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Review

Nijmegen breakage syndrome: clinical manifestation of defective response to DNA double-strand breaks

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Abstract

Nijmegen breakage syndrome is a rare autosomal recessive genetic disease belonging to a group of disorders often called chromosome instability syndromes. In addition to a characteristic facial appearance and microcephaly, patients suffering from Nijmegen breakage syndrome have a range of symptoms including radiosensitivity, immunodeficiency, increased cancer risk and growth retardation. The underlying gene, *NBS1*, is located on human chromosome 8q21 and codes for a protein product termed nibrin, Nbs1 or p95. Over 90% of patients are homozygous for a founder mutation: a deletion of five base pairs which leads to a framehift and protein truncation. The protein nibrin/Nbs1 is suspected to be involved in the cellular response to DNA damage caused by ionising irradiation, thus accounting for the radiosensitivity of Nijmegen breakage syndrome. We review here some of the more recent findings on the *NBS1* gene and discuss how they impinge on the clinical manifestation of the disease.

Keywords: Nijmegen breakage syndrome; Chromosomal instability; DNA double-strand breaks

1. Introduction

Nijmegen breakage syndrome (NBS) was first described in 1981 in Dutch patients, however, the majority of NBS patients live in Poland and the Czech republic and the Dutch patients may have had Bohemian ancesters who emigrated to Holland in the first half of the 17th century after the 30 Years War.

Patients with NBS have a characteristic facial appearance with a combination of receding forehead, receding mandible and prominent midface (Fig. 1a). In addition, most patients have epicanthal folds, large ears, and sparse hair. The facial features are perhaps even more striking since patients with NBS are also microcephalic, as illustrated by the head circumference growth chart shown in Fig. 1a. Mental retardation is generally mild, with most patients having an IQ within the normal range during early childhood, but with progressive retardation as they grow older. Most patients are born with microcephaly, but in 25% of patients, this becomes apparent only after a few months. Fig. 1b also shows the body-length charts for two boys with NBS; growth retardation seen in all patients with the majority in the 10th percentile, even though birth weight and size are usually normal. Short stature is apparent by 2 years of age, and is due to trunk shortening rather than lower extremity shortening. Body weight is proportional to body height.

The identification of the gene underlying NBS in 1998 as a gene involved in the repair of DNA double-strand breaks (DSBs) [1–3] seemed to offer an explanation for many of the clinical features (Fig. 2). Now 5 years later it is perhaps appropriate in this review to examine how well these explanations have been substantiated by further experimental findings.

2. Radiosensitvity, chromosomal breakage and DNA double-strand break repair

NBS patients are sensitive to ionising irradiation (IR) and its therapeutic use in undiagnosed patients can be fatal [4]. This radiosensitivity is, of course, reflected by an at least two-fold increase in cell death after an IR treatment of primary or immortalised NBS patient cells [5]. IR produces DNA DSBs as its most serious lesion and NBS cells are

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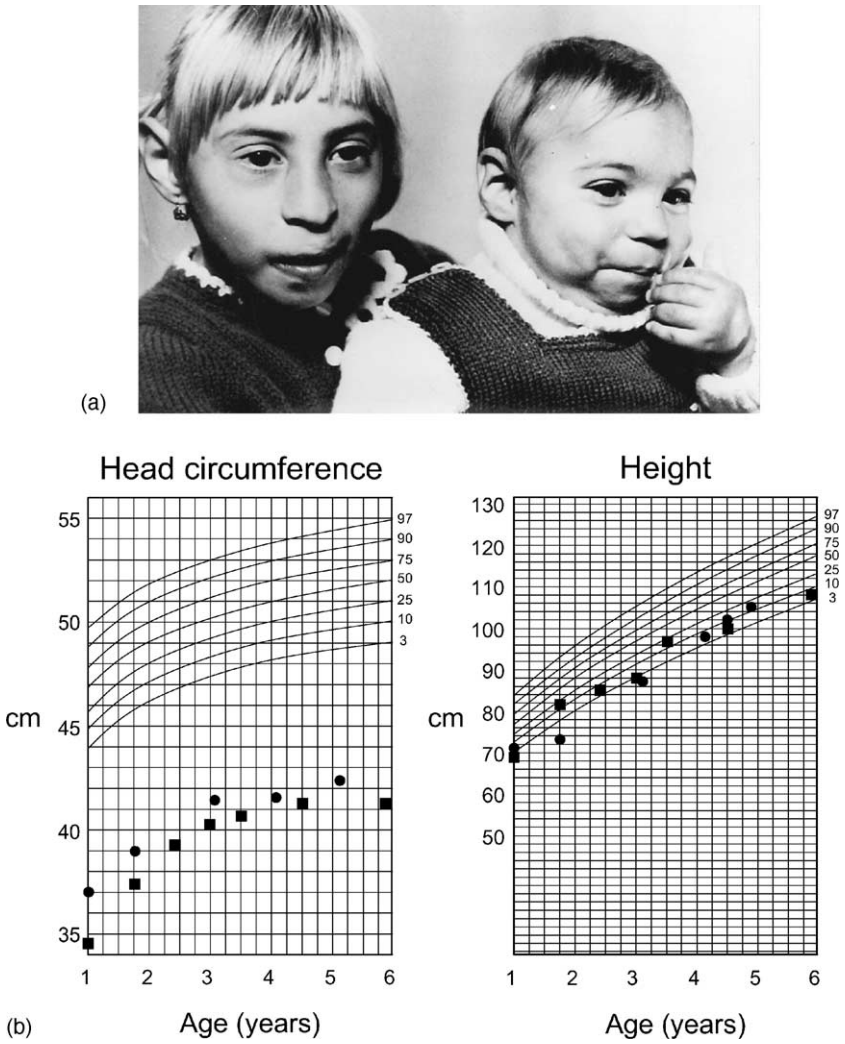

