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REVIEW

The role of NBS1 in DNA double strand break repair, telomere stability, and cell cycle checkpoint control

Ying Zhang[1], Junqing Zhou[1], Chang UK Lim[2]

[1]*Department of Environmental and Radiological Health Science, Colorado State University, Fort Collins, Colorado 80523, USA;* [2]*Cancer Center, Ordway Research Institute, Albany, New York 12208, USA*

The genomes of eukaryotic cells are under continuous assault by environmental agents and endogenous metabolic byproducts. Damage induced in DNA usually leads to a cascade of cellular events, the DNA damage response. Failure of the DNA damage response can lead to development of malignancy by reducing the efficiency and fidelity of DNA repair. The NBS1 protein is a component of the MRE11/RAD50/NBS1 complex (MRN) that plays a critical role in the cellular response to DNA damage and the maintenance of chromosomal integrity. Mutations in the *NBS1* gene are responsible for Nijmegen breakage syndrome (NBS), a hereditary disorder that imparts an increased predisposition to development of malignancy. The phenotypic characteristics of cells isolated from NBS patients point to a deficiency in the repair of DNA double strand breaks. Here, we review the current knowledge of the role of NBS1 in the DNA damage response. Emphasis is placed on the role of NBS1 in the DNA double strand repair, modulation of the DNA damage sensing and signaling, cell cycle checkpoint control and maintenance of telomere stability.

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Introduction

The genomes of eukaryotic cells are under continuous assault by environmental agents, including ultraviolet light, ionizing radiation, and reactive chemicals, as well as the byproducts of normal intracellular metabolism including reactive oxygen intermediates and inaccurately replicated DNA [1]. One of the most dangerous DNA lesions is the DNA double strand break (DSB), which usually leads to a cascade of cellular events, termed the DNA damage response. The DNA damage response involves sensing DNA damage, transduction of the damage signal to a network of cellular pathways, including cell cycle checkpoints, DNA repair, stress responses to telomere maintenance, and apoptosis, etc. [2] (Figure 1). Failure in the DNA damage response affect the level of cell survival, increase the frequency of gene mutation or chromosomal instability, and other cellular phenotypic abnormalities, which are the important mechanisms of carcinogenesis.

The *NBS1* gene contains 16 exons encompassing 48797 bps of genomic sequence on chromosome 8q21 [3-7]. The NBS1 protein is a component of the MRE11/RAD50/NBS1 complex (MRN) that plays a critical role in the cellular response to DNA damage and the maintenance of chromosomal integrity. The complete disruption of *NBS1* in mice is lethal [8, 9]. The hypomorphic mutations which lead to decreased function of the *NBS1* gene are responsible for Nijmegen breakage syndrome (NBS), a rare autosomal recessive hereditary disorder that imparts an increased predisposition to the development of malignancy [10-12]. In the Polish NBS registry, 18 of 48 patients had developed lymphomas by the age of 15 [13]. Other types of cancers are also reported in the *NBS1* compromised population [14]. Cells isolated from NBS patients exhibit cellular phenotypic alterations, very similar to those seen in ataxia telangectasia (AT) that is caused by the mutation of ataxia telangectasia mutated (*ATM*) gene; these alterations include

Correspondence: Ying Zhang
Tel: 01-970-491-0574;
E-mail: Ying.Zhang@colostate.edu

