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Deficiency of the DNA repair protein nibrin increases the basal but not the radiation induced mutation frequency *in vivo*

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Abstract

Nibrin (NBN) is a member of a DNA repair complex together with MRE11 and RAD50. The complex is associated particularly with the repair of DNA double strand breaks and with the regulation of cell cycle check points. Hypomorphic mutation of components of the complex leads to human disorders characterised by radiosensitivity and increased tumour occurrence, particularly of the lymphatic system. We have examined here the relationship between DNA damage, mutation frequency and mutation spectrum *in vitro* and *in vivo* in mouse models carrying *NBN* mutations and a *lacZ* reporter plasmid. We find that *NBN* mutation leads to increased spontaneous DNA damage in fibroblasts *in vitro* and high basal mutation rates in lymphatic tissue of mice *in vivo*. The characteristic mutation spectrum is dominated by single base transitions rather than the deletions and complex rearrangements expected after abortive repair of DNA double strand breaks. We conclude that in the absence of wild type nibrin, the repair of spontaneous errors, presumably arising during DNA replication, makes a major contribution to the basal mutation rate. This applies also to cells heterozygous for an *NBN* null mutation. Mutation frequencies after irradiation *in vivo* were not increased in mice with nibrin mutations as might have been expected considering the radiosensitivity of NBS patient cells *in vitro*. Evidently apoptosis is efficient, even in the absence of wild type nibrin.

Keywords

DNA damage; Mismatch repair; Haploinsufficiency

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Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.mrfmmm.2014.07.001>.

1. Introduction

Nijmegen Breakage Syndrome (NBS) belongs to a group of DNA-repair deficiency syndromes in which the mutated gene is directly involved in the repair of DNA double strand breaks (DSBs), in the case of NBS, the gene nibrin (*NBN*) [1]. Patients with NBS show symptoms such as immunodeficiency, sensitivity to ionising irradiation (IR) and an extreme risk for cancers, particularly of the lymphoreticular system [2]. The incidence for cancer among patients is approximately 1000 times higher than for the normal population [3]. Heterozygote carriers of mutations in *NBN* also have an apparently higher cancer risk, although cancer occurs much later and shows no obvious organ specificity [4]. Over 95% of NBS patients carry the major *NBN* mutation, a 5 bp deletion (c.657 661del5, p.K219fsX19), which leads to a truncated amino terminal fragment and, significantly, alternative translation from a cryptic start codon upstream of the deletion [5]. As a result, a 70 kDa carboxy-terminal protein fragment, p70-nibrin, is produced. This protein fragment maintains partial nibrin function [6,7]. In contrast, null mutations in mice are embryonically lethal due to massive apoptosis at the blastocyst stage [8,9].

Nibrin is a component of a highly conserved trimeric complex, together with MRE11 and RAD50, which is involved through its DNA binding and nuclease activities in the repair of DSBs by both non-homologous end joining (NHEJ) and homologous recombination (HR). In addition, its function in the DNA damage-induced activation of ATM contributes to the regulation of the cell cycle as part of the DNA damage response. Although the consequences of a failure in DSB repair for tumorigenesis are understandable, little is actually known about the correlation between DNA damage, mutation frequency and mutation type in the absence of wild type nibrin.

In order to analyse mutation frequencies and mutation spectra when nibrin is disrupted, we have utilised a conditional null mutant mouse [6] and a humanised NBS mouse which carries the human c.657 661del5 *NBN*-allele on a null mutant murine *Nbn* background [7]. Mice homozygous and heterozygous for nibrin mutations were examined since we have previously suggested that *NBN* is a haploinsufficient tumour suppressor gene [10]. We have bred these mice with a transgenic mutagenesis mouse harbouring 21 tandem copies of the bacterial *lacZ* gene, stably integrated on chromosome 11 [11]. The mutation frequency and mutation spectrum was determined in DNA extracted from fibroblasts or lymphatic tissues by rescuing the reporter plasmid and transformation of *E. coli*.

