Role of nibrin in advanced ovarian cancer

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Abstract
Nibrin is a protein coded by the *NBS1* gene which plays a crucial role in DNA repair and cell cycle checkpoint signalling. Nibrin apparently plays two different roles in ovarian cancer. Firstly, mutation in *NBS1* can be implicated in ovarian tumorigenesis. Secondly, in invasive tumours, high expression of nibrin mRNA or protein seems to correlate with a worse prognosis and worse response to treatment. All of these data indicate that nibrin could be involved in the clinical outcome of ovarian cancer patients and that it could be a potential target for this disease.

Key words: nibrin, ovarian cancer, trabectedin

Introduction

Nibrin (NBN, NBS1) is the product of the *NBS1* gene located in locus 8q21.3. This protein is a 754 amino acid polypeptide that acts together with MRE11 and RAD50 proteins to form the MRN complex. The MRN complex is involved in the recognition and the repair of double strand breaks (DSBs) through homologous recombination (HR) and non-homologous end-joining (NHEJ) pathways. It also activates the signalling cascades that lead to cell cycle control in response to DNA damage (Figure 1) [1]. Nibrin itself has no enzymatic activity, but mediates several protein-protein interactions that help the MRN complex to perform its many functions such as DSB recognition, DNA binding and DNA processing by activation of the MRE11 nuclease activity. Among the molecules interacting directly with nibrin are the ataxia telangiectasia mutated (ATM) kinase itself [2], the MDC1 adaptor protein [3, 4] and CtIP, a tumour suppressor protein that promotes processing of the DSBs by MRN [5], an effect which facilitates activation of the ATR-Chk1 signalling cascade and homologous recombination repair [5–8]. It was also demonstrated that nibrin interacts with phosphorylated histone γ-H2AX at sites of DSBs favouring the recruitment of the MRN complex. In addition, nibrin activates the cell cycle checkpoint and downstream molecules, including p53 and BRCA1 [9].

Mutations of the *NBS1* gene and tumorigenesis

Mutations of the *NBS1* gene have functional consequences for the biological activity of nibrin. Some *NBS1* mutations have been described to impair the binding to γ-H2AX and, thus, to hamper the localization of the MRN complex at the site of DNA damage [10, 11]. For example, the *NBS1* 553G>C polymorphism alters the BRCA1 C-terminal domain. This domain is important for interaction with histones and relocalisation of the MRN complex closer to the site of DNA damage; as a consequence, the GG genotype is associated with increased DNA damage [12]. Thus, it is now accepted that mutations in the *NBS1* gene will alter the DNA damage response (DDR).

At the clinical level, mutations in *NBS1* has been identified as the genetic basis of the Nijmegen Breakage Syndrome (NBS) [13–15]. Patients with NBS present with radiosensitivity, immunodeficiency and a higher predisposition to malignancies (mostly lymphomas and leukaemias) [15]. This has confirmed the role of nibrin as a tumour suppressor [16, 17]. The most frequent alteration in NBS patients is a homozygous 5-base pair deletion in exon 6 of the *NBS1* gene (657del5) called the "Slavic mutation" [15]. Nine other heterozygous mutations of the *NBS1* gene have been found to be associated with an increased risk of developing several cancers [18], including breast cancer,

