not possess any kinase activity (Suijkerbuijk et al., 2012a).

Mitotic kinases such as CDK1, Plk1, and Aurora B phosphorylate BubR1 at distinct sites. The outcome of the phosphorylation at each site is not fully understood, but is likely to be crucial in mitotic checkpoint signaling. BubR1 has two distinct functions in mitosis: regulation of chromosome congression and SAC (Elowe et al., 2010). Phosphorylation of BubR1 at serines and threnines of T680, T620, D670, D676, T680 are involved in tension sensing, kinetochore-microtubule interaction, and mitotic exit (Elowe et al., 2007; 2010; Huang et al., 2008; Suijkerbuijk et al., 2012a). Comparing the structure of yeast Mad3 with mammalian BubR1, it appears that the spindle checkpoint regulation may be a more ancient function and the role in the stabilization of kinetochore-microtubule attachment has evolved in vertebrates (Fig. 1). Apparently, the KARD domain, constituted of many Plk1-mediated phosphorylation sites are required for binding to PP2A-B56 , counteracting the excessive Aurora B kinase activity for chromosome alignment in metaphase (Kruse et al., 2013a; Suijkerbuijk et al., 2012a).

BubR1 IN SAC ACTIVATION, MAINTENANCE, AND SILENCING

Treatment of microtubule poisons result in hyperphosphorylation of BubR1, indicating that phosphorylations at multiple sites are important in exhibiting mitotic functions of BubR1. In addition to phosphorylation, BubR1 is acetylated at lysine 250 in prometaphase(Choi et al., 2009). Acetylated BubR1 functions as an inhibitor of APC/C and the deacetylated BubR1 becomes the substrate of APC/C. Polyubiquitination and subsequent degradation of deacetylated BubR1 are followed by cyclin B destruction, leading to anaphase onset and mitotic exit. When acetylation-mimetic mutant of BubR1 is expressed, it blocks the anaphase onset. On the contrary, the expression of acetylation - deficient

ABRC, School of Life Sciences, BK21 Plus KNU Creative BioReserach-Department of Biological Sciences and Institute of Molecular Biology and Genetics, Seoul National University,Seoul 151-742, Korea
*Correspondence: HL212@snu.ac.kr

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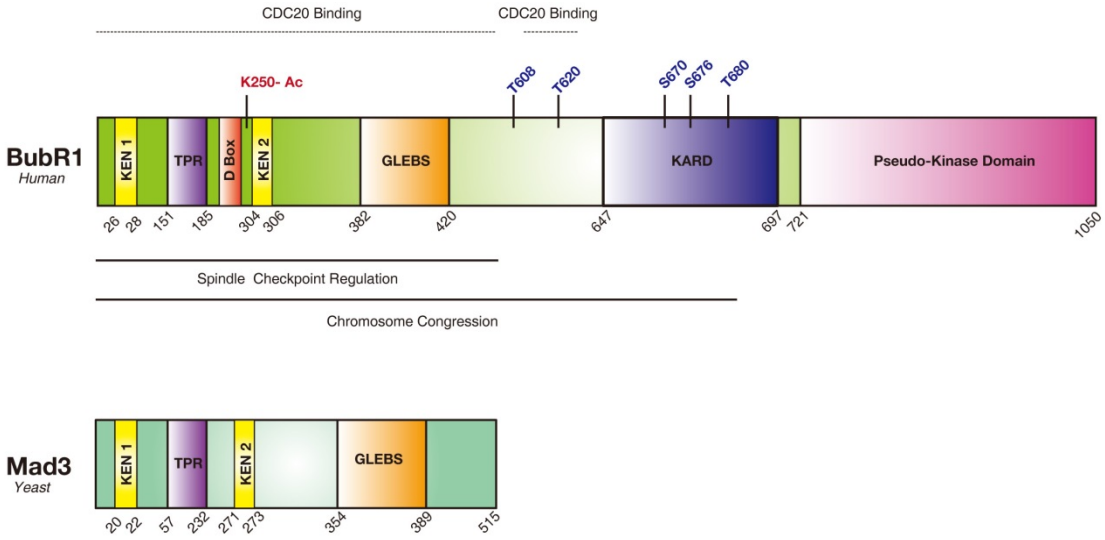

Fig. 1. Schematic illustration of human BubR1 and its ortholgue yeast Mad3. Functional domains, suggested functions, and reported phosphorylation and acetylation sites are marked, in comparison to size.

form allows shortening of mitotic timing with unequal chromosome segregation. The result indicates that acetylation/deacetylation of BubR1 determines the mitotic timing and modulates APC/C activity. Thus, BubR1 acetylation is a crucial part of SAC signaling (Choi et al., 2009).