We find that null mutation of *Nbn* leads to increased DNA damage *in vitro*, even in the absence of an external genotoxic treatment. Distinct mutation spectra indicate that the molecular origin of spontaneous and radiation induced base mutations is different. *In vivo* we find the basal mutation frequency measured in lymphatic tissue of the humanised NBS mice is approximately 2.5-fold higher than in control mice. We conclude that spontaneous DNA damage, incurred probably during replication, is a major source of mutations in nibrin deficient cells. Surprisingly, after irradiation, *in vivo* mutation frequencies were not significantly increased in the humanised NBS mice. This is attributed to efficient apoptosis even in the absence of wild type nibrin and suggests that spontaneous base mutations may have a more significant role in tumorigenesis in NBS patients than previously appreciated.

2. Materials and methods

2.1. Mice breeding and irradiation

For the *in vivo* experiments, the pUR288 *lacZ*-transgenic mouse line 30 [12], harbouring 21 copies of the bacterial *lacZ* gene, was crossbred with the humanised NBS mouse which harbours the human *NBN* gene with the 5 bp founder mutation on a null-mutant murine *Nbn* background (*Nbn*^{-/-} *NBN*^{del5}; [7]). The *lacZ*-transgenic mouse was also crossbred with our previously described cre recombinase inducible null mutant mouse (*Nbn*^{ins6/lox6}; [6]) and fibroblasts isolated for *in vitro* experiments in the complete absence of nibrin. All mice had a mixed C57BL/6/129Sv background. Geno-typing was conducted by PCR analysis on DNA isolated from tail biopsies of the mice. Whole-body irradiation of mice was performed with the Cs¹³⁷-irradiation device OB29/4 S/N 010 (Gamma-Service Medical GmbH) after sedation with Isoflurane. Mice were irradiated on 5 consecutive days with 0.5 Gy per day. Two days after the last irradiation animals were sacrificed and organs frozen in liquid nitrogen until extraction of DNA for the *lacZ* mutation assay.

2.2. Cell culture and cre recombinase treatment

Fibroblasts were derived from ear explants of control wild type mice (*lacZ-Nbn*^{+/+}), humanised mice (*lacZ-Nbn*^{-/-} *NBN*^{del5}) and heterozygous mice (*lacZ-Nbn*^{ins6/lox6}). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Life Technologies, Germany) supplemented with 5% glucose and 10% foetal calf serum (FCS). Cell culture conditions were 37 °C and 5% CO₂. Environmental oxygen was reduced to 4%. To generate homozygous null mutant fibroblasts *in vitro*, *lacZ-Nbn*^{ins6/lox6} cells were incubated in suspension on two consecutive days with 1 µM cre recombinase (Excellgen, Rockville) for 2 h. Loss of nibrin was determined by PCR and western blot analysis as previously described [6]. Fibroblast cultures were irradiated in 75 cm² flasks on ice with a single dose of 5 Gy and cells subsequently examined in the comet assay or *lacZ* assay.

2.3. Determination of mutation frequency and mutant classification

For the mutation frequency assay, cells were harvested 24 h after irradiation. The cell pellets were washed with PBS once and the DNA was extracted using standard methods. DNA was extracted from mouse organs flash frozen in liquid nitrogen until use. DNA was isolated by standard phenol/chloroform extraction from pulverised tissues treated previously with proteinase K.

Mutant frequencies were determined as described earlier [12]. Briefly, 10–20 µg of genomic DNA from *lacZ*-transgenic mice or fibroblasts was digested with *Hind*III and the *lacZ*-reporter plasmids recovered on magnetic beads pre-coated with a *lacZ/lacI* fusion protein. After washing, the plasmids were eluted with isopropylthio-β-galactoside (IPTG) and recircularised with T4-ligase. Plasmid DNA was electroporated into the *E. coli* C strain (*GalE*⁻, *LacZ*). To determine the mutation frequency, the bacteria were plated on two different agars containing either 5-Brom-4-chlor-3-indoxyl-β-d-galactopyranoside (X-gal) or phenyl-β-d-galactopyranoside (P-gal). As mutant plasmids will only grow on the selective P-gal plate, the mutation frequency is calculated as the number of mutants divided by the

number of total recovered plasmids (X-gal plate) multiplied by the appropriate dilution factor.

Mutant plasmids from the P-gal plate were transferred to 150 µl LB Medium in 96-well plates and cultured for at least 8 h before a colony-PCR for the *lacZ* gene was performed. PCR products were digested with *AvaI* and *HindIII* and the fragments were resolved on a 1% agarose gel. The mutation rate was corrected as previously described for *HindIII* star activity and cloning artefacts based on the restriction enzyme patterns [13]. Positive clones were sequenced directly or after mini preparation of plasmid DNA using the previously described overlapping primers in the *lacZ* sequence [14]. Identical mutations due to clonal expansion of cells were counted only once.