Fig. 1. Clinical manifestation of Nijmegen breakage syndrome. (a) Two brothers aged 1 and 7 with the characteristic facial features of the disorder. (b) Head circumference and body length growth-charts for two further male NBS patients. The photograph and charts were kindly provided by Professor Eva Seemanova.

sensitive to many other mutagens which produce DSBs or which make lesions repaired via DSBs as an intermediate: bleomycin, streptonigrin, etoposide, camptothecin, and the cross-linking agent mitomycin C [6,7]. In addition to clinical and cellular hypersensitivity to DSB-inducing agents, NBS is characterised by increased spontaneous and IR-induced chromosome breakage.

Chromosome instability is evident in stimulated T lymphocytes from patients with NBS, in addition to random breaks, specific translocations are frequently observed between the loci of the immunoglobulin and T-cell receptor genes on chromosomes 7 and 14 [5,8]. In cultured NBS fibroblast cell lines, there is an elevated frequency of chromatid breaks occurring at arbitrary sites [9,10]. Finally, telomere fusions leading to dicentric chromosomes are more frequent in NBS cells than controls. Following irradiation the frequency of chromosome aberrations is drastically increased in cultured NBS cells compared with normal cells, the damage being generally of the chromatid-type [9,11,12]. In contrast to this clear evidence for a DNA repair deficiency in NBS, attempts to define this deficiency by quantification of DNA repair by biochemical techniques have been unable to demonstrate a significant deficiency in DSB repair [7,13,14]. This may be due to technical limitations to the detection of DSBs, especially considering that even one unrepaired DSB can be lethal for an individual cell [15]. In addition, there are clearly multiple pathways for DSB repair and the use of alternative pathways may leave the NBS cell with ostensibly repaired DNA damage at the cost of an increased mutation rate.

The gene mutated in NBS was localised to chromosome 8q21 by linkage analysis in NBS families [16] and then identified by positional cloning [1,3]. Concurrently, the functional human orthologue of the yeast gene, *Xrs2*, was identified as the very same *NBS1* gene [2]. In yeast cells the product of the *Xrs2* gene is part of a trimeric complex

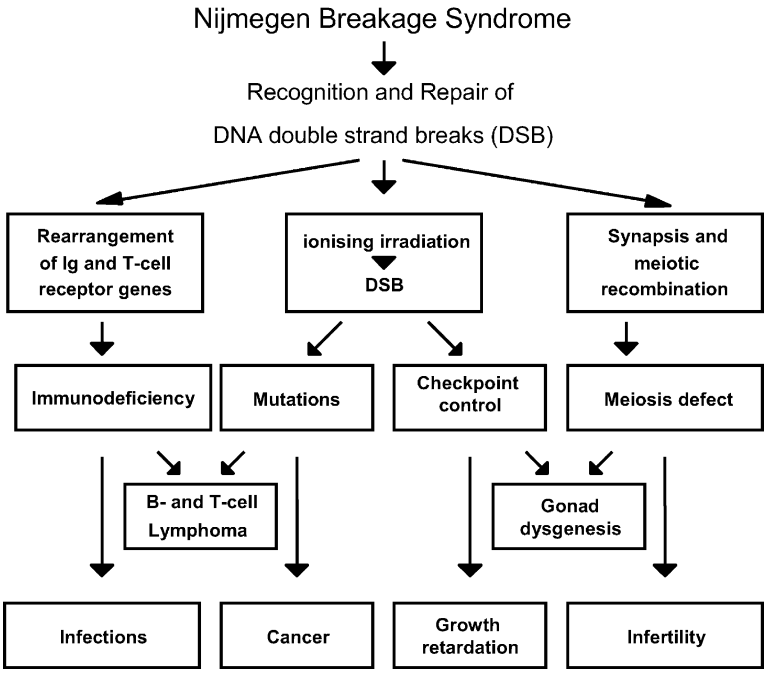

Fig. 2. The clinical characteristics of Nijmegen breakage syndrome and their potential relationship to double-strand break recognition and repair. This graphic from 1998 shows how the clinical features of NBS could be explained by the function(s) of nibrin/Nbs1. The current evidence for each association is discussed in the text.

