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BRCA2 is required for neurogenesis and suppression of medulloblastoma

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Defective DNA damage responses in the nervous system can result in neurodegeneration or tumorigenesis. Despite the importance of DNA damage signalling, the neural function of many critical DNA repair factors is unclear. BRCA2 is necessary for homologous recombination repair of DNA and the prevention of diseases including Fanconi Anemia and cancer. We determined the role of BRCA2 during brain development by inactivating murine *Brca2* throughout neural tissues. In striking contrast to early embryonic lethality after germ-line inactivation, *Brca2*$^{LoxP/LoxP}$*;Nestin-cre* mice were viable. However, *Brca2* loss profoundly affected neurogenesis, particularly during embryonic and postnatal neural development. These neurological defects arose from DNA damage as *Brca2*$^{LoxP/LoxP}$*;Nestin-cre* mice showed extensive γH2AX in neural tissue and p53 deficiency restored brain histology but lead to rapid formation of medulloblastoma brain tumors. In contrast, loss of the Atm kinase did not markedly attenuate apoptosis after Brca2 loss, but did partially restore cerebellar morphology, supporting a genomic surveillance function for ATM during neurogenesis. These data illustrate the importance of Brca2 during nervous system development and underscore the tissue-specific requirements for DNA repair factors.

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Introduction

Within the nervous system, appropriate responses to DNA damage are required to maintain homeostasis and prevent disease (McMurray, 2005; Lee and McKinnon, 2006). DNA double-strand breaks (DSBs) trigger a signalling cascade that leads to repair and resolution of the break, or as is frequent in the developing nervous system, apoptosis. The repair of DNA DSBs occurs via two mechanistically distinct pathways non-homologous end-joining (NHEJ) or homologous recombination (HR). Each pathway involves a distinct repertoire of repair enzymes and associated proteins. HR requires a group of RAD51-related proteins and a variety of other factors, including BRCA2, to ensure high-fidelity DNA repair using an undamaged homologous DNA template to replace an adjacent damaged one (Thompson and Schild, 2002; West, 2003). In contrast, NHEJ facilitates direct modification and ligation of the two DNA ends at the DSB. Efficient NHEJ requires among other factors, KU heterodimers (KU 70 and KU 80), DNA-PKcs, DNA ligase IV (LIG4) and XRCC4 (Lees-Miller and Meek, 2003; Lieber *et al*, 2003; Mills *et al*, 2003; O'Driscoll and Jeggo, 2006). Inactivation of either of these pathways in mice can lead to embryonic lethality or tumor development.

Broad insights illuminating DNA repair and nervous system development have been gained from mice in which gene targeting has disabled various DNA repair pathways (Friedberg and Meira, 2006). For example, inactivation of NHEJ in the mouse can lead to defective neurogenesis or brain tumors depending on the particular gene disruption and genetic background (Gao *et al*, 1998, 2000; Lee and McKinnon, 2002; Yan *et al*, 2006). Disruption of HR can also affect neural development (Deans *et al*, 2000; Orii *et al*, 2006), although when some key HR components such as Brca2 or Rad51 are inactivated, the result is early embryonic lethality during gastrulation (before neural development) (Lim and Hasty, 1996; Tsuzuki *et al*, 1996; Ludwig *et al*, 1997; Sharan *et al*, 1997; Suzuki *et al*, 1997). Moreover, DNA repair activity exhibits a clear tissue- and cell type specificity, as in the nervous system, HR only functions in proliferating cells, while NHEJ is particularly important in differentiated neural cells (Orii *et al*, 2006). Together, these data show that DNA DSB repair pathways operate in a complementary manner during neural development.

BRCA2 has a key role in HR and substantial DNA repair-associated defects result from its inactivation, including DNA damage hypersensitivity, chromosomal rearrangements and defective mammalian gametogenesis (Sharan *et al*, 1997; Tutt and Ashworth, 2002; Yang *et al*, 2002; Daniels *et al*, 2004). BRCA2 is a large protein of 3418 amino acids that physically interacts through its carboxyl terminus with RAD51, a protein essential for HR, and is responsible for the translocation of RAD51 to sites of DNA damage processing (Powell *et al*, 2002; Pellegrini and Venkitaraman, 2004; Shivji and Venkitaraman, 2004; Yang *et al*, 2005). BRCA2 has also been implicated in cell-cycle regulation via interaction with BRAF35 or Smad3 (Marmorstein *et al*, 2001; Preobrazhenska *et al*, 2002), and a role during an intra-S phase checkpoint has also been reported (Taniguchi *et al*, 2002). Brca2 functions as a tumor suppressor, as its loss confers susceptibility to breast, ovarian and brain tumors (Hughes-Davies *et al*, 2003; Offit *et al*, 2003; Shivji and Venkitaraman, 2004). The BRCA2-binding protein BALB2 is important for the tumor suppressor function of BRCA2 (Xia *et al*, 2006; Erkko *et al*, 2007; Rahman *et al*, 2007).

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Biallelic hypomorphic mutations of *BRCA2* are also responsible for some cases of Fanconi Anemia (FA), a rare autosomal recessive cancer susceptibility syndrome characterized by congenital abnormalities, progressive bone narrow failure and cellular hypersensitivity to DNA cross-linking agents (Kennedy and D'Andrea, 2005; Taniguchi and D'Andrea, 2006). FA results from inactivating mutations in any one of a multiprotein complex that functions during DNA repair (D'Andrea, 2003; Kennedy and D'Andrea, 2005). FA patients carrying *BRCA2* mutations exhibit a more severe phenotype compared with other FA groups, including predisposition to medulloblastoma brain tumors (Offit *et al*, 2003; Shivji and Venkitaraman, 2004). Defects in the BRCA2 partner-protein PALB2 can also result in FA (Reid *et al*, 2007; Xia *et al*, 2007).

Given the importance of BRCA2 during HR and the lethality associated with germ-line inactivation, we used conditional gene inactivation via Cre/LoxP technology to determine the requirement for Brca2 during neural development. Inactivation of *Brca2* resulted in microcephaly associated with defects in neurogenesis particularly during cerebellar development. Loss of Brca2 also led to medulloblastoma when p53 was disabled. Thus, Brca2 fulfils a critical role during nervous system development, highlighting the tissue-specific requirements for DNA repair during neurogenesis.

Results

Inactivation of Brca2 leads to microcephaly and cerebellar defects

To determine the requirement for Brca2 during development of the nervous system, we used a *Brca2* conditional mutant allele in which exon 11 was flanked by *LoxP* sites (Jonkers *et al*, 2001). To inactivate *Brca2*, we used *Cre* that was driven by the Nestin promoter, resulting in expression throughout the central and peripheral nervous systems from embryonic day 10.5 (Dahlstrand *et al*, 1995; Frappart *et al*, 2005). Similar to other reports using this Nestin-cre transgenic line (Graus-Porta *et al*, 2001; Frappart *et al*, 2005), we observed efficient deletion of *Brca2* throughout the nervous system, as determined using genomic DNA or RNA extracted from the mutant cerebrum or cerebellum (Supplementary Figure 1).

In contrast to early embryonic lethality after germ-line inactivation of *Brca2* (Ludwig *et al*, 1997; Sharan *et al*, 1997; Suzuki *et al*, 1997), $Brca2^{LoxP/LoxP}$;*Nestin-cre* mice (hereafter referred to as $Brca2^{Nes\text{-}cre}$) were viable and were relatively normal in overall appearance, size and behavior. However, inactivation of *Brca2* led to microcephaly as $Brca2^{Nes\text{-}cre}$ brains were smaller and weighed significantly less ($P<0.0001$) compared with littermate controls ($Brca2^{LoxP/LoxP}$ or $Brca2^{+/+}$;*Nestin-cre* littermates; hereafter referred to as $Brca2^{Ctrl}$) (Figure 1A–C). All $Brca2^{Nes\text{-}cre}$ brain structures were proportionally affected, including the hippocampus, cortex and olfactory bulb (Figure 1A). However, despite the effect on brain size, overall neural development was relatively normal and normal cortical lamination was present (Figure 1A). Notably, the *Brca2*-deficient cerebellum exhibited stunted foliation and lobule morphology (Figure 1D), and ectopic localization of some Purkinje cells was observed (Figure 1E), suggesting that migration defects might occur during $Brca2^{Nes\text{-}cre}$ cerebellar development. Although Cre expression in the brain can lead to microcephaly in some situations (Forni *et al*, 2006), on no occasion did we observe this with *Nestin-cre* mice that were wild type (WT) or heterozygous for the *Brca2* mutant allele.

As the phenotypic severity of many DNA repair mutant mice are rescued by associated *p53* inactivation (Gao *et al*, 2000; Lee and McKinnon, 2002), we generated $Brca2^{Nes\text{-}cre}$ mice in which *p53* was also inactivated. We found that *p53* inactivation significantly rescued the microcephaly of *Brca2*-deficient mice ($P<0.0001$; Figure 1B–D) and contributed to restoration of cerebellar structure (Figure 1D and E); *p53* heterozygosity also significantly improved cerebellar development, but had no significant effect on the occurrence of microcephaly (data not shown).