SAC activity can be divided into two phases: SAC signal generation and maintenance of SAC. First, Mad1 and Mad2 are recruited to unattached kinetochores. Then, Mad2 undergoes conformational change from open Mad2 (O-Mad2) to closed Mad2 (C-Mad2). C-Mad2 then binds to Cdc20, promoting the mitotic checkpoint complex (MCC) formation (Musacchio and Salmon, 2007), composed of Mad2, BubR1, Bub3, Cdc20. It is now known that Mad2 only loads Cdc20 to BubR1 and BubR1 is the true inhibitor for APC/C-Cdc20 (Han et al., 2013; Nilsson et al., 2008). It is now thought that the MCC formation is the key to sustain SAC by titrating out Cdc20 from APC/C (Musacchio and Salmon, 2007; Sudakin et al., 2001), and also by continuously degrading Cdc20 when SAC is on (Nilsson et al., 2008). Thus, MCC disassembly is the key to checkpoint silencing.

Progress has been made recently on the understanding of how MCC disassembly is promoted, leading to checkpoint silencing. APC15, one of the components of APC/C mediates the autoubiquitination of cdc20, resulting in the degradation and disassembly of MCC (Foster and Morgan, 2012; Mansfeld et al., 2011; Uzunova et al., 2012).

Deacetylation of BubR1 (Choi et al., 2009) leads to ubiquitination by APC/C and degradation of it, thus can also participate in the disassembly of MCC. Indeed, it was shown that cells isolated from the mouse heterozygous of acetylation-deficient allele (*K243R/+*) failed to retain MCC upon nocodazole treatment, whereas the checkpoint signal generation measured by Mad1/Mad2 localization at unattached kinetochores were intact. These results suggest that the early onset destruction of acetylation-deficient BubR1 may be a cue to disassembly of MCC (Park et al., 2013), and checkpoint silencing.

The role of p31 comet has been identified in SAC silencing: it binds to C-Mad2-Cdc20 complex, thereby titrating out Mad2, promoting the disassembly of MCC (Miniowitz-Shemtov et al., 2012; Teichner et al., 2011; Westhorpe et al., 2011; Yun et al., 2007). However, an elegant biochemical study have shown that it is BubR1-Cdc20 complex that truly inhibits APC/C activity, and extracting Mad2 from the complex does not inhibit APC/C to a potent level, suggesting that p31 comet's role in SAC silencing may be secondary in SAC silencing (Han et al., 2013).

FUNCTIONS OF BubR1 IN CHROMOSOME-SPINDLE ATTACHMENT

Loss of BubR1 results in the failure of the stable maintenance of chromosome-spindle attachment (Lampson and Kapoor, 2005). Loss of CENP-E, the plus end-directed motor protein that binds to BubR1 at kinetochore, leads to the increase of monotelic chromosomes near the pole (Kapoor et al., 2006). When compared to the loss of CENP-E, BubR1 loss leads to more profound defects in chromosome congression, indicating a direct role of BubR1 in kinetochore-microtubule (KT-MT) interaction than CENP-E. Furthermore, impaired kinetochore-microtubule attachment is restored in BubR1-depleted cells when Aurora B activity is inhibited, suggesting that BubR1 antagonizes Aurora B's activity in KT-MT attachment (Lampson and Kapoor, 2005).

Erroneous microtubule attachment is corrected by the activity of Aurora B. Located at the inner kinetochore, Aurora B phosphorylates multiprotein complexes at the outer kinetochore, the KMN network (KNL1-Mis12-Ndc80) and Sca1-Dam complexes, destabilizing the kinetochore-microtubule attachments (Chan et al., 2012; Cheeseman et al., 2006; Welburn et al., 2010). Once amphitelic attachment is achieved, Aurora B activity must be counteracted to stabilize KT-MT attachment and chromosome alignment at the metaphase plate (congression). In brief, KT-MT attachment and SAC are well harmonized in mitosis by the activity of Aurora B: SAC is on when chromosomes are not attached to microtubules in a bipolar manner (Aurora B active); and turns off when chromosomes-spindle attachment is satisfied (Aurora B inactive). Therefore, phosphatases PP1 (Kim et al., 2010) and PP2A (Tanno et al., 2010) have been suggested to antagonize Aurora B activity and promote chromosome congression.