together with Mre11 and Rad50, involved in DSB repair. In mammalian cells too, MRE11, RAD50 and the *NBS1* gene product, nibrin, also known as Nbs1 or p95, interact to form a complex. Experimental data from the yeast *S. cerevisiae* implicate the Mre11 complex in DSB repair by both non-homologous end joining (NHEJ) and homologous recombination (HR), the two major repair pathways (see the reviews by Frederick Alt, Michael Lieber, and Roland Kanaar in this issue). Much research has been aimed at elucidating the roles of the MRE11/RAD50/nibrin complex, hereafter, MRN complex, in DSB repair in humans and the particular functions of nibrin/Nbs1 within the complex, this is dealt with in detail in the reviews by Kenshi Komatsu and John Petrini in this issue. Generally speaking, examination of NHEJ in NBS patient fibroblasts [17], and NHEJ and HR in chicken DT40 cells with disrupted *NBS1* [18] or *MRE11* [19] genes is more supportive for a role of the human complex in homologous recombination rather than simple end joining.

Considerable attention has been paid to the enzymatic activities of the MRN complex, especially to the MRE11 protein, a 3′–5′ dsDNA exonuclease, an ssDNA endonuclease and dsDNA endonuclease. However, the relevance of these activities *in vivo* is uncertain even in *S. cerevisiae* [20]. Irrespective of its possible enzymatic properties, MRE11 is a DNA end-binding protein, and this activity is stimulated by RAD50 and nibrin/Nbs1 [21]. The molecular architecture of the RAD50/MRE11 complex bound to DNA has been examined by electron microscopy and X-ray crystallography [22–24] and indicates a bridge-like structure that could hold two DNA ends together as part of a DSB repair process and/or for marking and signalling the presence of a DSB.

Over 90% of all patients analysed to date are homozygous for a 5 bp truncating mutation, 657Δ5 [1]. Seven further truncating mutations have been identified in other patients (Fig. 2). These rarer mutations are located between nucleotides 657 and 1142 and are also predicted to truncate the nibrin/Nbs1 protein downstream of the N-terminal BRCT and FHA domains. The amino-terminal truncated protein of approximately 26 kDa is actually observed in cells from patients and heterozygotes with the 657Δ5 mutation as is, at much lower abundance, and so far only in EBV-transformed lymphoblastoid cells, a 70 kDa amino-terminal truncated fragment [25]. This fragment is produced by alternative initiation of translation at a start codon upstream of the deletion which brings it into frame. Similarly truncated proteins have been observed for the 835Δ4 and 900Δ25 mutations [25,26]. These proteins are shown schematically in Fig. 3 and arise presumably from downstream internal methionine codons in an acceptable context for ribosome entry. The first possible codons after the mutations are indicated in Fig. 3.

The significance of these nibrin/Nbs1 truncated products is that they may have partial activity and thus ameliorate the effects of an otherwise lethal mutation. The evidence for this comes from two knock-out mice which produce no truncated nibrin/Nbs1 and die early in embryonic development [27,28], as do mice with targeted inactivation of *MRE11* [29] and *RAD50* [30]. In contrast, two knock-out mice which do produce truncated nibrin/Nbs1 fragments (Fig. 3) are viable, albeit with symptoms of the human