Loss of Brca2 activates DNA damage-induced apoptosis

As p53 functions to signal DNA damage, we questioned if the phenotypic rescue resulting from p53 loss resulted from a block in DNA damage signalling to activate apoptosis. Therefore, to determine if Brca2 loss in the nervous system resulted in DNA damage, we assessed phosphorylated H2AX (γH2AX) levels in $Brca2^{Nes\text{-}cre}$ brains; γH2AX occurs in response to DNA DSBs and is a canonical marker for this type of damage (Rogakou *et al*, 1998; Fernandez-Capetillo *et al*, 2004). We found that the cells of the cerebellar external germinal layer (EGL) of mutant mice exhibited increased γH2AX foci (Figure 2A). These foci were concentrated in the outer EGL of the cerebellum that corresponds to the proliferative granule cell progenitors, suggesting that DNA DSBs resulting from Brca2 loss likely occurred during replication. This is consistent with a role for BRCA2 in stabilizing DNA structures at stalled replication forks (Lomonosov *et al*, 2003).

DNA damage during development of the nervous system can result in apoptosis (Lee and McKinnon, 2006). To determine if the DNA DSBs identified by γ-H2AX resulted in apoptosis, we used TUNEL or apoptosis-associated single-stranded DNA (ssDNA) assays (Frankfurt *et al*, 1996; Kawarada *et al*, 1998) to assess the WT and mutant developing cerebellum. We found that Brca2 inactivation resulted in apoptosis in the cerebellar EGL, while co-inactivation of p53 led to a significant decrease of apoptosis (Figure 2B and C). Apoptosis was restricted to proliferative granule cell progenitors and some early differentiating post-mitotic cells (that may have incurred sublethal damage during proliferation) in the $Brca2^{Nes\text{-}cre}$ EGL, as demarcated by immunostaining for Tag-1, a marker for premigratory granule cells (Supplementary Figure 2). However, some apoptosis still occurred in $Brca2^{Nes\text{-}cre};p53^{-/-}$ cerebella, indicating that while p53-dependent signalling accounts for most apoptosis in the Brca2-deficient cerebellum, there is some that is p53 independent. To confirm that the apoptosis assays specifically reflect apoptotic cells, we also quantified cells with pyknotic nuclei that are indicative of apoptosis. As shown in Figure 2D, the abundance of pyknotic cells reflects the number found using the TUNEL assay. Under the conditions used here, these distinct assay methods are accurate indicators of apoptosis and do not simply label damaged DNA. Both methods failed to show any positive apoptotic signal in various brain tissues up to 2 h after ionizing radiation treatment despite the presence of substantial levels of γ-H2AX in these tissues (data not shown). Finally, both ssDNA (Figure 2E) and TUNEL (not shown) positive signal were associated with pyknotic cells. Therefore, loss of Brca2

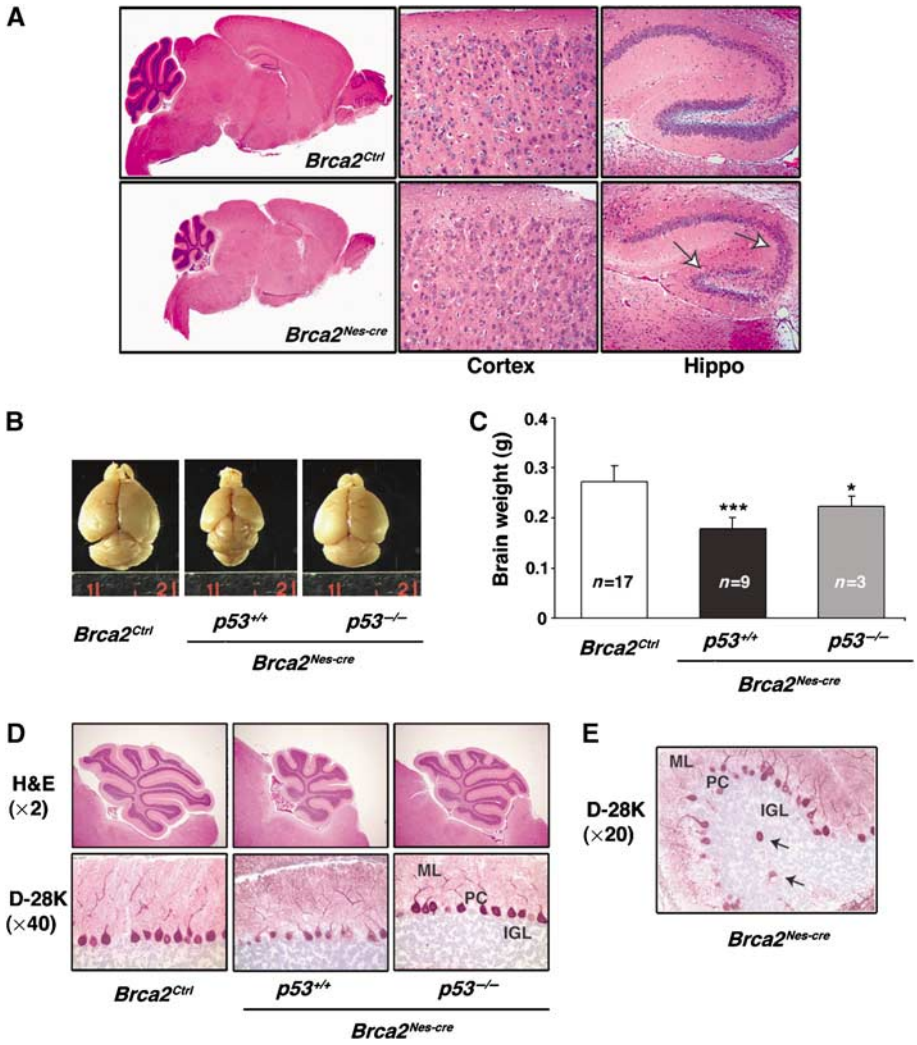

Figure 1 Brca2 loss leads to neurogenesis defects in a p53-dependent manner. (**A**) The *Brca2*^{LoxPLoxP}*;Nestin-cre* (*Brca2*$^{Nes\text{-}cre}$) mutant brain is substantially smaller than *Brca2*$^{+/+}$*;Nestin-cre* (*Brca2*^{Ctrl}) controls, although general morphology is intact. The cortex and the hippocampus (hippo) maintain relatively normal laminar structure (arrows). The cerebellum is markedly smaller in the mutant. (**B**) Reduced brain size at P21 in *Brca2*$^{Nes\text{-}cre}$ mice compared with that in *Brca2*^{Ctrl} mice. Rescue of cerebella size occurs in the *Brca2*$^{Nes\text{-}cre}$*;p53*$^{-/-}$ mice. (**C**) The relative brain weight of P7 *Brca2*$^{Nes\text{-}cre}$ and *Brca2*$^{Nes\text{-}cre}$*;p53*$^{-/-}$ compared with *Brca2*$^{+/+}$*;Nes-cre* (*Brca2*^{Ctrl}) shows rescue by p53 loss. (**D**) H&E staining of *Brca2*$^{LoxP/LoxP}$ (*Brca2*^{Ctrl}), *Brca2*$^{Nes\text{-}cre}$ and *Brca2*$^{Nes\text{-}cre}$*;p53*$^{-/-}$ brain sections at P21 reveals size reduction of the mutant cerebellum compared with WT (magnification $\times 2$). Calbindin (D-28K) staining shows the Purkinje cell layer is intact in the *Brca2*$^{Nes\text{-}cre}$ cerebellum (magnification $\times 40$), although ectopic localization of Purkinje cells occurs in some lobules (**E**, magnification $\times 20$). ML, molecular layer; PC, Purkinje cells; IGL, inner granular layer.

invokes a DNA damage response that can lead to apoptosis during development.

Brca2 inactivation promotes increased apoptosis but not proliferation defects

While apoptosis results from loss of Brca2, it is possible that proliferation defects also contribute to microcephaly. Therefore, we used bromodeoxyuridine (BrdU) incorporation to quantify proliferation, but found no statistically significant differences in BrdU incorporation between the mutant or WT genotypes at 90 min (Figure 3A) or 6 h (data not shown). Thus, Brca2 loss results in apoptosis, but does not appear to affect granule neuron progenitor proliferation.

Because *Brca2*-deficient cells *in vitro* can accumulate in G2 (Patel *et al*, 1998; Marmorstein *et al*, 2001), we examined cell-cycle progression using phosphohistone H3-specific immunostaining to identify cells in G2/M. We found a significant increase ($P = 0.0229$) of cells that were immunopositive for phosphorylated histone H3 in *Brca2*$^{Nes\text{-}cre}$ cerebellar EGL compared with control tissue (Figure 3B). We also found that the increase of G2/M cells after Brca2 loss was present in the *Brca2*$^{Nes\text{-}cre}$*;p53*$^{-/-}$ EGL, indicating that this cell-cycle block is independent of *p53* (Figure 3B). These data are consistent with DNA damaged cells activating a G2 arrest before either DNA repair or apoptosis (Kastan and Bartek, 2004; Sancar *et al*, 2004).