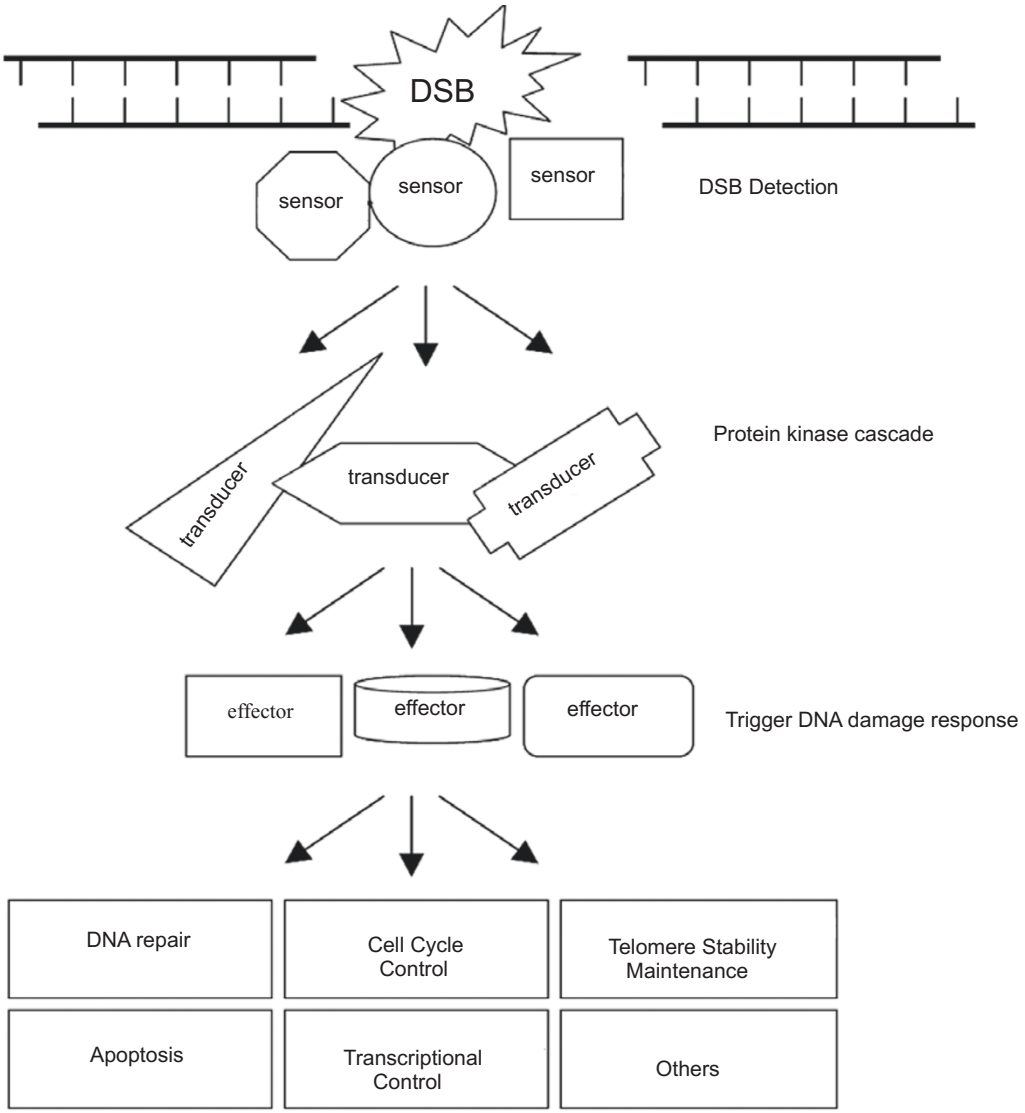

Figure 1 The DNA damage response network. The DNA damage such as DSB was detected by sensor(s). The sensor(s) then triggers the activation of a transducer system, which usually are a protein kinase cascade. The activated transducer(s) will amplify and diversify the signal by targeting a set of down stream effector(s), which leads to cellular events, such as DNA repair, cell cycle control, telomere stability maintenance, apoptosis, etc. Multiple sensors, transducers, and effectors participate in this cellular response. The final consequence of DNA damage in cells will be determined by types and magnitude of stress and the appropriateness of cellular response.

hypersensitivity to ionizing radiation (IR), chromosomal fragility, and abnormal cell cycle checkpoint regulation [3, 9, 15]. The down-regulation of NBS1 protein level by small interfering RNA (siRNA) leads to an increase in IR-induced mutation frequency in human lymphoblastoid cells [16]. Even though the severest form of NBS is recessive and of low frequency in the population, the incidence of NBS heterozygotes is estimated to be 1/177 in some southeastern areas in Europe [17]. Considering the report that mice heterozygous for NBN (the murine homologue of NBS1) developed a wide variety of tumors affecting the liver, mammary gland, prostate, and lung, in addition to lymphomas [8], the NBS1 partial deficiency in human could be a considerable threat to public health.