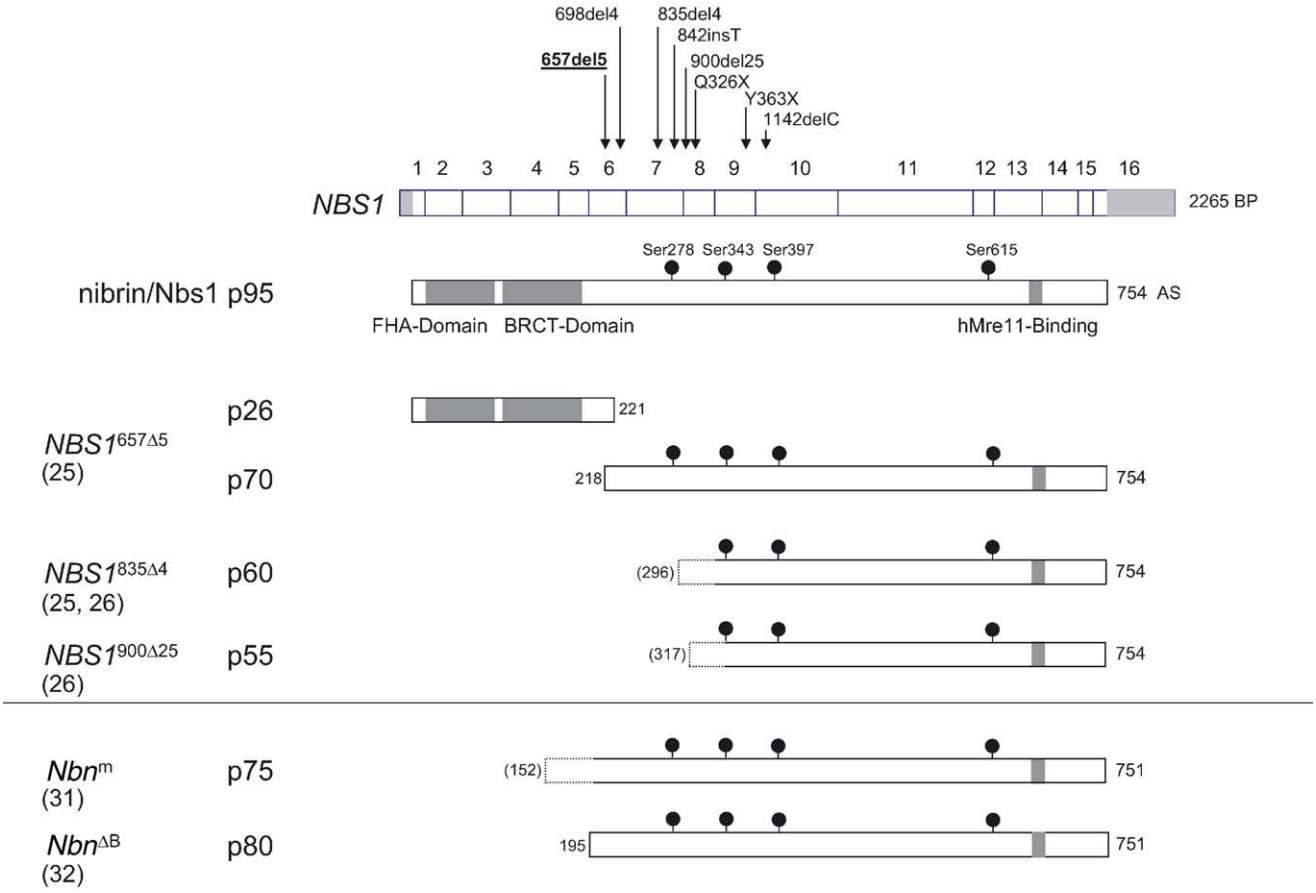

Fig. 3. The *NBS1* gene and its protein, nibrin/Nbs1. The exons of the *NBS1* gene are shown together with the sites of mutations found in NBS patients. Below the gene, the protein product nibrin/Nbs1, or p95, is drawn with the known domains and sites of serine phosphorylation by ATM. Nibrin/Nbs1 protein fragments can be detected in some patient cells and in the two viable mouse models of NBS. These truncated proteins are probably essential for viability. The amino acid numbering refers to regular nibrin/Nbs1 residues only, some novel amino acids may also be present due to frame shifts. Where unknown, the amino terminal end of a fragment is given as the first possible start codon in brackets. Only those nibrin/Nbs1 fragments are shown which have been actually observed by immunoprecipitation experiments, references to the literature are given in brackets below the gene mutation.

disease [31,32]. Examination of the sequence in the viable targeted mice indicates that, unlike 657Δ5 in the human mRNA, no upstream cryptic start codons are brought into the correct reading frame. Thus the observed truncated proteins presumably arise by initiation downstream of the mutation(s).

Although mice carrying hypomorphic *Nbn* mutations do survive, they are nevertheless radiation sensitive [31,32] and this may be related to the requirement for nibrin/Nbs1 phosphorylation by ATM as an early cellular response to IR [33,34]. ATM is the kinase deficient in patients with the chromosomal instability disorder, Ataxia telangiectasia (A–T), and it phosphorylates nibrin on serine 343 and at least three further serines (Fig. 3). Amino acid substitutions in the FHA and/or BRCT domains in nibrin result in failure of this phosphorylation [35]. Perhaps not surprisingly, the p70 nibrin/Nbs1 fragment which altogether lacks the BRCT and FHA domains is not phosphorylated after IR.

The FHA and BRCT domains of nibrin/Nbs1 may not be interacting directly with ATM to initiate phosphorylation at serines located downstream. These domains are also required for interaction of nibrin/Nbs1 with the histone H2AX, which is phosphorylated within minutes after IR at the sites of DSBs and can be visualised in the same sub-nuclear foci as nibrin by immunostaining [36]. It has recently been shown that the MRN complex is required for the activation of ATM and its own phosphorylation [37]. The same amino acid substitutions which prevent phosphorylation of nibrin/Nbs1 by ATM also prevent accumulation of the MRN complex in nuclear foci [35]. Thus the ability to associate with H2AX, via the FHA and BRCT domains, seems to be the prerequisite for efficient phosphorylation by ATM and thus the reason for failed phosphorylation of the p70 nibrin/Nbs1 fragment in NBS patient cells.