2.4. Single cell gel electrophoresis assay (comet assay)

The comet assay was performed under alkaline conditions as described [15]. Briefly, frosted-end slides were coated with one layer of 1% normal melting agarose (NMA) in PBS, dried overnight and then covered with a second layer of 0.5% NMA. 5×10^5 harvested fibroblasts were mixed with 0.7% low melting agarose and layered onto the prepared slides. Lysis carried out in the dark at 4 °C for 1 h followed by unwinding of the DNA under alkaline conditions for 20 min. Electrophoresis was for 20 min followed by neutralisation. After drying, slides were stained with 1 µM 4',6-diamidin-2-phenylindol (DAPI). Each data point is based on the analysis of at least 150 comets per coded sample. The scoring of the comets and the calculation of the tail moment ([percent of DNA in the tail] $\times$ [tail length]) was performed with the CASP software [16].

3. Results

3.1. Null and hypomorphic nibrin mutation increases replication based DNA damage and increases the induced mutation frequency in vitro

The trimeric Mre11/Rad50/Nibrin complex is involved in the detection and repair of DNA DSBs as indicated by the increased chromosomal breakage typical for NBS patient cells. As shown by the comet assay in Fig. 1, both our conditional null mutant mouse fibroblasts and the humanised mouse fibroblasts display a comparatively high initial comet tail moment in comparison to control cells ($p = 0.0424$, two-tailed Mann–Whitney *U* test). The alkaline comet assay used here detects not only single and double-strand breaks but also apurinic and apyrimidinic sites, which are thus alkali labile, and similarly, some damaged bases [17]. Surprisingly, DNA damage after irradiation with 5 Gy is only slightly increased for control cells and the null mutant cells, however, not at all for the humanised mouse cells (Fig. 1). This difference between null mutant cells and cells expressing the hypomorphic nibrin fragment may indicate the residual DNA repair capacity of the mutant fragment. These data suggest that spontaneous DNA damage, probably incurred during DNA replication, is increased in the absence of wild type nibrin and could contribute significantly to an increased mutation frequency.

To examine this question in more detail, we measured mutation frequency and mutation spectra *in vitro* for untreated and irradiated null mutant *Nbn* mouse cells using the *lacZ*

reporter assay. As detailed in supplementary Table S1a, the basal mutation frequency in null mutant *Nbn* mouse cells *in vitro* is 3.2×10^{-5} and rises slightly to 5.2×10^{-5} after 5 Gy irradiation. Control wild type cells had comparable mutation frequencies of 2.8×10^{-5} and 4.6×10^{-5} , respectively. Whilst wild type cells had mostly base substitutions and small indels as spontaneous mutations, the null mutant *Nbn* mouse cells had a large proportion (25%) of complex insertions and deletions involving mouse sequences (Fig. 2A). Such mutations are likely the result of erroneous homologous recombination or non-homologous end joining. A comparably high level of complex mutations in wild type cells was only seen after 5 Gy irradiation (Fig. 2B). Whilst such mutations are also found in null mutant *Nbn* mouse cells after irradiation, there is a striking increase in the proportion of base transversions in comparison to wild type cells (Fig. 2B).

Examining the specificity of base changes indicated that in unirradiated null mutant *Nbn* cells the transition G:C>A:T dominates whilst in irradiated cells the transversion C:G>A:T is particularly frequent (supplementary Table S1b). The transversions T:A>G:C and C:G>G:C were observed after irradiation of null mutant *Nbn* cells but not wild type cells. These characteristic mutation spectra indicate a distinct molecular origin for mutations incurred in the absence of wild type nibrin during normal cell growth or after a mutagenic treatment.

3.2. Hypomorphic mutation of nibrin leads to an increased basal mutation frequency *in vivo* with an increased proportion of single base transitions rather than transversions

Considering the increased mutation frequency measured *in vitro*, we turned to an *in vivo* analysis more relevant for the disease NBS which is characterised by lymphatic malignancies arising early in childhood with no known associated environmental stress [18]. We examined the major lymphatic organs of control mice and humanised mice for mutation frequency and spectrum using the *lacZ* assay (see supplementary Table S2a). As shown in Fig. 3, the mutation frequency was on average 10.5×10^{-5} in the spleen, bone marrow and thymus of control mice, a value comparable to that measured in other mice using the *lacZ* assay [19–21]. In comparison, the same organs in the humanised mice had more than double this mutation frequency at 23.9×10^{-5} ($p = 0.0018$ two-tailed Mann–Whitney *U* test).