Table 1. Different BubR1 mouse models and their characteristics

Strategy	Allele	Phenotype	Reference
Targeted disruption of the *BubR1* locus between exon 1 and 2 by gene-trapping method	$BubR1^{-/-}$	Failed to survive beyond day 8.5 in utero as a result of extensive apoptosis	
	$BubR1^{+/-}$	-Splenomegaly and abnormal megakaryopoiesis coupled with a decrease erythropoiesis -When challenged with azoxymethane (AOM), develop lung and intestinal adenocarcinomas	Wang et al. (2004)
	$BubR1^{+/-};Apc^{Min/+}$	Develops ten times more colonic tumor masses than *ApcMin*/+ with higher grade polyps; yet fewer polyps in small intestines.	Rao et al. (2005)
Hypomophic BubR1 expression: abnormal splicing of mutant *Bub1b* mRNAs cannot generate BubR1 protein (Wijshake et al., 2012)	$Bub1b^{H/H}$	-Aneuploidy -Features of aging: short lifespan, cachectic dwarfism, lordokyphosis, cataracts, loss of subcutaneous fat and impaired wound healing -Infertile -No spontaneous tumorigenesis	Baker et al. (2004)
Homologous Recombination mediated insertion of GTTA sequence in the murine BubR1 gene at position 2178	$BubR1^{GTTA/+}$	-Mimics nonsense mutation 2211insGTTA found in MVA patients -Mild aneuploidy -Reduced life span and carry age-related phenotypes: loss of skeletal muscle and fat -Increased incidence of lung tumor upon DMBA treatment	Wijshake et al. (2012)
Substitution of acetylation residue K243 to lysine in BubR1 allele by homologous recombination-mediated knock-in	$BubR1^{K243R/K243R}$	Embryonic lethal at E6.5	
	$BubR1^{K243R/+}$ *(K243R/+)*	-Heterozygous mutants exhibit spontaneous tumorigenesisone year after birth -Tumor incidence >40% -No developmental defect -No aging phenotype -Massive chromosome mis-segregation due to impaired KT-MT attachment combined with weakened SAC	Park et al. (2013)

BubR1 recruits B56α subunit of PP2A to the kinetochore, contributing to stable KT-MT attachment (Kruse et al., 2013b; Suijkerbuijk et al., 2012b). Notably, tension-sensitive phosphorylations by Plk1 at the KARD domain (S670, S676, T680) are required for binding PP2A-B56α to BubR1 and to be recruited to kinetochore (Fig. 1). PP2A activity at kinetochore antagonizes excessive activity of Aurora B in disassembly of microtubules at kinetochore, thus stabilizing KT-MT attachments and achieving chromosome congression(Kruse et al., 2013b; Suijkerbuijk et al., 2012b; Xu et al., 2013). Collectively, BubR1 links KT-MT attachment to SAC signaling.

In addition to phosphorylation by Plk1, BubR1 acetylation at K250 is also required for recruiting PP2A-B56α to the kinetochore. Interaction between BubR1 and PP2A-B56α is acetylation-dependent, and cells isolated from the mice heterozygous for acetylation-deficient BubR1 (*K243R/+*) exhibit severe problems in chromosome congression(Park et al., 2013). How acetylation at K250 is coordinated with Plk1's phosphorylation at KARD domain is not known. Nevertheless, the result suggests an interesting hypothesis that there may be an acetylation-phosphorylation code in sensing KT-MT attachments to SAC signaling, and finally to SAC silencing.

BubR1 MUTATION IN HUMAN DISEASE

Mosaic Variegated Aneuploidy (MVA) and/or Premature chromatid separation (PCS) are rare genetic disorders that display aneuploidy and premature sister chromatid separations (PMSC) in metaphase chromosome spreads. MVA patients are characterized by severe microcephaly, growth defects, mental retardation, and neoplasia (Jacquemont et al., 2002; Kawame et al., 1999; Matsuura et al., 2000).

Notably, BubR1 mutation is associated with MVA with cancer predisposition (de Voer et al., 2011; Suijkerbuijk et al., 2010). However, it is unclear whether specific mutations are linked with distinct phenotypes in MVA of heterologous symptoms. Taken together with the BubR1 mutations found in somatic cancers (Bolanos-Garcia and Blundell, 2011), it is noteworthy that dysfunctional BubR1 contributes to tumorigenesis.

BubR1 MOUSE MODELS IN CANCER PREDISPOSITION

Despite the frequent aneuploidy in human cancers, mutations in SAC genes are rare. The reason for this may be that SAC is essential in cell proliferation, therefore is detrimental when deleted. Indeed, homozygous deletions of genes coding for *Mad2*(Dobles et al., 2000), *Bub1*(Jeganathan et al., 2007), *BubR1* (Wang et al., 2004) in mice exhibit early embryonic le-

thality, indicating that severe mitotic failure are detrimental in proliferation.