In conclusion, the radiosensitivity of NBS patients can be clearly attributed to the role of nibrin/Nbs1 in the cellular response to IR. Whilst details of the repair pathway in which nibrin/Nbs1 functions remain to be fully clarified, its role as an early signalling response to IR is undisputed. This will be discussed further below in terms of the cell cycle. More

pertinent to the role of nibrin/Nbs1 in DSB repair may be the immunodeficiency of NBS patients.

3. Infections and immunodeficiency

NBS patients are prone to various infections, respiratory tract infections are particularly frequent with, in some cases, a fatal outcome. Agammaglobulinaemia has been reported for about one third of NBS patients whilst in others the humoral immune deficiency is more variable, deficiencies of IgA or IgG4, alone or in combination are, however, common. A minority of patients, about 10%, have normal Ig status [38,39]. Cellular immunity is more consistently deficient in NBS patients. T-lymphocyte proliferation in response to mitogenic stimuli is reduced in more than 90% of patients [39]. Whilst CD8+ cells are generally within the normal range, reduced proportions of CD3+ and CD4+ T cells are found in most patients. Consequently, the CD4+/CD8+ ratio is reduced. Mild to moderate lymphopenia has also been reported and in a study of such patients a failure of T cell regeneration in the thymus was postulated to be compensated for by non-thymic pathways [40].

The chromosome translocations frequently observed in isolated lymphocytes involve particularly the IgG and T-cell receptor genes on chromosomes 7 and 14. Since these chromosome translocations could be interpreted as the consequences of inaccurate V(D)J recombination events, this process has been examined by several reports. Furthermore, evidence from the yeast *S. cerevisiae* suggests a role for the trimeric complex, Mre11, Rad50, and Xrs2 in nonhomologous end joining (NHEJ) and the DNA repair components that are required for NHEJ are the same as required for V(D)J recombination.

Since nibrin/Nbs1 is the human orthologue of yeast Xrs2, the invovlment of the latter in NHEJ suggests that the immunodeficiency of NBS patients could potentially be ascribed to a disturbance of V(D)J recombination. Two findings support this suggestion. Firstly, V(D)J recombination is initiated by the RAG1 and RAG2 proteins which introduce double-strand breaks into Ig coding segments and produce DNA hairpin structures as an intermediate. In vitro, the Mre11/Rad50 complex has been shown to cleave DNA hairpins in an ATP-dependent fashion. This enzymatic activity is strongly promoted by the addition of nibrin/Nbs1 [21]. Secondly, immunofluorescence analysis of immature CD4+CD8+ double-positive thymocytes has indicated that nibrin/Nbs1 is found at the sites of active V(D)J recombination in the *TCR*αgene as a single fluorescent focus together with the histone H2AX [41]. This fluorescence pattern is distinct from the IR-induced foci discussed above.

Although these data can be assembled into an attractive hypothesis for the immunodeficiency of NBS patients, analyses of V(D)J in NBS patient cells has not been supportive. Sequencing of the appropriate segments amplified from NBS lymphoblastoid DNA has indicated normal immunoglobulin heavy chain rearrangement [42]. Similarly, the *CD3* sequences of endogenous Ig λL and κL chain gene loci cloned from NBS lymphocytes were not different to controls. Finally, plasmid reporter substrates have been used to examine V(D)J recombination frequencies and the quality of DNA-joins in NBS cells, both were found to be normal [13,17].

The deficiency of serum IgG and IgA characteristic for NBS together with essentially unaltered IgM levels suggest that the process of class switching in B-lymphocytes might be particularly affected by the lack of nibrin/Nbs1 [43]. Indeed, analysis of class switching in patient cells by Pan et al. [44] has supported this concept. The number of Sμ–Sα switch regions amplified from NBS patient B cells was considerably lower than that of controls, indicating a generally reduced level of switching. In addition, the sequences of the switch regions showed differences to controls and also to those of A–T patients in terms of sequence homology. The data on A–T indicated that when the normally predominant error-prone NHEJ system is not available, a process dependent on microhomology of 1–3 bp takes over. In NBS B cells the relatively long stretches of homology (10 bp) in amplified switch regions suggest that yet another pathway is possible and imply that the MRN complex is involved in the short-stretch microhomology pathway [44]. Finally, nibrin/Nbs1 and the phosphorylated histone, H2AX, have been found localised to the sites of class switch recombination in B lymphocytes [45] just as described above for nibrin/Nbs1 and H2AX in V(D)J.

That nibrin/Nbs1 indeed functions in immunoglobulin class switching has been substantiated by experiments using a conditional mouse model with in which a null mutation can be generated by Cre recombinase-induced disruption of the *Nbn* locus. When $Nbn^{+/-}$ isolated mouse B-cells were converted in vitro to $Nbn^{-/-}$ before stimulation to switch antibody expression, less than 50% were able to switch from IgM to IgG1 or IgG3 in comparison to untreated $Nbn^{+/-}$ cells [46].