In the absence of wild type nibrin in fibroblasts, irradiation leads to an increased proportion of base transversions (Fig. 2B), suggesting that this particular mutation can be attributed to the DSB repair deficiency caused by nibrin loss. In contrast, as shown in Fig. 4A, the increased basal mutation frequency in the humanised NBS mice *in vivo* is characterised by base transitions rather than transversions. Again, fewer complex mutations, the consequence of recombination events, are observed in the humanised mice than in control mice.

3.3. The *in vivo* radiation induced mutation frequency and mutation spectrum is not altered by hypomorphic mutation of nibrin

Considering the increased mutation frequency observed after irradiation in the null mutant fibroblasts, we examined the mutation frequency *in vivo* in the humanised mice after repeated low-dose irradiation. As detailed in supplementary Table S3 and shown in Fig. 5, mutation rates dropped in comparison to unirradiated humanised mice (supplementary Table

S2 and Fig. 3) from 22.12×10^{-5} to 5.91×10^{-5} in the thymus and from 25.65×10^{-5} to 14.14×10^{-5} in bone marrow. In the spleen, mutation frequencies were comparable with and without irradiation, 23.79×10^{-5} and 23.1×10^{-5} , however, significantly higher after irradiation than in the control mice ($p = 0.0087$, two-tailed Mann–Whitney U test). Although the proportions of base mutations (no change) and complex (size change) mutations varied amongst the three organs examined, they were in each case comparable between humanised mice and control mice (supplementary Table S3).

3.4. The *in vivo* basal mutation spectrum in the thymus of *Nbn* heterozygous mice resembles that of mice with a with a hypomorphic mutation in *Nbn*

Since heterozygous relatives of NBS patients have a significantly increased lifetime cancer risk [4] we were interested to see whether this is reflected in a measurably increased mutation frequency *in vivo* in mice carrying just one functional *Nbn* allele. In the *lacZ* assay, such heterozygous mice had an average mutation frequency of 14.3×10^{-5} in the three lymphatic organs examined, which is not significantly different from that of control mice (10.4×10^{-5}). However, the spectrum of mutations was indeed shifted from that of control mice (supplementary Table S2b). As shown in Fig. 4B, complex rearrangements, a mutation which represented 25% of mutations in the thymus of control animals were not found at all amongst the 4 heterozygous mice examined. In contrast, as in the case of *Nbn*^{-/-}*NBN*^{del5} mice, base transitions dominated the basal mutation spectrum.

4. Discussion

Although the spontaneous chromosomal breakage observed in lymphocytes and fibroblasts from NBS patients indicates increased DNA damage due to repair failure, most biochemical analyses find no gross deficiency in the rejoining of DSBs induced by ionising radiation [22,23]. This can reflect insensitivity of the assays used, but more likely stems from the fact that NBS cells have a hypomorphic mutation in the *NBN* gene and that a functionally partially active nibrin protein is indeed produced [6,7]. Using a conditional null mutant in murine cells we have previously shown extreme chromosomal breakage, massive cell cycle defects and rapid cell death in the complete absence of nibrin. However, exogenous expression of human nibrin with the hypomorphic mutation prolonged survival of null mutant cells *in vitro* [6]. Similarly, lymphocytes from the humanised mice expressing hypomorphic nibrin [7] show about half the chromosomal breakage observed in lymphocytes from our null mutant mice.

Although we did detect here a high level of DNA damage, DSBs, SSBs and alkali labile sites, in these null mutant cells, their mutation spectrum did not reflect erroneous repair of this kind of DNA lesion. Small deletions, due mainly to NHEJ, and complex rearrangements, due in general to HR, were not significantly increased in irradiated cells in comparison to unirradiated cells. However, the base mutation spectrum of irradiated null mutant *Nbn* cells differed clearly from that of untreated null mutant *Nbn* cells: transversions dominate in the irradiated cells, transitions in the untreated cells. Transversions have been previously reported as common in irradiated cells [24,25]. The transversions C:G>A:T and C:G>G:C, observed in the irradiated nibrin deficient cells are believed to originate from the

oxidation product of guanine, 8-oxo-deoxyguanosine and its own oxidation product, imidazolone, respectively [26]. Such oxidation products in DNA are expected by an indirect effect after formation of radicals from water through ionising radiation, indeed, for every DSB generated by ionising radiation there are approximately 100 damaged bases [27].