Apoptosis induced by Brca2 loss is present throughout neural development

To further assess the effects of Brca2 loss, we analyzed neural development at different developmental stages. We quantified BrdU-positive cells in the cerebellar primordia at E14.5 and similar to postnatal cerebellar development, we did not observe any differences in proliferation between control

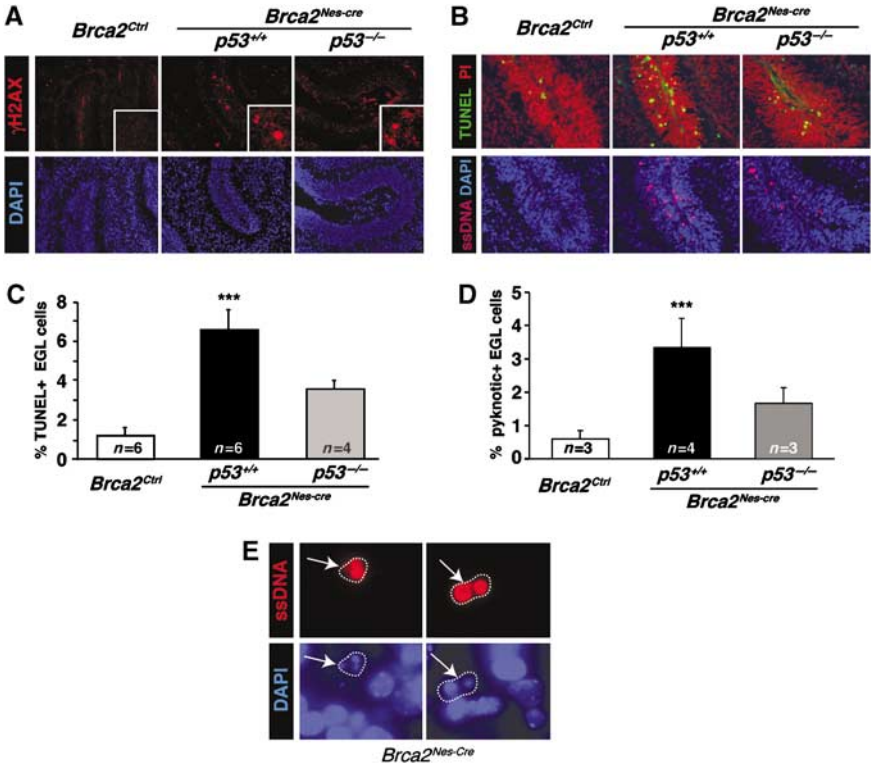

Figure 2 Brca2 loss leads to DNA damage and increased apoptosis in EGL granule cell progenitors. (**A**) Loss of *Brca2* leads to H2AX phosphorylation (γH2AX) in $Brca2^{Nes\text{-}cre}$ and $Brca2^{Nes\text{-}cre}$;$p53^{-/-}$ but not control; $Brca2^{Ctrl}$ ($Brca2^{Flox/Flox}$) P7 cerebellum (magnification ×20); insets show higher magnification of γH2AX (magnification ×40). (**B**) Compared with control $Brca2^{Ctrl}$ ($Brca2^{+/+}$;*Nestin-cre*) P7 cerebellum, apoptosis is widespread throughout the $Brca2^{Nes\text{-}cre}$ cerebellum, but is attenuated after associated loss of p53, as assessed by TUNEL or immunostaining for apoptosis-related single-stranded DNA (ssDNA) (magnification ×40). Quantification of TUNEL staining (**C**) or pyknotic nuclei (**D**) in $Brca2^{+/+}$;*Nes-cre* ($Brca2^{Ctrl}$) P7 EGL compared with $Brca2^{Nes\text{-}cre}$ showed a significant increase in apoptosis ($P<0.0001$), while apoptosis was attenuated in $Brca2^{Nes\text{-}cre}$;$p53^{-/-}$ tissue. (**E**) Apoptotic cells with typical nuclear morphology of pyknosis commonly show colocalization with biochemical apoptotic markers (ssDNA immunostaining) (magnification ×100).

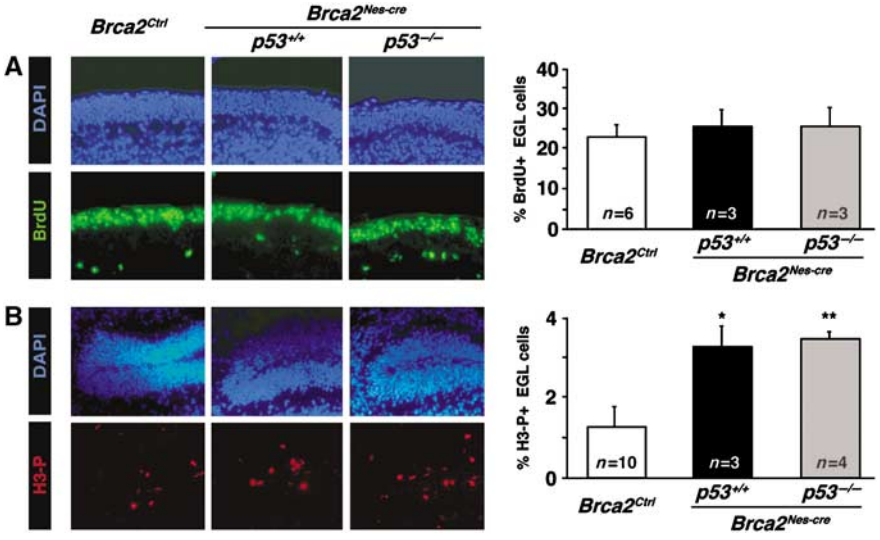

Figure 3 Apoptosis and proliferation analysis of $Brca2^{Nes\text{-}cre}$ cerebella. (**A**) Analysis of proliferation was determined after BrdU incorporation in control tissue and $Brca2^{Nes\text{-}cre}$;$p53^{+/+}$ and $Brca2^{Nes\text{-}cre}$;$p53^{-/-}$ EGL. Proliferation is not perturbed by Brca2 loss, as BrdU incorporation is similar between $Brca2^{Nes\text{-}cre}$ and $Brca2^{Ctrl}$. (**B**) Increased phosphorylated histone H3-positive cells were found in the $Brca2^{Nes\text{-}cre}$ and $Brca2^{Nes\text{-}cre}$;$p53^{-/-}$ EGL ($P<0.0001$). Asterisks indicate significant differences and *n* indicates the number of different cerebella analyzed from each genotype.

($Brca2^{+/+}$;*Nestin-cre*) and $Brca2^{Nes\text{-}cre}$ at this age (Figure 4A), although apoptosis was increased at this stage and remained elevated through postnatal development (Figure 4B).

We also analyzed other brain regions including the E14.5 neuroepithelium of the hindbrain, and again found no difference in proliferation, but there was an increase in apoptosis

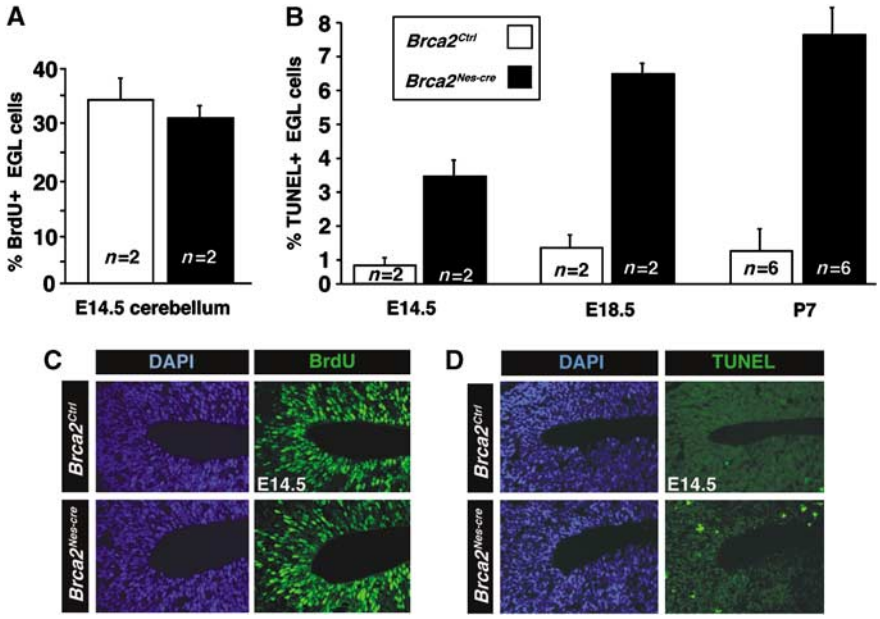

Figure 4 Developmental analysis of Brca2 loss. Proliferation and apoptosis were determined at various developmental times in *Brca2*^{Ctrl} and *Brca2*$^{Nes\text{-}cre}$ neural tissue. Analysis of apoptosis was performed using TUNEL and proliferation was determined after analysis of BrdU incorporation. (**A**) No difference in proliferation was found at early developmental times for the cerebellum. (**B**) Significantly increased apoptosis ($P<0.0001$) was found throughout neural development in mutant (*Brca2*$^{Nes\text{-}cre}$) tissue; *Brca2*^{Ctrl} was Br*ca2*$^{+/+}$*;Nestin-cre*. (**C**) Representative BrdU staining in the developing E14.5 hindbrain ventricular zone (magnification $\times 40$). (**D**) Representative TUNEL staining in the developing E14.5 hindbrain ventricular zone (magnification $\times 40$).

within this region of the central nervous system (CNS) (Figure 4C and D). Therefore, cell loss from apoptosis most likely accounts for the occurrence of microcephaly present in *Brca2*$^{Nes\text{-}cre}$ animals.