4. Lymphoma in NBS patients and cancer risk in heterozygous carriers

NBS patients have a high risk for developing cancer [47]. In the Polish NBS registry, 18 of 48 patients had developed lymphoma by the age of 15. Generally speaking, children with NBS suffer from malignancies otherwise rarely observed in childhood, The majority of lymphomas are non-Hodgkin's lymphomas (NHL), mainly diffuse large B-cell lymphomas (DLBL), which are unusual for childhood malignancy [48]. Peripheral T-cell lymphoma has been described in NBS and, again, is a malignancy of adulthood. Only four patients have been reported with typical childhood malignancies, 3 patients with lymphoblastic precursor T-cell lymphoma and 1 patient with a medulloblastoma.

Considering the chromosome instability of NBS, lymphomas in NBS probably originate from cells with chromosome translocations and so it might be expected that they would frequently show the chromosome 7/14 rearrangements often seen in peripheral blood cells. Although this analysis has yet to be conducted, rearrangements involving breakpoints in chromosome 14q32 have indeed been reported in A–T patients suffering from T-cell prolymphocytic leukaemia [49]. Reunions between chromosomes 14 and 7 are also characteristic for A–T.

Finally, the immunodeficiency of NBS patients itself may influence the characteristic spectrum of malignancies observed. Immunosuppresive therapy has been associated with an increased risk for NHL [50] and studies in mice have shown that age related changes in immune response and clonal B-cell expansions can be linked to B-cell neoplasia [51].

Whilst the increased cancer risk in NBS homozygotes is undisputed, the risk associated with heterozygosity is less clear. The original findings of Seemanova et al. [47] indicated a high frequency of neoplasia amongst relatives of NBS patients. Furthermore, molecular cytogenetic techniques have indicated that spontaneous chromosomal instability is increased in NBS heterozygotes, a finding that was not previously possible with conventional cytogenetics [52]. Since the cloning of the *NBS1* gene, several laboratories have examined the frequency of *NBS1* mutations in cancer patients: non-Hodgkin lymphoma [53–55], malignant lymphoma [56,57], acute lymphoblastic leukaemia [58], colorectal cancer [59] and breast cancer [60]. Generally these studies have been unable to prove a role for *NBS1* mutation in the pathogenesis of the particular cancers examined. In the case of acute lymphoblastic leukaemia, however, four amino acid substitutions, S93L, D95N, I171V and R215W, were found particularly in patients [58]. Three of these mutations are located in the FHA or BRCT domains. Inactivating mutations were not found on the second allele in these cells, suggesting that the amino acid substitutions have a dominant negative effect. In a further study of 20 breast cancer, ovarian cancer and adenocarcinoma cell-lines, several mutations and truncated nibrin/Nbs1 proteins were identified [61].

Further support for an increased cancer risk in *NBS1* heterozygotes has come from a study using gene targeting in the mouse [27]. The disruption of exon 6 of the mouse *Nbn* gene by insertion of a neomycin resistance cassette leads to embryonic lethality, suggesting that in contrast to hypomorphic mutations, this mutation leads to a null-mutant. Heterozygous carriers of the insertion mutation showed a significantly increased occurrence of solid tumours in a range of tissues in addition to lymphoma. Examination of the tumours gave no evidence for loss or mutation of the wild type allele so that haploinsuficiency is the presumed pathogenic mechanism. In contrast, for human heterozygotes, the possible existence of a truncated protein produced by alternative translation [25] and capable of interaction mith MRE11 would be compatible with a dominant negative mechanism.

Direct proof of an increased cancer risk in *NBS1* heterozygotes was obtained from a cohort study (“index-test-method”) based on the histories of about 250 persons from 21 Czech pedigrees. Each individual was personally interviewed and detailed information was collected. The items of relevance comprised: reproductive history; X-ray history; working place history; former and current health status and possible confounding factors. The “index cases” were the homozygous patients. With few exceptions (obligate heterozygotes), the blood relatives did not know their carrier status because they were interviewed before genotyping. Thus, the cancer incidence rates of carriers and non-carriers in the same families were compared. This is a statistically powerful bias-resistant approach, with the best possible comparison group in terms of matching for confounders. There were 13 cases of cancer among the heterozygous subgroup, as opposed to three cancer cases found among the normal homozygotes ($P < 0.01$). There was no difference in the age distribution and the socioeconomical status between the two groups. These studies confirm the earlier suggestion that *NBS1* gene carriers have an elevated cancer risk [47,62].