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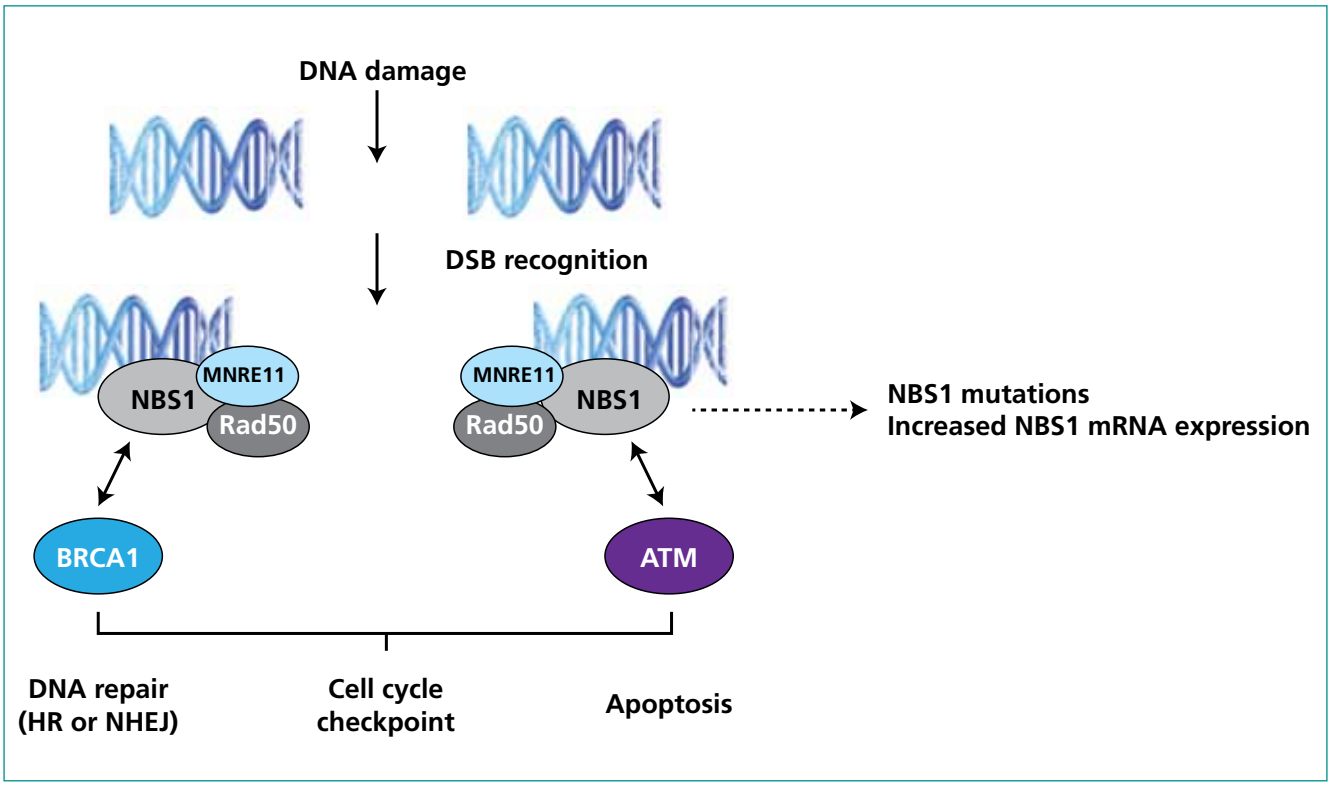

Fig. 1. Role of nibrin (NBS1) in DNA repair and cancer. The MRN complex acts as a sensor of DNA double strand breaks (DSB) and interacts with ATM, activating downstream molecules such as p53 and BRCA1. HR: homologous recombination; NHEJ: non-homologous end-joining.

colorectal cancer, prostate cancer, ovarian cancer, melanoma, medulloblastoma and primary glioblastomas. Among all mutations, the Slavic mutation, and the R215W and I171V mutations are the most frequent. For example, the Slavic mutation is more frequent in NHL (4.8%), melanoma (3.8%) and breast cancer (3.7%) patients [19]. Moreover, heterozygous carriers of this mutation present an increased risk of developing breast cancer. A high frequency of the missense R215W mutation is found in children affected by Hodgkin lymphoma (2.6%) and acute lymphoblastic leukaemia (2.1%) [18]. The missense mutation I171V has been associated with a 9-fold increased risk of breast cancer in Polish patients [20]. Similarly, the frequency of the I171V mutation is also high among patients with head and neck (6.1%) and larynx (2.3%) cancers [13].