Atm deficiency partially restores morphology of the Brca2$^{Nes\text{-}cre}$ *cerebellum*

Atm is required for DNA DSB-induced apoptosis in select neural populations (Lee *et al*, 2000; Sekiguchi *et al*, 2001; Kruman *et al*, 2004; Orii *et al*, 2006), although DNA damage from defective HR does not signal Atm (Orii *et al*, 2006; Adam *et al*, 2007). However, in those studies, the HR mutation was germ-line inactivation of Xrcc2, and resulted in early embryonic lethality. This early embryonic lethality precluded analysis of Atm signalling during later neural development. Therefore, to further determine Atm function after disruption of HR, we crossed *Brca2* mutant mice with *Atm*$^{+/-}$ mice and obtained *Brca2*$^{Nes\text{-}cre}$*;Atm*$^{-/-}$ animals. Notably, we found that *Atm* deficiency promoted partial recovery of cerebellar development (Figure 5A–C). However, in contrast to *p53* deficiency, *Atm* loss did not rescue the microcephaly resulting from Brca2 inactivation (Figure 5A–C). While *Atm* deficiency contributed to a reduction of apoptosis in the EGL (Figure 5D), it did not alter proliferation of the granule cell progenitors (Figure 5D). However, when we measured TUNEL-positive cells in the EGL, the statistical significance ($P=0.1219$) between the *Brca2*$^{Nes\text{-}cre}$ and the *Brca2*$^{Nes\text{-}cre}$*;Atm*$^{-/-}$ mice suggested that Atm loss contributes to the rescue of only a small fraction of the apoptotic cells in the *Brca2*$^{Nes\text{-}cre}$ EGL. This partial rescue of the *Brca2*$^{Nes\text{-}cre}$ cerebellum is consistent with a role for Atm after granule precursors exit the cell cycle, rather than a primary function in proliferating cells (Lee *et al*, 2001). ATM may therefore act as a backup surveillance to ensure cells containing DNA damage don't become incorporated into mature neural tissue.

Brca2 deficiency leads to defects in neural progenitor cell self-renewal and proliferation

The previous analyses indicate apoptosis is increased after loss of Brca2, leading to defective neural development, and both proliferative progenitor cells and early post-mitotic neurons are affected. To further investigate potential targets of *Brca2*-deficiency, we examined neurosphere cultures.

Neurospheres were established from *Brca2*^{Ctrl}, *Brca2*$^{Nes\text{-}cre}$ and *Brca2*$^{Nes\text{-}cre}$*;p53*$^{-/-}$ brains at E14.5 and P0 (Figure 6A and B). PCR analysis showed that there was efficient gene deletion in E14.5 *Brca2*$^{Nes\text{-}cre}$ neurospheres (data not shown). There was a substantial reduction in the number and size of neurospheres derived from the *Brca2*$^{Nes\text{-}cre}$ brains compared with those from *Brca2*^{Ctrl} or *Brca2*$^{Nes\text{-}cre}$*;p53*$^{-/-}$ brains (Figure 6A and B). When E14.5 neurospheres were cultured, the number of spheres was significantly reduced in *Brca2*-deficient animals after 7 days in culture ($P<0.0001$) (Figure 6B) compared with *Brca2*^{Ctrl} and *Brca2*$^{Nes\text{-}cre}$*;p53*$^{-/-}$ animals, potentially indicating less CNS stem/progenitor cells in *Brca2*-deficient animals, or alternatively, decreased proliferation or survival in culture. The *Brca2*-deficient neurospheres propagated less readily, as indicated by a smaller number of cells per sphere (Figure 6C). Notably, at later stages, we were also unable to isolate P0 *Brca2*-deficient neurospheres after two independent attempts (data not shown). However, inactivation of *p53* restored the number of *Brca2*$^{Nes\text{-}cre}$ neurospheres and number of cells per neurosphere after 7 days in culture of either E14.5 or P0 neural stem cells (Figure 6B–D). To determine why Brca2-deficient neurospheres were compromised, we analyzed apoptosis and proliferation. Similar to Brca2 loss *in vivo*, we found that

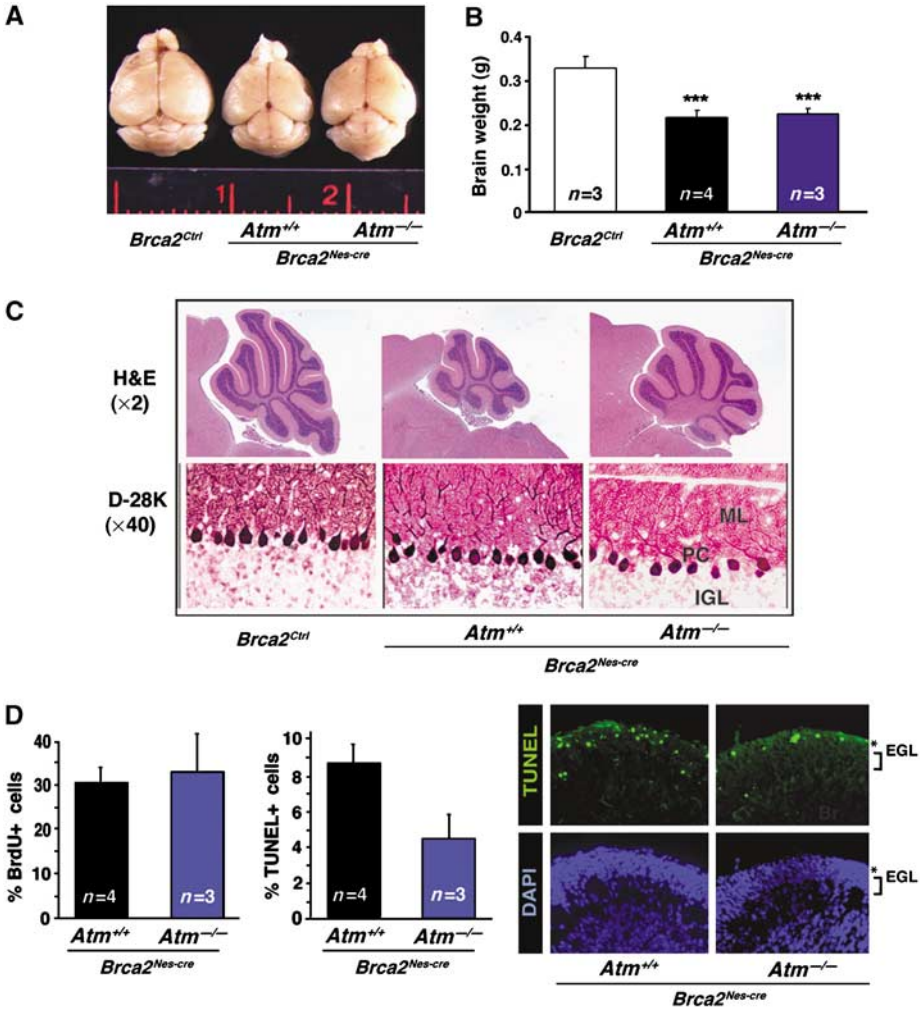

Figure 5 *Atm* inactivation restores cerebellar growth but not microcephaly in $Brca2^{Nes\text{-}cre}$ mice. (**A**) Partial rescue of cerebellar development in $Brca2^{Nes\text{-}cre}$ mice occurs when *Atm* is inactivated. (**B**) Comparison of brain weight at P7 between $Brca2^{+/+}$;*Nes-cre* ($Brca2^{Ctrl}$), $Brca2^{Nes\text{-}cre}$ and $Brca2^{Nes\text{-}cre}$;$Atm^{-/-}$ mice shows that loss of Atm does not restore microcephaly, as brain weight between $Brca2^{Flox/Flox}$ ($Brca2^{Ctrl}$) and $Brca2^{Nes\text{-}cre}$;$Atm^{-/-}$ is still significantly different ($P<0.0001$). (**C**) H&E staining of $Brca2^{Flox/Flox}$ ($Brca2^{Ctrl}$), $Brca2^{Nes\text{-}cre}$ and $Brca2^{Nes\text{-}cre}$;$Atm^{-/-}$ of P21 cerebellum sections shows that development is partially restored by *Atm* deficiency (magnification ×2); calbindin (D-28K) staining reveals the molecular layer (ML), the Purkinje cell layer (PC) and the inner granule layer (IGL), (magnification ×40). (**D**) Although proliferation is similar in the EGL of P7 $Brca2^{Ctrl}$, $Brca2^{Nes\text{-}cre}$ and $Brca2^{Nes\text{-}cre}$;$Atm^{-/-}$ cerebella, TUNEL is reduced in the post-mitotic region (parentheses) but not the proliferative layer (asterisk) (magnification ×40); *n* indicates the number of cerebella analyzed.

there was increased apoptosis in $Brca2^{Nes\text{-}cre}$ neurospheres, but we found reduced BrdU incorporation in neural progenitor cells *in vitro* (Figure 6E). However, *p53* inactivation restored normal proliferation and substantially reduced apoptosis in $Brca2^{Nes\text{-}cre}$;$p53^{-/-}$ neurospheres (Figure 6E and F). The discrepancy between proliferation defects *in vitro* but not *in vivo* after Brca2 loss may reflect culture stress activating cell-cycle checkpoints. Alternatively, as stem cells are a minor population, our BrdU assays may have missed this compartment in the tissue we examined. Overall, these data suggest that $Brca2^{Nes\text{-}cre}$ neural progenitors undergo increased rates of apoptosis and their loss may additionally contribute to the microcephaly observed in the $Brca2^{Nes\text{-}cre}$ nervous system.

