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Review

The importance of making ends meet: Mutations in genes and altered expression of proteins of the MRN complex and cancer

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ABSTRACT

The MRN protein complex, consisting of MRE1, RAD50 and NBS1, plays a crucial role in sensing DNA double-strand breaks (DSBs), and it is involved in cell cycle control. This makes the MRN complex an important guard of genome stability. Hypomorphic mutations in *NBS1* result in the Nijmegen breakage syndrome (NBS), which is characterized by, among other things, an increased predisposition to malignancies, especially leukemia/lymphoma. Relatives of NBS patients carrying heterozygous mutations are also more prone to cancer development. This review summarizes several studies searching for associations between heterozygous mutations in *NBS1*, *MRE11*, and *RAD50* and cancer and examining the levels of expression of proteins coded by these genes in tumor tissues. The results indicate that both decreased and increased expression of NBS1 may contribute to tumorigenesis, whereas overexpressed RAD50 has an anti-tumoric effect. *MRE11* and *RAD50* are also affected in tumors with microsatellite instability. However, the outcomes of association studies, which concerned primarily lymphomas/leukemias and breast cancer, were inconclusive. Heterozygous *NBS1* mutations and molecular variants 657del5, I171V, R215W and E185Q were most commonly analyzed. Among these, an association with cancer was found most frequently for 657del5 (in leukemia/lymphoma and breast cancer) and I171V (in leukemia, breast, head and neck and colorectal cancers); however, other studies gave contradictory results. For other *NBS1* as well as *MRE11* and *RAD50* variants, too little data were available to assess their role in cancer risk. Overall, the results suggest that heterozygous MRN complex mutations and molecular variants may contribute only to a limited fraction of tumors. This may be caused by several factors: various frequencies of the variants in specific populations, different criteria used for selection of control groups, possible effects of environmental factors, and potential interactions with variants of other low-risk genes. These issues, as well as the impact of the alterations on protein function, need to be addressed in future studies.

Contents

1. Introduction

Double-strand breaks (DSBs) are a particularly dangerous type of DNA damage because they pose a threat of genome rearrangements, disruption of replication and transcription, and finally apoptosis or carcinogenesis if the broken ends are not joined together. Therefore, it is vital that cells possess efficient mechanisms of DSB repair. DSBs can be induced by damaging agents such as ionizing radiation (IR) or chemicals (e.g. cisplatin, camptothecin, and neocarzinostatin) but also arise during regular cellular processes: replication, recombination in meiosis and rearrangements of immunoglobulin genes [1]. Eukaryotic cells have evolved

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two ways of DSB repair: non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is a simple mechanism that joins the two broken ends of DNA, guided only by a small (1–6 bp) region of sequence homology (microhomology). In contrast to this error-prone process, HR is more accurate because it relies on a template of homologous sequence, usually in the sister chromatid. In vertebrates NHEJ is more frequently used than HR, the latter being restricted to the late S and G2 phases. Proteins involved and reactions they carry out also differ between the two mechanisms [reviewed in 2].

The first and crucial step in DSB repair both through NHEJ and HR is recognition of DNA breaks. The nuclear MRN complex, consisting of MRE11, RAD50 and NBS1, is implicated to have an early role in the detection of DSBs and initial DNA ends processing [3]. MRE11 and RAD50 are highly conserved proteins from yeast to humans, while NBS1 shows only minor homology to the yeast XRS2 at the N-terminal end. The core of the complex is MRE11, a protein with nuclease activity interacting independently with RAD50 and NBS1. The main role of RAD50 is probably holding two DNA ends together. NBS1 interacts with phosphorylated histone H2AX (γ-H2AX) at DSBs and is phosphorylated by the ATM kinase [reviewed in 4–6]. The MRN complex is rapidly (within seconds) recruited to the sites of DNA lesions induced by ionizing radiation [7] but the dynamics of the IR-induced nuclear foci (IRIF) formation and interaction network of the proteins involved in early DSB sensing are not known in detail. There are two types of IRIFs: early and late foci. The MRN complex is recruited to the early foci independently of ATM or γ-H2AX and is probably the sensor of DSB. Moreover, this rapid and transient (10 min to 8 h after DNA damage induction) association of MRN with chromatin at the sites of DSB is required for ATM activation by autophosphorylation [8,9]. Activated ATM is then able to phosphorylate several effectors: BRCA1, 53BP1, CHK1, CHK2, H2AX, MDC1, SMC1 as well as NBS1. This initiates mechanisms of cell cycle checkpoints and DNA repair. The late IRIFs are more persistent (until at least 24 h after DSB induction) and larger as they encompass γ-H2AX-marked chromosome regions flanking the breaks. Recruitment of the MRN complex to the late foci depends on the interaction between NBS1 and γ-H2AX and requires MDC1 as a mediator [7, reviewed in 10,11]. Presence of the MRN complex at the sites of DNA lesions is required for activation of cell cycle control and DNA repair pathways as well as for regulation of chromatin remodeling and enzymatic repair [reviewed in 12].