The common transition, G:C>A:T, in unirradiated cells *in vitro* may be caused by the tautomer of guanine, enol guanine, which pairs with thymine during replication leading to G>A transitions unless detected by the mismatch repair pathway. In cells lacking the mismatch repair gene *Msh6*, the proportion of G:C>A:T transitions is strongly increased [21]. The transition T:A>C:G, previously reported as a consequence of UV radiation was not observed in the cells examined here.

The determination of mutation frequency *in vivo* demonstrated a significantly increased basal mutation rate in the humanised *Nbn*^{-/-}*NBN*^{del5} mouse compared to control mice in all three lymphatic organs analysed. The two-fold increase in mutation rate is comparable to the 1.2-fold increase found in the liver of NER deficient *Xpa*^{-/-} mice [28] or the 1.3-fold increase in NHEJ deficient *Ku80*^{-/-} mouse spleens [19]. In contrast, mismatch repair deficient *Mlh1*^{-/-} and *Pms2*^{-/-} mice had 18-fold and 13-fold more spontaneous mutations, respectively [29]. These differences illustrate dramatically the importance of mismatch repair proteins in removing base incorporation errors after replication.

The increased mutation rates reported for *Xpa* and *Ku80* mutants are not expected to be due to misincorporation during replication. In the case of *Nbn* mutants, however, replication errors may indeed play a role. The mutation spectrum in the thymus of *Nbn*^{-/-}*NBN*^{del5} mice showed an increased proportion of base mutations and a reduction in complex mutations and small deletions in comparison to control mice. Again the major base mutation was the transition, G:C>A:T. Since there were relatively few base transversions, the mutation so characteristic for mouse cells completely lacking nibrin, we can conclude that the p70-nibrin fragment generated from the hypomorphic *NBN*^{del5} allele is functional in at least this aspect of DNA repair.

The transition G:C>A:T can arise when enol-guanine mispairs during replication suggesting that replication errors are a major source of DNA mutation in *Nbn*^{-/-}*NBN*^{del5} mouse cells and, presumably, NBS patients too. Why should a hypomorphic mutation in a gene associated usually with the repair of DNA double-strand breaks lead to this type of mutation? The answer may be the involvement of nibrin in mismatch repair. Several lines of evidence have previously suggested that nibrin and the mismatch repair proteins are molecular partners. Firstly, the trimeric complex MRE11/RAD50/NBN is part of a larger complex, BASC, in which the major mismatch repair proteins, MLH1, MSH2 and MSH6 are also located [30]. Secondly, nibrin was found specifically bound to MLH1 in cells treated with temozolomide, an agent which generates the guanine adduct, O⁶-methylguanine [31]. Thirdly, MNNG treatment of cells leads to replication independent accumulation and association of PCNA and mismatch repair proteins accompanied by phosphorylation of chromatin-bound nibrin [32].

Although the exact role of nibrin in the recognition and repair of mismatches remains to be determined, the reports cited above suggest that mutation of nibrin could result in mismatch repair failure and a concomitant increase in transitions, such as G:C>A:T. Considering the lack of complex rearrangements and small deletions seen in humanised NBS mouse cells in the analyses reported here, the role of nibrin in DSB repair may well be less relevant for mutagenesis than its role in repair of mismatches during DNA replication.

The *in vivo* mutation frequency in the bone marrow and thymus of control and humanised mice was not significantly increased 7 days after irradiation. This clearly illustrates that elimination of damaged cells by apoptosis is functional in these organs in the presence of hypomorphic nibrin. Clearly nibrin is required for radiation induced apoptosis and the p70-nibrin product of the hypomorphic c.657 661del5 mutation is sufficient to uphold this function. Indeed, even enhanced apoptosis has been previously reported in irradiated NBS patient cells [33] and in a haploinsufficient mouse model [34]. Furthermore, the early embryonic lethality of *Nbn* null mutants was due to massive apoptosis [8]. Expression of null mutant *Nbn* specifically in the brain lead to cerebellar defects due to apoptosis of post mitotic neurons, an effect which was rescued on a p53^{-/-} background [35]. Interestingly apoptosis would seem to be somewhat less efficient in the spleen since there is a significantly higher mutation frequency in this organ in the humanised mouse than in controls. We propose that while the radiosensitivity of NBS patients can be attributed to the increased DNA damage response in terms of apoptosis, the increased cancer risk in the absence of environmental mutagenesis is due to the failure to repair spontaneous DNA damage, perhaps coupled with a specific apoptosis deficiency in the spleen.