Brca2 is required to suppress medulloblastoma

In the nervous system, inactivation of DNA DSB repair can lead to medulloblastoma (Lee and McKinnon, 2002; Holcomb *et al*, 2006; Yan *et al*, 2006), and *BRCA2* mutations in FANCD1 are also associated with this brain tumor (Offit *et al*, 2003). Therefore, to determine if *Brca2* functions as a tumor suppressor in the brain, we monitored tumor formation in five experimental groups over a period of 32 weeks: $Brca2^{Ctrl}$, $Brca2^{Nes\text{-}cre}$, $Brca2^{Nes\text{-}cre}$;$p53^{+/-}$, $p53^{-/-}$ and $Brca2^{Nes\text{-}cre}$;$p53^{-/-}$. Although $Brca2^{Nes\text{-}cre}$ mice exhibit a significantly shorter lifespan compared with control mice (Figure 7A) ($P<0.0001$), analysis of these mice did not reveal tumors (Table I). To determine the cause of the premature death of $Brca2^{Nes\text{-}cre}$ mice, we performed full necropsies and blood tests on 8-week-old $Brca2^{Nes\text{-}cre}$ animals. Although these animals did not exhibit obvious histological defects, hematological analysis revealed abnormalities in numbers of red blood cells and platelets in *Brca2*-deficient animals (data not shown). These results are consistent with aplastic anemia found in FA and explained by the occurrence of gene deletion in bone narrow using Nestin-cre (Betz *et al*, 1996).

However, from 10 weeks of age onwards, most $Brca2^{Nes\text{-}cre}$;$p53^{-/-}$ mice became moribund with medulloblastoma

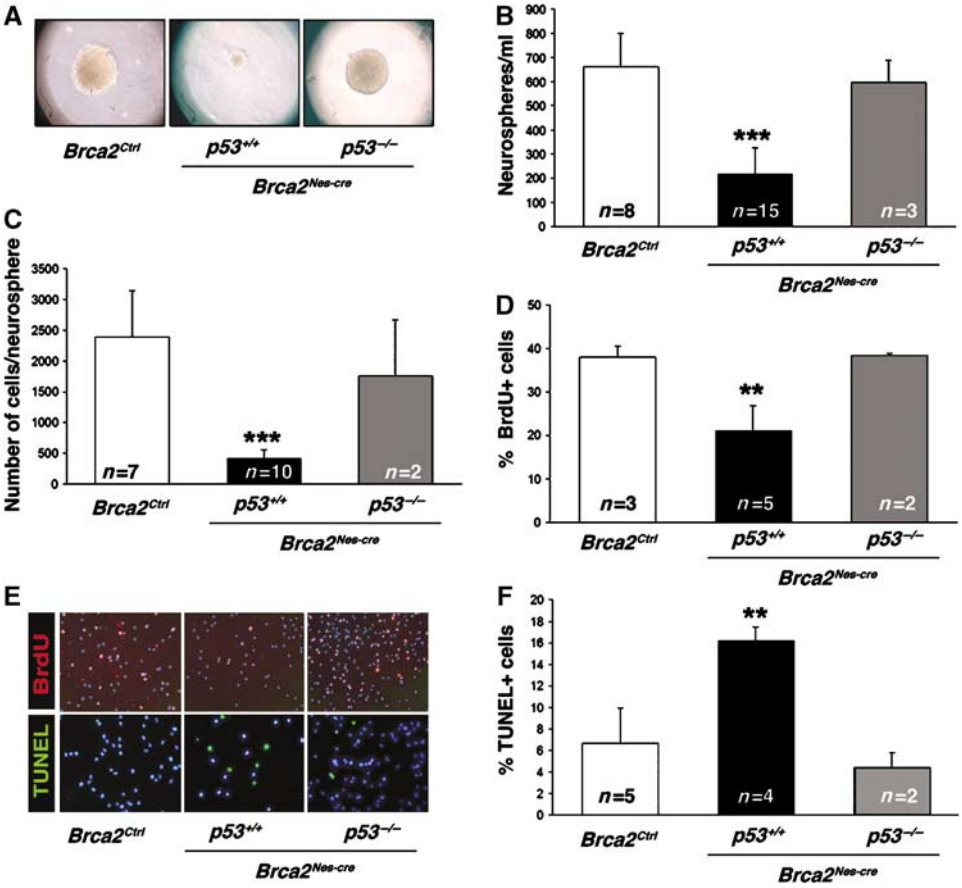

Figure 6 Analysis of *Brca2*$^{Nes\text{-}cre}$ neural progenitor cells. (**A**) Morphology of E14.5 *Brca2*^{Ctrl}, *Brca2*$^{Nes\text{-}cre}$ and *Brca2*$^{Nes\text{-}cre}$;*p53*$^{-/-}$ neurospheres after 7 days in culture. (**B**) Numbers of E14.5 neurospheres after an initial seeding of 2.5×10^5 cells/ml derived from control or mutant embryos. (**C**) Numbers of cells present in E14.5 neurospheres derived from control or mutant embryos. (**D**) BrdU and TUNEL staining of E14.5 neural progenitor cells 3 h after BrdU treatment. (**E**) Quantitation of BrdU-positive cells in the *Brca2*$^{Nes\text{-}cre}$ neural progenitor cell population compared with *Brca2*^{Ctrl}, and the respective number of TUNEL-positive cells (**F**). Asterisks indicate statistically significant differences; *n* indicates the number of individual cell lines analyzed.

($n = 19/23$; Table I and Figure 7A). Although *p53*$^{-/-}$ mice generally succumb to lymphoid tumors, in no case did we observe medulloblastoma in these mice ($n = 29$; Table I and Figure 7A). Notably, medulloblastoma also occurred in p53 heterozygous mice that were *Brca2*$^{Nes\text{-}cre}$ ($n = 34/47$), although with a significantly increased tumor latency compared with *Brca2*$^{Nes\text{-}cre}$;*p53*$^{-/-}$ mice ($\sim$13 weeks versus $\sim$21 weeks; $P < 0.0001$) (Figure 7A and B; Table I). Consistent with this, arrayCGH or spectral karyotyping (SKY) identified genomic rearrangements of chromosome 11 (on which *p53* resides) in *Brca2*$^{Nes\text{-}cre}$;*p53*$^{+/-}$ tumors (Figure 7C, Table I and Supplementary Figure 2). SKY analysis showed chromosome 11 translocations involved various other chromosomes (Figure 7C), while arrayCGH showed loss of chromosomal material spanning the region of chromosome 11 containing *p53* (Supplementary Figure 2). In contrast, no rearrangements or loss of chromosome 11 was found in *Brca2*$^{Nes\text{-}cre}$;*p53*$^{-/-}$ tumors, indicating that p53 loss underpinned tumorigenesis in the *Brca2*$^{Nes\text{-}cre}$;*p53*$^{+/-}$ mice (Supplementary Figure 2). Finally, PCR analysis performed on the same *Brca2*$^{Nes\text{-}cre}$;*p53*$^{+/-}$ samples showed a clear loss of heterozygosity of *p53*, even in samples which did not show major p53 genomic rearrangements (Figure 7D). Therefore, similar to FA, loss of Brca2 can lead to medulloblastoma.

Discussion

Repair of DNA via HR is critical for maintenance of genomic integrity, and BRCA2 is a central component of this pathway (West, 2003; Pellegrini and Venkitaraman, 2004). Cells lacking BRCA2 show pronounced genetic instability and susceptibility to DNA damaging agents, while *BRCA2* mutations predispose to breast, ovarian and prostate cancer (Tutt and Ashworth, 2002; Shivji and Venkitaraman, 2004). Initial *in vivo* studies revealed that germ-line deletion of *Brca2* in the mouse was lethal at E6 (Ludwig *et al*, 1997; Sharan *et al*, 1997; Suzuki *et al*, 1997; Patel *et al*, 1998; Jonkers *et al*, 2001). While this finding pointed to the critical importance of Brca2, the early lethality precluded spatiotemporal analysis of this protein's function during development. In this current report, we find that in contrast to germ-line inactivation, *Brca2*$^{Nes\text{-}cre}$ mice are viable. Notwithstanding this, it is clear that Brca2 fulfils an important role during neurogenesis, as *Brca2*$^{Nes\text{-}cre}$ mice are microcephalic and exhibit altered neural development arising from the effects of chronic genotoxic stress. Brca2 is also required to suppress the formation of medulloblastoma brain tumors. These data highlight the importance of Brca2 during normal neural development for maintaining genomic stability and uncover novel tissue-specific requirements for this DNA repair factor.