The human NBS1 protein is composed of three functional regions at the N-terminus, central region, and the C-terminus. The N-terminus contains a forkhead associated (FHA) and breast cancer C-terminus (BRCT) domain. The FHA/BRCT domain directly binds to the histone γ-H2AX, the phosphorylated form of H2AX as a result of the presence of DSBs, then recruits other members of the MRN complex (MRE11 and RAD50) to the proximity of DSB sites [18,

19]. The C-terminal region of NBS1 contains an MRE11 binding site [20, 21]. As mentioned above, null mutation of NBS1 is lethal, while the mice expressing C-terminal NBS1 survive to adulthood and exhibit many phenotypes similar to NBS patients [21, 22]. Consistent with that, the majority of NBS patients (homozygous 657del5) with the presence of the C-terminal NBS1 protein produced by this mutation are viable, which suggests that the maintenance of the MRE11 binding domain is essential for life [8, 23]. Several serine/glutamine motifs, consensus sequences of phosphorylation by ATM and ATM/RAD3-related (ATR), are found at the central region of NBS1. In particular, the serine residues at 278 and 343 are phosphorylated by ATM kinase in response to ionizing radiation both *in vitro* and *in vivo*, and such phosphorylation is responsible for intra-S phase checkpoint control [14, 15] and telomere maintenance [11, 13, 16].

Roles in DNA DSB repair

There are at least two major pathways to repair DSBs, termed non-homologous end joining (NHEJ) and homologous recombination (HR) [24, 25]. NHEJ is facilitated by DNA-dependent protein kinases (DNA-PK), composed of a catalytic subunit, DNA-PKcs, and the heterodimeric DNA binding regulatory complex Ku, which is composed of Ku70 and Ku80. The Ku heterodimer recognizes and binds the free DSB ends. DNA-PKcs is recruited and activated by the Ku complex. Artemis, a nuclease with endo- and exo-nuclease activity, is then recruited to the damage site via a direct interaction with DNA-PKcs and primes the site of damage. The DNA ligase IV/XRCC4 complex processes the DNA ends and initiates re-joining [26]. In HR, DNA ends are processed to produce 3-single strand tails, and then this single strand DNA invades the intact homologous chromosome (or another homologous intra-chromosomal sequence) with the aid of RAD51. After DNA synthesis, the Holliday structure is resolved, and DNA repair is completed [19].

The exact function of NBS1 in DNA repair is not fully understood. NBS1 protein itself does not have DNA binding and kinase activity, which are usually required in DNA repair and DNA damage signal transduction. However, the FHA/BRCT domain at its N-terminus binds to γH2AX, and this could be an important step to recruit the MRN complex to the proximity of DNA DSB. The other two components of MRN, MRE11 and RAD50 are usually located in the cytoplasm but relocate to nuclei following DSB inducing agents; this process is abolished in the absence of NBS1[3]. MRE11 possesses several biochemical properties which are required for DNA DSB repair, such as 3'-5' double-strand DNA exonuclease, single-strand DNA endonuclease, and DNA unwinding activity [27, 28]. RAD50 is a member of the structure maintenance of chromosome (SMC) protein family; it has two ATPase motifs at its N- and C-terminal ends and forms an anti-parallel homodimer with a flexible hinge region that may adopt a "V-like" conformation [29]. The "V-like" structure could serve as a bridge to hold together the broken ends of DSB and prevent them from floating away, as well as restrict the extent of nucleotide degradation of MRE11 to prevent excessive degradation (Figure 2) [3].

In *Schizosaccharomyces cerevisiae*, MRE11/RAD50/XRS2, a homologue for MRN, has been reported to be involved in both pathways [30-32]. The disruption of XRS2 leads to a deficiency in a special case of NHEJ termed V(D)J recombination, which is an important process during the development of the immune system to assemble a diverse array of immunoglobulin molecules and T-cell receptors [33]. XRS2 mutants show hypersensitivity to IR due to insufficient NHEJ and HR activity, as well as deficiency in meiotic recombination [3, 34].