Based on these data, heterozygous carriers should occur more frequently among cancer patients than in the general population. This hypothesis was tested in more than 1600 patients with malignant tumours from Poland. Among the patients screened, 16 carriers of the 657Δ5 mutation and 10 of the R215W variant were found while only 9 and 4 cases, respectively, were expected. The average age of carriers did not differ from that of non-carriers with the same tumours. Malignant tumours were reported in about 18% of close relatives (parents and siblings) of 14 657Δ5 mutation carriers interviewed, versus 8% in a large, approximately age-matched control group. These results confirm that heterozygous carriers of *NBS1* mutations indeed have an enhanced risk to develop malignant tumours, in particular melanoma, breast cancer, and colorectal cancers and, perhaps, also lymphoma [63].

5. Growth retardation and the cell cycle in NBS

Growth retardation is a cardinal symptom of NBS. Although birth weight and size are typically within the normal range, short stature is usually apparent by 2 years of age. Body weight remains proportional to body length [8]. A reduced proliferative capacity of patient cells could be inferred from this symptom and is supported by several studies. Mice with targeted hypomorphic mutations of *Nbn* are also growth retarded and fibroblasts isolated from these animals have a two to five-fold slower proliferation than controls [31]. Similarly, targeted mutation of the chicken gene in DT40 cells lead to a prolonged cell cycle and slow cell growth [18].

Disturbances of the cell cycle in NBS patient cells have been repeatedly reported although there is some controversy about the proficiency of cell cycle checkpoints in NBS. Several studies have examined the accumulation of p53 in

response to IR, most have found, as in A–T cells, delayed accumulation of p53 and subsequently reduced activation of p21 and other p53-dependent genes [9,64,65]. This finding would be expected to lead to a deficiency in the G1/S-Phase checkpoint, however, normal G1 arrest has been found in NBS fibroblast lines [66]. In lymphoblastoid NBS B-cells, G1 arrest was defective [14] or attenuated [9]. The data from mice carrying hypomorphic mutations in *Nbn* are particularly interesting in this respect. In one report, the p21 response to IR in mouse fibroblasts was indistinguishable from controls indicating that the G1/S checkpoint is normal [32]. In fibroblasts from other mice, entry into S-phase from G1 in un-irradiated cells was clearly reduced and this correlated, as expected, with a high basal level of p21 expression [31]. This was interpreted as a defect in the G1/S transition, leading albeit to a stronger rather than weaker checkpoint function [31]. A difference in G1 arrest was observed by Girard et al. depending on IR dose. At low doses, primary NBS fibroblasts showed a defective G1/S checkpoint which was not observed at higher doses [67].

Cells which enter G2 with damaged DNA remain in G2 until the damage has been repaired before entering mitosis. Thus a prolonged G2 phase is an indication of a functioning G2/M checkpoint. If the G2 phase accumulation persists then this is an indication that DNA damage has not been repaired. If cells do not remain in G2 but proceed to mitosis then this is an indication that the G2/M checkpoint is defective. The data for NBS cells are here, again, discrepant. Measuring the proportion of cells in mitosis after an IR treatment indicated a non functioning G2/M checkpoint in mouse fibroblasts with hypomorphic mutations in *Nbn* [32] whilst in EBV transformed B-lymphocytes from NBS patients the G2/M checkpoint was found to operate normally [68]. Accumulation in G2 in primary patient fibroblasts has been found to be comparable to controls [67] whilst in SV40 transformed fibroblasts [69] and in mouse fibroblasts [31] the mutation of *Nbn* has led to a longer G2 accumulation than in control cells. Clearly these differing results can in part be attributed to the use of different cell types and the difference in mutation between the human cells and the mouse cells.

One further analysis of lymphoblastoid NBS cells has indicated a striking defect in the G2/M checkpoint with NBS cells still entering mitosis one hour after a 1.5Gy irradiation [70]. In the same cells, the phosphorylation of CHK2 was found to be delayed. CHK2 is involved in the G2/M checkpoint through its inactivating phosphorylation of Cdc25c, which is required for formation of maturation promoting factor (MPF) and entry into mitosis. Attenuated phosphorylation of CHK2 after IR could, therefore, explain why NBS cells enter mitosis unchecked. CHK2 is phosphorylated by ATM, as is nibrin/Nbs1 itself. Phosphorylated nibrin/Nbs1, although not a kinase, promotes the phosphorylation of many target molecules by ATM, so also, CHK2 [35,70].

CHK2 may also be involved in a further major hallmark of NBS cells: radioresistant DNA synthesis (RDS). CHK2 inactivates the phosphatase, Cdc25A, required for activation of cyclinE/Cdk2 and thus for loading of Cdc45 onto DNA replication origins. However, the MRN complex is not believed to be involved in this particular CHK2 function [71,72]. A second pathway for inhibition of DNA synthesis after IR seems to involve SMC1, a cohesin protein. Efficient phosphorylation of SMC1 by ATM requires nibrin/Nbs1 [35]. When a dominant-negative mutant of SMC1, which cannot be phosphorylated, was over-expressed in normal cells, an RDS-phenotype was produced [73]. Apparently inhibition of DNA replication after IR is effected by independent pathways involving SMC1 or CHK2, the more severe RDS phenotype in A–T cells is explained by the dependence of both pathways on ATM, while nibrin/Nbs1 promotes only the SMC1 pathway [71].