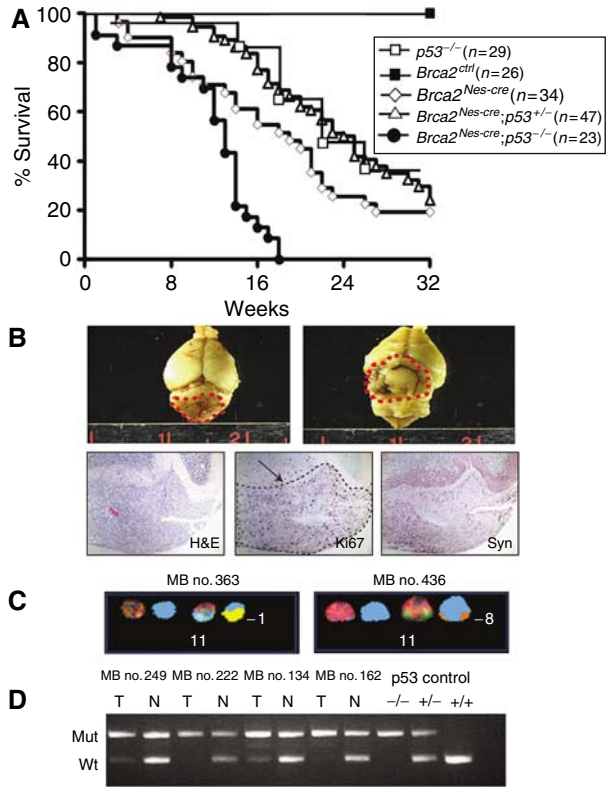

Figure 7 Medulloblastoma occurs in *Brca2*$^{Nes\text{-}cre}$ mice and is associated with *p53* deficiency. (**A**) Kaplan–Meier survival curves of *Brca2*-deficient mice. *Brca2*$^{Nes\text{-}cre}$ mice were monitored for viability, and >80% died by 32 weeks of age. *Brca2*$^{Nes\text{-}cre}$ mice with associated *p53*$^{-/-}$ or *p53*$^{+/-}$ mutations succumbed to medulloblastoma. Total animal numbers are indicated. (**B**) Examples of typical medulloblastomas in *Brca2/p53*-deficient mice, represented by a dashed line. Analysis of *Brca2*$^{Nes\text{-}cre}$;*p53*$^{-/-}$ medulloblastomas using H&E, Ki-67 or synaptophysin (magnification ×20). (**C**) SKY analysis of tumors showing translocations and genomic rearrangements on chromosome 11 in *Brca2*$^{Nes\text{-}cre}$;*p53*$^{+/-}$ tumors. (**D**) PCR analysis of p53 showing the loss of *p53* WT allele in *Brca2*$^{Nes\text{-}cre}$;*p53*$^{+/-}$; T represents tumor and N represents tail DNA from the same animal.

Table I Incidence, onset and chromosome 11 involvement of medulloblastoma in Brca2/p53-deficient mice

Genotype	Medulloblastoma/ total animals (incidence)	Onset (weeks) ± s.d.	Tumors and chromosome 11 loss
Brca2^{Ctrl}	0/26 (0%)	—	—
Brca2$^{Nes\text{-}cre}$	0/31 (0%)	—	—
P53$^{-/-}$	0/29 (0%)	—	—
Brca2$^{Nes\text{-}cre}$;*p53*$^{+/-}$	34/47 (72%)	20.53 ± 5.64	16/17 (94%)
Brca2$^{Nes\text{-}cre}$;*p53*$^{-/-}$	19/23 (83%)	13.26 ± 2.86	0/8 (0%)

Inactivation of many important DNA repair genes including *Brca2*, *Mre11*, *Nbs1* or *Rad51* disrupts gastrulation and therefore tissue formation, making broader biological interpretation of gene loss problematic (Friedberg and Meira, 2006). In other cases, disruption of DNA repair genes such as *Xrcc4*, *Lig4* or *Xrcc2* while also resulting in lethality do allow more substantial embryonic development, leading to phenotypes associated with DNA damage effects in specific tissue regions during development (Barnes *et al*, 1998; Frank *et al*, 1998; Gao *et al*, 1998; Orii *et al*, 2006). Bypassing embryonic lethality, via tissue-specific gene inactivation, provides unique functional insights and is an essential counterpoint for comparative analyses of other conditional DNA repair mouse mutants and myriad cellular *in vitro* studies.

This point is illustrated by comparing our current study with Brca2 to neural inactivation of Nbs1, where an overlapping but different phenotype was observed (Frappart *et al*, 2005). NBS1 is important for DNA DSB repair because Nijmegen Breakage syndrome individuals are radiosensitive, predisposed to tumors and isolated cells are hypersensitive to DNA DSB-inducing agents (Shiloh, 1997; Digweed and Sperling, 2004). Two important differences distinguish our current study from the *Nbs1*$^{Nes\text{-}cre}$ conditional mutant mice; these are the lack of overt proliferation defects and the occurrence of medulloblastoma in *Brca2*$^{Nes\text{-}cre}$ animals. Neural inactivation of *Nbs1* leads to substantial impairment of proliferation of granule neuron progenitor cells resulting in increased apoptosis in post-mitotic neurons primarily in the cerebellum (Frappart *et al*, 2005). However, the impressive phenotypic rescue of the *Nbs1*$^{Nes\text{-}cre}$ cerebellum by p53 loss does not result in medulloblastoma. This contrasting phenotype between DNA DSB repair factors could reflect different cellular roles, whereby NBS1 may be critical for monitoring DNA breaks during DNA replication possibly in collaboration with ATR (Pichierri and Rosselli, 2004; Stiff *et al*, 2005), while BRCA2 functions primarily to repair DNA DSBs during HR. Notably however, NBS1 is reportedly essential for HR (Tauchi *et al*, 2002; Yang *et al*, 2006), so the different phenotypes between *Nbs1*$^{Nes\text{-}cre}$ and *Brca2*$^{Nes\text{-}cre}$ may relate to cell- or tissue-specific effects, that the damage induced by Nbs1 loss is more severe or could indicate that NBS1 does not participate in HR in a physiological setting.

Although Brca2 is clearly important for maintenance of DNA integrity in proliferating granule neuron precursor cells, partial rescue of the *Brca2*$^{Nes\text{-}cre}$ cerebellar phenotype occurs in *Brca2*$^{Nes\text{-}cre}$;*Atm*$^{-/-}$ animals. Because Atm is not required for DNA damage signalling after disruption of HR *in vivo* (Orii *et al*, 2006; Adam *et al*, 2007), it is most likely that Atm-dependent apoptosis eliminates post-mitotic granule cells with unrepaired DNA damage (resulting from Brca2 loss during proliferation) to prevent migration of these cells to the inner granule layer. The kinase responsible for signalling DNA damage in the proliferative neural populations may well be the ATM-related kinase, ATR (Shiloh, 2003). While Atm involvement during cerebellar development in *Nbs1*$^{Nes\text{-}cre}$ mice has not been reported, the synthetic lethality between *Nbs1*$^{\Delta B/\Delta B}$ and *Atm*$^{-/-}$ mice (Williams *et al*, 2002) may make interpretation of this compound genetic background difficult, potentially further highlighting the different roles these respective DNA repair factors fulfil during development.

We also found that Brca2 was a potent tumor suppressor in the brain. When *Brca2*$^{Nes\text{-}cre}$ was introduced onto a p53 deficient background (either *p53*$^{+/-}$ or *p53*$^{-/-}$), most mice succumbed to brain tumors, resulting from enhanced DNA damage during proliferation in the EGL. Previous studies showed that medulloblastoma can occur when DNA DSB repair factors are disabled, as loss of NHEJ in collaboration with defective p53 signalling led to this type of tumor, although in those cases, no tumors were reported in *p53* heterozygotes (Gao *et al*, 1998, 2000; Lee and McKinnon, 2002; Yan *et al*, 2006). The high incidence of medulloblasto-

ma in *Brca2*$^{Nes\text{-}cre}$;*p53*$^{+/-}$ mice probably reflects the more severe effects of Brca2 disruption toward genomic integrity. Importantly, the human FA syndrome, which results from Brca2 mutations (FANCD1), also develops medulloblastoma (Offit *et al*, 2003), and microcephaly is a prevalent feature of FA (Gennery *et al*, 2004), indicating that Brca2 loss in the mouse recapitulates the neural aspects of this disease. Similar to other mutations common in human medulloblastoma, we found that the *Ptch1* was generally lost in the *Brca2*$^{Nes\text{-}cre}$; *p53*$^{-/-}$ medulloblastoma, while *N-Myc* was often amplified (data not shown). Loss of p53, or the p53 pathway, also features strongly in human medulloblastoma (Woodburn *et al*, 2001; Giordana *et al*, 2002; Lee *et al*, 2003; Frank *et al*, 2004), underscoring the relevance of the *Brca2*$^{Nes\text{-}cre}$; *p53*$^{-/-}$ model to human disease. Maintenance of genomic integrity by Brca2 is also likely to be critical for prevention of other brain tumor types in addition to medulloblastoma.

Our data help to clarify the relative developmental contributions of DNA damage response factors in a biological setting, and indicate that genomic stability is critical for proper neural development. As more tissue-restricted DNA repair mutant mice are generated, we will further determine the relationship between different DNA repair pathways during development, thereby providing a biological context for understanding the interplay of DNA repair factors and their role in preventing disease.

Materials and methods

Generation of mice with Brca2 deleted in the nervous system

The *Brca2*-floxed mice (Jonkers *et al*, 2001) were obtained from the MMHCC repository at the NIH: (http://mouse.ncifcrf.gov/available_strains.asp) and Nestin-cre transgenic mice (B6.Cg-Tg(Nes-cre) 1Kln/J; JAX #003771) were obtained from the Jackson Laboratory. These were interbred in order to obtain *Brca2*$^{LoxP/LoxP}$;*Nes-cre* mice. Mice used in this study resulted from backcrossing mixed C57BL/J $\times$ 129Ola for between two and four generations. During breeding, the *Nes-cre* transgene was routinely carried by the female to avoid germ-line *Brca2* disruption due to spurious Cre expression in the testis. The control group (*Brca2*^{Ctrl}) was consisted of the following genotypes: *Brca2*$^{LoxP/LoxP}$, *Brca2*$^{+/LoxP}$;*Nes-cre* or *Brca2*$^{+/+}$;*Nes-cre*. Genotyping for the mutant *Brca2* allele was as described (Jonkers *et al*, 2001), and the primers used for Cre genotyping were Cre-1: 5′-CGGTCGATGCAACGAGTGATG-3′ and Cre-2: 5′-CCAGAGACGGAAATCCATCGC-3′. Inactivation of p53 or Atm was achieved using *p53*$^{-/-}$ or *Atm*$^{-/-}$ mice (Herzog *et al*, 1998) and these were interbred with *Brca2*$^{LoxP/+}$;*Nes-cre* mice, and F1 mice were used to generate *Brca2*$^{Nes\text{-}cre}$;*p53*$^{-/-}$ or *Brca2*$^{Nes\text{-}cre}$;*Atm*$^{-/-}$.