In mammalian cells, by using a cell-free DNA fragment end joining assay *in vitro*, the addition of human Ku, DNA-PKcs, and Ligase IV/XRCC4 complex did not promote efficient joining of cohesive-ended DNA fragments to the level observed in crude human nuclear extract. However, the addition of a cell extract fraction, which contains MRN complex, restored the DNA fragment end-joining activity

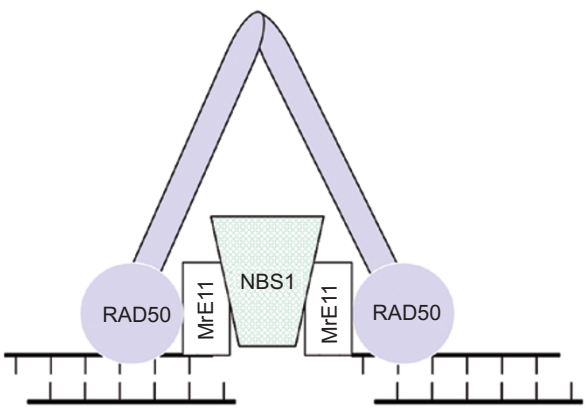

Figure 2 The schematic architecture of MRN complex. The triple MRN complex is composed of a dimers of RAD50 and MRE11, as well as a single NBS1 [28, 29, 114]. MRE11 and RAD50 are recruited to the proximity of DSB site by NBS1 through the interaction of histone γH2AX with the FHA/BRCT domain in the N-terminus of NBS1 (not shown). The RAD50 dimer binds to DNA duplex with its ATPase motifs and hold the broken ends together with its coiled-coil arms [28]. MRE11 associates with RAD50 and DNA duplex to prime broken ends with its nuclease activity. The MRE11 nuclease activity is modulated by NBS1 and RAD50.

to the level of the crude nuclear extract; suggesting a role of MRN in the process of DNA end-joining in human cells [35]. By using the similar assays as in [35], MRN was found to promote DNA fragment end joining activity *in vitro* via DNA ligase IV/XRCC4 complex and DNA-PKcs is not required for this pathway [36]. However, in chicken DT40 cells with a targeted disruption of the *NBS1* gene, the NBS1 deficient cells showed indistinguishable NHEJ events from those wild-type cells, as measured by a plasmid DNA end joining assay *in vivo* [9]. This is consistent with the observation that in cells from NBS patient carrying a mutated *NBS1* gene, the V(D)J recombination frequencies and the quality of signal and coding joining are comparable to wild-type controls [37]. The role of NBS1 in NHEJ is rather controversial when considering results obtained *in vitro* and *in vivo*, or in chicken and human cells, as mentioned above. Data obtained with *NBS1* mutated mice suggest the indirect involvement of NBS1 in V(D)J recombination. In *NBS1* mutated mice, when V(D)J recombination occurs at *TCR*-β and *TCR*-γ loci, the inter-chromosomal recombination is increased, whereas the intra-chromosomal recombination is not significant affected [21, 22]. This is consistent with the frequent chromosomal translocation observed at *TCR* loci in NBS1 patients [3]; suggesting that NBS1 plays a role in recognizing proper elements of V(D)J recombination by suppression of inter-chromosomal recombination, which could lead to gene translocation and genomic instability [3].

In the HR pathway, the *NBS1* knockout chicken DT40 cells showed a markedly reduction in HR events (sister chromatid exchange) following mitomycin-C treatment. Moreover, SCneo reporter assays revealed a dramatically reduced HR frequency of approximately 100-fold in NBS1 deficient DT40 cells, as compared to wild-type cells; this suggested that NBS1 is required in HR, at least in vertebrate cells [3, 9, 19].