There is evidence that the pathway disturbed in NBS interacts with the pathway affected in patients with the autosomal recessive chromosome instability disorder, Fanconi anaemia (FA). The Fanconi anaemia protein, FANCD2, is monoubiquitinated by a complex of proteins in response to DNA damage, mutations in these genes all lead to Fanconi anaemia. In addition, FANCD2 is phosphorylated by ATM in a nibrin/Nbs1-dependent fashion [74,75]. FANCD2 phosphorylation is related to the RDS phenotype of cells with biallelic mutations in *FANCD2*, a phenotype which is not found in cells mutated in the other FA genes. Phosphorylation of nibrin/Nbs1 by ATM is independent of FANCD2, placing this event upstream of FANCD2 phosphorylation. Whilst formation of subnuclear MRE11-foci after IR is normal in primary fibroblasts with *FANCD2* mutations, it is completely abrogated after immortalisation with SV40 large T-antigen [76]. Other viral oncoproteins have also been shown to interact with the MRE11-pathway [77], however, the additive effect of the viral oncogene large T-antigen and mutations in *FANCD2* suggests a specific interaction between FANCD2 and MRE11. The convergence of the FA and ATM/NBS pathways is discussed in greater detail in the review by Alan D'Andrea in this issue.

In summary, the role of nibrin/Nbs1 in the checkpoints of cell growth and division has been the subject of intensive research and has established nibrin/Nbs1 as a major player in the control of the cell cycle, whether independently of RAD50 and MRE11 remains to be demonstrated. Similarly, the implicit expectation that the nibrin/Nbs1 fragments of hypomorphic mutations can at least partially, and at least in some cells, uphold these checkpoint functions has not yet been proved.

6. Meiosis and infertility

Ovarian dysgenesis with primary amenorrhea and elevated gonadotrophin levels, has been described in several NBS patients [78,79]. Homologous recombination during meiosis is initiated by DSBs and yeast strains with mutations in *Mre11* or *Xrs2* are defective in meiotic recombination [80,81]. Thus ovarian dysgenesis in NBS could be the result

of meiotic failure. Nibrin/Nbs1 colocalises with MRE11 at the telomeres of meiotic chromosomes [82]. Nibrin/Nbs1 has previously been found associated with telomeres in cells [83,84] and the complementation of NBS patient cells with nibrin/Nbs1 is sufficient to correct their characteristically shortened telomeres [85]. What the specific role of nibrin/Nbs1 in meiotic chromosomes could be and how its failure leads to ovarian dysgenesis is still unclear.

With respect to ovarian dysgenesis the two mouse models are discrepant. Whilst one nibrin/Nbs1 knock-out has no negative effect on gonad function and fertility [32], in the other knock-out oogenesis is completely abolished in degenerated ovaries lacking oocytes and follicles [31]. In the latter case, male mice were normally fertile, this mouse seems to reflect more exactly the human phenotype in this respect.

7. Population genetics of NBS

The *NBS1* founder mutation, 657Δ5, is present in individuals of eastern, western, and southern Slavic origin. Thus, the original mutational event occurred while the Slavic people were still together in their homeland, the Pripet swamp, more than 1500 years ago. We found an unexpectedly high carrier frequency of this mutation under the new-born of five different Slavic populations: Czech Republic (8/1234), Poland (18/3569), Ukraine (Lvov, 5/908), Bulgaria (9/1002), and the sorbs (family studies, 8/326) [86]. These frequencies are much higher than that found, for example, in Berlin (1/900). If one excludes that this rather high allelic frequency is due simply to chance, then it must result from a difference in reproduction rate of the gene carriers. Our preliminary data suggest that the heterozygous mothers have a higher fertility due to a lower miscarriage rate. Since about 50% of all spontaneous abortions are due to aneuploidy this would imply that the *NBS1* heterozygotes have a lower non-disjunction rate compared to normal homozygous females [87].

The almost random distribution of *NBS1* heterozygotes in Czechia allows the estimation of the incidence of homozygotes at 1/68,000 live-births. The number of patients diagnosed, however, was 1/238,000, and thus clearly lower than expected. This discrepancy between expected and observed numbers of NBS patients is obviously due to underdiagnosis. The diagnosis in those patients observed was made at a relatively late age (mean 7.1 ± 4.5). At that time, between 14 and 37% of the patients had already developed a tumour [86]. The extreme radiosensitivity of NBS patients together with such a late diagnosis is a hidden danger as patients with a malignancy are subjected to chemo and radiotherapy which could lead to secondary tumours and even mortality. Therefore, it is absolutely essential to postulate criteria for the early and certain recognition of NBS in order to avoid the conventional treatment protocols in cases where a malignancy is already present.

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