Histological and immunohistochemical analyses

Histological analysis was carried out on 5 μm paraffin sections stained with hematoxylin and eosin (H&E), or by immunostaining on 10 μm cryosections, as described previously (Orii *et al*, 2006). Antibodies used were anti-calbindin D-28K, (1/500; Sigma-Aldrich) and anti-BrdU (1/500; Oxford Biotechnology). For the *in vivo* proliferation assays, newborn mice or pregnant females were injected intraperitoneally with BrdU (50 μg/g of body weight) (Sigma-Aldrich). Embryos or brains were removed 90 min or 6 h after injection and fixed in 4% PBS-buffered paraformaldehyde. Apoptosis was assessed by ssDNA immunoreactivity and TUNEL. Cryosections were incubated with ssDNA antibody (1:300; IBL Co.) overnight and visualized with indocarbocyanine (Cy3; Jackson ImmunoResearch) mounted with Vectashield containing DAPI (Vector Laboratories). TUNEL analysis was performed using cryosections with the ApopTag® fluorescein *in situ* apoptosis detection kit (Chemicon) according the manufacturer's instruction. For each genotype, at least 1000 cells from four different sections and two mice were counted using a Zeiss axioskop with epiflourescence. Images from matched fields were captured in Adobe Photoshop and cell numbers were scored. The γH2AX staining was performed on cryosections after antigen retrieval using polyclonal anti-γH2AX (phospho S139; 1/250; Abcam).

Neurosphere cultures

Culturing of neurospheres was essentially as described (Frappart *et al*, 2005). Briefly, neurospheres were obtained from dissociated E14.5 brains and grown in Dulbecco's modified Eagle's media (NutMix/F12)/B27 media (Invitrogen) supplemented with EGF (Peprotech) and bFGF (Peprotech).

Spectral karyotyping

Medulloblastoma primary tumors were collected 4 h after an intraperitoneal injection of colcemid (1.5 μg/g body weight) and single cell suspensions of medulloblastoma were subject to SKY analysis using a commercial SKY probe according to the SkyPaint hybridization and detection protocol (Applied Spectral Imaging). Pretreatment of samples used RNase A (100 μg/ml) for 1 h at 37°C and pepsin for 2 min at 20°C (50 μg/ml in 10 mM HCl), with counterstaining by 4′,6-diamidino-2-phenylindole (DAPI).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

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References

Adam J, Deans B, Thacker J (2007) A role for Xrcc2 in the early stages of mouse development. *DNA Repair (Amst)* **6:** 224–234

Barnes DE, Stamp G, Rosewell I, Denzel A, Lindahl T (1998) Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. *Curr Biol* **8:** 1395–1398

Betz UA, Vosshenrich CA, Rajewsky K, Muller W (1996) Bypass of lethality with mosaic mice generated by Cre-loxP-mediated recombination. *Curr Biol* **6:** 1307–1316

D'Andrea AD (2003) The Fanconi road to cancer. *Genes Dev* **17:** 1933–1936

Dahlstrand J, Lardelli M, Lendahl U (1995) Nestin mRNA expression correlates with the central nervous system progenitor cell state in many, but not all, regions of developing central nervous system. *Brain Res Dev Brain Res* **84:** 109–129

Daniels MJ, Wang Y, Lee M, Venkitaraman AR (2004) Abnormal cytokinesis in cells deficient in the breast cancer susceptibility protein BRCA2. *Science* **306:** 876–879

Deans B, Griffin CS, Maconochie M, Thacker J (2000) Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. *EMBO J* **19:** 6675–6685

Digweed M, Sperling K (2004) Nijmegen breakage syndrome: clinical manifestation of defective response to DNA double-strand breaks. *DNA Repair (Amst)* **3:** 1207–1217

Erkko H, Xia B, Nikkila J, Schleutker J, Syrjakoski K, Mannermaa A, Kallioniemi A, Pylkas K, Karppinen SM, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW, Haber DA, Grip M, Reiman M, Jukkola-Vuorinen A, Mustonen A, Kere J, Aaltonen LA, Kosma VM, Kataja V, Soini Y, Drapkin RI, Livingston DM, Winqvist R (2007) A recurrent mutation in PALB2 in Finnish cancer families. *Nature* **446:** 316–319

Fernandez-Capetillo O, Lee A, Nussenzweig M, Nussenzweig A (2004) H2AX: the histone guardian of the genome. *DNA Repair (Amst)* **3:** 959–967

Forni PE, Scuoppo C, Imayoshi I, Taulli R, Dastru W, Sala V, Betz UA, Muzzi P, Martinuzzi D, Vercelli AE, Kageyama R, Ponzetto C (2006) High levels of Cre expression in neuronal progenitors cause defects in brain development leading to microencephaly and hydrocephaly. *J Neurosci* **26:** 9593–9602

Frank AJ, Hernan R, Hollander A, Lindsey JC, Lusher ME, Fuller CE, Clifford SC, Gilbertson RJ (2004) The TP53-ARF tumor suppressor pathway is frequently disrupted in large/cell anaplastic medulloblastoma. *Brain Res Mol Brain Res* **121:** 137–140

Frank KM, Sekiguchi JM, Seidl KJ, Swat W, Rathbun GA, Cheng HL, Davidson L, Kangaloo L, Alt FW (1998) Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. *Nature* **396:** 173–177

Frankfurt OS, Robb JA, Sugarbaker EV, Villa L (1996) Monoclonal antibody to single-stranded DNA is a specific and sensitive cellular marker of apoptosis. *Exp Cell Res* **226:** 387–397

Frappart PO, Tong WM, Demuth I, Radovanovic I, Herceg Z, Aguzzi A, Digweed M, Wang ZQ (2005) An essential function for NBS1 in the prevention of ataxia and cerebellar defects. *Nat Med* **11:** 538–544

Friedberg EC, Meira LB (2006) Database of mouse strains carrying targeted mutations in genes affecting biological responses to DNA damage version 7. *DNA Repair (Amst)* **5:** 189–209

Gao Y, Ferguson DO, Xie W, Manis JP, Sekiguchi J, Frank KM, Chaudhuri J, Horner J, DePinho RA, Alt FW (2000) Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. *Nature* **404:** 897–900

Gao Y, Sun Y, Frank KM, Dikkes P, Fujiwara Y, Seidl KJ, Sekiguchi JM, Rathbun GA, Swat W, Wang J, Bronson RT, Malynn BA, Bryans M, Zhu C, Chaudhuri J, Davidson L, Ferrini R, Stamato T, Orkin SH, Greenberg ME, Alt FW (1998) A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. *Cell* **95:** 891–902

Gennery AR, Slatter MA, Bhattacharya A, Barge D, Haigh S, O'Driscoll M, Coleman R, Abinun M, Flood TJ, Cant AJ, Jeggo PA (2004) The clinical and biological overlap between Nijmegen Breakage syndrome and Fanconi Anemia. *Clin Immunol* **113:** 214–219

Giordana MT, Duo D, Gasverde S, Trevisan E, Boghi A, Morra I, Pradotto L, Mauro A, Chio A (2002) MDM2 overexpression is associated with short survival in adults with medulloblastoma. *Neuro-oncol* **4:** 115–122

Graus-Porta D, Blaess S, Senften M, Littlewood-Evans A, Damsky C, Huang Z, Orban P, Klein R, Schittny JC, Muller U (2001) Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. *Neuron* **31:** 367–379

Herzog KH, Chong MJ, Kapsetaki M, Morgan JI, McKinnon PJ (1998) Requirement for Atm in ionizing radiation-induced cell death in the developing central nervous system. *Science* **280:** 1089–1091

Holcomb VB, Vogel H, Marple T, Kornegay RW, Hasty P (2006) Ku80 and p53 suppress medulloblastoma that arise independent of Rag-1-induced DSBs. *Oncogene* **25:** 7159–7165

Hughes-Davies L, Huntsman D, Ruas M, Fuks F, Bye J, Chin SF, Milner J, Brown LA, Hsu F, Gilks B, Nielsen T, Schulzer M, Chia S, Ragaz J, Cahn A, Linger L, Ozdag H, Cattaneo E, Jordanova ES, Schuuring E, Yu DS, Venkitaraman A, Ponder B, Doherty A, Aparicio S, Bentley D, Theillet C, Ponting CP, Caldas C, Kouzarides T (2003) EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. *Cell* **115:** 523–535

Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A (2001) Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat Genet* **29:** 418–425

Kastan MB, Bartek J (2004) Cell-cycle checkpoints and cancer. *Nature* **432:** 316–323

Kawarada Y, Miura N, Sugiyama T (1998) Antibody against single-stranded DNA useful for detecting apoptotic cells recognizes hexadeoxynucleotides with various base sequences. *J Biochem (Tokyo)* **123:** 492–498