Roles in cell cycle checkpoint control

NBS1 has proved to be an essential modulator in cell cycle checkpoint control, which is an important part of the DNA damage response. NBS cells exhibit defects in cell cycle checkpoints after exposure to IR or radiomimetic drugs. One of the striking checkpoint defects is in the intra-S-phase checkpoint; the failure of the intra-S-phase checkpoint leads to radio-resistant DNA synthesis (RDS), in which NBS cells continue DNA replication despite the presence of radiation-induced DNA damage [3, 38, 39]. There are three parallel pathways to modulate the intra-S-phase checkpoint control [3, 29, 40-42]. The first is ATM-CHK2-CDC25A-CHK2 pathway [40]. In this pathway, IR first activates ATM, then ATM activate CHK2, a cell cycle checkpoint kinase, most probably by phosphorylation at thr 68 [43], then activated CHK2 phosphorylates CDC25A, another cell cycle signaling kinase at ser 123, which leads to a phosphorylation of CDK2 at tyr 15 and thr 14 and a disruption of cyclin E/CDK2 kinase which causes the intra-S-phase checkpoint [40]. NBS1 hase been reported to be required for the activation of ATM and CHK2 after IR [15, 44-48]. The down-regulation of NBS1 by siRNA leads to a decreased activation of CHK2 after exposure to IR [16]. Thus, NBS1 could be involved in regulating intra-S-phase checkpoint control via ATM and CHK2. However, the RDS is more severe in AT cells than in NBS cells, and while some reports showed that CHK2 activation was normal in NBS cells [49], others showed that NBS1 was required in CHK2 activation; this suggested that factors other than ATM and CHK2 might also be involved in intra-S-phase checkpoint control.

The second is a pathway involving ATM, NBS1, and a cohesin protein SMC1 [51]. The effective phosphorylation of SMC1 at ser 957 and ser 966 leads to an activation of an intra-S-phase checkpoint and RDS, which depends on phosphorylation at ser 278 and ser 343 of NBS1 by ATM; this clearly indicated a role of NBS1 in the intra-S-phase checkpoint control [51].

The third is the ATM/FANCD2 pathway [52]. In this pathway, ATM phosphorylates ser 222 of FANCD2 (mutated in the chromosomal instability syndrome Fanconi's anemia) and this event activates the intra-S-phase checkpoint. This FANCD2 phosphorylation requires the phosphorylation at ser 343 of NBS1 by ATM [52, 53].

NBS1 may also become involved in G1 checkpoint control although there is some controversy about that. A deficiency in G1 arrest was observed after low doses of IR in primary NBS fibroblasts but was not observed at high doses [54]. In addition, the expression of *p53* and *p21* in NBS fibroblast cell lines, which should lead to G_1 cell cycle arrest, was found to be attenuated after IR [55]. In B-lymphoblastoid NBS cells, G_1 arrest was also found to be defective or attenuated [56, 57]. However, some other researchers reported that normal G_1 checkpoint control was present in NBS fibroblast cell lines [55, 58]. In a mouse model with a mutated *NBN* (the murine homologue of *NBS1*), the p21 response in fibroblast cells to IR was found to be indistinguishable from normal control [22].

The reports for NBS cells and the G_2 checkpoint are also discrepant. Lymphoblastoid cells derived from NBS patients, unlike normal cells, fail to stop their entry into mitosis immediately after IR and activate CHK2, which suggests a G_2 checkpoint defect. The defect of G_2 checkpoint and CHK2 activation were rescued by the ectopic expression of a wild-type *NBS1* [15]. NBS lymphoblastoid cells also showed a defect in replication stalling induced

G_2 checkpoints and the activation of CHK1, c-jun and p53 [50]. The degradation of MRE11 in Hela cells also leads to a failure of the G_2 checkpoint after IR treatment [45]. A disruption of G_2 checkpoints in mouse fibroblastoid cells with a mutation in *NBN* was also reported [59] while the EBV transformed B-lymphoblastoid cells from NBS patients the G_2 checkpoint was found to be normal [41, 60]. Taken together, even though there is some evidence to show that NBS1, and/or the MRN complex is involved in the control of G_1 and G_2 checkpoints, there is some discrepancy among studies. A possible explanation for this discrepancy is the redundant pathways in checkpoint control, the different cell types tested, and the possible differences among *NBS1* mutations used in those studies.

Roles in telomere stability maintenance

Telomeres are highly specialized nucleoprotein structures that maintain genomic stability by stabilizing and protecting the ends of linear chromosomes. Telomeric DNA is composed of tandem arrays of a 5-26 bp repetitive G-rich sequences that are oriented 5'-to-3' towards the chromosome end; these also serve as binding sites for specific telomere associated proteins [61-63]. Because conventional replication machinery cannot synthesize new DNA to the very end of a linear chromosome, replication results in progressive erosion of telomeric DNA[64, 65]. Activation of telomerase, a cellular ribonucleoprotein reverse transcriptase responsible for elongating one strand of the telomere, prevents the gradual loss of sequence from chromosome ends [61, 66, 67].