Mice knockout of *BubR1* allele are lethal in early embryonic stage; heterozygous mice are viable and display megakaryopoiesis with aneuploidy but not spontaneous cancers (Wang et al., 2004). Paradoxically, gradual reduction of BubR1 level in mice of hypomorphic*BubR1* allele exhibit senescence and premature aging phenotype but not spontaneous cancer (Baker et al., 2004). Apparently, both haploinsufficient(Wang et al., 2004) and hypomorphic*BubR1* allele display aneuploidy, indicating that aneuploidy alone does not impose on tumorigenesis. Notably, mice heterozygous of *CENPE*-null allele (Weaver et al., 2007) imply that aneuploidy can be oncogenic to a certain degree, but is tumor suppressive when combined with genetic insults. Indeed, aneuploidy in mammalian cells impose to proliferation defects and metabolic abnormalities, suggesting that the burden of having to produce too much nucleic acids and proteins are toxic to cellular fitness (Williams et al., 2008), consistent with the phenotypes observed from Down's syndrome (Table 1).

However, when *BubR1*haploinsufficient mice are crossed to $APC^{Min/+}$ mice, tumorigenesis in the large intestine increases to ten-fold, with the decrease of the polyps in the small intestines (Rao et al., 2005), suggesting that there is more than aneuploidy in *BubR1* mutation, compared to *CENP-E*haploinsufficiency. It might be related to the observation that the depletion of BubR1 has far more profound effects in chromosome congression, compared to CENP-E depletion as mentioned above (Lampson and Kapoor, 2005).

Reduction of BubR1 protein level predisposes to early onset aging in mice (Baker et al., 2004), which is also observed in the monoallelicinsertional mutation of BubR1 at nucleotide 2118 position (Wijshake et al., 2012). This allale ($BubR1^{GTTA/+}$)was created after the genetic mutation in one of the MVA patients. However, the mutation lies after the KARD domain (Fig. 1) and thus affects the C-terminus pseudokinase domain, which might not have crucial functions in SAC. Nevertheless, $BubR1^{H/H}$ and-$BubR1^{GTTA/+}$ mice links *BubR1* mutation with aging-related MVA phenotype, albeit the absence of tumor formation (Summarized in Table 1).

Notably, cancer predisposition is observed in mice heterozygous of acetylation-deficient BubR1 allele (K243R/+): *K243R/+* mice are succumbed to high incidence of spontaneous tumorigenesis, most abundantly lymphoma (Park et al., 2013). In fact, this is the first mouse model in SAC components that a single mutation is associated with high incidence tumorigenesis.

It is important to speculate the difference of *K243R/+* mice from those of other *BubR1* mouse models in cancer predisposition. Critical difference may rely on the fact that acetylation of K250 (K243 in mice) has dual roles: one in stable chromosome-spindle attachment and the other in the stable maintenance of MCC until checkpoint is satisfied. Homozygous mutations are detrimental that it exhibits embryonic lethality at embryonic day 6, even before the homozygous deletion of BubR1 allele (Table 1).

In K243R/+ heterozygous cells, the wild-type and mutant protein co-exist in 1:1 ratio; K243R protein localizes to the kinetochore as much as the wild-type BubR1 does, inhibiting the recruitment of PP2A-B56α to kinetochores, thus *K243R* allele is a dominant negative allele. In comparison, BubR1 in $BubR1^{+/-}$ cells localized more to the kinetochores with the decrease of the cytosolic portion, therefore the defect was not as severe as *K243R/+*, explaining the absence of spontaneous tumorigenesis in $BubR1^{+/-}$ mice. It is worth mentioning that the acetylation of BubR1 at K250 requires BRCA2, hence BubR1 acetylation is a tumor suppressive mechanism (Choi et al., 2012). *K243R/+* mice represent the *BRCA2*-deficiency in mitotic failure and tumorigeneis.

CONCLUSION

What makes *K243R/+* mice tumor-prone and others don't? The answer may line in that the massive chromosome mis-segregation resulting from combined effects of failure in KT-MT attachment and weakened SAC instigated genetic instability, thus cancer in K243R/+ mice. As observed from *BubR1*haploinsufficiency, cells try to cope with the reduced level of BubR1 in mitosis. However, the early onset aging phenotypes in hypomorphic*BubR1* mouse and the $BubR1^{GTTA/+}$ mice suggest that reduced level of BubR1 may have effects in interphase, leading to senescence and aging.

Recently, it was shown that BubR1 acetylation status modulates the outgrowth of dendrite in neuronal cells (Watanabe et al., 2014) and also lifespan (North et al., 2014), indicating that BubR1 has many more functions than unerstood. Nevertheless, lessons from *K243R/+* mice indicate that BubR1 mutations linked with its critical functions in mitosis can predispose to tumorigenesis. It will be important to assign different human genetic mutations with different functions of BubR1.

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