Nibrin and ovarian cancer

In ovarian cancer, the Slavic mutation was reported in 1–2% of cases and is associated with a clear cell histology type [21, 22]. The germ line 1142delC *NBS1* loss of function mutation has been also found in serous ovarian carcinoma [23]. There is also evidence that a heterozygous state for this deletion may contribute to enhanced predisposition to ovarian cancer [24]. Loss of heterozygosity (LOH) for the Slavic mutation is associated with a loss of nibrin expression detected by immunohistochemistry (IHQ) [13]. Allelic losses on chromosome 8q have not previously been reported for ovarian cancer [25, 26]; in fact, amplification at 8q has been observed frequently [27, 28].

The Cancer Genome Atlas (TCGA) research network compiled detailed molecular datasets on more than 500 advanced-stage, high-grade serous ovarian carcinomas and identified an expression profile signature that was associated with survival [29]. In addition, besides the expected mutations in p53 or BRCA1/2, there was a remarkable degree of genomic disarray that apparently was correlated with a high prevalence of mutations and promoter methylation in putative DNA repair genes, including homologous recombination components. Extraction and analysis of differential gene expression data of 151 DNA repair genes from TCGA database resulted in a molecular score based on the expression of 23 genes involved in platinum-induced DDR pathways [30]. Because of the significant association between high levels of nibrin mRNA and a worse OS observed in the TCGA database, nibrin was included in the 23-gene signature to build their molecular score [30]. Improved survival was associated with the high-scoring group (high *vs* low scores: 5-year OS, 40%

vs 17%, p<0.001). The high-scoring group also showed better complete response rate, recurrence-free survival, and PFS (p<0.001 for all). Importantly, these findings were validated in two additional datasets of advanced-stage ovarian cancer patients treated with platinum-based chemotherapy and demonstrated that a molecular score based on expression of genes that are involved in platinum-induced DDR could provide prognostic for survival when it is composed of genes from relevant DNA repair pathways [30].

More recently, a retrospective IHQ analysis of 13 proteins related to cell proliferation, cell cycle checkpoint signalling, and DNA repair was performed in 139 diagnostic samples from patients with ovarian cancer. These patients have participated in the phase III randomized study (OVA-301) that have compared trabectedin in combination with doxorubicin hydrochloride/pegylated liposomal doxorubicin (PLD) *versus* PLD alone after failure of platinum-based chemotherapy [31]. In the subset of patients with serous histology (N = 114) a statistically significant correlation between high levels of nibrin and lower ORR (p=0.03), shorter PFS (p=0.007) and shorter OS (p=0.01) was observed. The results of a multivariate analysis, including clinical variables (age, race, ECOG PS score, treatment arm, prior taxane therapy, platinum-free interval, baseline CA-125 levels, baseline liver/lung involvement, ascites at baseline, and the presence of bulky disease) and molecular variables (protein expression levels of Ki-67, p53, ATM, CHK1, CHK2, BRCA1, BRCA2, DNA-PK, ERCC1, FANCD2, nibrin, RAD50 and XPA), showed that, besides the predictable clinical variables, high level of nibrin was independently correlated with a worse PFS (HR=1.02; 95% CI: 1.01–1.03; p=0.001) and OS (HR=1.01; 95% CI: 1.00–1.02; p=0.006) [32].

Conclusions

All these data indicate that nibrin could play different roles in ovarian cancer. Firstly, mutation in *NBS1* may be implicated in ovarian tumorigenesis in epithelial serous carcinomas. This could be related to alterations in the DNA damage response that is generated because of a deficient recruitment of the MNR complex to DSB sites. On the other hand, in invasive tumours, high expression of nibrin mRNA or protein seems to correlate with a worse prognosis and worse response to treatment. All these data warrant further efforts (i) to develop new techniques to detect nibrin, and (ii) to investigate the potential use of treatments aimed to decrease the expression of nibrin in ovarian cancer patients, in order to sensitize them to treatment.

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