Telomeres "cap" chromosome ends, preventing them from being processed in the same way as broken DNA ends. DSBs normally lead to arrested growth and attempts by the cell to repair the ends [68, 69]. Functional telomeres turn this response into an appropriate response that acts to retain genomic integrity [61]. Therefore, it is not surprise that DNA DSB repair proteins may play an important role in telomere capping. In normal cells, the background level of DSBs are very low, about 0.05 DSBs/cell [70]. However, DNA repair associated proteins are found to participate in capping 92 new telomere ends, which is equivalent to 46 DSBs, far in excess of the background number of DSBs [64]. This suggests that DNA repair proteins are involved not only in joining the broken DSBs, but also in preventing the inappropriate joining of telomere ends. A set of proteins which mainly involved in the NHEJ pathway have been demonstrated to prevent telomere dysfunction; these include DNA-PKcs [64, 71-77], Ku70 [73, 75-77], Ku80 [73, 78-80], XRCC4 [80], and Artemis [81, 82].

Accumulating evidence demonstrates that the MRN complex is required for the maintenance of telomere length in mammals, plants and yeast [29, 31, 83-87]. In yeast, the XRS2 complex is involved in at least two pathways in telomere elongation modulation [3, 29, 88, 89]. First, it is thought to generate 3' ssDNA at the telomere, which is essential for the recruitment and subsequent action of telomerase. Second, it is involved in the homologous recombination mediated pathway to elongate telomeres, which involves recombination between tracts of telomere repeats but is not dependent on the function of telomerase [90]. In primary fibroblast cells isolated from NBS patients, accelerated telomere shortening during *in vitro* culture has been observed. Neither the introduction of NBS1 nor the catalytic subunit of telomerase, TERT, alone, has restorative effect on telomere length. Whereas, the co-expression of NBS1 and TERT leads to a significantly greater telomere length, implying that MRN functions in generating the 3'-overhangs and TERT can then replicate the telomeres by using this G-rich strand as a primer [42, 87]. However, in T and B cell lines derived from different NBS patients, the telomere length maintenance was found to be intact [91]. Due to the fact that a 70 kDa NBS1 variant which contained the MRE11 binding site was present in those patients, the MRE11-binding domain could be sufficient for the telomere length maintenance by NBS1 [91]. Because NBS is caused by a polymorphism of *NBS1* gene, it is reasonable to speculate that different polymorphisms could result in the presence of different NBS1 protein fragments in NBS patients. Thus, the specificity of telomere abnormality could be related to the specificity of individual NBS1 fragments.

In telomerase negative cancer cells, telomere length is maintained by an alternative lengthening of telomeres (ALT). The telomere associated nuclear body, which is dependent on the promyelocytic leukemia protein (PML), is often found in the nucleus of ALT cells. The PML nuclear bodies appear at exactly the same time as the activation of the ALT mechanism during cell immortalization and contain HR associated proteins, such as RPA, RAD51, and RAD52, which suggests that PML may function through a recombination mechanism in ALT [42]. NBS1 was found to be co-localized with PML, as well as to be associated with a nuclear dot-associated PML-binding protein, SP100, by the BRCT-domain at its C-terminus [92]. NBS1 also functions in recruiting other recombination proteins, including RAD50, MRE11, and BRCA1 to PML nuclear bodies [59, 93]. The above mentioned observations suggest a role of NBS1 in the ALT pathway.

In human cells, NBS1 was found to associate with TRF2, an important telomere function modulator, in a cell cycle specific manner [94]. Indirect immunofluorescence demonstrated that RAD50 and MRE11 presented at interphase telomeres, whereas NBS1 was associated with TRF2 and