In an epidemiological study of relatives of NBS patients we have previously reported an increased tumour risk for heterozygotes [4]. Similarly, in many, but not all reports, heterozygous *NBN* mutations have been found more frequently amongst cancer patients than in the general population [36–38]. Examining mice heterozygous for a null mutation, *Nbn*^{ins6}, indicated an increased tumour occurrence without loss of the remaining wild type allele [8]. Similarly, the same *Nbn*^{ins6} mutation on a mammary tumour prone MMTV-neu background lead to the development of highly metastatic mammary tumours [34]. We have examined here the mutation frequency in mice heterozygous for a null mutation in *Nbn* and although we find no evidence for an increased basal mutation rate, we do see a shift in mutation spectrum in comparison to wild type mice. This shift, a reduction in the proportion of complex mutations and an increase in base transitions, is practically identical to the spectrum of the humanised mice *in vivo* and very similar to that of *Nbn* null mutant fibroblasts *in vitro*. This finding supports our concept of NBN as a haploinsufficient tumour suppressor gene [10] and suggests furthermore that the failure to repair spontaneous DNA lesions is a major contributor to the increased cancer risk not only in NBS patients but probably also in heterozygous carriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

DNA damage in untreated and irradiated *Nbn* null mutant and control fibro-blasts. The columns give DNA damage as measured with the comet assay (comet tail moment) for murine fibroblasts with the given genotypes. Cells were either untreated (-) or irradiated 10 minutes before lysis with 5 Gy (+). At least 150 nuclei were measured per sample. The chart shows the average results of three experiments, the error bars give the standard deviation.

Fig. 2.

Basal and induced mutation in *Nbn* null mutant fibroblasts. Conditional null mutant *Nbn*^{ins6/lox6} fibroblasts carrying the *lacZ* reporter were treated with cre recombinase to induce homozygosity for *Nbn* null mutations before measuring mutation frequency of the *lacZ* reporter as described in Materials and Methods. Mutation spectra were determined by sequencing of rescued *lacZ* plasmids (A) Basal mutation rate in wild type control mouse cells and *Nbn* null mutant mouse cells. (B) Mutation rate in wild type control mouse cells and *Nbn* null mutant mouse cells 24 h after irradiation with 5 Gy. Full data are given in supplementary Tables S1 and S2.

Fig. 3.

Basal *in vivo* mutation frequency in spleen, thymus and bone marrow for *Nbn*^{+/+} and *Nbn*^{-/-} *NBN*^{del5} mice. Control mice (*Nbn*^{+/+}, white boxes) and humanised mice (*Nbn*^{-/-} *NBN*^{del5}, grey boxes) carrying the *lacZ* reporter were examined for mutation frequency as described. The box plot shows the results from 3 to 5 control and 4 to 6 humanised mice. The mean and median are indicated.

Fig. 4. Basal *in vivo* thymus mutation spectra in control, humanised and heterozygous NBS mice. Mutations were determined by DNA sequencing of plasmids rescued from the thymus of mice carrying the *lacZ* reporter as in the experiments shown in Fig. 3. (A) Control mice (*Nbn*^{+/+}) and humanised mice (*Nbn*^{-/-} *NBN*^{del5}). (B) Heterozygous *Nbn*^{+/-} mice. The legend gives the four types of mutation detected.

Fig. 5.

Irradiation induced *in vivo* mutation frequency in spleen, thymus and bone marrow for *Nbn*^{+/+} and *Nbn*^{-/-}*NBN*^{del5} mice. Control mice (*Nbn*^{+/+}, white boxes) and humanised mice (*Nbn*^{-/-}*NBN*^{del5}, grey boxes) carrying the *lacZ* reporter were examined for mutation frequency as described. The box plot shows the results from 3 to 6 control and 3 to 5 humanised mice. The mean and median are indicated.