Apart from sensing DSBs, the MRN complex has several other functions: it is involved in DNA replication [13,14], recombination during meiosis [15], telomere maintenance [16,17], V(D)J recombination of immunoglobulin genes [18,19], control of S and G2/M cell cycle checkpoints [20–24] and induction of apoptosis [25,26]. Null mutations in the *NBS1*, *MRE11* and *RAD50* genes in mice result in embryonic lethality [27–29] revealing the crucial role of the MRN complex for cell proliferation. In humans hypomorphic mutations in *NBS1* and *MRE11* lead to Nijmegen breakage syndrome (NBS) and ataxia telangiectasia-like disease (ATLD), respectively, which phenotypically resemble ataxia telangiectasia—a neurodegenerative disease resulting from dysfunctional ATM protein kinase [30]. NBS is a rare autosomal recessive disorder characterized by microcephaly, chromosomal instability, immunodeficiency, hypersensitivity to ionizing radiation and increased risk of malignancies. Most of the NBS patients have a 657del5 mutation (so called Slavic mutation) which results in translation of a short N-terminal protein with FHA/BRCT domains, which recognizes γ-H2AX, and a C-terminal protein capable of interacting with MRE11 and RAD50, thus maintaining some functions of the full-length NBS1 protein. ATLD patients have ataxia, neurodegeneration, frequent translocations of chromosomes 7 and 14 in T-lymphocytes and increased radio-sensitivity but cancer predisposition has not been reported [reviewed in 31]. So far mutations in *RAD50* have not been linked with any complex disorder.

The involvement of the MRN complex in DNA repair and cell cycle checkpoints signaling suggests that mutations disrupting the functionality of NBS1, MRE11 and RAD50 may lead to genome instability and carcinogenesis. Evidence from NBS patients and mice carrying heterozygous or hypomorphic mutations [32,33] support this supposition. Also an increased cancer risk has been reported in relatives of NBS patients carrying heterozygous *NBS1* mutations [34]. These observations led to several studies examining the association of known mutations in *NBS1* with cancer and searching for new alterations in the genes of the MRN complex in tumors. Expression levels of NBS1, MRE11 and RAD50 proteins in malignant tissues have also been measured. This review summarizes the results of those studies.

2. Expression of NBS1, MRE11 and RAD50 in tumors

Altered expression of the MRN complex proteins has been observed in tumors. Results of several studies indicate that both reduced and increased levels of NBS1 protein may play role in carcinogenesis. In NBS patients the amount of the 70 kDa C-terminal fragment of NBS1 is correlated with cancer occurrence. Significantly more patients with low 70 kDa NBS1 levels in their lymphoblastoid cell lines developed cancer (in most cases lymphomas) when compared with patients with high 70 kDa NBS1 expression [35]. In cancer patients without NBS low expression of NBS1 was also observed in 46/99 (46%) of breast carcinomas. Decreased levels of MRE11 and RAD50 were less frequent (31 and 28%, respectively) but there was a good correlation in the protein levels within the majority of individual tumors. Low expression of the MRN complex proteins was associated with deregulated levels of ATM and P53. Furthermore, cytoplasmic expression was detected in seven cases for NBS1, four for RAD50 and one for both NBS1 and RAD50 [36], whereas in normal cells the MRN complex shows nuclear localization [37]. Nuclear and cytoplasmic staining for NBS1 in various tumor tissues but expression levels comparable to those in corresponding normal tissues were reported by Sakata et al. [38]. In benign breast lesions reduced expression of the MRN complex proteins was also observed but significant differences between the myoepithelial and luminal compartments occurred (low levels of NBS1 were found in 11/15 and 6/15 cases, respectively; MRE11: 9/15 and 3/15; RAD50: 5/15 and 2/15). Again, a correlation in the levels of the three proteins was observed [39].

Decreased MRN levels could promote tumor development through less efficient DSB repair in combination with impaired checkpoint signaling and an apoptotic defect. DNA damage alone would not be enough to promote cell transformation as properly functioning cell cycle checkpoints and apoptosis would prevent cells from proliferation. However, as intra-S phase checkpoint and induction of apoptosis have been shown to depend on a proper MRN complex-ATM cooperation [25,26], cells with reduced MRN levels could divide regardless of DNA damage possibly leading to carcinogenesis.

On the other hand, increased levels of NBS1 were found in tumors as well. Screening of 29 non-small lung cell cancer, 13 hepatoma and 10 esophageal cancer samples showed increased expression of NBS1 in 52, 48 and 40% of the samples compared to corresponding normal tissues [40]. Overexpression of NBS1 in certain types of tumors is a prognostic marker of advanced malignancy and shorter survival period. In uveal melanoma NBS1 immunostaining intensity was strongly correlated with tumor severity and metastatic risk. The 6-year actuarial survival was also significantly lower in the high NBS1 group than in the low NBS1 group (22% *vs.* 100%) [41]. Similarly,