telomeres in S-phase, but not in G1 and G2. In mammalian cells, the telomeres end in a large duplex loop (t-loop). Closure and stabilization of the t-loop is thought to occur by the invasion of the telomeric 3'-overhang into the duplex telomeric repeat tract, creating a stable heteroduplex at the base of the loop; that can be viewed as a recombinational event. TRF2 plays an important role in the process of strand invasion and T-loop stabilization. The formation of the telomeric t-loop is required after telomere replication, and the stabilization of the telomeric t-loop is continuously required throughout the cell cycle. Considering the role of MRE11 and RAD50 in HR, it is not surprising to see that MRE11 and RAD50 become involved in this process by functioning in forming and stabilizing telomeric t-loop throughout the whole cell cycle [94]. However, the transient recruitment of NBS1 to telomeres only in S-phase suggests a role of NBS1 in telomere replication. NBS1 functions in potentiating DNA-helicase and endonuclease activity of MRE11. Thus, the recruitment of NBS1 to telomeric MRE11/RAD50 may regulate a helicase mediated unpairing of the t-loop base. This process would open the t-loop, perhaps facilitating progression of DNA replication machinery to the end of chromosome [94].

Our recent results showed that the down regulation of NBS1 protein level by siRNA transfection also results in an increase of a telomeric abnormality called telomere association, a phenomenon in which telomeres of the same or different chromosomes are observed in unusually close proximity in metaphase spreads [16]. The mechanism of the increased telomere association by NBS1 deficiency is not fully understood. It may related with the functional roles of NBS1/MRN in ALT mediated telomere elongation modulation [42, 59, 92, 93] and the inter-chromosomal recombination of the repetitive telomeric tracts [16].

Modulation of PIKK family

PI3K-like protein kinases (PIKKs) are a conserved family of proteins from yeast to human, most of which possess a serine/threonine kinase activity. All of these proteins contain a domain with motifs that are typical of the lipid kinase phosphatidylinositol 3-kinase (PI3K), so they are termed PIKKs [95]. PIKKs respond to various stresses by phosphorylating key proteins in the corresponding response pathways; thus, they trigger numerous processes including cell cycle checkpoint control and DNA repair [95-100], depending on the spectrum of their downstream substrates. In mammalian cells, the PIKKs members DNA-PKcs, ATM, and ATR are known to be involved in DNA damage response. Whereas ATM and DNA-PKcs responds primarily to DSBs, ATR respond to ultraviolet (UV) light, DSBs, and stalled replication forks [95, 96]. Recent studies suggest that NBS1 is required for the activation of the DNA damage pathway of PIKKs family members ATM, ATR, and DNA-Pkcs; direct experimental evidence has been obtained for ATM and ATR [44-48, 50, 101].

The similarity of clinical manifestation and cellular phenotypic characteristics between NBS and AT patients, namely immunodeficiency, radiation sensitivity, chromosomal instability, and cancer predisposition [7, 9, 102, 103] suggests some possible links between NBS1 and ATM. NBS1 was first recognized as a downstream substrate of ATM based on the evidence that ATM phosphorylated NBS1 at ser 278 and 343 in the central region [15, 104, 105]. Phosphorylation of NBS1 by ATM could play some roles in major ATM-mediated pathways, the intra-S and G2/M cell cycle checkpoints [105]. Furthermore, NBS1 appears to facilitate ATM-mediated phosphorylation of several ATM substrates, such as the checkpoint kinase CHK2, the chromatin remodeling protein SMC, CHK1, and MRE11 [15, 51, 54, 106-108]. However, the fact that MRN complex adherence to the sites of DSB is independent of ATM make people question that ATM acts upstream of NBS1. Uziel *et al* found that after treatment with the radiomimetic chemical neocarzinostatin, ATM activation measured by the phosphorylation at ser1981 was defective in NBS1 compromised cells. Consistent with that, the nuclear retention of ATM (a sign of chromosomal binding) and the activation of ATM substrates, such as CHK2, P53, and HDM2 were also defective or attenuated in cells with an NBS1 deficiency [48]. Carson *et al* utilized the adenoviral proteins E1b55k/E4orf6 to disrupt the MRN complex in human cells, and showed that after treatment with DSB inducing agents, the infected cells had a marked reduction in ATM phosphorylation, ATM-dependent G2/M checkpoint abrogation, and the ATR-mediated DNA damage response deficiency [45]. Similar results were obtained by Horejsi *et al*, who investigated the timing and magnitude of ATM activation by phosphorylation at ser1981 of ATM and ser 966 of the ATM substrate SMC1 in *NBS1* proficient and deficient human fibroblast cells [101]. After 2 Gy IR, NBS1 proficient cells showed a rapid phosphorylation of ATM at ser1981 and the substrate. However, the activation of ATM was abrogated or attenuated in NBS1 deficient cells. The activation of ATM and radiosensity was rescued via retrovirally mediated reconstitution of wild-type NBS1, but not NBS1 lacking the MRE11 binding domain [101], which support the view that DNA processing activity of MRE11 is also required for ATM activation [47].