Kennedy RD, D'Andrea AD (2005) The Fanconi Anemia/BRCA pathway: new faces in the crowd. *Genes Dev* **19:** 2925–2940

Kruman II, Wersto RP, Cardozo-Pelaez F, Smilenov L, Chan SL, Chrest FJ, Emokpae Jr R, Gorospe M, Mattson MP (2004) Cell cycle activation linked to neuronal cell death initiated by DNA damage. *Neuron* **41:** 549–561

Lee Y, Barnes DE, Lindahl T, McKinnon PJ (2000) Defective neurogenesis resulting from DNA ligase IV deficiency requires Atm. *Genes Dev* **14:** 2576–2580

Lee Y, Chong MJ, McKinnon PJ (2001) Ataxia telangiectasia mutated-dependent apoptosis after genotoxic stress in the developing nervous system is determined by cellular differentiation status. *J Neurosci* **21:** 6687–6693

Lee Y, McKinnon PJ (2002) DNA ligase IV suppresses medulloblastoma formation. *Cancer Res* **62:** 6395–6399

Lee Y, McKinnon PJ (2006) Responding to DNA double strand breaks in the nervous system. *Neuroscience* doi: 10.1016/j.neurosciece.2006.07.026

Lee Y, Miller HL, Jensen P, Hernan R, Connelly M, Wetmore C, Zindy F, Roussel MF, Curran T, Gilbertson RJ, McKinnon PJ (2003) A molecular fingerprint for medulloblastoma. *Cancer Res* **63:** 5428–5437

Lees-Miller SP, Meek K (2003) Repair of DNA double strand breaks by non-homologous end joining. *Biochimie* **85:** 1161–1173

Lieber MR, Ma Y, Pannicke U, Schwarz K (2003) Mechanism and regulation of human non-homologous DNA end-joining. *Nat Rev Mol Cell Biol* **4:** 712–720

Lim DS, Hasty P (1996) A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol Cell Biol* **16:** 7133–7143

Lomonosov M, Anand S, Sangrithi M, Davies R, Venkitaraman AR (2003) Stabilization of stalled DNA replication forks by the BRCA2 breast cancer susceptibility protein. *Genes Dev* **17:** 3017–3022

Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A (1997) Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev* **11:** 1226–1241

Marmorstein LY, Kinev AV, Chan GK, Bochar DA, Beniya H, Epstein JA, Yen TJ, Shiekhattar R (2001) A human BRCA2 complex containing a structural DNA binding component influences cell cycle progression. *Cell* **104:** 247–257

McMurray CT (2005) To die or not to die: DNA repair in neurons. *Mutat Res* **577:** 260–274

Mills KD, Ferguson DO, Alt FW (2003) The role of DNA breaks in genomic instability and tumorigenesis. *Immunol Rev* **194:** 77–95

O'Driscoll M, Jeggo PA (2006) The role of double-strand break repair—insights from human genetics. *Nat Rev Genet* **7:** 45–54

Offit K, Levran O, Mullaney B, Mah K, Nafa K, Batish SD, Diotti R, Schneider H, Deffenbaugh A, Scholl T, Proud VK, Robson M, Norton L, Ellis N, Hanenberg H, Auerbach AD (2003) Shared genetic susceptibility to breast cancer, brain tumors, and Fanconi anemia. *J Natl Cancer Inst* **95:** 1548–1551

Orii KE, Lee Y, Kondo N, McKinnon PJ (2006) Selective utilization of nonhomologous end-joining and homologous recombination DNA repair pathways during nervous system development. *Proc Natl Acad Sci USA* **103:** 10017–10022

Patel KJ, Yu VP, Lee H, Corcoran A, Thistlethwaite FC, Evans MJ, Colledge WH, Friedman LS, Ponder BA, Venkitaraman AR (1998) Involvement of Brca2 in DNA repair. *Mol Cell* **1:** 347–357

Pellegrini L, Venkitaraman A (2004) Emerging functions of BRCA2 in DNA recombination. *Trends Biochem Sci* **29:** 310–316

Pichierri P, Rosselli F (2004) The DNA crosslink-induced S-phase checkpoint depends on ATR-CHK1 and ATR-NBS1-FANCD2 pathways. *EMBO J* **23:** 1178–1187

Powell SN, Willers H, Xia F (2002) BRCA2 keeps Rad51 in line. High-fidelity homologous recombination prevents breast and ovarian cancer? *Mol Cell* **10:** 1262–1263

Preobrazhenska O, Yakymovych M, Kanamoto T, Yakymovych I, Stoika R, Heldin CH, Souchelnytskyi S (2002) BRCA2 and Smad3 synergize in regulation of gene transcription. *Oncogene* **21:** 5660–5664

Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Easton DF, Stratton MR (2007) PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* **39:** 165–167

Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, Neveling K, Kelly P, Seal S, Freund M, Wurm M, Batish SD, Lach FP, Yetgin S, Neitzel H, Ariffin H, Tischkowitz M, Mathew CG,

Auerbach AD, Rahman N (2007) Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. *Nat Genet* **39:** 162–164

Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* **273:** 5858–5868

Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* **73:** 39–85

Sekiguchi J, Ferguson DO, Chen HT, Yang EM, Earle J, Frank K, Whitlow S, Gu Y, Xu Y, Nussenzweig A, Alt FW (2001) Genetic interactions between ATM and the nonhomologous end-joining factors in genomic stability and development. *Proc Natl Acad Sci USA* **98:** 3243–3248

Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P, Bradley A (1997) Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* **386:** 804–810

Shiloh Y (1997) Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. *Annu Rev Genet* **31:** 635–662

Shiloh Y (2003) ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* **3:** 155–168

Shivji MK, Venkitaraman AR (2004) DNA recombination, chromosomal stability and carcinogenesis: insights into the role of BRCA2. *DNA Repair (Amst)* **3:** 835–843

Stiff T, Reis C, Alderton GK, Woodbine L, O'Driscoll M, Jeggo PA (2005) Nbs1 is required for ATR-dependent phosphorylation events. *EMBO J* **24:** 199–208

Suzuki A, de la Pompa JL, Hakem R, Elia A, Yoshida R, Mo R, Nishina H, Chuang T, Wakeham A, Itie A, Koo W, Billia P, Ho A, Fukumoto M, Hui CC, Mak TW (1997) Brca2 is required for embryonic cellular proliferation in the mouse. *Genes Dev* **11:** 1242–1252

Taniguchi T, D'Andrea AD (2006) Molecular pathogenesis of Fanconi anemia: recent progress. *Blood* **107:** 4223–4233

Taniguchi T, Garcia-Higuera I, Xu B, Andreassen PR, Gregory RC, Kim ST, Lane WS, Kastan MB, D'Andrea AD (2002) Convergence of the fanconi anemia and ataxia telangiectasia signaling pathways. *Cell* **109:** 459–472

Tauchi H, Kobayashi J, Morishima K, van Gent DC, Shiraishi T, Verkaik NS, vanHeems D, Ito E, Nakamura A, Sonoda E, Takata M, Takeda S, Matsuura S, Komatsu K (2002) Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells. *Nature* **420:** 93–98

Thompson LH, Schild D (2002) Recombinational DNA repair and human disease. *Mutat Res* **509:** 49–78

Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M, Matsushiro A, Yoshimura Y, Morita T (1996) Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc Natl Acad Sci USA* **93:** 6236–6240

Tutt A, Ashworth A (2002) The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. *Trends Mol Med* **8:** 571–576

West SC (2003) Molecular views of recombination proteins and their control. *Nat Rev Mol Cell Biol* **4:** 435–445

Williams BR, Mirzoeva OK, Morgan WF, Lin J, Dunnick W, Petrini JH (2002) A murine model of nijmegen breakage syndrome. *Curr Biol* **12:** 648–653

Woodburn RT, Azzarelli B, Montebello JF, Goss IE (2001) Intense p53 staining is a valuable prognostic indicator for poor prognosis in medulloblastoma/central nervous system primitive neuroectodermal tumors. *J Neuro-oncol* **52:** 57–62

Xia B, Dorsman JC, Ameziane N, de Vries Y, Rooimans MA, Sheng Q, Pals G, Errami A, Gluckman E, Llera J, Wang W, Livingston DM, Joenje H, de Winter JP (2007) Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. *Nat Genet* **39:** 159–161

Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM (2006) Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol Cell* **22:** 719–729

Yan CT, Kaushal D, Murphy M, Zhang Y, Datta A, Chen C, Monroe B, Mostoslavsky G, Coakley K, Gao Y, Mills KD, Fazeli AP, Tepsuporn S, Hall G, Mulligan R, Fox E, Bronson R, De Girolami U, Lee C, Alt FW (2006) XRCC4 suppresses medulloblastomas with recurrent translocations in p53-deficient mice. *Proc Natl Acad Sci USA* **103:** 7378–7383

Yang H, Jeffrey PD, Miller J, Kinnucan E, Sun Y, Thoma NH, Zheng N, Chen PL, Lee WH, Pavletich NP (2002) BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science* **297:** 1837–1848

Yang H, Li Q, Fan J, Holloman WK, Pavletich NP (2005) The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA–ssDNA junction. *Nature* **433:** 653–657

Yang YG, Saidi A, Frappart PO, Min W, Barrucand C, Dumon-Jones V, Michelon J, Herceg Z, Wang ZQ (2006) Conditional deletion of Nbs1 in murine cells reveals its role in branching repair pathways of DNA double-strand breaks. *EMBO J* **25:** 5527–5538