The direct evidence of the physical protein to protein interaction between MRN and ATM, as well as the modulation of the kinase activity ATM was obtained by Lee and Paull [46]. By using purified MRN and ATM protein expressed in a baculovirus system, they carried out *in*

vitro kinase activity and gel filtration assays. The results demonstrated that MRN stimulates the kinase activity of ATM *in vitro* toward its substrates p53, CHK2, and histone H2AX. MRN makes multiple contacts with ATM and stimulates ATM activity by facilitating stable binding to the substrates. To further investigate the role of MRN in the activation of ATM signaling in DSBs, Lee and Paull used a similar assay to test ATM binding to DNA fragments by MRN [47]. The results show that the binding of MRN to DNA is ATM independent, whereas ATM is associated with DNA only when MRN was associated. Furthermore, the unwinding of DNA ends by MRN is essential for ATM stimulation. These results indicated that ATM activation by DSB through MRN complex occurred by facilitating ATM-DNA binding and the unwinding of the DNA duplex [47]. Stiff et al [50] reported that NBS1 also facilitated ATR-dependent phosphorylation. NBS1 deficient cell lines show a similar defect in ATR phosphorylation of CHK1, c-jun and p53 in response to UV irradiation- and hydroxyurea (HU)-induced replication stalling. NBS1 deficient cells also showed an impaired ubiquitination of FANCD2 after HU treatment, which is ATR dependent. Following HU-induced replication arrest, NBS1 and ATR deficient cells showed a similarly impaired G_2/M checkpoint arrest and an impaired ability to restart DNA synthesis at stalled replication forks. Moreover, NBS1 deficient cells failed to retain ATR in the nucleus following HU treatment and extraction [50]. A recent finding by Falck *et al* [44] revealed a conserved C-terminal motif in NBS1 that serves to recruit activated ATM to the sites of DNA damage and promote the phosphorylation of ATM substrates, leading to the events of the DNA damage response. Interestingly, the motif shares sequence homology to the C-terminus of Ku80 and ATRIP. Ku80 and ATRIP are the partner proteins which are required to recruit PIKK family member DNA-PKcs and ATR to the sites of DNA damages, respectively [109-113]. This motif is required for Ku80 and ATRIP interactions with ATR and DNA-PKcs and the modulation of their functions. Interestingly, the synthesized NBS1 C-terminal peptide with this motif interacts with DNA-PKcs as measured by a peptide pull down assay, suggesting a common mechanism of the activation of PIKKs, as well as the possible extension of the role of NBS1 in PIKKs activation [44]. In other words, NBS1 may also play an important role in modulating other PIKKs not limited to ATM and ATR; DNA-PKcs could be a reasonable candidate for that.

Conclusions

NBS1 is a component of the MRN complex and plays an important role in DNA damage response. It modulates the DNA damage signal sensing by recruiting PIKK protein family members ATM, ATR, and probably DNA-PKcs to the DNA damage sites and activating their functions. It can also recruit MRE11 and RAD50 to the proximity of DSBs by an interaction with γH2AX through the BRCT/FHA domain at its C-terminus; thus, NBS1 functions in DNA DSB repair both in the NHEJ and HR pathways. NBS1 also functions in telomere length maintenance by generating the 3' overhang which serves as a primer for telomerase dependent telomere elongation. In addition, NBS1 co-localizes with PML and functions in recruiting recombination proteins to PML to modulate ALT pathway. NBS1 is a major player in the control of intra-S-phase checkpoint and there is some evidence that NBS1 is involved in G_1 and G_2 checkpoints. The roles of NBS1/MRN encompass DNA damage sensor, signal transducer, and effector, which enable cells to maintain DNA integrity and genomic stability.

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